

108 E Green Meadows Road, Suite 9 Columbia, MO 65203 PH 573.443.4100 FAX 573.443.4140 www.geosyntec.com

December 27, 2013

Shellie Chard-McClary Water Quality Division Director Oklahoma Department of Environmental Quality P.O. Box 1677 Oklahoma City, OK 73101-1677 <u>Shellie.Chard-McClary@deq.ok.gov</u>

## Subject:Statement of QualificationsOklahoma Scenic Rivers Joint Phosphorus Criteria Study

Dear Ms. Chard-McClary:

On behalf of Geosyntec Consultants, we are pleased to present you and your fellow committee members with this statement of qualifications to develop the data and science necessary for a successful conclusion of the Oklahoma Scenic Rivers Joint Phosphorus Criteria Study. Geosyntec has assembled a team of scientific and academic experts who are well-qualified to conduct this study and prepare an objective final report on water quality data and literature and, the relationships between phosphorus concentrations and ecological response levels in the Oklahoma designated Scenic Rivers.

Geosyntec Consultants has teamed with PhycoTech, University of Missouri, University of Toledo, and Portland State University to provide a technical team with deep experience. Our team has recent, relevant and practical experience in assessing phosphorus threshold response levels, evaluating algal species composition and biomass production, and following the EPA's stressor-response relationships to derive criteria that address the harmful effects of excess nutrients on waterbodies.

Our team does not have any potential conflicts of interest. No team members are based in Arkansas or Oklahoma, were engaged in the 2005 lawsuit brought by Oklahoma against poultry companies in Arkansas, or have financial involvement with any of the committee members.

The Geosyntec team offers proven water quality standards development experience, nationally recognized stressor-response researchers, a robust quality assurance program for any disputed outcomes, experienced data collection and laboratory professionals, and an interdisciplinary group to support a diverse criteria derivation approach. Further, Geosyntec has a proven track record of delivering project outcomes on time and within budget, even for projects with aggressive schedules.

Page 2

We appreciate the opportunity to submit this statement of qualifications. Please call Chris Zell, our proposed Project Manager, at 573.499.5442, or Adrienne Nemura our proposed Project Director, at 734.476.0357, with any questions regarding our submittal. We would sincerely welcome the opportunity to further discuss our qualifications and submit a proposal to support the Joint Commission in finalizing nutrient criteria in the Oklahoma designated Scenic Rivers.

Sincerely,

Chris Zell, PH Senior Consultant

Adrimi Vernun

Adrienne Nemura, P.E. Associate

cc: Dr. Song Qian, University of ToledoMr. Daniel Obrecht, University of MissouriDr. Yangdong Pan, Portland State UniversityDr. Ann St. Amand, PhycoTech

## **Statement of Qualifications**

Prepared for the Oklahoma Scenic Rivers Joint Study Committee States of Arkansas and Oklahoma



Geosyntec<sup>▶</sup> consultants





December 27, 2013

**Contacts:** Christopher C. Zell, PH Geosyntec Consultants, Inc. 573-499-5442 CZell@Geosyntec.com

Adrienne Nemura, P.E. 734.476.0357 ANemura@Geosyntec.com

## **Table of Contents**

Introduction	1
Joint Study Requirements Overview	1
Project Team	3
Geosyntec Consultants	3
PhycoTech	5
University of Missouri Limnology Laboratory	5
University of Toledo	6
Portland State University	6
Organization and Management Structure	7
Research Team Qualifications	9
Key Personnel	9
Additional Staff	13
Benefits from Selecting Geosyntec	15
Similar Projects	16
Example Reports and Journal Articles	21
Summary	23

Appendix 1: Representative Reports and Journal PublicationsAppendix 2: Additional Project ExperienceAppendix 3: Resumes



#### INTRODUCTION

Geosyntec Consultants (Geosyntec) is pleased to present this Statement of Qualifications to the Joint Study Committee consisting of representatives of the states of Arkansas and Oklahoma. The committee's objective in issuing this Request for Statement of Qualifications is to identify qualified firms that will be invited to submit a proposal to effectively and efficiently assist the committee in determining the appropriate phosphorus threshold(s) to protect the designated uses of the Scenic Rivers.



Specifically, the study is intended to determine "the total phosphorus threshold response level, in milligrams per liter (mg/L), at which any statistically significant shift occurs in algal species composition or algal biomass production resulting in undesirable aesthetic or water quality conditions in the Designated Scenic Rivers" would be expected. The timeframe for completing the study is 33 months (April 2014 to December 2016) and will likely include: synthesis of existing data, development of sampling plans, data collection, analysis, criteria derivation, and reporting tasks. Geosyntec and our team members, PhycoTech, the University of Missouri Limnology Laboratory, and academics from the University of Toledo and Portland State University, are dedicated to providing the committee with the resources and technical expertise required to meet the study objectives and timeframe of the study.

In the sections below we outline: (1) our understanding of the Joint Study requirements; (2) our understanding of the Illinois River system; (3) our proposed project team (including our organization and management structure); and (4) our research team qualifications, including the expertise and benefits the Geosyntec team brings to the committee and the study. We also present some of our team's experience in conducting similar projects, including example summary reports and peer-reviewed journal publications.

#### JOINT STUDY REQUIREMENTS OVERVIEW

The spring-fed headwaters of the Illinois River are situated in the Ozark Highlands of northwest Arkansas. The Illinois River flows west into northeast Oklahoma (i.e., multi-jurisdictional waterbody) and is the primary tributary of Tenkiller Ferry Reservoir (i.e., Lake Tenkiller) located approximately 100 miles downstream of the headwaters. This upper segment of the Illinois River is designated as a Scenic River by the State of Oklahoma under the Oklahoma Scenic Rivers Act.

Water quality data collected by investigators in the 1980's indicated that some degradation of Lake Tenkiller and the Illinois River may be occurring as a result of nutrient enrichment. Following several subsequent investigations, the Illinois River was listed by the State of Oklahoma as not meeting aesthetic uses due to excessive total phosphorus (TP). In 2002, the Oklahoma Water Resources Board (OWRB) promulgated a numeric criterion for TP in the Illinois River of 0.037 mg/L expressed as a 30-day geometric mean. In response to concerns over the TP criterion and other matters, the U.S. Environmental Protection Agency (US EPA) negotiated an agreement between the states of Oklahoma and Arkansas titled 'Statement of Joint Principles and Actions' that sets forth consensus water quality improvement measures and criteria evaluation milestones for the Illinois River. In February 2013, another agreement titled the 'Second Statement of Joint Principles and Actions' was signed by the states of Oklahoma and Arkansas to administer a three-year water quality study (*i.e.,* Joint Study) to quantify ecological changes in response to phosphorus concentrations in the Illinois River. The study seeks to quantify cause and an effect, which is often, termed a 'Stressor-Response' study. The primary objective of this study will be to quantify, characterize, or bound (i.e., multiple lines of evidence) the TP threshold(s) at which a statistically significant change occurs in algal species composition or biomass production that results in undesirable aesthetic or water quality conditions in the Illinois River.

Nested within the primary objectives above are several key questions that Geosyntec team members routinely address during water quality standards studies. Some of these key questions include:

- What are the appropriate statistical confidence levels and hypothesis tests for precautionary ecological thresholds?
- What level of deviation from a natural or least-disturbed state corresponds to a meaningful change in ecological structure and function such that designated uses are not attained (i.e., the US EPA Biological Condition Gradient)?
- Does a threshold developed to achieve aquatic life use protections under the fishable goal of the Clean Water Act correspond to protections needed for Scenic River and antidegradation compliance?
- What empirical sample size or model simulation length is needed to confidently determine magnitude, frequency, and duration components of the water quality criteria?
- What are the sources of phosphorous and how controllable are they?

To achieve the study's primary objective and assist in answering key questions, Geosyntec has assembled an interdisciplinary research team of scientists, statisticians, and engineers that have a proven track record in stressor-response analysis, aquatic ecology, and water quality standards studies.

The Geosyntec team will work with the Joint Study committee to develop an optimized and consensusbased study design that may feature one or more of the following investigative components:

- Pilot studies such as rapid periphyton surveys to characterize variances, estimate sample size, support development of conceptual models, and bound environmental gradients;
- Identification of least-disturbed reference streams and river reaches to establish baseline phosphorus levels;
- Collection of algal biomass and composition data in the Illinois River and reference reaches to characterize seasonal patterns;
- Model driven data collection and continuous simulation of periphyton dynamics;
- Discrete source in-situ nutrient manipulations (whole stream) using nutrient infused substrata; and
- Flow-through enclosure manipulations (i.e., mesocosms) along a gradient of nutrient dosage regimes.

We understand that time is of the essence. Our project team is committed to meeting the timeline and objectives of the Joint Study. Our extensive consulting experience, combined with the expertise of our academic partners, makes Geosyntec the ideal choice for meeting a tight schedule and working with stakeholders to facilitate understanding of this complex situation.

SOQ: Oklahoma Scenic Rivers Joint Phosphorus Criteria Study

SOQ: Oklahoma Scenic Rivers Joint Phosphorus Criteria Study

#### **PROJECT TEAM**

Our project team will be led by Geosyntec Consultants, and supported by subconsultants including PhycoTech, the University of Missouri Limnology Laboratory, and academics from the University of Toledo and Portland State University. The following sections provide a brief synopsis of each institution. The team's organization and management structure as well as qualifications of individual team members follows.

### **Geosyntec Consultants**

Geosyntec is a specialized consulting and engineering firm that works with private and public sector clients to address new ventures and complex problems involving our environment, natural resources, and civil infrastructure. Geosyntec has more than 1,000 engineers,

scientists, and related technical and project support personnel located in 60 offices throughout the U.S. Geosyntec regularly coordinates with top researchers around the country including faculty from North Carolina State University, the University of Alabama, Humboldt State University, Portland State University and the University of Toledo, among others, to bring leading-edge technologies and services to our clients. Geosyntec is pleased to note that over 75% of our technical staff hold advanced degrees in an engineering or environmental science discipline, an industry leading metric.

Since our founding in 1983, we have built top tier specialty practices to meet our clients' needs in:

- Environmental studies and remediation
- Water resources
- Natural resources assessment and restoration
- Engineering and design for environmental, geotechnical, and structural infrastructures
- Safety and risk evaluation, planning and mitigation

We are nationally known for our technical leadership, broad experience, and exceptional client service.

Geosyntec has built its Water and Natural Resources practice to address our clients' most challenging regulatory, sustainability, and restoration issues. Our clients include a variety of infrastructure project sponsors and resource agencies including state and local agencies, utilities, developers, municipalities, and agriculture/silviculture entities. We also consult with federal agency officials, including those at the US EPA, the Natural Resources Conservation Service, and the Federal Energy Regulatory Commission. We frequently provide our services for public interest foundations and a wide range of community stakeholders.

Our professionals continue to develop new technology applications and capabilities. Our applied research partnerships with leading universities, National Oceanic & Atmospheric Administration (NOAA), National Aeronautics and Space Administration (NASA), US EPA, Federal Highway Administration (FHWA), U.S. Geological Survey (USGS), U.S. Army Corps of Engineers (USACE), the Water Environment Research Foundation (WERF), the National Cooperative Highways Research Program (NCHRP) and others are producing better methods for the in situ remediation of recalcitrant chemicals in the environment; management of urban watersheds to reduce pollutant loadings; protection of endangered species from the impacts of stormwater runoff; design and construction of industrial and municipal waste disposal



Geosyntec<sup>▶</sup>

consultants

facilities; geotechnical analysis and design of transportation facilities; and application of data acquisition and management technologies.

Geosyntec also has considerable experience working with technical oversight committees as part of projects. Examples include:

- As part of an Integrated Planning study for Seattle Public Utilities, Geosyntec worked with a five member technical panel of academics and leading professionals to come to consensus on the technical approach for evaluating the performance of stormwater projects to meet Consent Decree requirements.
- Geosyntec is currently working on six projects, and have completed five over the past few years for the National Cooperative Highway Research Program (NCHRP), which is part of the National Academies. Each of these projects was initiated by a panel of academic, government agency and private industry experts in the transportation and environmental industries. The panel's role with each project is ensuring the project objectives are met and the resulting guidance document(s) meet their industry needs. The projects have involved nutrient fate and transport in linear corridors; watershed approaches to stormwater management and phosphorus management, among other topics.
- Geosyntec worked with an independent panel of experts during their review of a proposed stormwater best management practice (BMP) retrofit project to comply with EPA mandated treatment of PCBs coming from an industrial facility in Washington State.
- A large industrial client in Southern California was required to meet strict effluent limits from their property and wanted to implement an Engineered Natural Treatment System (ENTS) to comply with NPDES effluent limits. The client and Geosyntec, with the Regional Water Quality Control Board's review, assembled a stormwater Expert Panel consisting of six members from academia and consulting. The panel's role was to review and provide recommendations on the site-specific design criteria and ENTS designs. This work occurred at a fast pace with extensive public involvement.

Geosyntec uses state-of-the-practice tools such as remote sensing, innovative water quality models, sampling designs based on statistical analyses, hydromodification assessments, hydrodynamic models, and endangered species investigations to assess water quality impacts and develop appropriate solutions.

We are known for our innovative work in stormwater and surface water quality management; water quality standards, regulations, and policies; Total Maximum Daily Load (TMDL) studies (development and implementation); technical guidance manuals; hydromodification assessments and mitigation; watershed management; hydrology and hydraulics; flood modeling; river, estuary, lake and reservoir hydrodynamic and water quality modeling; integrated surface and groundwater modeling and management; surface and ground water monitoring and modeling; and adaptive management.

Geosyntec maintains a multidisciplinary staff of highly trained ecological professionals, fisheries scientists, wetland specialists, and engineers with extensive experience addressing issues and impacts to natural resources. Our practical solutions are based on a portfolio of projects conducted for a diversity of clients including private companies and all levels of government.

Our practitioners excel in the areas of water quality monitoring and modeling of nutrients and other pollutants in aquatic systems. This includes phytoplankton and periphyton species dynamics; site specific criteria; watershed modeling; and statistical analysis of water quality and environmental data. We also have extensive experience in large watershed hydrology and pollutant load modeling, TMDL development and implementation, use attainability analyses, regulatory compliance and planning; and technical review of regulatory policy changes.

## PhycoTech

PhycoTech is the only commercial lab in North America to utilize a unique proprietary permanent mounting technique for archiving and preparing water samples for algal enumeration.

Dr. Ann St. Amand, president and algal taxonomist, has 29 years of experience and has processed over 33,000 samples from both freshwater and marine systems from all over North America. She also is Part Coordinator for Part 10000, Biological Examination, and Joint Task Group (JTG) Chair for Section 10200, Plankton, for Standard Methods for Examination of Water and Wastewater. PhycoTech normally processes between 2,000 and 3,000 samples per year. We have processed several state wide surveys in the Mid-West and Florida. PhycoTech also consults with federal and state agencies, including the US EPA, the USGS, the Bureau of Reclamation, and the USACE on experimental design and quality assurance /quality control (QA/QC) issues. We process samples for general water quality, zooplankton, macroinvertebrates, as well as for invasive, toxic and taste/odor producing algae.

PhycoTech utilizes its proprietary, custom written data management software, ASA (Aquatic Sample Analysis) system. This unique, powerful program tracks samples from receipt to data delivery within the same software program, providing a myriad of diversity and water quality indices (including all of the indices contained within the Rapid Bioassessment Protocol, US EPA). With ASA, we are able to provide the most complete data available commercially. Our equipment includes several research grade Olympus microscopes equipped with differential interference contrast (DIC), Phase, reversion-induced LIM protein (RIL) and epifluorescence, as well as full fume hood facilities for ash free dry weight determinations and acid cleaning diatom samples.

PhycoTech has a strong commitment to educational outreach and maintains three educational saltwater reef tanks in the Saint Joseph School District. We partner with Upton Middle School sixth grade teachers on the UpStream project, a hands-on stream ecology program with in-class and field modules and a 7<sup>th</sup>-8<sup>th</sup> grade after-school club; Science Olympiad and Salmon in the Classroom.

### University of Missouri Limnology Laboratory

The School of Natural Resources (SNR) in the University of Missouri (MU) is one of the leading educational institutions in the nation emphasizing an integrated approach to natural resource management. SNR is a division of the College of Agriculture, Food and Natural Resources. SNR programs are ranked among the top 10 percent of similar programs nationally. With approximately 500 undergraduates and 50 faculty members,



PhycoTech, Inc.

SNR is noted for excellent education, strong professional orientation, active student organizations and outstanding advising.

One of SNR's facilities includes the Anheuser-Busch Natural Resources Building which houses MU's Limnology Laboratory. Researchers at the laboratory are involved in quantifying factors regulating the abundance and distribution of algal biomass in freshwater systems such as reservoirs, lakes, streams and rivers, and to evaluate the strength of these relationships at various spatial and temporal scales. The lab has generated one of the largest long-term data sets focused on trophic state conditions in lentic systems, with over 250 water bodies having been monitored, some for up to 30 years. The lab has experience with analyzing samples from low nutrient systems, having performed nutrient analyses for the Ozark Scenic Riverways located in southern Missouri.

## **University of Toledo**

The University of Toledo's Department of Environmental Sciences is an interdisciplinary group of ecologists and geologists whose research and teaching interests address human impacts on the environment, Earth surface processes, and ecosystem science. We are committed to providing all students with an excellent educational experience that includes real-life problem solving, and field and laboratory work. Our department was

founded in 2000 with the primary goal of expanding interdisciplinary research on the Earth and our environment. In 2008, we were accepted into the prestigious Association of Ecosystem Research Centers. Each year about 50 graduate students receive training in their department and about 40 percent of them are supported by extramural grants. We provide both MS and Ph.D. degrees and strive to give students the training they need to go on to careers in research, natural resource management, other environmental fields, and industry. Students work closely with a faculty advisor and committee. Graduate students become part of a laboratory group that provides an environment of peer-enriched learning and experience.

## **Portland State University**

Portland State University is recognized throughout the world for programs like Urban Planning, Social Work, and Environmental Studies that directly engage the community, and aim our students

towards the creation of a better, more sustainable world. The goal of the Department of Environmental Science and Management (ESM) is to conduct high quality research on, and teach effectively about, the effects of and solutions to critical anthropogenic stressors to ecosystems.

The ESM Department focuses on the study of the interactions between society and the physical, chemical, ecological, and biological processes that structure and maintains ecosystems. Our work is critical to understanding and developing sustainable ecosystems, human societies, and economies. The ESM Department studies the processes that link terrestrial and aquatic ecosystems, consequences of human alteration of those linkages, and development of policies to manage human interaction with the environment. We conduct research by studying organisms and specific linkages and processes across systems and by studying interactions between organisms, processes, and linkages in a specific ecosystem or watershed, such as the Columbia River Basin. We teach the concepts of ESM to precollege and professional as well as to undergraduate and graduate students.





6

#### **ORGANIZATION AND MANAGEMENT STRUCTURE**

In addition to having a strong research team, Geosyntec understands the importance of having a strong management team in place for successful project delivery. Geosyntec regards technical competence as a key factor in managing a project. We also believe that a highly-qualified technical project manager must be supported by other personnel to provide the leadership, organization, financial management and effective communication needed for success of complex projects.

Throughout the lifetime of the project, Geosyntec will work closely with the committee and our subconsultants to ensure project meets the committee's objectives and that issues potentially affecting progress and budget are communicated as quickly as possible. This early communication will help ensure that problems can be avoided or minimized and, if the need arises, a collaborative adjustment to the project can be implemented. Geosyntec recognizes that good communication is the key to good project management and is committed to working with the committee at all levels to ensure a satisfactory outcome. Geosyntec will provide regular biweekly status reports to the committee's project manager. Other meetings (including webinars) will be held as needed for project coordination or to discuss emerging issues. Our project management approach involves the successful implementation of:

- Project organization, leadership, and subconsultant management;
- Communication and coordination;
- Scope, cost, and schedule monitoring and control;
- Peer review and quality control of project deliverables; and
- Health and safety

Chris Zell will serve as Project Manager (PM) and the main point of contact for the committee. He will develop the project scope and budget, oversee its implementation, and coordinate across the technical leads to oversee and monitor the scope, budget, and schedule using systematic project management controls. Mr. Zell's duties include, but are not limited to, allocation of staff to project tasks, scheduling, periodic review of project performance, and keeping regular communication with the committee.

Adrienne Nemura, P.E. will serve as Project Director and will be responsible for overall quality assurance and cost control, and for assuring the project is carried out to the committee's satisfaction. Additionally, Ms. Nemura will support the PM on technical and project implementation matters so as to provide for a second point of contact between Geosyntec and the committee when the PM is unavailable.

Julie Caprio will serve as lead for Quality Assurance for the project. Ms. Caprio's role will be to review data collected in the field, ensure the Quality Assurance project plan is developed and implemented, and review final work products and interim products to so that the committee receives the highest quality deliverables. Her more than 25 years of experience in project management and quality assurance make her the ideal person for leading quality assurance for this project.

The team assembled for this project brings the depth and breadth of experience and expertise necessary to perform this work efficiently and professionally. An organizational chart with project roles for key personnel staff is provided below followed by brief biographies (biosketch) that describe their background, experience, and key attributes. Additional professional technical support staff are available to assist each key task leader with all elements of this project and are listed below in the staff table.





#### **RESEARCH TEAM QUALIFICATIONS**

## **Key Personnel**

Below are brief biographical summaries for the project manager, project director, and key personnel; and their project role. Two-page resumes containing additional details of each team member's attributes (years of experience and past project experience) are included in Appendix 3.

<b>Chris Zell, MS, PH</b> Geosyntec Title: Senior Consultant		<b>Proj</b> e Year	ect Role: Project Manager & Experimental Design Lead / 15 s of Relevant Experience
Education: MS, Natural Resources Certifications: Professional Hydrologist	<b>Biosketch:</b> Mr. Zell maintains a diverse background in environmental and regulatory analysis that includes coordination of Missouri's water quality standards program, lead technical developer of water quality-based permit limits and waste load allocations (125+), and principal investigator of nutrient, bacteria, and dissolved oxygen TMDL studies. Mr. Zell continues to develop and implement regulatory modeling and policy analysis solutions to meet emerging water quality challenges of Geosyntec's clients including development of the only site-specific water quality standards in Missouri approved by EPA Region 7. In many of these projects, Chris was responsible for project management, task lead, work deliverables, and schedule and budget compliance. He is an effective oral and written communicator and strives to exceed expectations.		
Adrienne Nemura, MS, PEProject Role: Project Director / 29 YearsGeosyntec Title: AssociateExperience		<b>Project Role: Project Director</b> / 29 Years of Relevant Experience	
Education: MS, Civil Engineering Certifications: Professional Engineer	<b>Biosketch:</b> Ms. Nem information to help i She began her caree James and Appoma Governments she w governments, three regional water quali Subcommittee. She estuaries and helped nuisance blue-green numerous fate and t estuaries; critically r state agencies to ev criteria; and has wo attends and support Association of Clear Council.	intra infor er at attox was stat ity is was d dev alga crans revie valua orkec ts th n W	s expertise is in translating scientific studies and engineering m technical and policy decisions under the Clean Water Act. t the Virginia Water Control Board as the modeler for the k Rivers. At the Metropolitan Washington Council of responsible for serving as the liaison between 18 local te water quality agencies and EPA Region 3 on local and ssues, including serving on the Chesapeake Bay Modeling s the chief modeler for the Potomac and Anacostia River velop site-specific kinetics for sediment oxygen demand and al growth. In private consulting she has conducted or led port studies of nutrients and bacteria in streams, rivers and wed numerous TMDLs; represented clients in working with the appropriate water quality standards, including nutrient I on other Clean Water Act issues. Ms. Nemura routinely e Association of Clean Water Administrators, the National ater Agencies, and the US Conference of Mayors Water

consultants

		Conducting	
Julia Klens Capr Geosyntec Title	<b>io, MA, MBA</b> : Associate	<b>Project Role: Quality Assurance Lead</b> / 25 Years of Relevant Experience	
Education: MBA, Quality Management MA, Organizational Management Certifications: ASQ Certified Quality Manager Certified Environmental Field Sampler NQA-1 Lead Auditor	<b>Biosketch:</b> Ms. Caprio is an Associate specializing in Quality Assurance and has over 25 years of experience in the environmental field. Currently she specializes in project quality management, preparation and review of quality assurance project plans (QAPPs); quality assurance management plans (QMPs), field sampling plans, data verification, data evaluation, data validation, QA audits including laboratory audits and project on-site field audits. Her data validation experience includes chemical, radiological and geotechnical parameters for media including but not limited to sediment, soil, groundwater, surface water, biota, soil vapor and air monitoring. Ms. Caprio also provides both in-house quality assurance training through the various office locations within Geosyntec and outside quality assurance training for clients. She has over 20 years of experience as an analytical chemist in environmental and biotechnology laboratories including laboratory management, data management, quality control/quality assurance, and supervision of wet chemistry, gas chromatography, and high performance liquid chromatography departments.		
Marc Leisenring Geosyntec Title	Aarc Leisenring, MS, PEProject Role: Data Analysis Lead / 12 Years of RelevaGeosyntec Title: Senior EngineerExperience		
Education: MS, Civil & Environmental Engineering Certifications: Registered Civil Engineer	<ul> <li>Biosketch: Mr. Leisenring has 12 years of water resources and urban stormwat quality project experience that includes a focus on urban hydrology, water quality and BMP research and performance. Mr. Leisenring has a strong background in the analysis and summary of spatial and temporal environmental data with the ability manage and query large databases and perform advanced statistical analysis techniques. He is an effective oral and written communicator, provides support and direction to junior staff, and strives to exceed the expectations of his clients are colleagues.</li> </ul>		
Song Qian, PhD University of To Professor, Depa Environmental S	ledo Title: Assistant rtment of Sciences	<b>Project Role: Stressor Response Lead</b> / 15 Years of Relevant Experience	
Education: PhD, Environmental Sciences MS, Statistics	<b>Biosketch:</b> Dr. Qian H environmental and ecolo ecological risk assessme and consulting, is focu modeling both for rese teaching career coveri management, environm assessment. Dr. Qian	has been engaged in the research and practices of ogical statistics, water quality modeling and assessment, and nt for over 15 years. His work, including teaching, research, used on environmental and ecological data analysis and arch and for environmental management. He has a long ng environmental science, water quality modeling and ental and ecological data analysis and modeling, and risk is known for his statistical skill reflected in his published	

textbook on environmental and ecological statistics and his upcoming book on the

consultants

statistical issues in setting and evaluating the compliance of environmental standards, modeling phosphorus retention in the everglades wetlands, detecting and quantifying ecological thresholds, watershed modeling for TMDL development, drinking water standard compliance study, effects of urbanization on stream ecosystem, environmental engineering, and various ecological topics. His research is focused on the development and adaptation of statistical modeling methods that are suitable for applied problems. He developed the Bayesian hierarchical model for the US EPA for assessing drinking water standard compliance; introduced the "hockey stick" model as a tool for developing numerical phosphorus criterion for the everglades; applied the seasonal trend analysis using loess for assessing long term trends in nutrient concentrations in the Neuse River Basin; developed the Bayesian SPARROW model, introduced the multilevel models to study the effects of urbanization on stream ecosystem; introduced the use of the change point model as a tool for nutrient criterion development; and introduced the use of several advanced statistical tools (such as multinomial regression, zero-inflated regression) for analyzing species compositional data. Dr. Qian has published over 60 peerreviewed journal articles, and numerous book chapters and conference presentations.

Rob Annear, PhD, PE	Project Role: Modeling Lead / 16 Years of Relevant
Geosyntec Title: Senior Engineer	Experience

Education:	Biosketch: As a water resources engineer, Dr. Annear is principally involved in
PhD, Civil & Environmental Engineering MS, Civil Engineering <b>Certifications:</b> Environmental Engineer (PE) Graduate Certificate in Hydrology	hydrodynamic and water quality modeling with a focus on regulatory permits and requirements, stormwater management, surface water system assessments, TMDL development and implementation, Endangered Species Act (ESA), nutrient criteria studies, and water quality management for multiple uses (supply, salmon, recreation etc.). He has over 16 years of experience in the development and calibration of hydrodynamic and water quality models (1-D, 2-D, and 3-D) throughout the U.S. His experience includes reviewing 2-D hydrodynamic and sediment transport and fate & transport models of riverine and estuarine systems. He has also served as an Expert Witness in cases involving hydrology; water rights; and hydrodynamic, sediment transport and chemical fate and transport modeling. He is Affiliated Faculty at Portland State University, teaching undergraduate and graduate courses and has more than 10 years of experience teaching water quality modeling training workshops. Dr. Annear has considerable experience in leading multidisciplinary teams of professionals, managing projects, budgets, work flow processes, quality control and assurance, on call contracts and complex project implementation and developing monitoring plans and conducting field work. He has conducted numerous peer reviews of surface water models for agencies such as the U.S. EPA, Oregon DEQ, WA Dept. of Ecology, and the U.S. Bureau of Reclamation and has also served as a reviewer for various water resource and hydrologic journals and has served on EPA national water quality grant review panels.

Randy Crawford, MA		<b>Project Role: Data Collection Lead</b> / 35 Years of Relevant Experience		
Education: MA, Biology	<ul> <li>Biosketch: Mr. Crawford has more than 35 years of experience conducting and managing water quality and biological monitoring assignments throughout Missouri, including Big River Experience. Prior to joining Geosyntec Consultants, Mr. Crawford managed a group of 15 environmental professionals and technicians responsible for all the Missouri Department of Natural Resources (MDNR) water quality and biological monitoring. Since joining the firm, he has successfully managed some of Geosyntec's most intense data collection efforts.</li> </ul>			
<b>Steve Layman,</b> I Geosyntec Title	PhD Associate	<b>Project Role: Aquatic Ecology Lead</b> / 32 Years of Relevant Experience		
<b>Education:</b> PhD, Biological Sciences MS, Ecology	<b>Biosketch:</b> Dr. Layman, a senior environmental scientist focuses on the characterization, assessment, and mitigation of potential impacts to water resources associated with urban and industrial development projects. He specializes in applying aquatic biology and ecosystem management principles to water resources projects in the Southeast and eastern North America. He leads ecological assessments and manages projects associated with watershed assessments, water resources permitting, National Environmental Policy Act (NEPA) reviews, NPDES compliance, Federal Energy Regulatory Commission (FERC) hydropower licensing, ESA compliance, and ecosystem management.			
Daniel Obrecht, MSProject Role: Nutrients Laboratory Lead / 20 YearUniversity of Missouri Title: Senior Research Specialist, Fisheries & Wildlife SciencesProject Role: Nutrients Laboratory Lead / 20 Year				
Education: MS, Fisheries & Wildlife	<b>Biosketch:</b> Mr. Obrech laboratory for over 23 y aspects of water quality implementation of qua report writing. Mr. Obr processes. He has condu- distribution of algal biom between nutrients and a included a long-term stu- the physiography and hu- research examining the lakes along with metric among-system variation through March 2008 M nutrient criteria for rese- member of the scientific He was the lead auth presented to Missour	t has worked for the University of Missouri limnology ears. During this time he has garnered experience in many or monitoring, including the design of monitoring programs, lity control and assurance measures, data analyses, and echt focuses his research on limnology and aquatic ecology ucted research to quantify factors regulating abundance and hass in freshwater systems and to determine the relationship algal biomass in lakes and reservoirs. His research has also udy showing the trophic state of Missouri reservoirs reflects uman alteration of their drainage basins. He also conducted proportion of cropland cover in the catchment of artificial is of morphology and hydrology account for much of the in both phosphorus and nitrogen. From October 2005 Ar. Obrecht took part in the development of Missouri's ervoirs. He served initially as a stakeholder and then as a c committee that fashioned Missouri's approach which was i's Clean Water Commission and submitted to EPA.		

Ann St. Amand, PhD, CLP		Project Role: Algae Laboratory Lead / 33 Years of Relevant	
PhycoTech Title: President		Experience	
Education: PhD, Aquatic Biology BSc, Ecology Certifications: Certified Lake Professional	Experienceation:Biosketch: Ann St. Amand, President of PhycoTech Inc. coordinates Part 1000AquaticBiological Examination of Standard Methods. Dr. Amand has been involved in managing lakes across the U.S. since 1990, specializing in aquatic sample analys with an emphasis on freshwater phytoplankton, periphyton and zooplankton. Sh has processed over 33,000 freshwater and marine aquatic samples in her career and has co-chaired a workshop on freshwater algal identification at the annual NALN symposium since 1991. She also serves on several technical and education committees at the local and national level, including the Indiana Blue-Green Alg Task Force, the NALMS Blue-Green Initiative, and the UpStream program with the S Joseph, Michigan public school district. Dr. Amand has been a NALMS Certified Lak Professional since 2003. She earned her B.Sc. in ecology from Purdue University an her Ph.D. from the University of Notre Dame in Aquatic Ecology. In addition, sh completed two postdoctoral positions, one in surface water/groundwate interactions and the other in PCB interactions in stream systems.		
Yangdong Pan, PhDProject Role: Aquatic Ecologist / 30 Years of RelevePortland State University Title: Chair, Environmental Science andExperienceManagementFrom the second s			
<b>Education:</b> PhD, Biology MS, Biology	<b>Biosketch:</b> Dr. Pan chairs the Department of Environmental Science and Management at Portland State University. His research centers on water resource science and conservation. Specifically he uses algal assemblages to monitor and assess ecological risk in freshwater ecosystems including both lotic and lentic systems. He and his associates have participated in several national surface water quality programs such as the US EPA's Environmental Monitoring and Assessment Programs (EMAP) in the Mid-Atlantic Region and in the western U.S. with a leading role on algal indicators development. Recently, he has been collaborating with Chinese environmental professionals on several water-quality projects in the Yangtze Delta region including drinking water protection for the city of Shanghai. He teaches two graduate-level courses on univariate and multivariate environmental and biological data analysis.		

## Additional Staff

In addition to the Key Personnel listed above, the following additional Geosyntec staff will be available to support this project, as needed, to meet project objectives. If selected, the need for, and level of involvement, will be identified during negotiation of the scope of work.

Name	Credentials	Years of Experience		Project Role
		Geosyntec	Total	
Tom Wallace	MS, Environmental Health Science	4	33	Assistant Project Manager
Misty Huddleston	PhD, Natural Resources; MS, Wildlife & Fisheries Sciences; Certified Wetland Delineator	1	10	Ecological Data Collection and Analysis
Cristin Corless Krachon	MS, Environmental Health Sciences	13	15	Ecological Data Collection and Analysis
Nick Muenks	BS, Biology; Hydrologic Technician II	4	12	Hydrologic Data Collection
Keith Tolson	PhD, Toxicology; MS, Food Science and Human Nutrition	9	19	Water Quality Standards Development
David Carani	MS, Natural Resources; MPA, Public Administration; Aquatic Macroinvertebrate Identification Program certification		10	Water Quality Standards Development
Dan Pankani	Pankani BS, Civil Engineering & Computer Science; Registered Civil Engineer		15	Data Management, Database Development
Cathy Crea	rea MSc, Mathematics & Statistics		9	Statistical Analysis
Tom Fendley BS, Civil Engineering; Professional Engineer		2	30	Ecological Data Collection and Analysis
Paul Hobson	Paul Hobson MS, Civil Engineering; Engineer-in-Training		9	Statistical Analysis, Data Management, Database Development
Cara Poor, PhD	PhD, Civil Engineering; MS, Environmental Engineering; Civil Engineer		15	Water Quality Standards Development
Aaron Poresky	BS, Civil Engineering; BS, aron Poresky Environmental Engineering; Civil Engineer		9	Water Quality Modeling Support, Water Quality Standards Development

consultants

Name	Credentials	Years of Experience		Project Role
		Geosyntec	Total	
Brian Apple	MS, Environmental Systems; Engineer-in-Training	1	12	Water Quality Modeling Support
Adam McGuire	BS, Environmental Resources Engineering; Engineer-in- Training	1	14	Water Quality Modeling Support

#### **Benefits from Selecting Geosyntec**

We understand that some field data collection, data analyses and interpretation will be needed to conduct a study to quantify ecological changes in response to phosphorus concentrations in the Illinois River. The committee needs to feel confident that the study will be conducted using good science and will result in a final report with relevant guidance on the phosphorus criteria components and threshold level(s). We also understand that adequate time must be provided to the committee and others to review and comment on the draft report(s) to help make sure that statements are technically justified and are appropriately qualified. Having conducted several multi-year ecological studies, the team fully understands the necessary coordination, logistics, and quality assurance needed to produce findings that are robust and durable. Further, we readily acknowledge and appreciate the significance of conducting such a study and the implications to both states as they work to cost-effectively improve and protect water quality in the Illinois River system.

Geosyntec has assembled a team of key experts to conduct the study in an efficient and cost-effective manner. The team consists of professional staff that possesses a unique combination of talent, regional experience, and nationallyrecognized expertise in water quality standards and nutrient criteria development. These characteristics, coupled with the strength of our project management, health & safety, and quality assurance systems, allow Geosyntec to understand the needs of the committee and bring to bear the best practices in a manner that will meet project objectives.

We have included experts in aquatic ecology focusing on diatoms and periphyton responses to phosphorus and nutrient loading; water quality modeling; algal community composition testing; and ecosystem stressor-response to shifts in nutrient levels. The team has extensive experience in field data collection; data

#### **Geosyntec Team**

- Interdisciplinary Team Supports Diverse Criteria Derivation Approach
- Proven Water Quality Standards Development Experience
- Nationally Recognized Stressor-Response Researchers
- Robust Corporate Quality Assurance Program for Potentially Disputed Outcomes
- Experienced Professional Data Collection and Laboratory Team

analyses, including statistical analyses and threshold response; numerical modeling; numeric criteria development and management; quality assurance for field data collection; and regulatory compliance. Our team provides key experts with a deep bench of expertise to support the project.



As a national leader in water and natural resources management with intimate knowledge of challenges facing the Illinois River watershed, Geosyntec is positioned to deliver effective, high-quality services to the committee. The expertise of our project team, combined with the experience gained working on projects, such as the Ecosystem Threshold Technical Review in the State of Connecticut and Numeric Nutrient Criteria Review in Ohio, will help ensure a high level of service to the committee. Furthermore, our extensive quality assurance planning and regulatory experience allows us to develop a project plan that will meet the multi-state project objectives. Finally, our regional presence and past experience in water quality standards and nutrient impacts will facilitate collaboration and will allow us to quickly respond to project challenges that may occur.

#### SIMILAR PROJECTS

The following table provides brief descriptions of similar projects performed by the Geosyntec team. These are further described in Appendix 2.

Regulatory	Support and Biomonitoring	Team Staff: Chris Zell		
Services	nt: City of Dontonyillo			
Owner Cilei	nt: City of Bentonville,			
AIKalisas				
Scope	The City retained Geosyntec i	to apply our technical and regulatory expertise to satisfactorily		
	Geosyntec reviewed the histor	L. Working alongside the City of Bentonvine management team,		
	quality assessment staff to b	etter characterize aquatic life conditions in Town Branch. In		
	addition to providing biologica	I and water quality monitoring services, Geosyntec assessed the		
	potential challenges of meeting	g stringent phosphorus waste load allocation targets in municipal		
	stormwater runoff. The project	ct consisted of working closely with ADEQ staff to re-evaluate the		
	need for additional phosphorus	s removal to assure that the City's resources are not expended on		
	expensive treatment plant upg	rades that may have little if any water quality benefit.		
	Following an in-depth review	of both the TMDL and available historical data, Geosyntec		
	identified several significant data gaps that should be addressed to more accurately determine			
	Geosyntec developed a quality assurance project plan (OAPP) and conducted an assossment of			
	water quality and the aquatic community in Town Branch and previously identified reference			
	streams. The assessment included collections of periphyton, continuous water levels and flows.			
	and macroinvertebrates using multiple methods. Geosyntec evaluated the data collected during			
	the preliminary assessment.	Geosyntec continues to work closely with the City and ADEQ to		
	resolve the issues regarding the	e TMDL.		
Analysis of	Phytoplankton – Diatom	Team Staff: Ann St. Amand		
Only				
Owner Clie	nt: Massachusetts			
Departmen	t of Environmental Protection			
Scope	Prepared slides from preser	ved stream periphyton samples collected by the client and		
	provide analysis reports for D	atoms as well.		

Geosyntec<sup>></sup>

Analysis an	d I.D. of Phytoplankton and	Team Staff: Ann St. Amand	
Periphyton			
Owner Clier	nt: Purdue University		
Scope	Prepared slides from preserve	ed water samples collected by the client and provide analysis	
	reports. Results were used i	n the Baseline Ecological Investigation Report LTB CKD Release	
	Site which was prepared for the	ne US EPA.	
Evaluation	of Periphyton-Environmental	Team Staff: Yangdong Pan	
Gradients i	n Western Streams		
Owner Clier	nt: U.S. Environmental		
Protection	Agency		
Scope	As part of the US EPA's Enviro	nmental Monitoring and Assessment Program (EMAP) Western	
	Pilot Survey, more than 1,500	) stream/river sites in the 12 western US states were selected	
	using a spatially balanced p	robabilistic design. In each site, algai assemblages, water	
	characterized in this study	we enumerated and identified benthic algal assemblages	
	performed OA/OC on algal s	pecies compositional data, and compared different US EPA's	
	algal sampling protocols.	Ve then evaluated algae-environmental relationships in the	
	western US streams/rivers u	ising different statistical methods to develop diatom traits,	
	indicators for assessment of b	iological condition and indicators for diagnosing stressors.	
Algae Biom	onitoring and Assessment of	Team Staff: Yangdong Pan	
Central Cal	ifornia Coast Watersheds		
Owner Clie	nt: California State University		
Monterey E	, Bay		
Scope	Nutrient enrichment is a le	eading cause of water quality impairment and threatens	
	beneficial uses in many stre	ams of the Central California Coast. The purpose of this	
	project was to produce prec	dictive models and an algae index of biotic integrity (IBI) to	
	help develop reference-and	effects-based nutrient targets and to help develop biological	
	criteria that reflect the effect	ts of nutrients on streams.	
Narrative N	Iutrient Criteria and	Team Staff: Adrienne Nemura	
Phosphoru	s Limits		
Owner Clier	nt: Connecticut Municipalities		
Scope	The Connecticut Department of	of Energy and the Environment (DEEP), in response to pressure	
	from US EPA Region 1, develop	ed and revised a "Phosphorus Reduction Strategy for Inland Non-	
	Tidal Waters". The municipalities wanted to know if the state's methodology was scientifically		
	sound or whether there was justification for less stringent phosphorus limits (0.7 milligrams per liter). The municipalities also wanted assistance in working with the state to develop an		
	alternative strategy to meet the intent of the state legislation. Geosyntec was retained to review		
	and comment on the state's methodology on behalf of the Cities of Danbury and Meriden and		
	the Towns of Wallingford and Southington. The state agreed to issue permits with interim limits		
	the municipalities could meet and final effluent limits based on the state's existing methodology,		
	with language that these limits could be revised based on new information. The state legislature		
	directed that DEEP work with stakeholders. A coordinating committee has been established to		
	update the state's strategy. The coordinating committee is being supported by a nonpoint source		
	workgroup, and the Connecticu	t Academy of Science and Engineering.	
	<b>C 1</b>		



Ohio Troph	ic Index Condition Review	Team Staff: Adrienne Nemura	
and Implementation			
Owner Clier	<i>wner Client:</i> City of Lima, Ohio		
Scope	The Ohio Environmental Protection Agency (OEPA) has been developing a trophic index		
	condition (TIC) to aid the state	in assessing attainment of aquatic life uses and address concerns	
	about excessive instream nutri	ent concentrations. OEPA conducted early stakeholder outreach	
	to obtain comments on the pro	posed TIC and draft implementation procedures. The City of Lima	
	was concerned the TIC might	be adopted into the state's water quality standards regulations	
	was retained to review and co	ment on the state's methodology on behalf of the City of Lima	
	The state agreed to form a ter	chnical advisory group and Geosyntec is representing the state's	
	small POTWs on the TAG.		
Site-Specifi	c Dissolved Oxygen Criteria	Team Staff: Chris Zell	
and Waste	load Allocation Modeling		
Study			
Owner Cliei	nt: City of Blue Springs,		
Missouri			
Scope	To accommodate anticipated g	rowth around the Kansas City metropolitan area, the City of Blue	
	Springs (City) planned to expan	d and upgrade their wastewater treatment facility (WWTF), which	
	discharges into Sni-A-Bar Cre	ek. Perennial reaches of Sni-A-Bar Creek were included in	
	Missouri's impaired waters list	due to low dissolved oxygen (DO) concentrations. Geosyntec and	
	during summer conditions as a	result of shallow depths, high residual sodiment evygen demand	
	low reagration and transients	stagnant features such as beaver dams and backwater areas. To	
	permit expansion of a WWTF t	hat discharges to impaired river reaches, state regulatory policies	
	require intensive water quali	ty study and evaluation of attainable conditions. Geosyntec	
	evaluated water quality condi	tions within the basin, developed site-specific dissolved oxygen	
	targets, and performed water	quality modeling to assist in wastewater planning and permitting	
	efforts. Geosyntec prepared and implemented a site-specific DO criteria study in conjunction		
	with the Waste Load Allocation (WLA) studies. Using GIS, Geosyntec worked to select suitable		
	reference streams that best e	xhibited natural DO conditions in the region and confirmed the	
	choices with physical and biolo	gical habitat assessments. Geosyntec then conducted extensive,	
	long-term, diel DO measurem	ents and field studies. Results from the study confirmed that	
	and ultimately formed the ba	eciuded attainment of Missouri's DO chiefia on a consistent basis	
	Missouri's first antidegradation	reviews	

Main Ditch	n Aquatic Life Use	Team Staff: Chris Zell	
Attainability Analysis			
Owner Client: City of Poplar Bluff,			
Missouri			
Scope	Main Ditch is a channelized irrigation canal that was dredged and straightened during the early 1900's to reduce flooding and improve agricultural production in the southeast region or Missouri. Intermittent reaches of Main Ditch receive treated effluent from the City of Popla Bluff, Missouri. Historically, periodic and intensive water quality surveys indicated that dissolved oxygen (DO) concentrations in Main Ditch were frequently below the 5.0 milligram per lite (mg/L) water quality criterion necessary to support aquatic life use designations. As a result, the Missouri Department of Natural Resources (MDNR) initiated total maximum daily load (TMDL activities in 2002. The draft TMDL, issued in 2005, concluded that the city needed to implement significant, and potentially unaffordable, upgrades to their wastewater treatment facility to attain water quality standards in Main Ditch. The city retained Geosyntec to review the technica validity of these conclusions and evaluate the applicability of implementing use attainability analysis (UAA) and site-specific DO criteria flexibilities in the context of the TMDL conclusions Geosyntec successfully coordinated and lead technical workgroup meetings between state federal, and local regulatory agencies which resulted in a consensus-based approach to UA/ development activities. The Missouri Clean Water Commission approved Geosyntec's site specific dissolved oxygen criteria for Main Ditch in 2012. When these criteria are confirmed by the US EPA the city will save over \$50 million in unnecessary treatment upgrades.		
Two-Mile	Two-Mile Prairie Stream Evaluation Team Staff: Chris Zell		
<i>Owner Client:</i> City of Ashland, Missouri			
Scope	The Two-Mile Prairie region bet area located within ecologically s ecoregion. Geosyntec conducted to evaluate the relative contribu- predicted dissolved oxygen and a the FOEA, Geosyntec extensively flow conditions to reliably and ac conducted several intensive wat (including bottom algae, or per verification. To create a more re- conducted during both summe continuously monitored best-av- dissolved oxygen in the study re- creator of the Qual2K water que documented the significant im- dissolved oxygen balances and per decrease wastewater treatment of	ween Columbia and Ashland, Missouri is a growing suburban sensitive watersheds on the northern edge of the Ozark Plateau la First-Order Error Analysis (FOEA) of previous modeling efforts ation of individual model parameters to the overall variance of ammonia concentrations. To address uncertainties identified in evaluated stream travel time and hydrogeometry under varying ccurately describe model hydraulic parameters. Geosyntec also ere quality surveys during which continuous and discrete data eriphyton) were collected to aid in model calibration and obust dataset and increase model accuracy, these studies were er and winter seasons. Geosyntec identified, verified, and vailable reference streams to determine highest attainable egion. Geosyntec worked closely with Dr. Steve Chapra, co- ality model, on the Two-Mile Prairie project. Geosyntec also pact that low periphyton densities have on shallow-stream roposed site-specific DO criteria which, when implemented, will costs for the City.	

The following table provides a list of very brief project snapshots of nutrient-related projects performed by the Geosyntec team. Full page project descriptions are provided in Appendix 2 for those projects marked with an asterisk and underlined in the table below.

Project Name	Client	Description and Notable Accomplishments	
Missouri Innovative Nutrient Trading (MINT) Project	USDA-NRCS	The MINT project resulted in a nutrient trading policy document that features a comprehensive analysis of clean water act policy challenges in the context of real-world trading scenarios. The report can be found at <u>http://www.geosyntec.com/UI/Default.aspx?m=ViewProject&amp;p=208</u>	
Evaluating and Practicing Innovation (EPIC)	USDA-NRCS	This project focused on the development of conceptual and statistical study designs and engineering plans to support performance monitoring of multiple agricultural Best Management Practices (BMPs). Future phases will include monitoring and modeling of BMPs to support development of sizing criteria.	
<u>Town Branch</u> <u>Phosphorus</u> <u>TMDL*</u>	City of Bentonville, AR	Initial TMDL conclusions were did not fully incorporate or consider existing water quality and biological conditions. Geosyntec revised the TMDL to reflect a more data-driven phosphorus requirement and is currently negotiating the revision with EPA Region 6.	
Bear Creek Nutrient TMDL	City of Kirksville, MO	Incorporated EPA's Integrated Urban Planning approach into an implementation plan to meet nutrient TMDL requirements and guide infrastructure upgrade schedules.	
Cahokia Canal Dissolved Oxygen and Nutrient TMDL	City of Collinsville, IL	Provided a technical review of the impairment decision methodology, modeling activities, and waste load allocations proposed by the state agency. As a result of these efforts, the TMDL was rescinded.	
<u>Ohio Numeric</u> <u>Nutrient Criteria</u> <u>Review*</u>	City of Lima, OH MWH America, Inc.	Reviewed Ohio EPA's proposed numeric nutrient criteria development approach (trophic index criterion or TIC). Geosyntec found a number of shortcomings that must be addressed before the TIC is implemented and recently submitted comments to that effect.	
<u>Connecticut</u> <u>Phosphorus</u> <u>Reduction Strategy</u> <u>Review*</u>	CT Cities and Towns Barnes and Thornburg	Reviewed the state's approach for developing low phosphorus effluent limits for wastewater treatment plants and found that it could be improved based on the best available science. As a result of our review, the state agreed to postpone implementing the limits and consider a new strategy.	
Cave Springs Branch Evaluation	Simmons Foods, Inc. Conner and Winters	Reviewing the technical basis for the nutrient TMDL developed by the state. The initial review indicates that the TMDL conclusions were based on an incorrect assessment of the biological community	
Missouri Water Quality Standards Review	City of Springfield, MO St. Louis MSD	Reviewed and provided comments on Missouri's recently proposed water quality standards and effluent regulations. The state has revised the proposals as a result of these comments.	
Atherton Regulatory Review	Little Blue Valley Sewer District HDR Engineering	To assist the District's long-term wastewater treatment planning efforts, prepared a series of memos outlining anticipated future regulatory requirements for the facility. The memos included a discussion of likely new ammonia, nutrient, bacteria, and wet- weather regulations expected to impact the District's NPDES permit.	

Client **Description and Notable Accomplishments Project Name** Provided a technical review of the TMDL and found that nutrient Mound Branch City of Butler, waste load allocations were unachievable and needed additional **Dissolved Oxygen** and Nutrient MO refinement. As a result, the agency modified the TMDL and agreed **TMDL** Review to extend the City's NPDES permit compliance schedule. To address existing TMDL requirements, conducted a nutrient Cooper Creek City of Branson, mixing zone evaluation, performed a peer review of a riverine Antidegration MO nutrient-algal water quality model, conducted an antidegradation Review review, and derived effluent limits for the City's treatment facility. Initial TMDL-based nutrient limits and implementation schedules Stinson Creek City of Fulton, were not achievable or affordable for the City. Successfully Regulatory MO negotiated an extended schedule of compliance for the City's permit based on modeled nutrient sensitivity analyses using the QUAL2K Negotiations **HDR Engineering** water quality model.

#### **EXAMPLE REPORTS AND JOURNAL ARTICLES**

The Request for Statement of Qualifications specified that a minimum of "[t]wo examples of summary reports for similar projects, or peer-reviewed journal publications" be included. The table below provides a list of 16 reports or publications authored by our team members that are relevant to this project. Appendix 1 below provides copies of the 16 documents.

Кеу	Title of Report or	Authors/Key	Relevance to This Project	
#	Journal Publication	Personnel		
1	Review of Connecticut Methodology to Establish Phosphorus Limits for Municipal POTWs, Connecticut Department of Energy & Environmental Protection, 2012.	Adrienne Nemura, Rob Annear	Conducted a critical review of the scientific and technical merit of how the state of Connecticut developed their phosphorus criteria using ecosystem thresholds.	
2	An evaluation of light intensity functions for determination of shaded reference stream metabolism, Journal of Environmental Management, 2011.	Chris Zell	Computation of daily photosynthesis values, reference stream identification and analysis, assessed relationship between dissolved oxygen and community photosynthesis.	
3	Rationale for Missouri's Proposed Nutrient Criteria Rule, Report to Missouri Department of Natural Resources, 2008.	Daniel Obrecht	Analyzed lake data and developed a decision making matrix for nutrient criteria and phosphorus management.	

Key #	Title of Report or Journal Publication	Authors/Key Personnel	Relevance to This Project
4	Development of diatom indicators of ecological conditions for streams of the western US, Journal of the North American Benthological Society, 2008.	Yangdong Pan	The species composition of benthic diatoms were related to environmental conditions in streams in the western US to develop diatom traits and indicators for diagnosing stressors including nutrients such as phosphorus and nitrogen
5	Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment, Journal of the North American Benthological Society, 1996.	Yangdong Pan	Benthic diatoms and water chemistry were sampled from 49 stream sites in the Mid- Atlantic Highlands region of the U.S. to evaluate the use of diatoms as indicators of environmental conditions in streams. Patterns of diatom species distributions in relation to environmental variables such as pH, turbidity and total phosphorus were determined using canonical correspondence analysis.
6	Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment, Hydrobiologia, 2004.	Yangdong Pan	Assessed the importance of spatial scales and land-use on diatom assemblages in the agricultural Willamette Valley, Oregon. Periphyton, water chemistry, and physical habitat conditions were characterized for 25 streams.
7	<i>Efficacy of molecular DNA</i> <i>methods for confirming</i> <i>species identifications on</i> <i>morphologically variable</i> <i>populations of toxin-producing</i> <i>Anabaena (Nostocales),</i> Lake and Reservoir Management, 2007.	Ann St. Amand	Conducted detailed analysis of algae samples collected from three lakes to identify algal species composition and then conduct a species confirmation using molecular DNA methods.
8	Baseline Ecological Investigation Report for the Little Traverse Bay CKD Release Site, Technical Report to U.S. EPA Region 5, 2009.	Ann St. Amand	Conducted a detailed baseline investigation of the aquatic community in Little Traverse Bay.
9	Algae Analysis Report and Data Set, 2012.	Ann St. Amand	An example of the detailed data analysis and interpretation of algae sample data. Includes speciation, indices, and biomass.
10	Statistical Considerations in Assessing the Compliance of Water Quality Criteria.	Song Qian	Presents and discusses statistical issues related to the compliance of numerical water quality criteria that cannot be directly measured and sample statistics were used instead.

consultants

Key #	Title of Report or Journal Publication	Authors/Key Personnel	Relevance to This Project
11	Two statistical methods for the detection of environmental thresholds, Ecological Modeling, 2003.	Song Qian	Two statistical methods for the detection of environmental thresholds are presented using macroinvertebrate and phosphorus data.
12	Importance of landscape variables and morphology on nutrients in Missouri reservoirs	Daniel Obrecht	Conducted research connecting land use with nutrient loading to receiving waters and the resulting water quality impacts.
13	Responses in the James River Arm of Table Rock Lake, Missouri (USA) to point-source phosphorus reduction	Daniel Obrecht	Studied the impact of phosphorus reductions to receiving water due to a sanitary treatment plant upgrade and resulting changes in algal biomass and concentration.
14	Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management	Daniel Obrecht	Examined the impacts of land use and more specifically nonpoint source loading of nutrients to receiving water nutrient concentrations. The result indicate hydrology and hydraulic flushing play a role in resulting nutrient concentrations and should be considered in setting nutrient criteria.
15	Role of contemporary and historic vegetation on nutrients in Missouri reservoirs: implications for developing nutrient criteria	Daniel Obrecht	Additional research showing vegetation cover (i.e. historic vs. contemporary land use) play a role in nutrient concentrations in the receiving water and its implications for setting nutrient criteria.
16	Chlorophyll maxima and chlorophyll: Total phosphorus ratios in Missouri reservoirs	Daniel Obrecht	This research examined the relationship between peak algal biomass and total phosphorus concentrations.

## SUMMARY

Geosyntec is pleased to present this Statement of Qualifications for the Oklahoma Joint Phosphorus Criteria Study. Our team understands and fully appreciates the importance of this project and the need to efficiently assist the committee in determining whether to raise, lower, or leave as is the Oklahoma Scenic River Water Quality Standard for Phosphorus. Geosyntec is committed to providing the committee with the resources and technical expertise required to meet the specific objectives of the study.

Geosyntec has assembled a team of key experts from PhycoTech, University of Missouri, University of Toledo, and Portland State University. Our team possesses an excellent combination of talent, understanding of water quality issues in the region and nationally-recognized expertise in water quality standards and nutrient criteria development. These characteristics, coupled with the strength of our

SOQ: Oklahoma Scenic Rivers Joint Phosphorus Criteria Study

project management, health & safety, and quality assurance systems, allow Geosyntec to understand the committee's needs and provide the best value to meet project objectives.

Our interdisciplinary team supports the ability to consider a variety of criteria development approaches as data analyses are undertaken and we have proven water quality standards development experience. The team includes nationally recognized stressor-response researchers with field, laboratory, modeling, and statistical analyses expertise. Our team includes experienced professional data collection and laboratory team members to help ensure that the highest quality data are collected in the field and are appropriately interpreted. Geosyntec has also included a robust corporate quality assurance program which can be invaluable if there are disputed outcomes.

We appreciate the opportunity to submit this statement of qualifications. Please call Chris Zell, our proposed Project Manager, at 573.499.5442, or Adrienne Nemura, our proposed Project Director, at 734.476.0357, with any questions regarding our statement of qualifications. We would very much appreciate the opportunity to provide a proposal to conduct the Oklahoma Scenic Rivers Joint Phosphorus Criteria Study.

## Appendix I – Reports and Journal Publications

- 1. Review of Connecticut Methodology to Establish Phosphorus Limits for Municipal POTWs, Connecticut Department of Energy & Environmental Protection, 2012.
- 2. An evaluation of light intensity functions for determination of shaded reference stream metabolism, Journal of Environmental Management, 2011.
- *3. Rationale for Missouri's Proposed Nutrient Criteria Rule,* Report to Missouri Department of Natural Resources, 2008.
- 4. Development of diatom indicators of ecological conditions for streams of the western US, Journal of the North American Benthological Society, 2008.
- 5. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment, Journal of the North American Benthological Society, 1996.
- 6. Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment, Hydrobiologia, 2004.
- 7. Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing Anabaena (Nostocales), Lake and Reservoir Management, 2007.
- 8. Baseline Ecological Investigation Report for the Little Traverse Bay CKD Release Site, Technical Report to U.S. EPA Region 5, 2009.
- 9. Algae Analysis Report and Data Set, 2012.
- 10. Statistical Considerations in Assessing the Compliance of Water Quality Criteria

- 11. Two statistical methods for the detection of environmental thresholds, Ecological Modeling, 2003.
- 12. Importance of landscape variables and morphology on nutrients in Missouri rivers, Canadian Journal of Fisheries and Aquatic Sciences, 2004.
- 13. Responses in the James River Arm of Table Rock Lake, Missouri (USA) to the point-source phosphorus reduction, Verhandlungen des Internationalen Verein Limnologie, 2005.
- 14. Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management, Lake and Reservoir Management, 2008.
- 15. Role of contemporary and historic vegetation on nutrients in Missouri reservoirs: implications for developing nutrient criteria, Lake and Reservoir Management, 2009.
- 16. Chlorophyll maxima and chlorophyll: Total phosphorus ratios in Missouri reservoirs, Lake and Reservoir Management, 2011.



2395 Oak Valley Dr., Suite 110 Ann Arbor, MI 48103 PH 734.332.8004 FAX 734.332.8063 www.geosyntec.com

## Memorandum

Date:	03 July 2012	
To:	Fredric P. Andes, Esq.	
Copies to:	Rob Annear, David Carani, John Christiansen (Geosyntec)	
From:	Adrienne Nemura	
Subject:	Review of Connecticut Methodology to Establish Phosphorus Limits for Municipal POTWs	
	Geosyntec Project: MOM003	

#### **OVERVIEW**

Geosyntec staff have reviewed the "Phosphorus Reduction Strategy for Inland Non-Tidal Waters" (Strategy) that the Connecticut Department of Energy and Environmental Protection (DEEP) is using as a basis to calculate total phosphorus limits for 43 publicly owned treatment works (POTWs) in the state<sup>1</sup>. We have also reviewed publications by Dr. Song Qian<sup>2</sup> and several scientists from the US Geological Survey (USGS). These publications raise concerns regarding the technical validity and regulatory applicability of the foundation of DEEP's strategy.

Our conclusion is that this strategy is not based on the best available science and that DEEP did not follow generally accepted procedures to ensure that their work is scientifically sound. This strategy should therefore not be used to establish National Pollutant Discharge Elimination System (NPDES) permits. Furthermore, the methodology that DEEP used to develop the proposed NPDES limits should be available for review and discussion. We recommend DEEP engage in a scientific review of its strategy to determine if a different nutrient reduction strategy for restoring and protecting aquatic life uses in Connecticut's streams and rivers is more

ctscienceanalysis070312

<sup>&</sup>lt;sup>1</sup> <u>http://www.ct.gov/dep/cwp/view.asp?a=2719&q=474130&depNav\_GID=1654</u> (accessed Jun. 25, 2012). The 43 facilities are listed in the "Presentation from the March 2011 NEAEB Conference".

 $<sup>^{2}</sup>$  Dr. Song Qian was formerly with Duke University and will be with the University of Toledo starting August 1, 2012.

appropriate. We further recommend DEEP engage in a dialogue on developing permitting guidance to implement any state nutrient reduction strategy.

Specific observations about DEEP's nutrient reduction analysis and permitting approach follow. References are available on request.

#### (1) THE UNDERLYING ANALYSIS RELIES ON A CONDITIONAL PROBABILITY APPROACH AND DOES NOT DEMONSTRATE CAUSE AND EFFECT.

The scientific approach used in the Strategy is based on a new analytical technique, Threshold Indicator Taxa Analysis known as TITAN (Baker and King 2010). That technique uses programming to correlate changes in biological species (such as macroinvertebrates or diatoms) to suspected causal variables (such as percent imperviousness of watershed cover or total phosphorus) without demonstrating a physical or biological basis for the correlation. As such, the technique is a "conditional probability" approach which "is based on the assumption that as nutrient concentrations increase, the likelihood of an impact on some negative response increases"<sup>3</sup>.

EPA's Science Advisory Board (SAB) indicated that application of conditional probability by itself was problematic with respect to developing instream nutrient criteria<sup>4</sup>. This is because there can be a lack of a cause and effect relationship between the stressor and response<sup>5</sup>. The SAB stated:

"The empirical stressor-response framework described in the Guidance is one possible approach for deriving numeric nutrient criteria, but the uncertainty associated with estimated stressor-response relationships would be problematic if this approach were used as a "stand alone" method because statistical associations do not prove cause and effect. We therefore recommend that the stressor-response approach be used with other available methodologies in the context of a tiered approach where uncertainties in different approaches are recognized, and weight-of-evidence is used to establish the likelihood of causal relationships between nutrients and their effects for criteria derivation. In this regard, we recommend that EPA more clearly articulate how this particular guidance fits within the Agency's decisionmaking and regulatory processes and, specifically, how it relates to and complements EPA's

<sup>&</sup>lt;sup>3</sup> http://n-steps.tetratech-ffx.com/PDF&otherFiles/stat\_anal\_tools/conditional%20prob\_final.pdf

<sup>&</sup>lt;sup>4</sup> In the context of DEEP's analysis, "nutrient" is considered to be phosphorus only. The state's strategy does not address nitrogen or micro-nutrients such as silica which can be important for algal growth.

<sup>&</sup>lt;sup>5</sup> In the case of the DEEP strategy, the stressor is phosphorus (only) and the responses are decline in abundance of a desirable diatom taxon or increase in abundance of an undesirable diatom taxon.

other nutrient criteria approaches, technical guidance manuals, and documents." (EPA SAB 2010).

#### (2) THE ENRICHMENT FACTOR (EF) APPROACH USED BY DEEP INCORRECTLY ASSUMES THE APPARENT CORRELATION BETWEEN PHOSPHORUS AND DIATOM SPECIES COMPOSITION REPRESENTS A DIRECT CAUSE-EFFECT RELATIONSHIP.

In a draft document titled, "Interim Phosphorus Reduction Strategy for Connecticut Freshwater Non-tidal Waste-Receiving Rivers and Streams," DEEP stated that "causal links between anthropogenic inputs of phosphorus and aquatic life response were identified to focus the analysis on direct responses of aquatic life to excess phosphorus" (Becker 2011a). Geosyntec is not aware of supporting information or studies by DEEP that document the statistical process by which these causal links were identified. It appears, based on the available information, the premise for DEEP's nutrient management approach is an assumption that the diatom community structure is directly linked to ambient phosphorus levels - as measured by the calculated enrichment factor (EF) rather than a scientific demonstration of cause and effect.

Although phosphorus loadings may possibly be correlated to diatom community structure, correlation does not indicate causation. As DEEP itself has indicated (Becker 2011a, 2011b), factors other than phosphorus may be influencing shifts in the diatom community. For example, the general scientific consensus is that nitrogen, not phosphorus, "may have more importance as a limiting element of biomass in streams than in lakes" (EPA 2000). Specifically with regards to diatoms, silica is often regarded as the limiting nutrient. There are also environmental and habitat-related factors that may significantly influence algal dynamics in Connecticut's receiving streams. These factors include shading, temperature, hydrologic influences, changes in substrate, and grazing impacts, as well as the effects of recent nitrogen reduction efforts. Until the apparent diatom shift is evaluated in the context of these factors, the analysis is incomplete. If DEEP has evaluated these factors in their analysis of the diatom data, it should be provided to stakeholders and the scientific community for review and comment. Otherwise there is significant cause for questioning the assumption that the entire diatom community structure is directly linked to only phosphorus.

#### (3) GIVEN THE SENSITIVITY OF THE ALLEGED RELATIONSHIP BETWEEN THE ENRICHMENT FACTOR (EF) AND DIATOM RESPONSE, DEEP'S METHODOLOGY NEEDS TO UNDERGO PEER REVIEW.

DEEP's methodology for deriving EFs is documented in Becker and Dunbar (2009). This document does not address many of the steps recommended in EPA's guidance to "help ensure the utility, scientific soundness, and defensibility of the models and their output for [environmental] decision making" (EPA 2009). These steps include procedures for model development (whether the model is sufficiently complex, properly constrained, and verified from a coding perspective); model evaluation (including scientific peer review, sensitivity and uncertainty analysis); and model application (including application of multiple models and model post-audit). Geosyntec recommends that the EF methodology be peer reviewed to ensure the calculation of the EFs are appropriate and the methodology is being appropriately applied for decision making.

DEEP stated during the March 6, 2012 and April 11, 2012 community presentations that their methodology indicates the following change points in the diatom community (Day 2012):

Enrichment		Increase over
Factor (EF)	Description of Change	EF = 1.9
1.9	Algae starts to change	
6.0	Algae increases dramatically	3.2X
8.4	Upper limit where algae definitively increase	4.4X

Because the change points are numerically close, the uncertainty in the calculation of the EF needs to be evaluated. Geosyntec is not aware of information where DEEP has documented the uncertainty associated with their calculation of the EFs in their methodology.

Becker and Dunbar (2009) indicate the EFs are derived from calculations that depend on the land use within a watershed (agriculture, forest, and urban) and wastewater treatment plant loads. The state included water and wetland land cover in forest because "wetlands function like forests by filtering nutrient loads to surrounding stream" (page 3). The significance of this assumption is not discussed. The report also indicates that land cover categories were condensed using the 2002 UCONN Clear Land Use dataset (Version 1) and that the state condensed eleven land cover groups into three. The appropriateness of using the 2002 land use dataset and the states' method for condensing the land uses into three categories should be reviewed.

DEEP apparently tested their methodology by comparing the calculated export coefficient load to an "actual" total daily load for 8 monitoring locations (see Table 4 of Becker and Dunbar 2009). DEEP indicated the "actual" load is based on a minimum of "[f]our samples taken during the target 'growing season' (April through October) from 1985 – 2007 under varying flow conditions at a site." Geosyntee is not aware of information that shows how well the "actual" daily load calculation represents the average daily load conditions that are used to correlate EFs to the diatom data. Comparison of these "actual" total loads to a total load that is derived with a continuous simulation of a hydrologic and hydraulic model of the watershed is recommended so there is greater confidence in the estimate of the "actual" load.

# (4) DEEP'S NUTRIENT MANAGEMENT APPROACH DOES NOT CONSIDER THE OVERALL BIOTIC COMMUNITY.

The fundamental premise underlying the DEEP approach is that the prescribed phosphorus reductions will control diatom community shifts and therefore protect designated beneficial uses. We are not aware of any data or studies definitively linking phosphorus levels or diatom shifts to aquatic life use (macroinvertebrates and fishes) impairments in Connecticut. As DEEP (Becker and Dunbar 2009) discusses in their nutrient management technical support document, "the alteration of plant communities in turn alters the composition of other aquatic life communities such as benthic invertebrates and fish that rely on aquatic plant communities for food and habitat." We agree that changes in stream plant communities can affect organisms in higher trophic levels. However, DEEP has not demonstrated that phosphorus levels or shifts in the diatom community alone are linked to negative impacts on the overall structure and function of the overall biotic community in the receiving streams. If the purpose of DEEP's approach is to protect aquatic life use attainment, then direct measurements of the macroinvertebrate and fish phosphorus levels will correspond to a healthy aquatic community; indicating that phosphorus reductions are not appropriate.

# (5) DEEP DOCUMENTATION ON HOW THE TITAN FRAMEWORK WAS IMPLEMENTED IS NOT AVAILABLE IN THE PUBLIC RECORD.

If DEEP intends to use a new approach to develop permit limits, it is critical that affected stakeholders be able to review the basis for the new strategy. Geosyntec has not been able to locate documents on DEEP's approach other than information presented in PowerPoint Slides and a draft white paper that is seven pages long (Becker, 2011b). DEEP should provide a more detailed and robust report describing the data collected, documenting how the TITAN model was implemented, and describing the interim results in the process of applying TITAN.

#### (6) DEEP SHOULD ENGAGE IN A SCIENTIFIC DISCUSSION ABOUT CONCERNS OVER THE TITAN METHODOLOGY.

The technical papers that were used to develop the DEEP strategy have been strongly criticized by other researchers, including Dr. Song Qian and several scientists from the US Geological Survey (USGS). The approach documented in these papers is a program that analyzes data to

detect ecological community "thresholds" where there is an undesirable shift in the ecological community based on a stressor. These community thresholds are then used in a regulatory framework to help manage the ecosystem. There are published papers raising serious issues regarding the technical validity and regulatory applicability of this approach that DEEP has not yet addressed.

Dr. Qian and the USGS researchers have been very critical of the use of thresholds for ecological management decisions. For example, the research (Qian 2012) has been critical of the TITAN framework and its ability to predict thresholds on the individual taxa and ecosystem level. This research has identified several artifacts in the TITAN framework that influence the ability of the method to identify individual taxa thresholds. This deficiency is inherent in DEEP's use of the methodology, since it presupposes that a decline in individual taxa is inherently bad for the ecosystem.

# (7) THE EMPHASIS ON INDIVIDUAL TAXA VERSUS COMMUNITY STRUCTURE IS INAPPROPRIATE.

There are inherent problems with analyzing individual taxa, such as characterizing the distributions of rare taxa and the high variability associated with taxa abundances and occurrences. This makes it difficult to model responses and extract change points based on individual taxa. Other metrics such as an integrated insect test [Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa richness] integrate information not considered in TITAN (Cuffney, et al. Oct. 2011). Based on the DEEP presentations, it is clear that the state is trying to understand aquatic ecosystem thresholds across environmental gradients and how this information can be used to drive nutrient loading reductions in CT streams. TITAN does not adequately address the need to understand aquatic ecosystem shifts and has inherent problems with trying to understand change points across multiple species.

#### (8) THERE ARE INHERENT ISSUES WITH THE TITAN FRAMEWORK EVEN IF IT IS APPLIED TO CORRELATE NUTRIENT CONCENTRATIONS WITH ALGAL ABUNDANCE.

The TITAN framework is not a statistical model since no probability distribution assumption was made for the response variable(s). As a result, TITAN should not be considered a statistical model, but rather a data transformation and processing code (Qian 2012). It is likely that the TITAN analysis has calculated a threshold that is not correct. Because of the points raised below, TITAN could produce inconsistent and unpredictable results, particularly with more or
less data. Other statistical models should at least be applied to determine if the TITAN analysis is reliable.

The TITAN framework is supposed to identify change point (of discontinuities) in an otherwise smooth curve (environmental gradient). TITAN does not define a response variable or a probability distribution function. The impact behind the split (change) point is dependent on the underlying model, which is poorly defined in this framework (Qian 2012). When two groups of data are defined by a split point (defined by maximum difference between IndVal of two data groups) along an environmental gradient, the statistical model is a step-function. As a result the TITAN framework is only good for detecting responses along an environmental gradient that are represented by a step function. There is no guarantee that any taxa abundance data will follow this behavior.

Baker and King (2010) limited the sampling permutations to 250 and this was not supported statistically, largely because the underlying distribution of the two data groups is not defined. Qian (2012) noted when the sample size is large, the 250 permutations may be insufficient to characterize the null hypothesis distribution and this is especially true when the underlying abundance data distribution is highly skewed (meaning the data includes large or small values). As a result, there is a high level of uncertainly due to a high variance in all the statistics related to the test, including the z-score utilized by Baker and King (2010).

The TITAN framework selects the split point along a truncated environmental gradient by using the largest z-score (using IndVal) but the process used introduces a systematic bias (Qian 2012). This occurs because in the permutation test, the estimated IndVal mean and standard deviation values for each divided group are functions of the sample size of each group. When the difference in sample size between two groups is large, the variance is large and the unevenness in sample size between the two data groups is dependent on the location of the split point. When the split point is near the end of a truncated gradient, the permutation variance is the largest. When the split point is in the middle, the permutation variance is smallest. The resulting zscores for split points near both ends of the gradient tend to be smaller than they should be if the null hypothesis of no clustering is true. As noted above, the high variability in permutation estimated statistics and the small number of permutations result in very different split points being identified when TITAN is used several times with the same underlying data set. Overall, inconsistent and unrepeatable results are generated from TITAN.

## (9) THE TITAN FRAMEWORK DOES NOT CAPTURE THE UNDERLYING RESPONSE MODEL BETWEEN NUTRIENT CONCENTRATIONS AND ALGAL ABUNDANCE.

DEEP used TITAN to establish a state strategy for nutrient control using a pre-supposed relationship between phosphorus and diatom abundance. This approach does not follow the Science Advisory Board's recommendations to use the conditional probability approach with a weight-of-evidence approach (EPA SAB 2010). It also does not help ensure that the desired response will be attained. This is because "[t]he individual and aggregate change points in TITAN do not provide any information on the form of the response nor do they provide any parameters that might be used to predict responses in other situations so they do not provide as much information [as alternative models]... Because the TITAN method derives change points without determining the underlying response model, much of the ecological and management significance of the response is lost" (Cuffney, et al. Oct. 2011).

Although TITAN identifies change points along an environmental gradient, without an understanding of the overall response model that results in the change point, then it becomes difficult to manage the environmental gradient to impact the change point.

## (10) THE TITAN FRAMEWORK CAPTURES ONLY STEP FUNCTION RESPONSES BETWEEN ENVIRONMENTAL GRADIENTS AND ALGAL ABUNDANCE.

It is not known whether DEEP confirmed that the diatoms used to establish the thresholds exhibited a step-function relationship to the EFs, which is the only relationship that TITAN can evaluate. If the diatom abundance data (for individual taxon) exhibit a relationship other than a step-function, then TITAN will likely result in an erroneous split point (Qian 2012). This is because TITAN identifies false thresholds for other relationships (linear, hockey stick) or where the uncertainty in the observed abundance is large (Qian 2012). Even if the underlying relationship can be approximated by a step function, if the uncertainty in the observed abundance data is large, then TITAN will also likely fail (Qian 2012). The thresholds calculated with TITAN using different species could be an artifact of the TITAN framework - it is not known if DEEP conducted any evaluations to confirm that this is not the case with the diatoms studied (such as use of alternative models to confirm the relationships).

Qian (2012) provided examples of linear, step function and hockey stick response types of abundance data along a gradient. His review of these response types using TITAN showed further investigation is needed to explain change points (hockey stick) or lack thereof (linear). A permutation test results in a p-value of 0 confirming the selected split point as a "threshold." The bootstrapping technique used to estimate the confidence interval of the selected split point uses

subsamples of possible split points. Each bootstrap sample will result in a selected split point that is larger or smaller than the observed split point and tends to be skewed towards one end of the gradient. The result is these two "tests" give a false impression of a distinct split point, but the result is in fact an artifact of the program. The end result is the TITAN program will result in identifying erroneous split points for all response types except the step function model. TITAN is also noted to fail when the uncertainty in the observed abundance is large (Qian 2012).

## (11) THE TITAN FRAMEWORK HAS A HIGH LEVEL OF UNCERTAINTY IN IDENTIFYING CHANGE POINTS.

The bootstrap method used in TITAN has been identified as inappropriate for the split point analysis, and therefore there is higher uncertainty associated with this method than other methods. The bootstrapping technique is used to obtain an approximation of the sampling distribution of the parameter of interest and involves substituting random samples for the target population with random samples from the existing data. According to Qian (2012), the bootstrap method is not appropriate for a split point problem [citing Bühlmann and Yu (2002) and Banerjee and McKeague (2007)]. This is because the bootstrap method estimated standard deviation is always smaller than the true standard deviation for a split point problem which leads to erroneously narrow confidence intervals. Based on the available documentation, it does not appear that DEEP adequately characterized this uncertainty in establishing and applying its nutrient reduction strategy.

### (12) THE TITAN FRAMEWORK DOES NOT CAPTURE COMMUNITY RESPONSE.

The TITAN approach is inappropriate for community response (the z-score is calculated for a particular species as a measure against the null hypothesis). Summing of these individual z-scores is inappropriate, as the sum is only meaningful for tests that share the same null hypothesis. Based on the available documentation, it is not clear that DEEP has shown that there is a relationship between the aquatic life community and the individual taxon of diatoms that were used to establish the EF change point where adverse impacts on the community supposedly occur.

Qian (2012) noted that the synchronicity of the thresholds for different species abundances is likely an artifact of the TITAN program for two reasons. The first is the species split point identified using z-scores was shown to be biased to the middle of the data. Second, the distribution of sites along the environmental gradient is biased towards the low end of the environmental gradient. This can be shown for abundance data with various levels of noise in the data. When the noise level is zero, the IndVal increases along the gradient with the highest

value at the truncated end of the gradient. As the noise level increases, the pattern of IndVal is the same for all cases but the z-score moves closer to the center of the data (Qian 2012). This results in what appears to be a high degree of synchronicity in split points among different species.

The z-score for a particular taxon is the permutation test as a measure against the null hypothesis (e.g., no clustering in abundance of the taxon along the environmental gradient) which is specific to each particular taxon. Qian (2012) noted the z-scores are for different tests with different null hypotheses and the sum of the z-scores is only meaningful for tests that share the share the same null hypothesis. In the TITAN framework, the z-score is calculated for each taxon separately and therefore for different null hypotheses. As a result the z-score should not be combined (for evaluating community thresholds), as the summation is meaningless.

### (13) DEEP HAS NOT DEMONSTRATED THAT THE ENRICHMENT FACTOR (EF) APPROACH IS LINKED TO BENEFICIAL AQUATIC LIFE USES.

We understand that the Connecticut biological condition gradient<sup>6</sup> has only been calibrated for macroinvertebrates (Gerritsen and Jessup 2007) and not to diatoms or EFs. As a result, it is not clear how applying this nutrient management approach will ensure beneficial use attainment in the receiving waters. Biological condition gradient calibrations notwithstanding, the approach used by DEEP is only intended to identify shifts in the structure of one element (diatoms) of the biotic community. It was not intended to identify changes in community function, as is required to demonstrate non-attainment with the Connecticut water quality standards. Aquatic communities are composed of a complex set of interactions between communities and among trophic levels. Even if the diatom shift identified in DEEP's model were found to be valid, it is an incomplete evaluation of overall biotic community structure and function.

## (14) THE DEEP DIATOM AND ENRICHMENT FACTOR (EF) APPROACH IS INCONSISTENT WITH CONNECTICUT'S COMPREHENSIVE ASSESSMENT AND LISTING METHODOLOGY (CALM).

The Connecticut CALM (DEEP 2011) approach describes the procedures used by DEEP to evaluate attainment with water quality standards. According to the CALM document, methods for determining aquatic life use support include evaluating macroinvertebrate and fish data, conventional physical and chemical criteria, chronic toxicity, the history of catastrophic events,

<sup>&</sup>lt;sup>6</sup> The Connecticut biological condition gradient is described at

http://www.ct.gov/dep/lib/dep/water/water\_quality\_standards/ct\_bio\_cg\_faq.pdf

and stream flow impacts on the biotic community (Table 1-2). The CALM approach does not mention the use or interpretation of either EFs or diatom community structure in making attainment decisions. Because these factors cannot be used to make attainment decisions, it is not clear how diatom structure indices or EFs can be used as the primary driver for deriving permit limits.

## (15) THERE MAY BE FLAWS IN HOW DEEP HAS USED ITS METHODOLOGY TO DERIVE EFFLUENT LIMITS.

In the draft NPDES permits that have been issued, DEEP is proposing a weekly and monthly total phosphorus concentration for April 1 to October 31. They have also proposed language stating that if a seasonal averaged load limit, in pounds per day, is exceeded for "any two consecutive calendar years or any two of three consecutive calendar years, the permittee shall develop and submit for the review and approval of the Commissioner a plan to reduce future Total Phosphorus in the effluent" (DEEP 2012).

To our knowledge, DEEP has not provided information to justify how these permit limits are necessary and appropriate for measuring compliance with the proposed strategy. It is important to consider the relationship of the averaging period used in the permit limits to the averaging of the underlying data in the TITAN calculations. Based on the available documentation, it would appear that the seasonal averaged load limit is the most appropriate expression of the current strategy. Even with this limit, it is not clear how exceeding the enrichment factor calculation in two consecutive years or in any two of three consecutive years is linked back to the underlying data analysis. Further, did DEEP consider whether this seasonal averaged load should be considered as a seasonal median load to reflect that the distribution of total phosphorus concentrations in the effluent is likely to be skewed and log-normal?

The use of a 30-day averaging period to establish weekly and monthly average concentration limits needs to be documented and discussed. How do these values translate back to the seasonal averaged load? It is likely the distribution of phosphorus loads in the treatment plant effluent is skewed and log-normal, due to the flow and total phosphorus relationships. For distributions that deviate from normality, having infrequent large values (right-skewed), the arithmetic mean is a biased estimator of central tendencies. Therefore, it would appear that if monthly or weekly limits are needed, compliance with a proposed criterion should be measured using the median statistic, or geometric mean, of applicable phosphorus data.

We also note that water quality criteria are typically described by three numeric characteristics: magnitude, excursion frequency, and duration (averaging period). DEEP has not provided the rationale for establishing the proposed permit limits.

\* \* \* \* \*

#### REFERENCES

- Baker, M.E., and R.S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution 1:25-37.
- Banerjee, M. and I.W. McKeague. 2007. Confidence sets for split points in decision trees. The Annals of Statistics, 35 (2): 543–574.
- Becker, M.E. 2011a. The Science Behind the CT Statewide Interim Phosphorus Management Strategy in Non-Tidal Waste Receiving Rivers and Streams: As Applied in the Quinnipiac River Basin. Presentation to Town of Wallingford. Jul. 8, 2011.
- Becker, M.E. 2011b. Interim Phosphorus Reduction Strategy for Connecticut Freshwater Non-Tidal Waste-Receiving Rivers and Streams. CT Department of Environmental Protection. Bureau of Water Protection and Land Reuse: Planning and Standards Division. Apr. 21, 2011. <u>http://www.eli.org/pdf/CT\_P\_Mgmt\_Interim.pdf</u>
- Becker, M.E. and L. Dunbar. 2009. Connecticut Methodology for Freshwater Nutrient Management Technical Support Document. Last revised: Jun. 9, 2009. <u>http://www.ct.gov/dep/lib/dep/water/water\_quality\_standards/nutrient\_mgmt\_tech\_fdraf</u> <u>t\_699.pdf</u>
- Bühlmann, P. and B. Yu. Analyzing bagging. The Annals of Statistics, 30 (4): 927–961, 2002.Cuffney, T. F., S. S. Qian, R. A. Brightbill, J. T. May, and I. R. Waite. 2011 A Response to King and Baker: limitations on threshold detection and characterization of community thresholds, Ecological Applications, 21:2840-2845.
- Connecticut Department of Energy and Environmental Protection (DEEP). 2012. Draft Municipal NPDES Permit ID CT0100536 for the Town of Southington, CT. Jun. 6, 2012.
- Cuffney, T.F., R.A. Brightbill, J.T. May, and I.R. Waite. 2010. Response of benthic macroinvertebrates to environmental changes associated with urbanization in nine metropolitan areas, Ecological Applications, 20(5), 2010, pp. 1384-1401.

Day, D. 2012. Personal communication.

- EPA Science Advisory Board (SAB). 2010. Ecological Processes and Effects Committee (FY 2009) Augmented for Review of Nutrient Criteria Guidance. April 27, 2010. <u>http://yosemite.epa.gov/sab/sabproduct.nsf/0/e09317ec14cb3f2b85257713004bed5f/\$FIL</u> <u>E/EPA-SAB-10-006-unsigned.pdf</u>
- EPA. 2009. Guidance on the Development, Evaluation, and Application of Environmental Models. Office of the Science Advisor, Council for Regulatory Environmental Modeling. EPA/100/K-09/003. March 2009. http://www.epa.gov/crem/library/cred\_guidance\_0309.pdf
- EPA. 2000. Nutrient Criteria Technical Guidance Manual Rivers and Streams. Office of Water, July 2000. <u>http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/2009\_04\_22\_</u> <u>criteria nutrient guidance rivers rivers-streams-full.pdf</u>
- Gerritsen and Jessup. 2007. Calibration of the Biological Condition Gradient for High Gradient Streams of Connecticut, Prepared for U.S. EPA Office of Science and Technology, Connecticut Department of Environmental Protection, By Tetra Tech, Inc. Aug. 2007.
- Qian, S.S. 2012. TITANic Journey, Songs Blog, May 23, 2012. http://songqiansblog.blogspot.com/2012/05/titanic-journey.html
- Qian, S.S. and T.F. Cuffney. 2012. A Critique of the Use of Indicator Species Scores for Identifying Thresholds in Species Responses, Journal of the Society of Freshwater Science, to be published.

Provided for non-commercial research and education use. Not for reproduction, distribution or commercial use.



(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Journal of Environmental Management 97 (2012) 69-77

Contents lists available at SciVerse ScienceDirect



## Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

#### Short communication

# An evaluation of light intensity functions for determination of shaded reference stream metabolism

## Chris Zell<sup>a,\*</sup>, Jason A. Hubbart<sup>b,c</sup>

<sup>a</sup> Geosyntec Consultants, 1123 Wilkes Blvd., Suite 400, Columbia, MO 65201, USA
 <sup>b</sup> Department of Forestry, University of Missouri, Columbia, MO 65211, USA
 <sup>c</sup> Department of Soils, Environmental and Atmospheric Sciences, University of Missouri, Columbia, MO 65211, USA

#### ARTICLE INFO

Article history: Received 15 January 2011 Received in revised form 21 August 2011 Accepted 7 December 2011 Available online xxx

*Keywords:* Whole stream metabolism Dissolved oxygen Reference stream

#### ABSTRACT

The performance of three single-station whole stream metabolism models were evaluated within three shaded, seasonally hypoxic, Missouri reference streams using high resolution (15-minute) dissolved oxygen (DO), temperature, and light intensity data collected during the summers (July-September) of 2006–2008. The model incorporating light intensity data consistently achieved a lower root mean square error (median RMSE =  $0.20 \text{ mg L}^{-1}$ ) relative to models assuming sinusoidal light intensity functions (median RMSE = 0.28 mg L<sup>-1</sup>) and constant diel temperature (median RMSE = 0.53 mg L<sup>-1</sup>). Incorporation of site-specific light intensity into metabolism models better predicted morning DO concentrations and exposure to hypoxic conditions in shaded study streams. Model choice significantly affected (p < 0.05) rate estimates for daily average photosynthesis. Low reaeration (pooled site mean 1.1 day $^{-1}$  at 20 °C) coupled with summer temperatures (pooled site mean = 25.8 °C) and low to moderate community respiration (site median 1.0–3.0 g  $O_2 m^{-2} day^{-1}$ ) yielded diel dissolved oxygen concentrations near or below critical aquatic life thresholds in studied reference streams. Quantifying these process combinations in best-available or least-disturbed (i.e., reference) systems advances our understanding of regional dissolved oxygen expectations and informs environmental management policy. Additional research is warranted to better link landscape processes with distributed sources that contribute to community respiration.

© 2011 Elsevier Ltd. All rights reserved.

#### 1. Introduction

Dissolved oxygen (DO) is essential for sustaining aerobic metabolisms of aquatic biota (Heath, 1995; Kramer, 1987; Smale and Rabeni, 1995), and as such, is an accepted indicator of aquatic ecosystem health (Hynes, 1971; Smith, 1982). The availability of cost-effective and reliable high-frequency DO measurement systems (i.e., data sondes) is increasing, thus allowing agencies and scientists to obtain these devices and more accurately assess the water quality status of fresh and saline waters (Glasgow et al., 2004; Wetzel and Likens, 2000). As a consequence, workers equipped with high resolution data capture systems have identified several waterways as having DO concentrations below default water quality criteria established by environmental management agencies including state designated reference streams (as per Hughes et al., 1986), coastal streams in Georgia (Todd et al., 2009; Utley et al., 2008), and bottomland waters in Louisiana (Ice and

\* Corresponding author. Tel.: +1 573 443 4100.

E-mail address: czell@geosyntec.com (C. Zell).

Sugden, 2003). Water quality reference streams have been used by agencies to set regional goals or criteria for key water quality indicators, including DO (Gallant et al., 1989; LDEQ, 2008). Effective management of water resources relies on establishing criteria targets that are protective of biota and achievable within leastdisturbed conditions (i.e., reference streams). As reference stream conditions may guide regional management initiatives, understanding the processes that yield hypoxic conditions is imperative to assure proper aquatic resource management.

It is understood that time-variable interactions between community photosynthesis (P), respiration (R), and reaeration processes produce sinusoidal oscillations characteristic of diel DO profiles (O'Connor and Di Toro, 1970; Odum, 1956). Collectively, individual processes of biochemical oxidation, plant (algae, macrophyte) respiration, and sediment oxygen demand (SOD) yield community respiration whereas oxygen produced by algae and aquatic macrophytes yield community P values (Chapra, 1997). Mass transfer exchange of oxygen between the stream and atmosphere is governed by reaeration processes that are largely governed by in-stream hydraulic properties (Moog and Jirka, 1998; Rathbun, 1977) and surface wind currents (Chu and Jirka, 2003;

<sup>0301-4797/\$ —</sup> see front matter  $\odot$  2011 Elsevier Ltd. All rights reserved. doi:10.1016/j.jenvman.2011.12.006

Wanninkhof, 1992). Reaeration processes export oxygen from the water column during periods of supersaturation and import oxygen during undersaturated conditions. Quantifying individual DO processes discussed above is often pursued through a combination of direct measurement (APHA, 1995; Truax et al., 1995; Hauer and Lamberti, 2006; Kilpatrick et al., 1989), sophisticated mechanistic models (Chapra et al., 2009), and analysis of diel DO profiles (see summary in Wang et al., 2003).

Diel profile analyses that yield community estimates (i.e., whole stream metabolism) of P, R, and reaeration were first documented by Odum (1956). Since that time several researchers have explored the application and development of the single-station diel analysis approach. The Delta Method (DM) approach (Chapra and DiToro, 1991; McBride and Chapra, 2005) is a widely applied singlestation method used to estimate P and R from diel profiles. However, Butcher and Covington (1995) and Loperfido et al. (2009) identified situations in shallow streams where the assumption of constant diel water temperature and DO saturation reduces the applicability or accuracy of the DM. A less frequently evaluated assumption of the DM includes a half-sinusoid light intensity function that co-symmetrically forces community photosynthesis. While Chapra and DiToro (1991) explicitly stated that the DM is not applicable to canyon streams, the effects of truncated light (and concomitant photosynthesis) on metabolism estimates was not explored. In addition to topographic shading, riparian canopy shading is well documented to attenuate light reaching stream surfaces (Broadmeadow and Nisbet, 2004; DeNicola et al., 1992; DeWalle, 2010, 2008). Incorporating the time variable attenuation of light from shading sources is needed to better characterize whole stream metabolism within shaded stream environments.

Riparian zones provide several benefits to aquatic ecosystem health including habitat diversity, temperature buffering, and filtering or retention of contaminants (Allen, 2004; Gregory et al., 1991; Naiman and D'Camps, 1997). For these reasons, the presence of a riparian corridor of appropriate width and vegetative density is valued when identifying streams that represent leastdisturbed or best-available conditions (i.e., reference stream) for a region (Hughes et al., 1986; Stoddard et al., 2006; Huggins and Dzialowski, 2005; Rabeni et al., 1997). For streams with riparian corridors that truncate light intensity functions, diel profile studies that incorporate site-specific light information such as Holtgrieve et al. (2010), Parkhill and Gulliver (1998) and Portielje et al. (1996) are expected to improve characterization of photosynthetic inputs and therefore whole metabolism estimates.

The objective of our work was to better understand the relative importance of light in determining whole stream metabolism and to quantify rate parameters that produce hypoxic DO regimes. Improving scientific understanding of diurnal DO model predictions is needed to support effective management of aquatic ecosystems. To meet our objective, we evaluated the performance of three single-station stream metabolism methods in shaded reference streams to identify the best model for hypoxic streams. Each method featured a different set of assumptions governing light and photosynthesis forcing functions. With the exception of Holtgrieve et al. (2010) that valued light data in determining reaeration, previous studies including site-specific light profiles (e.g., Portielje et al., 1996; Parkhill and Gulliver, 1998) have not examined the relative importance of light data in determining rate parameters, or time-variable DO behavior. Understanding the role of light in determining rate parameters is important in distinguishing hypoxic conditions caused by shading and low photosynthetic input compared to low reaeration or high respiration. Therefore, we used data obtained from a multi-year summer season investigation of three Missouri reference streams using a high resolution (15minute) data scheme to evaluate the performance of three stream metabolism frameworks: the DM (Chapra and DiToro, 1991), the temperature-dependant approach of Butcher and Covington (1995) 'BC', and an approach incorporating site-specific light intensity functions (LIF approach) similar to the model developed by Portielje et al. (1996).

#### 2. Materials and methods

#### 2.1. Study area

Dissolved oxygen regimes within three Missouri reference streams (Fig. 1) were monitored during summer baseflow conditions from 2006 to 2008. Monitored reference segments and watershed areas of East Fork Crooked River (EFCR, 222–241 km<sup>2</sup>), Heaths Creek (HC, 147–194 km<sup>2</sup>), and Little Drywood Creek (LDC, 124–319 km<sup>2</sup>) are situated within Central Irregular Plains (EFCR, LDC) and Interior River Valley (HC) Ecoregions (Level III, US EPA, 2000) and located within, or intersecting, the Missouri counties of Ray (EFCR), Pettis (HC), and Vernon (LDC). A 6.3 km segment of EFCR is a 5th order reference reach underlain by sandstone, shale, limestone and poorly drained by Wabash silty clay soils (Detroy and Skelton, 1983; SCS, 1986). Heaths Creek is 21.2 km segment of a 5th order reference stream situated within an unglaciated watershed and predominantly overlain by 1.5 m or more of moderately drained Dockery silt loam (SCS, 1995). Radley silty clay loams underlay monitored segments of Little Drywood Creek (SCS, 1977), a 30.3 km, 5th order prairie reference stream. The 30-year (1979-2009) annual average cumulative precipitation for cooperative gages #234904 (EFCR), #237632 (HC), and #235987 (LDC) is 104, 109, and 114 cm, respectively (MRCC, 2010).

While point source discharges are not known to affect reference segments, moderate cropland densities of 53% (HC), 32% (EFCR), and 24% (LDC) obtained from 2005 Thematic Mapper imagery support a 'least-disturbed' (Stoddard et al., 2006) characterization of study reaches. Fine channel substrates and woody debris predominates glide-pool mesotypes in EFCR and LDC. Heaths Creek is a riffle-pool system featuring large pools contained by limestone and shale bedrock outcroppings and short riffles. Aquatic



Fig. 1. Location of studied reference streams within Missouri's Ecological Drainage Unit (EDU) framework (Sowa et al., 2007).

macrophytes were infrequently observed during summer sampling within main channel areas of study streams. Riparian zones typically exceed 18 m in width and are composed of a brush and deciduous tree mixture. Chemical and physical characteristics of monitored reference streams are listed in Table 1.

#### 2.2. Monitoring framework

Surface light intensity, DO, and water temperature data were collected at 15-minute intervals at multiple locations in each stream (HC: 3 sites, EFCR: 2 sites, LDC: 2 sites) from early July through early September of 2006 (LDC, HC), and 2007-2008 (all sites). Continuous in-stream water quality data were obtained with Yellow Springs Instruments (YSI) 600 or 6600 series data sondes calibrated and maintained weekly according to manufacturer specifications and validated using drift correction procedures outlined by Wagner et al. (2000). Surface light intensity profiles, measured at 15-minute intervals with UA-002-64 series HOBO sensors (lumens m<sup>-2</sup>), were deployed directly above data sondes and cleaned weekly. Measurements made in quanta, radiometric, or in this case, photometric units are highly correlated (Hauer and Hill, 2006) and therefore suitable to characterize the temporal shape of light intensity functions. Open-field light intensity was measured at 15-minute intervals at one site per stream to characterize riparian canopy light attenuation and thus deviation from sinusoid assumptions.

Weekly thalweg grab samples for the following parameters were collected at one site per stream during sonde deployment and are used to provide context for metabolism estimates obtained from DO analyses: 20-day carbonaceous biochemical oxygen demand (CBOD-20), total ammonia-nitrogen (NH<sub>3</sub>N), total nitrogen (TN), total phosphorus (TP), suspended chlorophylla (SChl<sub>a</sub>). Five replicate scrapings from available natural substrates were collected weekly from one site per stream within a 100 m nearest DO and light instrumentation to characterize benthic chlorophyll-a (BChl<sub>a</sub>). Methods according to APHA (1995) were used to quantify grab sample variables. Weekly openchannel discharge and 15-minute pressure corrected stream level data were collected at one location per stream over the study period using SOLINST 3001 series level transducers. Physical stream dimensions were characterized from equally spaced transects (n = 9 per site) collected in 2007 during comparable low-flow conditions for each stream.

#### Table 1

Chemical and physical characteristics of three studied Missouri reference reaches (July–September, 2005–2008). All values reported as site medians with the exception of bed gradient.

Parameter	Unit	EFCR <sup>a</sup>	HC <sup>a</sup>	LDC <sup>a</sup>
Benthic chlorophyll-a	mg Chl <sub>a</sub> m <sup>-2</sup>	4	39	16
Periphyton bottom coverage	%	5	20	5
Sestonic chlorophyll-a	$\mu g L^{-1}$	6	14	10
20-day carbonaceous	${ m mg}~{ m L}^{-1}$	2	4	4
biochemical oxygen demand				
Ammonia–Nitrogen	mg $L^{-1}$	0.03	0.03	0.03
Total nitrogen	mg $L^{-1}$	0.842	0.880	0.767
Nitrite + Nitrate nitrogen	mg $L^{-1}$	0.229	0.086	0.065
Total phosphorus	$\mu g L^{-1}$	105	160	62
Wetted width	meters	4.3	8.8	6.7
Level	meters	0.4	0.5	0.4
Survey streamflow	m <sup>3</sup> sec <sup>-1</sup>	0.19	0.018 <sup>b</sup>	0.010 <sup>b</sup>
Study reach bed gradient	percent	0.05	0.09	0.04
(1:24 K topographical map)				

<sup>a</sup> EFCR: East Fork Crooked River, HC: Heaths Creek, and LDC: Little Drywood Creek.

 $^{\rm b}$  Includes values below measurement threshold of 0.0283  $\rm m^3~sec^{-1}$  that are calculated as 0.0283/2.

#### 2.3. Stream metabolism modeling

Three single-station DO analysis methods were used to estimate metabolism parameters in each of three streams at the location in each stream that featured continuous stream level, continuous light, nutrient, and algae data. Methods DM, BC, and LIF include the Delta Method (Chapra and DiToro, 1991), Butcher and Covington (1995), and a modified version of Portielje et al. (1996), respectively. A brief discussion of DM, BC, and LIF methods is provided as follows. The single-station approach does not consider reach length as a forcing variable and therefore applies where spatial DO deficit gradients are negligible. Spatial gradients in DO deficit are assumed negligible where aquatic plants are distributed uniformly over the distance of  $>3U/k_a$ , where U = velocity (m day<sup>-1</sup>) (Chapra and DiToro, 1991), a condition achieved within our study streams.

#### 2.3.1. The Delta Method (DM)

The DM assumes the deficit mass-balance can be described by:

$$\frac{dD}{dt} + k_a D = R - P(t) \tag{1}$$

Where D = DO deficit (mg O<sub>2</sub> L<sup>-1</sup>), t = time (days),  $k_a = reaeration rate (day<sup>-1</sup>), R = community respiration rate (mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>), and P (<math>t$ ) = community photosynthesis rate (mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>) at time t. The DM assumes diel stationarity of respiration rate, reaeration, and temperature while describing P(t) with a half-sinusoid function as:

$$P(t) = P_{av}\left(\frac{\pi T}{2f}\right) \sin\left(\frac{\pi t}{f}\right), \ 0 \le t \le f$$
(2)

$$\mathbf{P}(t) = \mathbf{0}, \ \mathbf{f} \le t \le \mathbf{T} \tag{3}$$

Where  $P_{av} = daily$  average community photosynthesis rate  $(mg O_2 L^{-1} day^{-1})$ , T = period (1 day), and f = photoperiod (days). A piecewise DM solution summarized in Chapra and DiToro (1991) for light and dark intervals can be numerically optimized to derive estimates for P, R, and  $k_a$ .

#### 2.3.2. The Butcher and Covington method (BC)

The BC model is an ordinary differential equation (ODE) presented below as Equation (4). The BC differs from the DM in that P, R, and  $k_a$  are temperature-dependent (i.e., not stationary). Temperature dependence in the BC approach is modeled using van't Hoff Arrhenius constants of 1.07, 1.08, and 1.02 for P, R, and  $k_a$ , respectively. Using observed deficit (D) data as the initial condition, the BC model is applied as a forward finite divided difference to propagate changes in D while a central difference is used to describe changes in DO saturation concentration ( $C_s$ ):

$$\begin{aligned} D_{t+1} &= D_t - k_a g(t) D_t \Delta t + Rh(t) \Delta t - P(t) i(t) \Delta t + C_s, (t+1) \\ &- C_s, (t-1), \quad 0 \leq t \leq f \end{aligned} \tag{4}$$

Where g (*t*), h (*t*), and i (*t*) are temperature dependent correction factors for  $k_a$ , R, and P, respectively, using Arrhenius constants listed above. During dark periods  $f \le t \le T$ , P (*t*) is zero in Eq. (4). During daylight hours, Butcher and Covington (1995) assume that P(*t*) can be described by Eq. (2).

#### 2.3.3. The Light Intensity Function (LIF) approach

Use of light intensity data to force P(t) instead of Eq. (2) characterizes the LIF approach as described in Eqs. (5)–(7). Eq. (5) describes photosynthesis as:

$$P(t) = G_{\max}F(I)i(t)\Delta t$$
(5)

Where  $G_{max}$  = maximum photosynthesis rate at 20 °C (mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>), *F*(I) = dimensionless fraction of  $G_{max}$  according to the Smith (1936) framework as follows:

$$F(\mathbf{I}) = \frac{\left(\frac{\mathbf{I}}{\mathbf{I}_{\mathbf{k}}}\right)}{\sqrt{1 + \left(\frac{\mathbf{I}}{\mathbf{I}_{\mathbf{k}}}\right)^2}}$$
(6)

Where I = surface light intensity (in lumens m<sup>-2</sup>) at time t and I<sub>k</sub> is the Smith light parameter (lumens m<sup>-2</sup>). The LIF method includes a respiration inhibition factor,  $F_{\text{ox}}$ , to account for shifts in electron receptor use and therefore better predict low DO concentrations as follows:

$$F_{\rm ox} = 1 - e^{-K_{\rm ox}O} \tag{7}$$

Where  $F_{ox}$  = dimensionless fraction of respiration in response to low DO, O = DO at time step *t*-1, and  $K_{ox}$  = inhibition parameter (L mg O<sub>2</sub><sup>-1</sup>). An ODE describing the LIF is provided as:

$$D_{t+1} = D_t - k_a g(t) D_t \Delta t + RF_{ox} h(t) \Delta t - G_{max} F(l) i(t) \Delta t$$
  
+  $C_s, (t+1) - C_s, (t-1)$  (8)

To compare LIF results that calculate  $G_{max}$  to BC and DM production estimates that often use the average daily rate, P (*t*) output as Eq. (5) was converted to P<sub>av</sub> (from Chapra, 1997):

$$P_{av} = \frac{\int\limits_{0}^{T_{p}} P(t)dt}{T_{p}}$$
(9)

Where  $T_p = daily period (1 day)$ .

Diel temperature adjusted stationarity was assumed for photosynthesis, respiration, and reaeration parameters. Model parameters were allowed to change on a day to day basis to account for changes in light regime, stream hydraulics, and recovery from flood scour.

A mean daylight intensity profile was calculated for each stream to compare measured open field and stream surface light profiles with sinusoids assumed by DM and BC methods. The mean daylight intensity profile is the arithmetic mean of measurements obtained at each 15-minute interval per site (n = 10 days per site) and is expressed as a single intensity value for each 15-minute period throughout the day. A sinusoidal light intensity profile was developed for each site according to:

$$I = I_{Lmax} \sin\left(\frac{t\pi}{f}\right) \tag{10}$$

where  $I_{Lmax}$  = mean maximum light intensity (kilolux) and  $I_L$  = light intensity (kilolux) at time *t*.

#### 2.4. Performance testing

Minimized root mean square error (RMSE, Eq. (11)) between predicted and observed DO deficits for randomly selected days at EFCR, HC, and LDC were obtained through parameter adjustment using unconstrained optimization routines in Microsoft Excel.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (D_{\text{m}} - D_{\text{p}})^2}{n}}$$
(11)

Where  $D_m$  = measured DO deficit and  $D_p$  = predicted DO deficit.

Diel analyses were conducted for n = 30 paired (same site, same day) method comparisons from randomly selected days during

baseflow conditions at the site on each of three streams where stream level and flow data were collected. The RMSE statistic is also used as the basis for comparing performance of metabolism models in our evaluation. While some authors have constrained optimization, or rate parameter estimation, by using a predictive reaeration equation (McTammany et al., 2003; Wang et al., 2003; Portielje et al., 1996), values obtained are often unreliable as these formulae may perform poorly outside the setting in which they were derived (Gualtieri et al., 2002; Melching and Flores, 1999; Moog and Jirka, 1998). Therefore, P, R, and reaeration were determined through optimization of Eq. (8).

#### 3. Results and discussion

#### 3.1. Light intensity function outcomes

Stream surface and open field light intensity measurements deviated from symmetric sinusoids at all sites as illustrated in Fig. 2. The effect of riparian shading is evident at LDC and EFCR where light intensity is truncated in the morning and afternoon (Fig. 2). Photoperiod average daylight stream surface intensity relative to open field measurements was greatest at HC (44%) followed by 32% and 30%, at LDC and EFCR, respectively. Pairwise comparison of open field, stream surface, and sinusoidal light intensities (n = 62 daylight pairs) were significantly different (p < 0.01, Fig. 2) from one another within monitored reference reaches. Therefore, measured light intensity profiles differed in shape and magnitude in comparison to sinusoid assumptions.

Peak light intensity at the stream surface occurred 1.6 h after solar noon on average (n = 10) at HC as a result of canopy and streambank shading. However, HC received significantly (p < 0.05) more total light at the stream surface (mean total = 2446 kilolux) than either LDC (1533 kilolux) or EFCR (1170 kilolux). In addition, average daily photosynthesis (Pav) values (Table 2) at each site were positively correlated ( $r^2 = 0.99$ , p = 0.06, df = 2) with mean total illuminance at each site, suggesting that a combination of topographic, canopy, and streambank shading processes may be limiting primary production within monitored reaches. Canopy light limitation of periphyton biomass and/or calculated Pav has been documented in several studies (Hill et al., 1995; Hill and Knight, 1988; Lowe et al., 1986; Mulholland et al., 2001; Young and Huryn, 1999). Daily comparisons of Pav and incident illuminance were considerably more variable ( $r^2 = 0.24$ , p < 0.01, df = 29) in our study streams and may be attributable to water column attenuation from suspended materials (Lloyd et al., 1987; Stefan et al., 1983) or changes in photosynthetic efficiency in turbid or reduced light conditions (Parkhill and Gulliver, 2002). Well quantified relationships between water column light availability, photosynthetic adaption, and stream metabolism are not well established. However, a recent light quality study by Julian et al. (2008) could supply a quantitative framework to link metabolism with light characteristics. Julian et al. (2008) identified riparian shading and water column depth as most correlated with benthic light availability in small and large study streams, respectively. Water surface processes such as reflection were relatively constant in both reaches studied by Julian et al. (2008) with 92%-93% of incident light entering the water column. In our study we incorporated the effects of cloud and riparian attenuation by measuring light at the stream surface. On that basis, we conclude that variation in the relationship between  $P_{av}$  and illuminance in our study streams is more related to water column processes, including reduced light adaptation, than atmospheric attenuation. We suggest the light budgeting framework of Julian et al. (2008) shows great promise as a tool to investigate water column light and

C. Zell, J.A. Hubbart / Journal of Environmental Management 97 (2012) 69-77



**Fig. 2.** Open field (gray) and stream surface (black) average daylight intensity profiles (n = 10 measurements per 15-min interval) compared to approximate sinusoid function for three Missouri reference streams.

stream metabolism relationships, and thus may supply impetus for future investigations.

#### 3.2. Performance evaluation

The LIF method resulted in a consistently lower and less variable RMSE than the DM or BC (Fig. 3). Curve-fitting error was reduced successively along a site-specific data gradient. The DM which included only high resolution (15-min) DO deficit data achieved a median RMSE (mRMSE) of 0.53 mg L<sup>-1</sup>. A mRMSE = 0.20 mg L<sup>-1</sup> was obtained using the LIF model, which specified diel light

intensity and temperature regimes (Fig. 3). Curve-fitting performance gained through use of light intensity data and model parameterization in the LIF model approached DO instrumentation accuracy of  $\pm 0.2 \text{ mg L}^{-1}$ , suggesting that the LIF model structure does not exacerbate predictive uncertainty beyond measurement error. The reliability of metabolism estimates as reflected by goodness-of-fit (see example in Fig. 4) is well documented in multiple studies including Loperfido et al. (2009), Parkhill and Gulliver (1998) and Portielje et al. (1996). In other approaches (see reviews by; Butcher and Covington, 1995; Frankforter et al., 2010; McTammany et al., 2003, 2007; Mulholland et al., 2001, 2005; Wilcock et al., 1998), this reliability is either depicted graphically or not specified. Portielje et al. (1996) obtained model efficiencies  $r^2 > 0.9$  from 38% to 75% of the time over a two year period (n > 30). A model efficiency of 0.9 was exceeded 93% of the time for n = 30 randomly selected site days with the LIF model in this study. The Parkhill and Gulliver (1998) photorespiration model achieved standard deviations between measured and predicted DO values from 0.12 mg L<sup>-1</sup> to 0.49 mg L<sup>-1</sup>. Loperfido et al. (2009) obtained low RMSEs (<0.15 mg L<sup>-1</sup>) for n = 2 days of data at three locations. Models proposed by Holtgrieve et al. (2010), Parkhill and Gulliver (1998), and Loperfido et al. (2009) fit their respective datasets with error comparable to, or less than, error yielded by the LIF model in this study. Differences between datasets (e.g., sample size, variability, timing, or magnitude) mask performance that solely reflects differences in model structure. For example, the BC model used by Loperfido et al. (2009) achieved low RMSEs ( $<0.15 \text{ mg L}^{-1}$ ) computed from a single stream over a two day deployment period. In comparison, the BC method was outperformed by the LIF model in our analysis and achieved an RMSE of 0.15 mg  $L^{-1}$  or less in just 10 of 30 comparisons across multiple streams and years. Additional sources of variation (i.e., multiple streams and years) present in our dataset may confound inferences of a superior model structure from RMSE metrics presented in other studies.

Pairwise comparison of same-site, same-day estimates of  $P_{av}$  for the DM, BC, and LIF methods were significantly different (p < 0.01). Average daily respiration ( $R_{av}$ ) estimates did not differ significantly (p > 0.05) between methods (Table 2) and exhibited greater variance (F = 0.52,  $\sigma^2 = 8.47$ , p < 0.05) compared to  $P_{av}$  ( $\sigma^2 = 4.39$ ). Sites having the highest and lowest estimates of  $P_{av}$  and  $R_{av}$  also featured the highest and lowest median phytoplankton concentrations and periphyton densities (Tables 1 and 2). The site featuring a temporally-skewed light intensity profile (HC) as a result of topographic shading (cliff wall) had the highest RMSE regardless of analysis method, but was best fit by the LIF approach.

Reaeration is the process whereby oxygen is exchanged between the water column and the atmosphere. The reaeration rate, expressed as either day<sup>-1</sup> or as a mass-transfer velocity in m day<sup>-1</sup>, is positive when the water column is undersaturated (D > 0) and negative (i.e., DO is evaded) when the water is supersaturated (D < 0) (Chapra, 1997). High rates of oxygen transfer to the water column act as a buffer against large deficits and low DO (see Eq. (1)). Therefore, it is well accepted that streams with lower reaeration rates are more sensitive to oxygen demanding substances and processes (Bush, 1972; Langbein and Durum, 1967). Reaeration rates in our study were not significantly different (p > 0.05) when compared by site. Across streams, reaeration rates between methods differed pairwise (p < 0.05) between the LIF model (mean = 1.5 day<sup>-1</sup> at 20 °C) and the DM (mean = 0.9 at 20 °C). The mean BC rate ( $k_a = 1.0 \text{ day}^{-1}$  at 20 °C) was not significantly different from either the DM or LIF. Overall, calculated reaeration rates in study streams were low (pooled mean = 1.1 day<sup>-1</sup> at 20 °C,  $\sigma$  = 0.92, n = 90; 3 sites × 10 days × 3 methods). No diel method vielded reaeration values that were

## Author's personal copy

#### C. Zell, J.A. Hubbart / Journal of Environmental Management 97 (2012) 69-77

### 74 **Table 2**

<sup>a</sup> Site: $n = 10$ , comparisons per site	<sup>b</sup> Method	$^{c}P_{av} (mg O_2 L^{-1} day^{-1})$	${}^{c}R_{av} (mg O_2 L^{-1} day^{-1})$	$^{c}k_{a} (mg \; O_{2} \; L^{-1} \; day^{-1})$	P/R ratio
EFCR: $DO_{mean} = 4.8 \text{ mg } \text{L}^{-1}$ ,	DM (RMSE = 0.29)	3.0	3.2	0.3	1.0
$DO_{min} = 4.1 \text{ mg L}^{-1}$	BC(RMSE = 0.22)	2.1	6.4	1.7	0.3
	LIF (RMSE $= 0.11$ )	2.0	6.1	1.6	0.4
		(Site mean $= 2.4$ )	(Site mean $= 5.2$ )	(Site mean $= 1.2$ )	(Site mean $= 0.6$ )
HC: $DO_{mean} = 6.1 \text{ mg } L^{-1}$ ,	DM (RMSE = 0.80)	6.5	7.5	1.2	0.9
$DO_{min} = 4.3 \text{ mg L}^{-1}$	BC (RMSE = 0.54)	4.3	5.0	0.7	0.9
5	LIF (RMSE $= 0.40$ )	4.1	6.3	1.9	0.7
		(Site mean $= 5.0$ )	(Site mean $= 6.3$ )	(Site mean $= 1.3$ )	(Site mean $= 0.8$ )
LDC: $DO_{mean} = 5.0 \text{ mg } L^{-1}$ ,	DM (RMSE = 0.59)	5.3	6.9	1.1	0.8
$DO_{min} = 3.6 \text{ mg L}^{-1}$	BC (RMSE = 0.35)	3.1	4.7	0.8	0.7
5	LIF (RMSE $= 0.26$ )	2.8	4.8	1.0	0.6
		(Site mean $-3.7$ )	(Site mean $-55$ )	(Site mean $-0.9$ )	(Site mean $-0.7$ )

Optimized parameter rate estimates (at 20 °C) from three single-station whole stream metabolism models evaluated within three shaded Missouri reference streams during July–September, 2006–2008.

<sup>a</sup> EFCR: East Fork Crooked River, HC: Heaths Creek, LDC: Little Drywood Creek.

<sup>b</sup> DM: Delta Method, BC: Butcher and Covington model, LIF: Light Intensity Function model, RMSE: Root Mean Square Error.

<sup>c</sup> P<sub>av</sub>: Average Daily Photosynthesis, R<sub>av</sub>: Average Daily Respiration, k<sub>a</sub>: Average Daily Reaeration.

consistently and significantly correlated with daily average flow. This may be explained by episodic wind driven reaeration during low-flows when wind and streambed shear transitionally compete for dominance of the mass-transfer process (Chu and Jirka, 2003). Alternatively, low correlation between flow and reaeration could be attributed to the predominance of low-flows measured during the study and flow being a surrogate for hydraulic characteristics (e.g., depth, velocity etc.) often related to measured reaeration rates (Melching and Flores, 1999; Moog and Jirka, 1998). Notwith-standing reaeration variability discussed above, it is worth noting that despite variability in reaeration value, relative site mean reaeration values ranked directly with bed gradient (Table 1), a key variable in reaeration predictions (Moog and Jirka, 1998).

The timing of diel DO minima and maxima is influenced by reaeration processes (Chapra and DiToro, 1991). In low reaeration systems, DO minima typically occur later in the morning and maxima further from solar noon (Chapra and DiToro, 1991). As a result of a) low reaeration and b) early morning shading (photosynthesis truncation), minimum DO concentrations occurred 2.2, 2.8, and 3.4 mean hours after sunrise at HC, EFC and LDC study sites, respectively. Termed the 'Approximate' Delta



**Fig. 3.** Root mean square error from n = 30 method comparisons using 15-min continuous sensing data (July–September, 2006–2008) obtained from three Missouri reference streams, where DM is Delta Method, BC is Butcher and Covington model, and LIF is Light Intensity Function model.

Method (ADM), McBride and Chapra (2005) fit solutions of the full DM with computationally less intensive multivariate logistic regression models that utilize photoperiod and lag time  $(\phi)$ between solar noon and deficit minima to calculate reaeration. Confirmation of reaeration rates estimated in this study with ADMderived values is confounded because variable temperature, light, and Arrhenius effects interacted to produce centroid photosynthesis values at 0.3, 0.5, and 1.1 mean hours past solar noon for sites EFC, LDC, and HC, respectively. Application of the ADM to measured data in this study yields mean reaeration estimates of 3.1 (HC), 3.5 (LDC), and 5.8 (EFCR) day<sup>-1</sup> at 20 °C; values above those estimated by curve-fitting optimization (Table 2). It is noteworthy that the duration of photosynthetic input (photoperiod), a key ADM variable, may effectively be truncated by shading (Fig. 2). Overestimation of reaeration through explicit (vs. optimized) application of the DM (or ADM) was also observed by Butcher and Covington (1995), suggesting that the consequences of DM assumptions should be carefully considered when conducting waste assimilation studies.

The relative rate of primary production ( $P_{av}$ ) and respiration ( $R_{av}$ ) characterize whole metabolism as either autotrophic (P/R > 1) or heterotrophic (P/R < 1) and is a fundamental descriptor of aquatic ecosystem energy flow (Odum, 1956; Vannote et al., 1980; Hauer and Lamberti, 2006). Our study streams were net



Fig. 4. Example single-day method calibration comparison (Little Drywood Creek, Missouri) for 17 July 2006.

heterotrophic (site mean P/R ratios < 1.0, Table 2) although the degree of heterotrophy was significant (p < 0.05) only between sites HC and LDC, and sites HC and EFCR. Across streams, each model produced a significantly (p < 0.01) different heterotrophic P/R ratio. On a daily comparison basis, there was trophic agreement (all methods greater, or lesser, than 1.0 on the same site-day) among methods in only 18 of 30 days (68%). Our results suggest that model choice could affect trophic classification (autotrophic or heterotrophic) if applied to a short data record.

In our study, the LIF model produced the lowest estimate of  $P_{av}$  and P/R ratio relative to the DM or BC models. This result is reasonable given the truncated photoperiod (Fig. 2) available for photosynthetic production. To compensate for the relatively low  $P_{av}$ , achieve mass-balance, and fit observed DO data the LIF model produced the highest relative reaeration rate. A similar result was determined by Holtgrieve et al. (2010) where differences in the assumed functional relationship between light and photosynthesis produced distinctly different estimates of  $P_{av}$ ,  $R_{av}$ , and  $k_a$ . Correctly apportioned mass-balances are needed for inverse problems where metabolism parameters are used as calibration targets in process-based water quality models (Chapra, 1997) or to describe ecosystem function (i.e., P/R ratio).

The LIF model (RMSE =  $0.40 \text{ mg L}^{-1}$ ) performed better than the BC model (RMSE =  $0.54 \text{ mg L}^{-1}$ ) in Heaths Creek where deviations from symmetric light intensity profiles were most pronounced. Across streams however, the LIF model slightly reduced RMSE by 0.08 mg  $L^{-1}$  relative to the BC model (Fig. 3), a result attributed to streams in this study being heterotrophic and likely less sensitive to refined representations of photosynthesis functions. However, relatively small differences in daily RMSE can be significant with respect to management implications. For example, Fig. 4 shows that while daily BC and LIF RMSEs differ by only 0.11 mg  $L^{-1}$ , the ability of the LIF to better predict the lowest DO concentrations (critical to aquatic life) during early morning periods is evident. In addition, the ability of the LIF model to better predict the shape of DO profile (Fig. 4) is likely to provide more reliable rate parameter estimates, particularly reaeration (Holtgrieve et al., 2010). For streams where early morning shade delays photosynthetic inputs, models incorporating site-specific light profiles are needed to predict minimum DO concentrations and exposure to hypoxic conditions.

#### 3.3. Metabolism yielding low dissolved oxygen

Diel minimum concentrations approaching concentrations presumed critical for aquatic life (Chapman, 1986; Smale and Rabeni, 1995) are reported for representative sites listed in Table 2. Inspection of Eq. (1) provides a fundamental insight into water column DO processes; when DO is removed from the water column via respiration at a rate exceeding that of photosynthesis, DO deficit will increase unless reaeration is sufficiently high to mitigate losses. Increased deficit generally results in lower DO, except during periods of cooling temperatures where increases in DO saturation may offset a small induced deficit ( $\sim 0.7 \text{ mg L}^{-1}$ ,  $\sim \Delta T = 5$  °C in this study). During days and sites evaluated, R<sub>av</sub> was greater than average Pav, saturation concentration was relatively low (mean  $C_{sat} = 8.1 \text{ mg L}^{-1}$ ), and reaeration rates were well below central tendency database values reported by Melching and Flores (1999) and Moog and Jirka (1998). Consequently, DO was well below saturation and approached critical concentrations. Interestingly, diel minimum DO concentrations were generally observed two or more hours after sunrise suggesting that reduced photosynthesis input from shading, in the presence of low reaeration, may influence the magnitude and duration of critical deficits.

To compare community respiration rates estimated by the LIF model in this study to recent literature, conversion of volumetric model values (as mg  $O_2 L^{-1} day^{-1}$  at 20 °C) to areal units (as g O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> at 20 °C) is necessary. Conversion was accomplished by multiplying daily average pressure-corrected stream level (in meters) by volumetric rates. Community respiration estimated by the LIF model in this study (HC = 3.0, EFCR = 1.5, LDC = 1.0 as g  $O_2 m^{-2} day^{-1}$  at 20 °C) are not unusually greater than values cited in recent literature. In a stream metabolism study featuring 33 sites (n > 2 days per site) within 4 mixed landuse catchments across the U.S., Frankforter et al. (2010) calculated site median respiration values ranging from 0.03 to 36 g  $O_2$  m<sup>-2</sup> day<sup>-1</sup> (median of all sites = 5.0 g  $O_2 m^{-2} day^{-1}$ ), but did not report associated DO minima. Forested streams or corridors studied by Bott et al. (2006), McTammany et al. (2003), and Menninger et al. (2008) generated community respiration values comparable to, or exceeding, values calculated in this study. More extensive lists of community respiration values are provided in Bowie et al. (1985). McTammany et al. (2003) and Sinsabaugh (1997).

Low concentrations of CBOD and ammonia-nitrogen (Table 1) suggest that community respiration in studied reference streams is primarily the result of plant respiration and SOD processes. Studies by Fuss and Smock (1996) and Todd et al. (2009) indicate that SOD may be responsible for the majority of community respiration in blackwater, low DO systems. The upper range of SOD expected from sandy soils is 1.0 g O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (Chapra, 1997) and would account for 100% of the median community respiration estimated at LDC presuming complete coverage of oxygen demanding bottom deposits. Generation of SOD occurs in response to organic matter flux to the streambed (DiToro et al., 1990). Process-based investigations are needed to quantitatively link organic matter budgets (e.g., Webster and Meyer, 1997), community respiration, landscape activities, and DO regimes in studied reference streams. Determination of community metabolism through these method comparisons (i.e., weight of evidence) is a first step towards quantifying metabolism yielding low DO concentrations in least-disturbed reference streams.

#### 4. Conclusions

This study compared whole stream metabolism estimates generated with three single-station diel DO models within three shaded, and seasonally hypoxic, reference streams. The LIF model incorporating continuous light intensity data consistently achieved better goodness-of-fit (median RMSE =  $0.2 \text{ mg L}^{-1}$ ) compared to models assuming sinusoidal light-intensity profiles. Given that daily average stream surface light intensity was significantly less than open-field measurements and sinusoid approximations, the improved performance of the LIF model is not unexpected; however providing evidence of this improvement is novel. Estimates of  $P_{av}$  were significantly different (p < 0.05) between diel analysis methods while  $R_{av}$  estimates were not.

Reaeration rates estimated by all methods were generally low (pooled mean = 1.1 day<sup>-1</sup> at 20 °C) within reference streams. Explicit methods such as the Approximate Delta Method yielded reaeration values greater than those derived through numerical optimization. Differences between explicit and optimized estimates of reaeration are attributed to delays in peak photosynthetic input caused by shading and Arrhenius effects. In combination with low reaeration and summer temperatures, low to moderate community respiration values yielded DO concentrations at or below levels presumed necessary for aquatic life protection (5.0 mg L<sup>-1</sup> in Missouri). Our research suggests that process combinations that occur seasonally (i.e., low reaeration) may limit the achievability of default DO criteria targets for bottomland or prairie streams during warm-weather, low-flow conditions. Identification of these hydroclimatic constraints on regional DO

expectations advances our scientific understanding and informs environmental management policy.

Use of light intensity functions improved the ability of a whole stream metabolism model to characterize the magnitude, duration, and frequency of DO concentrations in shaded study streams; and therefore should be considered a key component for future investigations in shaded systems. Researchers have identified SOD as a primary contributor to community respiration in hypoxic blackwater streams; a process likely important in Missouri reference streams during shallow low-flow conditions. Studies quantifying generation, transport, and delivery processes that yield in-stream SOD are warranted to understand the role of human activities in determining historic, current, and future SOD and DO regimes in reference stream settings.

#### Acknowledgments

This work was funded by the U.S. Environmental Protection Agency Environmental Water Resources and Assessment Project (EWRAP) grant administered by Robert Bacon with the Environmental Resources Coalition in Jefferson City, Missouri. This paper would not be possible without the continued diligence and dedication of David Carani, John Christiansen, Abby Lynn, and Nick Muenks. Mr. Zell is thankful for the guidance and support provided by Trent Stober and his coauthor during this project. The authors are grateful for comments provided by anonymous reviewers whose insights improved the quality of this article.

#### References

- Allen, J., 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284.
- American Public Health Association, APHA, 1995. Standard Methods for the Examination of Water and Wastewater, nineteenth ed. (Washington D.C).
- Bott, T., Newbold, J., Arscott, D., 2006. Ecosystem metabolism in piedmont streams: reach geomorphology modulates the influence of riparian vegetation. Ecosystems 9, 398–421.
- Bowie, G., Mills, W., Porcella, D., Campbell, C., Pagenkopf, J., Rupp, G., Johnson, K., Chan, P., Gherini, S., Chamberlin, C., 1985. Rates, Constants, and Kinetics Formulations in Surface Water Quality Modeling. EPA/600/3-85/040, second ed. U.S. Environmental Protection Agency, Athens, GA.
- Broadmeadow, S., Nisbet, T., 2004. The effects of riparian forest management on the freshwater environment: a literature review of best management practices. Hydrol. Earth Syst. Sci. 8, 286–305.
- Bush, A., 1972. A five-minute solution for stream assimilative capacity. J. Wat. Pollut. Control Fed. 44, 1453–1456.
- Butcher, J., Covington, S., 1995. Dissolved oxygen analysis with temperature dependence. J. Env. Eng. 121, 756–759.
- Chapman, G., 1986. Ambient Water Quality Criteria for Dissolved Oxygen. EPA 440/ 5-86-003. U.S. Environmental Protection Agency, Office of Water, Washington D.C.
- Chapra, S., DiToro, D., 1991. Delta method for estimating primary production, respiration, and reaeration in streams. J. Env. Eng. 117, 640–655.
- Chapra, S., Pelletier, G., Tao, H., 2009. QUAL2K A Modeling Framework for Simulating River and Stream Water Quality, Version 2.11: Documentation and Users Manual Givid and Environmental Face Dont. Tuffs: University, Moderal MA
- Manual. Civil and Environmental Eng. Dept., Tufts University, Medford, MA. Chapra, S., 1997. In: Clark, B., Damstra, D., Bradley, J. (Eds.), Surface Water Quality Modeling. McGraw-Hill, Boston, MA.
- Chu, C., Jirka, G., 2003. Wind and stream flow induced reaeration. J. Env. Eng. 129, 1129–1136.
- DeNicola, M., Hoagland, K., Roemer, S., 1992. Influences of canopy cover on spectral irradiance and periphyton assemblages in a prairie stream. J. N. Am. Benth. Soc. 11, 391–404.
- Detroy, M., Skelton, J., 1983. Hydrology of Area 38, Western Region, Interior Coal Province Iowa and Missouri. Water Resources Investigations Open-file Report 82–1014. U.S. Geological Survey, Rolla, Missouri and Iowa City, Iowa.
- DeWalle, D., 2008. Guidelines for riparian vegetative shade restoration based upon a theoretical shaded-stream model. JAWRA 44, 1373–1387.
- DeWalle, D., 2010. Modeling stream shade: riparian buffer height and density as important as buffer width. JAWRA 46, 323–333.
- DiToro, D., Paquin, P., Subburamu, K., Gruber, D., 1990. Sediment oxygen demand model: methane and ammonia oxidation. J. Env. Eng. 116, 945–986.
- Frankforter, J., Weyers, H., Bales, J., Moran, P., Calhoun, D., 2010. The relative influence of nutrients and habitat on stream metabolism in agricultural streams. Env. Mon. Assess. 168, 461–479.

- Fuss, C.L., Smock, L.A., 1996. Spatial and temporal variation of microbial respiration rates in a blackwater stream. Fresh. Biol. 36, 339–349.
- Gallant, A., Whittier, T., Larsen, D., Omernik, J., Hughes, R., 1989. Regionalization as a Tool for Managing Environmental Resources. EPA/600/3–89/060. U.S. Environmental Protection Agency, Office of Research and Development, Corvalis, Oregon.
- Glasgow, H., Burkholder, J., Reed, R., Lewitus, A., Kleinman, J., 2004. Real-time remote monitoring of water quality: a review of current applications, and advancements in sensor, telemetry, and computing technologies. J. Exp. Mar. Biol. Ecol. 300, 409–448.
- Gregory, S., Swanson, F., McKee, W., Cummins, K., 1991. An ecosystem perspective of riparian zones. BioSci. 41, 540–551.
- Gualtieri, C., Gualtieri, P., Doria, G., 2002. Dimensional analysis of reaeration rate in streams. J. Env. Eng. 128, 12–18.
- Hauer, F., Hill, W., 2006. Temperature, light, and oxygen. In: Hauer, F., Lamberti, G. (Eds.), Methods in Stream Ecology, second ed. Academic Press, Burlington, MA, pp. 103–118.
- Hauer, F., Lamberti, G. (Eds.), 2006. Methods in Stream Ecology, second ed. Academic Press, MA.
- Heath, A., 1995. Water Pollution and Fish Physiology, second ed. Lewis, Boca Raton, FL, 359 pp.
- Hill, W., Knight, A., 1988. Nutrient and light limitation of algae in two northern California streams. J. Phycol. 24, 125–132.
- Hill, W., Ryon, M., Schilling, E., 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology 76 (4), 1297. Holtgrieve, W., Schindler, D., Branch, T., A'mara, Z., 2010. Simultaneous quantifica-
- Holgheve, W., Schnader, D., Branch, L., A mara, Z., 2010. Simultaneous quantification of aquatic ecosystem metabolism and reaeration using a Bayesian statistical model of oxygen dynamics. Limnol. Oceanogr. 55, 1047–1063.
- Huggins, D., Dzialowski, A., 2005. Identification and Quantification of Reference Conditions Associated with Lotic Ecosystems of the Central Plains and Surrounding Regions: A Summary of Approaches and Factors and Regional Approach. Report No. 134. Kansas Biological Survey, Lawrence, KS.
- Hughes, R., Larsen, D., Omernik, J., 1986. Regional reference sites: a method for assessing stream potentials. J. Env. Mgmt. 10, 629–635.
- Hynes, H., 1971. The Biology of Polluted Waters. Univ. of Toronto Press.
- Ice, G., Sugden, B., 2003. Summer dissolved oxygen concentrations in forested streams of northern Louisiana. S. J. Appl. For. 27, 92–99.
- Julian, J., Doyle, M., Stanley, E., 2008. Empirical modeling of light availability in rivers. J. Geophys. Res. 113 (G03022), 1–16.
- Kilpatrick, F., Rathbun, R., Yotsukura, N., Parker, G., DeLong, L., 1989. Determination of Stream Reaeration Coefficients by Use of Tracers. Book 3, Chapter A18. USGS Techniques of Water Resources Investigations, Denver, Colorado.
- Kramer, D.L., 1987. Dissolved oxygen and fish behavior. Env. Bio. Fishes 18, 81–92. Langbein, W., Durum, W., 1967. The Aeration Capacity of Streams. Geological Survey Circular 542. U.S. Geological Survey, Washington, D.C.
- Lloyd, D.S., Koenings, J.P., La Perriere, J.D., 1987. Effects of turbidity in fresh waters of Alaska. N. Am. J. Fish. Mgmt. 7, 18–33.

Loperfido, J., Just, C., Schnoor, J., 2009. High-frequency diel dissolved oxygen stream data modeled for variable temperature and scale. J. Env. Eng. 135, 1250–1256.

- Louisiana Department of Environmental Quality (LDEQ), 2008. Use Attainability Analysis of Barataria and Terrebonne Basins for Revision of Dissolved Oxygen Water Quality Criteria. Water Quality Assessment Division, Baton Rouge, Louisiana.
- Lowe, R., Golladay, S., Webster, J., 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. J. N. Am. Benth. Soc. 5, 221–229.
- McBride, G., Chapra, S., 2005. Rapid calculation of oxygen in streams: approximate delta method. J. Env. Eng. 131, 336–342.
- McTammany, M., Webster, J., Benfield, E., Neatrour, M., 2003. Longitudinal patterns of metabolism in a southern appalachian river. J. N. Am. Benth. Soc. 22, 359–370.
- McTammany, M., Benfield, E., Webster, J., 2007. Recovery of stream ecosystem metabolism from historical agriculture. J. N. Am. Benth. Soc. 26, 532–545.
- Melching, C., Flores, H., 1999. Reaeration equations derived from U.S. geological survey database. J. Env. Eng. 125, 407–414.
- Menninger, H., Palmer, M., Craig, L., Richardson, D., 2008. Periodical cicada detritus impacts stream ecosystem metabolism. Ecosystems 11, 1306–1317.
- Midwest Regional Climatic Center, MRCC, 2010. Annual Data Download for Cooperative Stations 234904 (Lexington 3 N), 237632(Sedalia WTP), and 235987 (Nevada WTP) (Champaign, IL).
- Moog, D., Jirka, G., 1998. Analysis of reaeration equations using mean multiplicative error. J. Env. Eng. 124, 104–110.
- Mulholland, P., Fellows, C., Tank, J., Grimm, N., Webster, J., Hamilton, S., Martia, E., Ashkenas, L., Bowden, W., Dodds, W., McDowell, W., Paul, M., Peterson, B., 2001. Inter-biome comparison of factors controlling stream metabolism. Fresh. Biol. 46, 1503–1517.
- Mulholland, P., Houser, J., Maloney, K., 2005. Stream diurnal dissolved oxygen profiles as indicators of in-stream metabolism and disturbance effects: Fort Benning as a case study. Ecol. Indic. 5, 243–252.
- Naiman, R., D'Camps, H., 1997. The ecology of interfaces: riparian zones. Annu. Rev. Ecol. Evol. Syst. 28, 621–658.
- O'Connor, D., Di Toro, D., 1970. Photosynthesis and oxygen balance in streams. J. Sanit. Eng. Div. 96, 547–571.
- Odum, H.T., 1956. Primary production in flowing waters. Limnol. Oceanogr. 1, 102-117.

C. Zell, J.A. Hubbart / Journal of Environmental Management 97 (2012) 69-77

- Parkhill, K., Gulliver, J., 1998. Application of photorespiration concepts to whole stream productivity. Hydrobiologia 389, 7–19.
- Parkhill, K., Gulliver, S., 2002. Effect of inorganic sediment on whole-stream productivity. Hydrobiologia 472, 5–17.
- Portielje, R., Kersting, K., Lijklema, L., 1996. Primary production estimation from continuous oxygen measurements in relation to external nutrient input. Water. Res. 30, 625–643.
- Rabeni, C., Sarver, R., Wang, N., Wallace, G., Weiland, M., Peterson, J., 1997. Development of Regionally Based Biological Criteria for Streams in Missouri. Cooperative Fish and Wildlife Research Unit, University of Missouri.
- Rathbun, R.E., 1977. Reaeration coefficients of streams-state-of-the art. J. Hydr. Div., ASCE 103, 409–424.
- Sinsabaugh, R.L., 1997. Large-scale trends for stream benthic respiration. J. N. Am. Benth. Soc. 16, 119–122.
- Smale, M., Rabeni, C., 1995. Hypoxia and hyperthermia tolerances of headwater stream fishes. Trans. Am. Fish. Soc. 124, 698–710.
- Smith, E., 1936. Photosynthesis in relation to light and carbon dioxide. Proc. Natl. Acad. Sci. 22, 504–511.
- Smith, L., 1982. Introduction to Fish Physiology. T.F.H. Publications, Neptune, New Jersey, 346 pp.
- Soil Conservation Service, SCS, 1977. Soil Survey of Vernon County, Missouri. United States Department of Agriculture, Soil Conservation Service.
- Soil Conservation Service, SCS, 1986. Soil Survey of Clay and Ray Counties, Missouri. United States Department of Agriculture, Soil Conservation Service.
- Soil Conservation Service, SCS, 1995. Soil Survey of Pettis County, Missouri. United States Department of Agriculture, Soil Conservation Service.
- Sowa, S., Annis, G., Morey, M., Diamond, D., 2007. A gap analysis and comprehensive conservation strategy for riverine ecosystems in Missouri. Ecol. Mono. 77, 301–334. Stefan, H., Cardoni, J., Schiebe, F., Cooper, C., 1983. Model of light penetration in
- a turbid lake. Water. Resour. Res. 19, 109–120. Stoddard, J., Larsen, D., Hawkins, C., Johnson, R., Norris, R., 2006. Setting expectation
- for the ecological condition of streams: the concept of reference condition. Ecol. App. 16, 1267–1276.

- Todd, M., Vellidis, G., Lowrance, R., Pringle, C., 2009. High sediment oxygen demand within an instream swamp in southern Georgia: implications for low dissolved oxygen levels in coastal blackwater streams. JAWRA 45, 1493–1507.
- Truax, D.D., Shindala, A., Sartain, H., 1995. Comparison of two sediment oxygen demand measurement techniques. J. Env. Eng. 121, 619–624.
   United States Environmental Protection Agency, US EPA, 2000. Level III Ecoregions
- United States Environmental Protection Agency, US EPA, 2000. Level III Ecoregions of the Continental United States. National Health and Environmental Effects Research Laboratory, Corvallis, Oregon.
- Utley, B., Vellidis, G., Lowrance, R.R., Smith, M.C., 2008. Factors affecting sediment oxygen demand dynamics in blackwater streams of Georgia's coastal plain. JAWRA 44, 742–753.
- Vannote, R., Minshall, G., Cummins, K., Sedell, J., Cushing, C., 1980. The river continuum concept. Can. J. Fish. Aq. Sci. 37, 130–137.
- Wagner, R., Mattraw, H., Ritz, G., Smith, B., 2000. Guidelines and Standard Procedures for Continuous Water-Quality Monitors: Site Selection, Field Operation, Calibration, Record Computation, and Reporting. Water-Resources Investigations Report 00–4252. U.S. Geological Survey, Reston, VA.
   Wang, H., Hondzo, M., Xua, C., Poole, V., Spacie, A., 2003. Dissolved oxygen
- Wang, H., Hondzo, M., Xua, C., Poole, V., Spacie, A., 2003. Dissolved oxygen dynamics of streams draining an urbanized an agricultural catchment. Ecol. Model. 160, 145–161.
- Wanninkhof, R., 1992. Relationship between wind speed and gas exchange over the ocean. J. Geophys. Res., C: Oceans Atmos. 97 (C5), 7373–7382.
- Webster, J., Meyer, J., 1997. Organic matter budgets for streams: a synthesis. J. N. Am. Benth. Soc. 16, 141–161.
- Wetzel, R., Likens, G., 2000. Limnological Analyses, third ed. Springer-Verlag New York, Inc, 429 pp.
- Wilcock, R., Nagels, J., McBride, G., Collier, K., Wilson, B., Huser, B., 1998. Characterisation of lowland streams using a single-station diurnal curve analysis model with continuous monitoring data for dissolved oxygen and temperature. NZ J. Mar. Fresh. Res. 32, 67–79.
- Young, R., Huryn, A., 1999. Effects of land use on stream metabolism and organic matter turnover. Ecol. App. 9, 1359–1376.

## RATIONALE FOR MISSOURI'S PROPOSED NUTRIENT CRITERIA RULE – DRAFT II March 4, 2008

## Table of contents

Nutrient Criteria Background and Timeline	2
The Nutrients and Algae	2
Nutrient Forms, Algal Estimators, Units of Measure and Methodologies	3
EPA Approaches to Nutrient Criteria	5
Missouri's Lakes and Reservoirs	6
Relationships between Water Quality Parameters in Missouri's Reservoirs	10
Proposed Nutrient Criteria Approach for Missouri	12
Regional Divisions	13
Influence of Morphology, Hydrology and Historic land Cover	14
Reference Reservoirs	16
Phosphorus Rule	19
Nitrogen Background	21
Nitrogen Rule	23
Chlorophyll Background	24
Chlorophyll Rule	26
Literature Cited	29

## Tables

Comparison of criteria based on EPA approaches	5
Statistics for reservoir morphology and watershed measurements	7
Statistics for water quality measurements in Missouri reservoirs	8
Reference reservoir phosphorus data	.18

## Figures

Distribution of phosphorus values in Plains reservoirs	8
Distribution of phosphorus values in Ozark Highlands reservoirs.	
Distribution of phosphorus values in Ozark Border reservoirs.	
Chlorophyll-phosphorus relationship in Missouri reservoirs.	
Chlorophyll-nitrogen relationship in Missouri reservoirs	
Phosphorus-nitrogen relationship in Missouri reservoirs	
Secchi transparency-chlorophyll relationship in Missouri reservoirs	12
Map of eco-regions	13
Influence of residence time on predicted phosphorus	15
Observed-predicted phosphorus in Plains Region	15
Observed-predicted phosphorus in combined Ozark regions	16
Prediction, 75 <sup>th</sup> and 10 <sup>th</sup> percentile lines	17
Phosphorus matrix with action zones	
Phosphorus matrix divided into sub-zones	
Breakdown of Zone A	19
Breakdown of Zone B	
Breakdown of Zone C	21
Maximum-mean chlorophyll relationship in Missouri reservoirs	25
Frequency of extreme chlorophyll values	

## Nutrient Criteria Background and Timeline

In 1998 the U.S. Environmental Protection Agency (EPA) published the *National Nutrient Strategy for Development of Regional Nutrient Criteria* in response to renewed concerns about nutrient enrichment of the nation's surface waters (Kennedy 2001). The impetus for moving forward with criteria development was rooted in the fact that ~50% of surface water impairments reported by states were attributed to excess nutrients and the related biological growth (EPA 2000). EPA set as a goal for states to have nutrient criteria rules in place by 2003 (EPA 1998). The difficultly inherent in developing nutrient criteria rules was punctuated when no states were able to meet the 2003 deadline.

In October 2003, a document entitled *Developing nutrient criteria for Missouri lakes* was presented to Missouri Department of Natural Resources (MDNR). This report, written by University of Missouri (MU) limnologists Dr. M.F. Knowlton and Dr. J.R. Jones, represented a review of water quality in Missouri's lakes and their professional opinion concerning the development of nutrient criteria for the state. In summary, Knowlton and Jones felt there was "too little information on the relationship between nutrients and specific water quality impairments (except for reduced water clarity) with which to formulate numerical criteria for nutrients specific to statutorily protected water uses" (Knowlton and Jones 2003). A starting point, it was noted, might be to set phosphorus criteria to maximize water clarity to the extent achievable for individual lakes. Factors such as lake depth, hydrology and traditional land use within watersheds could be used to predict achievable water clarity (Knowlton and Jones 2003).

In July 2005, MDNR and EPA agreed on a nutrient criteria plan for Missouri that had as a goal the promulgation of rules governing criteria in lakes and reservoirs by the end of 2006 (MDNR 2005). The first Nutrient Criteria Stakeholders meeting was held in October 2005, with meetings occurring on a monthly basis. By December 2006 a number of options had been discussed by the stakeholders for setting criteria for lakes and reservoirs in the state, but none were acceptable to the stakeholders group as a whole. In January 2007 a scientific sub-committee was formed to focus on developing well reasoned and defendable approaches to nutrient criteria. The sub-committee met on a monthly basis and slowly pieced together the current proposed approach. As the approach was developed, reports back to the stakeholders took place. This rationale represents the combined work of both the sub-committee and the stakeholders group in developing a workable plan for Missouri that meets EPA requirements.

## The Nutrients and Algae

Phosphorus and nitrogen are the two nutrients listed as causal variables to be addressed by states in the development of criteria (EPA 2000). Aquatic ecologists have long known the importance of these two nutrients in regulating plant growth in lakes, with one or both nutrients often limiting plant biomass (Sakamoto 1966). Both phosphorus and nitrogen are naturally occurring in the environment, and the nutrients themselves are not a direct danger to the aquatic life within the lake or to humans using the lake for recreation. The nutrients act as fertilizers in lakes, just as they do in the terrestrial system. The aquatic plant growth of most concern is the proliferation of algae that accompanies excess nutrients.

Algae are plant-like organisms suspended in the water column or attached to substrate. They are mostly microscopic, though individual cells can form colonies which may be seen as clumps or "surface scums" in the open water or as mats of green growth on the bottom. Algae are an important part of the lake ecosystem, making up the base of the food web which fixes solar energy into usable organic matter for other aquatic life. While algae are important for a healthy lake, excessive algae can negatively impact water quality. Large algal blooms affect aquatic life by causing considerable fluctuations in dissolved oxygen levels within the lake. During the day an algal bloom will give off a large amount of oxygen as a byproduct of photosynthesis, leading to high concentrations of dissolved oxygen. At night the algae and other aquatic life use oxygen through respiration. This respiration along with decomposition of organic matter can reduce dissolved oxygen levels low enough to harm aquatic life (e.g. fish kill). Also, algal blooms can reduce the recreational value of a lake by decreasing water clarity (EPA 2000).

While nutrients and algae are naturally occurring in our lakes, the inherent background level of nutrients found within a water body varies. The natural background level of nutrients is not of concern to EPA, instead the focus of criteria development is excess nutrients associated with human influences in the watersheds.

## Nutrient Forms, Algal Estimators, Units of Measure and Methodologies

There are various forms of both phosphorus and nitrogen in natural waters. Some nutrient forms are dissolved while others are bound to inorganic materials or integrated within organic matter. The proposed criteria will focus on what is commonly known as total phosphorus and total nitrogen. Simply put, we are not concerned with the individual forms of the nutrients, but in the total amount of the nutrients inclusive of all forms.

Calculating algal biomass is both time-consuming and costly so algal biomass is often estimated by measuring the photosynthetic pigment chlorophyll. EPA lists chlorophyll as the primary response variable that states should focus on during nutrient criteria development (EPA 2000). This rationale and the proposed rule will use the measure of total chlorophyll as a surrogate for algal biomass.

In this rationale and in the proposed rule all phosphorus, nitrogen and chlorophyll values are presented using the same unit of measure, micrograms per liter ( $\mu$ g/L). Because we are interested in the total amounts of phosphorus and nitrogen, reported values represent the weight of the element of interest and not the weight of a compound

(ex. a phosphorus value of 10  $\mu$ g/L would equate to 10  $\mu$ g of phosphorus and not 10  $\mu$ g of PO<sub>4</sub>). It should be noted that micrograms per liter is often considered the equivalent to parts per billion (ppb).

The majority of water quality data used in the development of nutrient criteria originated from the MU limnology laboratory, and were produced following Quality Assurance Project Plans (QAPP) approved by MDNR and EPA. Data generated in the future for the purpose of nutrient criteria compliance should be a product of the same methods or methods that can be shown to produce comparable data. The following are the laboratory methods used by MU for analyses of nutrients and chlorophyll:

Total Phosphorus is measured using the ascorbic acid method (method 4500-P E) after persulfate digestion (method 4500-P B5) as presented in *Standard Methods for the Examination of Water and Wastewater* (APHA 1995).

Total Nitrogen is measured using the Second Derivative Method (Crumpton *et al.* 1992) after persulfate digestion.

Total Chlorophyll is measured fluorometrically after extraction in ethanol (Knowlton 1984, Sartory and Grobbelaar 1986).

Nutrients and algal chlorophyll are highly variable on the temporal scale in Missouri's reservoirs (Knowlton *et al.* 1984, Knowlton and Jones 2006b). Chlorophyll varies the most, with individual reservoir maximum and minimum values differing on average by a factor of 22. By comparison, maximum and minimum phosphorus values differ on average by a factor ~7, while nitrogen varies by an average factor ~4 (Knowlton and Jones 2003). Because of this high variability, sufficient monitoring is required to ensure that calculated mean values for nutrients and chlorophyll are truly representative of reservoir water quality (Knowlton and Jones 2006a, Knowlton and Jones 2006b). Past research on Missouri's reservoirs indicates that appropriate monitoring should include data from at least four summers, with at least four samples collected during each summer (Knowlton and Jones 2006b). This level of monitoring will allow the state to meet EPA's recommendation that "the method of data gathering for compliance should be near as possible to that used to establish the criteria" (EPA 2000).

Because of the natural variability in nutrient and chlorophyll data, geometric averaging has been used in the development of the proposed rule. This technique for defining the central tendency is less influenced by extreme values than simple arithmetic averaging. The science sub-committee recommends the use of geometric mean calculations for all future nutrient criteria efforts.

## EPA Approaches to Nutrient Criteria

EPA's *Nutrient Criteria Technical Guidance Manual* lists determination of reference conditions as a cornerstone to nutrient criteria development (EPA 2000). In short, EPA suggests states monitor water quality in the least impacted/most pristine lakes and reservoirs within the state. Nutrient levels in the reference water bodies could be used either as part of the process of criteria development or the final criteria could be set based on reference lake data (EPA 2000). If states use reference data to set criteria, EPA recommends the 75<sup>th</sup> percentile of the data distribution as the criteria (EPA 2000).

A second approach that uses data distribution to identify criteria is suggested if states do not have a sufficient number of reference lakes and reservoirs. This approach focuses on data from all monitored water bodies within the state. EPA recommends using the 25<sup>th</sup> percentile of this data distribution as a parallel to the 75<sup>th</sup> percentile of reference data (EPA 2000).

A third option is for states to use ecoregional data that has been assembled by EPA. This is an option that would allow states with little or no historic monitoring of lakes and reservoirs to be able to generate criteria. The approach is to simply use the 25<sup>th</sup> percentile of data from the ecoregion distribution. A comparison of phosphorus, nitrogen and chlorophyll criteria based on these three different approaches is shown in Table 1.

These three approaches suffer the same major short-coming; all reservoirs within a region are held to the same criteria without regard to the morphological, hydrological or biological differences among the reservoirs. These "one size fits all" approaches could fail to protect those reservoirs that currently have exceptional water quality (if criteria values are set too high), target some reservoirs for unobtainable nutrient reductions (if criteria are set too low), or a combination of the two problems (if criteria are set at a moderate level).

	rubie 1.77 companion of potential officina based off three baggeoted El 77 approaches.				
Region	Approach	TP (µg/L)	TN (µg/L)	CHL (µg/L)	
	Reference	58	820	22.9	
Plains	Population	31	675	11.5	
	Ecoregion*	40	660	5.6	
Ozork	Reference	41	660	19.7	
Ozark	Population	20	495	5.2	
Doruer	Ecoregion	30	615	9.1	
Ozork	Reference	26	490	8.2	
Uzark	Population	9	275	2.3	
	Ecoregion	24	500	6.1	

Table 1.	A com	parison	of potential	criteria	based on	three su	iggested	EPA ap	proaches.
----------	-------	---------	--------------	----------	----------	----------	----------	--------	-----------

\*A few reservoirs in the northwest corner of the state would fall into a separate EPA ecoregion. Criteria for these reservoirs would be: phosphorus 55  $\mu$ g/L, nitrogen 965  $\mu$ g/L and chlorophyll 18.8  $\mu$ g/L.

The stated goal of nutrient criteria is to protect the designated uses that are impaired by elevated levels of algal biomass (EPA 2000). One conceivable approach to developing nutrient criteria would be to identify the various impairments to lake uses, tie those impairments to algal biomass, and then correlate the algal biomass to phosphorus and nitrogen concentrations. In theory, lakes and reservoirs meeting nutrient criteria would not have algal biomass that would cause the various use-impairments.

The science sub-committee chose not to pursue this approach for setting nutrient criteria because use-impairments can be difficult to identify and correlating impairments to specific nutrient levels can be impracticable. Uses in which the identification of impairments can be difficult include: resident and migratory wildlife habitat, storm and flood storage attenuation, industrial process and cooling water, and irrigation. For uses where impairments can be identified the impairments do not necessarily relate to measures of algal biomass. In drinking water reservoirs the presence of disagreeable tastes and odors can be considered as an impairment, but not all taste and odors are directly related to algae. Inorganic chemicals such as reduced species of iron, manganese and sulfur can be the source of taste and odor problems, as can organic chemicals associated with bacteria (Knowlton and Jones 2003). Not all algae produce taste and odors, and when taste and odor problems associated with algae do occur, the problems are a factor of algal speciation and not the over-all algal biomass.

Another problem with using the impairment-based approach to developing nutrient criteria is the fact that Missouri's reservoirs support multiple uses. Two of the most common uses are fishing and swimming, which differ greatly in terms of the algal biomass associated with optimal water quality. A reservoir with exceptional water clarity might be considered perfect for swimming, but would lack the algal growth to maximize fish production. On the other hand, the best fishing lakes in Missouri are never crystal clear.

## Missouri's Lakes and Reservoirs

Missouri has an extremely diverse population of lakes and reservoirs, with approximately 1800 water bodies >10 acres in surface area. The majority of lakes in Missouri are constructed impoundments (reservoirs), with the natural lakes generally being limited to oxbow lakes and "blew holes" located within river flood plains. The state currently lists 458 water bodies as classified waters.

About a third of the classified waters (141 of 458) are reservoirs that have been monitored sufficiently to allow for adequate description of average phosphorus, nitrogen and algal chlorophyll concentrations. Data from these monitored water bodies were used in the process of developing the proposed criteria. The following is a brief review of the range of both the factors that are important in influencing water quality (Table 2) and the observed long-term water quality (Table 3). The goal of this review is to communicate the diversity of Missouri's water resources.

Missouri's reservoirs span an extremely wide range of morphological conditions (Table 2). This range is somewhat skewed by the fact that along with moderate sized "community" lakes, the data set contains a dozen reservoirs that are over 2000 acres in size. When these large reservoirs are removed from the analyses, we still find a very diverse collection of reservoirs, with surface areas, dam height and volume spanning 6 to 1576 acres, 15 to 139 feet, and 48 to 27,680 acre/feet, respectively.

Watershed size also varies considerably even after the large reservoirs are removed from the data set, with a range of 83 to 174,000 acres. Along with size, watershed land cover differs greatly in Missouri. In the 141 watersheds analyzed, the current amount of forest land cover ranges from 0 to 95% of the watershed, grassland ranges 0 to 78%, crop ranges 0 to 74% and urban ranges from 0 to 96% (Jones *et al.* 2004). While current land cover is not to be used in setting nutrient criteria (EPA, per comm), research has shown that current land cover plays a strong role in determining reservoir water quality (Jones *et al.* 2004, Jones *et al.* 2008).

and watershed measurements.			
	Minimum	Maximum	Median
Surface Area (acres)	6	53,814	103
Dam Height (feet)	15	252	45
Volume (acre/feet)	48	2,700,000	1,675
Watershed Area (acres)	83	>4,000,000	2,516
Residence Time* (months)	0.08	108	10.5

Table 2. Minimum, maximum and median values for important reservoir morphology and watershed measurements.

\*Residence time is a hydrological calculation that describes the average amount of time it takes for a reservoir's inflow to equal the reservoirs's volume.

Just as reservoir morphology and watershed characteristics vary greatly in Missouri reservoirs, so does water quality. Some of Missouri's reservoirs are categorized as oligotrophic (low plant productivity) and have very low nutrient and chlorophyll concentrations. These reservoirs are best described as clear and blue. On the other end of the spectrum, some of Missouri's reservoirs are hypereutrophic (extremely high plant productivity) and have excess nutrients concentrations and very high chlorophyll values. These reservoirs tend to have very low water clarity, are green in color, and may have "surface scums" of algae.

In monitored reservoirs, long-term geometric mean phosphorus concentrations range from 6 to 170  $\mu$ g/L (Table 3). Nitrogen spans an order of magnitude among Missouri's reservoirs, with geometric mean values ranging from 200 to 2235  $\mu$ g/L, while geometric mean chlorophyll values range from 1.1 to 56.7  $\mu$ g/L.

	Minimum	Maximum	Median
Phosphorus (µg/L)	6	170	39
Nitrogen (µg/L)	200	2235	723
Chlorophyll (µg/L)	1.1	56.7	14.2

Table 3. Minimum, maximum and median long-term geometric mean values from 141 monitored reservoirs.

In order to provide a visual representation of the variability found in Missouri's reservoirs, geometric mean phosphorus concentrations are shown as bar plots (Figure 1 - 3). The reservoirs have been divided according to ecoregion in order to show that even when regional differences are accounted for, there is still substantial variation in water quality. Data from the Plains Region of the state, which consist of northern and western Missouri, is shown in Figure 1. Each horizontal bar represents an individual reservoir in this region, with geometric mean phosphorus scaled along the x-axis. In the Plains Region, reservoirs have phosphorus levels that range from 14 -170  $\mu$ g/L (Figure 1). In the Ozark Highlands Region (southern Missouri) geometric mean phosphorus ranges from 6 - 59  $\mu$ g/L (Figure 2), a notably smaller range than measured in the Plains Region. The Ozark Border Region of the state is a transitional zone between the Plains and the Highlands. Phosphorus concentrations in this region range from 12 - 87  $\mu$ g/L (Figure 3). All three regions display considerable among-system variability, with minimum and maximum phosphorus means differing by a factor of 12, 10 and 7 in the Plains, Highlands, and Border regions, respectively.



Figure 1. Distribution of geometric mean phosphorus values for monitored reservoirs in the Plains Region.



Figure 2. Distribution of geometric mean phosphorus values for monitored reservoirs in the Ozark Highlands Region.



Figure 3. Distribution of geometric mean phosphorus values for monitored reservoirs in the Ozark Border Region.

## Relationships between Water Quality Parameters in Missouri's Reservoirs

Missouri has a wealth of water quality data on its reservoirs and numerous scientific articles have been published concerning nutrients and their relationship to algal chlorophyll (Knowlton *et al.* 1984, Jones and Knowlton 1993, Knowlton and Jones 1995, Jones *et al.* 1998, Jones and Knowlton 2005, Knowlton and Jones 2006a, Knowlton and Jones 2006b). Algal chlorophyll shows strong correlations to both phosphorus and nitrogen in Missouri reservoirs (Figures 4 & 5). As nutrient concentrations increase across the range of values found in the state, there is a predictable increase in the amount of algal chlorophyll.



Chlorophyll correlates to both phosphorus and nitrogen because the two nutrients tend to co-vary strongly in Missouri's reservoirs (Figure 6). Reservoirs with low levels of phosphorus tend to have low concentrations of nitrogen, while reservoirs with high levels of phosphorus have high concentrations of nitrogen. This correlation between phosphorus and nitrogen occurs because reservoir and watershed characteristics (including anthropogenic activities) that are important factors in determining water quality influence both nutrients. Because of this tendency for nutrient concentrations to increase concurrently, algal growth in most Missouri reservoirs is not strongly limited by either nutrient individually, but instead may be considered as being co-limited by both nutrients.



Figure 6. The relationship between nitrogen and phosphorus in Missouri's reservoirs. Symbols represent geometric mean values from individual reservoirs and the line represents the average relationship between the two parameters. [ $r^2 = 0.82$ ]

Chlorophyll shows a curvilinear relationship to water clarity (measured as Secchi depth). This relationship has two distinct arms where water clarity responds to changes in algal chlorophyll in very different fashion (Figure 7). When chlorophyll levels are low (<6  $\mu$ g/L) the relationship between transparency and chlorophyll is nearly vertical, with dramatic changes occurring in water clarity associated with relatively small increases or decreases in algal chlorophyll concentrations (Figure 7). When chlorophyll concentrations are >12  $\mu$ g/L the two parameters display a flat relationship, with water clarity changing very little even when chlorophyll concentrations display substantial shifts. The inflection point, where the relationship changes, occurs when chlorophyll concentrations are between 6 - 12  $\mu$ g/L.



Figure 7. The relationship between algal chlorophyll and Secchi transparency values in Missouri's reservoirs. Symbols represent geometric mean values from individual reservoirs and the solid line represents the average relationship between the two parameters. Vertical dashed lines provide an estimate of the inflection zone.  $[r^2 = 0.49]$ 

## Proposed Nutrient Criteria Approach for Missouri

The science sub-committee proposes an approach that:

- Separates reservoirs by ecoregion, to reduce the risk of comparing water bodies built in landscapes with different geology, soils and topography.
- 2) Uses reservoir morphology and hydrology to differentiate reservoirs with varying water quality potential based on factors that were determined when the water bodies were constructed (e.g. volume, watershed area).
- Determines the range of expected phosphorus concentrations in reservoirs that have nominal human impact within their watershed. This component is a modification to EPA's reference approach.

By using this multi-faceted approach the scientific sub-committee was able to create a matrix that will aid in decision making concerning phosphorus criteria. Actions concerning nitrogen and chlorophyll will also be based on the individual reservoir's location in the matrix. Target nitrogen and chlorophyll values will be determined using the target phosphorus values.

The proposed rule will be applicable for sites located in deep water near the dam for all reservoirs in the Plains, Ozark Border and Ozark Highlands regions. The proposed rule and this rationale do not address nutrient criteria in Missouri's natural lakes (oxbows and blew holes) or any man-made reservoirs that are located within the Big Rivers Region (Figure 8). Criteria for these water bodies will be developed at a later time. Criteria for secondary sites on reservoirs with surface areas >2000 acres will also be developed at a later time to address the spatial variability in water quality observed in these large reservoirs (Jones and Novak 1981, Jones and Kaiser 1988, Knowlton and Jones 1995, Obrecht *et al.* 2005).

## Regional Divisions

For the purpose of setting nutrient criteria, the scientific sub-committee suggests the state be divided into four ecoregions, which differ in terms of geology, topography, and historic land cover (Figure 8). These factors influence reservoir water quality and should be taken into consideration when setting nutrient criteria. The four suggested ecoregions are:

<u>Plains Region</u> which is located in northern and western Missouri. This region consists of rolling hills that historically had substantial prairie land cover.

<u>Ozark Highlands Region</u> which is located in southern Missouri. This region is described as having steep topography, with historic forest land cover. Soils are often thin with exposed bedrock.

<u>Ozark Border Region</u> is a transitional zone between the plains and highlands. This region has mixed topography and historically had mixed land cover.

<u>Big Rivers Region</u> consists of southeast Missouri's boot heel and floodplains along Missouri and Mississippi rivers. This region is characterized by flat topography and was historically inundated by periodic flooding.



Figure 8. Map of ecoregions.

## Influence of Morphology, Hydrology and Historic Land Cover

Reservoir water quality is a function of nutrient inputs from point and nonpoint sources as well as reservoir morphology and hydrology. The relative importance of different reservoir and watershed characteristics in describing cross-system variation in phosphorus concentrations was investigated using multiple regression analysis. Results of this analysis indicate that three factors were significant in predicting phosphorus concentrations in Plains Region reservoirs: 1) proportion of the watershed that was historically prairie, 2) residence time (a measure of hydrology) and 3) dam height. In the Ozark Border and Ozark Highlands regions, only dam height was significant in predicting phosphorus concentrations. Using the results from the analysis, a formula was developed for predicting reservoir phosphorus levels for each region.

Plains: Predicted TP = (% historic prairie/4) + (16/residence time) + (570/dam height in feet)
Ozark Border: Predicted TP = (740/dam height in feet) + 15
Ozark Highlands: Predicted TP = (740/dam height in feet) + 5

Historic prairie land cover represents the inherent nutrient levels in the soils in which Plains reservoirs were built. Reservoir nutrient concentrations increase with the portion of the watershed that was historic prairie. Historic prairie land cover was compiled by Jim Harlan (MU geography) based on data collected during the original state survey (circa 1815-1850).

Residence time is the theoretical time it takes water to move through the reservoir and is calculated by dividing the reservoir's volume by the annual amount of inflow. A short residence time equates to high in-reservoir nutrient levels because there is a greater inflow volume (which transport nutrients from the watershed) relative to reservoir volume. Along with less initial dilution of inputs, a short residence time also means less loss of nutrients through sedimentation.

Research has shown the important role that residence time has in influencing nutrient concentrations in Missouri reservoirs (Jones et al 2004, Jones et al 2008). If other factors such as reservoir depth and nonpoint source inputs (represented by % watershed in crop land cover) are held constant, residence time has a predictable influence on nutrient concentrations. This pattern can be seen in Figure 9 where % crop is scaled along the x-axis and predicted phosphorus is scaled on the y-axis. Each line within the figure represents a different residence time (measured in months). For any given level of crop, the predicted phosphorus concentration increases as residence time decreases. This finding suggests that a short residence time can limit a reservoir's potential to reach a low phosphorus concentration, even if a dramatic reduction in nonpoint source pollution is achieved through watershed management.

Dam height is a substitute for reservoir depth, and is an important factor in determining reservoir water quality because shallow reservoirs are more influenced by internal processes such as mixing of the water column. If all other factors were held constant, a decrease in dam height would lead to an increase in nutrient concentrations.

Relationships between predicted phosphorus values and the geometric mean phosphorus values are shown in Figures 10 and 11. Plains Region reservoirs are shown in a separate graph (Figure 10) because the factors used to predict phosphorus for this region differed from the factor used for the other two regions. Data from the Ozark Highlands and Ozark Border regions have been combined into one graphic because the same factor (dam height) was important in predicting phosphorus in both of these regions (Figure 11). It is worth noting that the relationship for the Plains Region is stronger than that of the combined Ozark Border and Ozark Highlands. The increased ability to predict phosphorus in the Plains relates to the fact that three independent factors were used to make predictions as opposed to only one factor. Also, the range of phosphorus values in the Plains Region is twice that measured in the other two regions, leading to Figures 10 and 11 being scaled very differently.

Figure 9. The influence of Residence Time (RT) and crop land cover (as % of watershed) on predicted phosphorus levels in Plains Region reservoirs. Note as RT (months) decreases predicted phosphorus increases for all levels of % crop.



Figure 10. Prediction Line for phosphorus in Plains Region reservoirs. Symbols represent data from individual reservoirs. The line represents the relationship between predicted phosphorus based on reservoir and watershed characteristics (x-axis) and geometric mean phosphorus values (y-axis).  $[r^2 = 0.58]$ 



Figure 11. Prediction Line for phosphorus in Ozark Border and Ozark Highlands regions. Symbols represent data from individual reservoirs. The line represents the relationship between predicted phosphorus based on reservoir and dam height (x-axis) and geometric mean phosphorus values (y-axis). The constant values are 15 for the Ozark Border Region and 5 for the Ozark Highlands Region. [ $r^2 = 0.26$ ]

## Reference Reservoirs

The scientific sub-committee decided to use an adaptation of EPA's reference approach to complement the predictive model created using reservoir morphology and hydrology. Reservoirs selected as reference water bodies were not meant to reflect pristine watershed conditions, but instead represent watersheds with relatively low human impact. The goal was to determine the range of in-reservoir phosphorus concentrations that could be expected when watersheds contain nominal human influence. The scientific sub-committee felt that some human influence should be allowable in reference watersheds as most of Missouri's reservoirs were built after 1960 (Knowlton and Jones 2003) and therefore are fairly recent modifications to the landscape. Many of Missouri's classified water bodies are community reservoirs that were built near the towns they serve and by virtue of location, have human impact within the watershed. Given these facts, the scientific sub-committee decided to define reference conditions using the following guidelines, adapted from EPA's guidance document (EPA 2000) and Dodds *et al* (2006):

- Less than 20% of watershed currently in combined urban and crop land cover
- No permitted point sources or permitted CAFOs within the watershed

 At least 50% of current watershed in grass land cover for Plains Region, at least 50% of current watershed in forest land cover for Ozark Highlands Region, and at least 50% of current watershed in combined forest and grass land cover for Ozark Border Region

There number of reservoirs that met these criteria for each ecoregion ranged from 7 to 23 (Table 4). Phosphorus concentrations for reference reservoirs were ordered from lowest to highest and the 10<sup>th</sup> and 75<sup>th</sup> percentiles of the range of values were calculated. Horizontal lines were then added to the observed-predicted phosphorus plot to represent the 10<sup>th</sup> and 75<sup>th</sup> percentile phosphorus values (Figure 12). The 10<sup>th</sup> percentile line is referred to as Site Specific Value in the proposed rule. The 75<sup>th</sup> percentile line is referred to as the Reference Line in the proposed rule and in this rationale. The use of the lines (Predicted, Reference and 10<sup>th</sup> percentile) and the intersections of the lines creates three main zones in the matrix which will be used to determine the appropriate action when data from individual reservoirs are plotted using predicted and long-term geometric mean phosphorus values (Figure 13). The three main zones can be further broken down into eight sub-zones (Figure 14).



Figure 12. Prediction Line for Plains Region with 10<sup>th</sup> and 75<sup>th</sup> percentile lines delineated.

Region	n	Range	10 <sup>th</sup> %	75 <sup>th</sup> %	
Plains	7	14 – 72	20	58	
Ozark Border	7	12 – 51	16	41	
Ozark Highlands	23	6 - 32	9	26	

Table 4. The phosphorus ranges,  $10^{th}$  and  $75^{th}$  percentile values for reference reservoirs in each region. Phosphorus values in  $\mu g/L$ .



Predicted Phosphorus

Figure 13. Phosphorus matrix with the three zones of action labeled.



Predicted Phosphorus

Figure 14. A further breakdown of the matrix into sub-zones.
## Phosphorus Rule

## Zone A

**Location within matrix** – Long-term geometric mean phosphorus concentration is below the 10<sup>th</sup> Percentile Line (sub-zone A1) or Predicted phosphorus value is below the 10<sup>th</sup> Percentile Line, while long-term geometric mean phosphorus concentration is above the 10<sup>th</sup> Percentile Line (sub-zone A2) - Figure 15.

**Water Quality Standard** – Site specific criteria set at current long-term geometric mean phosphorus concentration.

Action Taken - No action taken.

**Rationale** – Reservoirs located in sub-zone A1 have the lowest nutrient and algal chlorophyll concentrations, along with the highest water clarity within the region. Small increases in nutrient levels would lead to increased algal chlorophyll and decreased water clarity (Figure 7) in these reservoirs. Changes in water clarity could impact recreational uses in these reservoirs. Reservoirs located in sub-zone A2 are predicted to have low phosphorus concentrations, and should be kept from further nutrient enrichment. Phosphorus reduction will not be implemented because these reservoirs have phosphorus concentrations that are within the range found in the reference reservoirs (note: there are only a handful of reservoirs that fall into sub-zone A2, most of which are above the 10<sup>th</sup> percentile line by only a few micrograms per liter.



**Predicted Phosphorus** 

Figure 15. Breakdown of Zone A.

## Zone B

**Location within matrix** – Long-term geometric mean phosphorus concentration and predicted phosphorus concentration are between 10<sup>th</sup> Percentile Line and Reference Line (sub-zones B1, B2 or B3) or Long-term geometric mean phosphorus is above Reference Line, but below Prediction Line (sub-zone B4) Figure 16.

**Water Quality Standard** – Phosphorus criteria for reservoirs in sub-zones B1 and B3 will be set at the Reference Value, while reservoirs in sub-zones B2 and B4 will have site-specific criteria set at the reservoir's Predicted phosphorus value.

Action Taken - No action taken.

**Rationale** – Reservoirs located in sub-zones B1, B2 and B3 have long-term geometric mean phosphorus values that are comparable to the majority of regional reference reservoirs, therefore phosphorus levels are deemed acceptable. Reservoirs located in sub-zone B4 are above the Reference Line, but below the Prediction Line indicating less phosphorus than expected given reservoir and watershed characteristics, therefore current values are acceptable.



Predicted Phosphorus

Figure 16. Breakdown of Zone B.

## Zone C

**Location within matrix** – Long-term geometric mean phosphorus value is above the regional Reference Line and Prediction Line (sub-zones C1 and C2) Figure 17.

**Water Quality Standard** - Reservoirs in sub-zone C1 will have the regional Reference Value as the water quality standard, while reservoirs located in sub-zone C2 will have site specific phosphorus criteria set at the reservoir's Predicted Value.

**Action** – Reduce phosphorus concentration to Reference Value (sub-zone C1) or Predicted Value (sub-zone C2).

**Rationale** – Reservoirs in Zone C have more phosphorus than predicted and more phosphorus than the majority of reference reservoirs within the region. These reservoirs are at the highest risk for algal blooms that could cause impairments to aquatic life and recreational uses.





Figure 17. Breakdown of Zone C.

## Nitrogen Background

While phosphorus is often mentioned as the most important predictor of algal biomass in lakes and reservoirs, aquatic ecologist have long known the importance of nitrogen to algal growth (Naumann 1929, Sakamoto 1966, Elser *et al.* 1990). In Missouri, algal chlorophyll correlates to both phosphorus and nitrogen (Figures 4 & 5) explaining 84% and 77% of the cross-system variability in chlorophyll values respectively (Knowlton and Jones 2003). Because nitrogen is an important causal variable in determining algal chlorophyll, the U.S. Environmental Protection Agency has requested that states address both phosphorus and nitrogen in nutrient criteria plans (EPA 2000).

Research has shown the importance of both phosphorus and nitrogen in promoting algal growth. Work by McCauley *et al.* (1989) indicated that the chlorophyll growth per unit phosphorus increased as the ratio of nitrogen to phosphorus (N:P) increased. That is, nitrogen had a positive influence on algal chlorophyll even when the N:P ratio was high enough to suggest that nitrogen was not limiting algal growth (McCauley *et al.* 1989). In another study, a review of data from 133 lakes indicated that chlorophyll showed the strongest relationship to both phosphorus and nitrogen when the N:P ratios were between 23:1 and 28:1 (Prairie *et al.* 1989).

These findings deviate somewhat from the theory that N:P ratios can be used to identify which nutrient limits algal growth. Sakamoto (1966) was the first to suggest the use of N:P ratio as a gauge, citing an N:P ratio of <10:1 would indicate nitrogen limitation of algal growth, while an N:P ratio >17:1 would be indicative to phosphorus limitation. Other scientists followed Sakamoto with different suggested cut-points (Dillion and Rigler 1974, Downing and McCauley 1992, Levine and Schindler 1992), which underlines the fact that these cut-points are inexact.

A situation where N:P ratios are not successful as a predictor of the limiting nutrient is in lakes and reservoirs that have low nutrient levels (Downing and McCauley 1992). These water bodies may be, by default, co-limited by both nutrients because concentrations are so low.

If N:P ratios can be used to predicted nitrogen limitation, then in theory the ratios could also be used to predict the dominance of nitrogen fixing blue-green algae in lakes. Lakes with low N:P ratios would be perfect environments for algae that have the ability to fix atmospheric nitrogen because these species would have a competitive advantage over species that cannot fix atmospheric nitrogen. Blue-green algae are considered to be the least desirable algae because they can form "surface scums" that reduce aesthetics, they are poor food for other aquatic life, some species can cause taste and odors, and some species produce toxins (EPA 2000). Results from whole-lake experiments in Canada support the theory of blue-green algae dominance in lakes with low N:P ratios (Schindler 1977), as does a review of published data (Smith 1983). Other researchers have suggested that additional factors are important in regulating blue-green algae dominance, and some lakes with low N:P ratios do not suffer from blue-green algal dominance (Knowlton and Jones 1996).

One reason for the discrepancy in the use of N:P ratio as a predictor of nutrient limitation or blue-green algae dominance is the fact that algal populations can be quite diverse. During summer 2000, MU sampled 60 reservoirs from across the state for algal identification. On average, each monitored reservoir had 28 species of algae, with a range of 18 - 40 species (unpublished data).

Research has shown that the optimal N:P ratio varies among algal species, with reported lows near 4:1 and highs near 28:1 (Smith 1982). While 4:1 and 28:1 probably represent the extreme ends of the continuum, it is obvious that diverse algal

requirements coupled with high species diversity interferes with the use of N:P ratios as predictors of nutrient limitation or blue-green algal dominance.

## Nitrogen Rule

## Zone A

**Water Quality Standard** - Reservoirs located in Zone A of the matrix (Figure 15) will have site-specific nitrogen criteria that are set at the current long-term geometric mean nitrogen value.

Action - No action taken.

**Rationale** - Reservoirs located in Zone A have the lowest nutrient and algal chlorophyll concentrations, along with the highest water clarity within the region. Small increases in nutrient levels would lead to increased algal chlorophyll and decreased in water clarity (Figure 7) in these reservoirs. Changes in water clarity could impact recreational uses in these reservoirs. Site-specific nitrogen criteria set at current long-term values will offer a level of protection from nutrient enrichment.

Nitrogen reductions will not take place if N:P ratios are above 20:1 because the use of N:P ratio to predict the limiting nutrient is not applicable at these low nutrient levels. It is also worth noting that most low-nutrient reservoirs in Missouri have forested watersheds, and runoff from these watersheds is expected to have a relatively high N:P ratio (Downing and McCauley 1992).

## Zone B

**Water Quality Standard** - Reservoirs located in Zone B of the matrix (Figure 16) shall have nitrogen criteria set at either 20 times the Reference phosphorus value (sub-zones B1 and B3) or 20 times the Predicted phosphorus value (sub-zones B2 and B4). **Action** - Nitrogen reduction if a reservoir's current long-term geometric mean nitrogen value is greater than 20 times the Reference phosphorus value (sub-zones B1 and B3) or 20 times the Predicted phosphorus value (sub-zones B1 and B3) or 20 times the Predicted phosphorus value (sub-zone B2 and B4). If geometric mean nitrogen value is less than 20 times the appropriate phosphorus value (Reference or Predicted) then no action taken.

**Rationale** - Reducing nitrogen concentrations to achieve an N:P ratio of 20:1 will move reservoirs below the optimal range of N:P ratios identified by Prairie *et al.* (1989) and should result in lower algal chlorophyll levels. Maintaining N:P ratios at 20:1 will reduce the risk of pushing reservoirs into nitrogen limitation and reduce the risk of creating environments that favor blue-green algal growth. Target nitrogen values are based on the Reference and Predicted phosphorus values because reservoirs in Zone B of the matrix have these values as water quality standards. The scientific sub-committee felt that setting nitrogen target values to existing geometric mean phosphorus values would be inappropriate given the potential for these values to fluctuate in the future.

## Zone C

**Water Quality Standard** - Reservoirs located in Zone C of the matrix (Figure 17) shall have nitrogen criteria set at either 20 times the Reference phosphorus value (sub-zone C1) or 20 times the Predicted phosphorus value (sub-zone C2).

**Action** – Reduce nitrogen if a reservoir's current long-term geometric mean nitrogen value is greater than 20 times the Reference phosphorus value (sub-zone C1) or 20 times the Predicted phosphorus value (sub-zone C2). If geometric mean nitrogen value is less than 20 times the appropriate phosphorus value (Reference or Predicted) then no action will be taken.

**Rationale** - Reservoirs located in Zone C of the matrix are targeted for phosphorus reductions to either the Reference or Prediction value, therefore these values will be used to calculate the nitrogen target values. Reducing nitrogen to a 20:1 N:P ratio should aid in the reduction of algal chlorophyll in these reservoirs. This is especially true for those reservoirs where phosphorus reductions will be accomplished by implementing best management practices (BMPs). The BMPs could, along with reducing phosphorus, also reduce the erosional runoff from the watershed into the reservoir. Reductions of inorganic suspended solids (from erosional runoff) would create an improved light environment within the reservoir which in turn could promote algal growth. It is possible that reductions of only phosphorus (and the accompanying reduction in ISS) would lead to higher algal chlorophyll levels even though phosphorus concentrations have been decreased. Reductions in nitrogen levels accompanying the reduction in phosphorus should aid in achieving the overall goal of lower algal chlorophyll levels.

## Chlorophyll Background

Algae are an important component in a healthy aquatic ecosystem, providing both dissolved oxygen and energy to the rest of the aquatic food web. Numerous studies have shown that fish productivity is positively correlated with moderate to high levels of algal biomass. A study of mid-west reservoirs indicated that sport-fish yield is maximized when chlorophyll levels are ~20 - 50  $\mu$ g/L (Knowlton and Jones 2003). This finding is supported by research at Auburn University which indicates that chlorophyll concentrations of 40 - 60  $\mu$ g/L correlates to optimal sport-fish production (Maceina 2001).

It is also known that fisheries are not at their healthiest in systems that have excessive algae. An lowa study showed that while total fish catch per unit effort increased positively with chlorophyll levels, there was a shift from game fish production to rough fish production such as carp (Egertson and Downing 2004). The lowa study also found that increasing chlorophyll had a negative effect on some game fish species such as bluegill and black crappie. Another problem associated with excessive algal growth in terms of fishery health is the potential for fish kills associated with widely oscillating dissolved oxygen levels (EPA 2000). We know that reservoirs need algae in order to have healthy fisheries, that fish production may be optimized at a moderate to high level of algal biomass, and that excess algae can have a negative impact on the fishery. What we do not know is the exact point when the positive relation between aquatic life and algal biomass becomes a negative relation. One reason this tipping point is difficult to define is because various studies relating the health of the fishery to chlorophyll use different methods of measuring fish health. Studies have looked at total fish biomass, sport fish biomass, fish growth/productivity, and catch per unit effort. The use of various ways of measuring the health of the fishery prevents easy comparisons among studies. A second factor to consider is that not all fish species have the same optimal water quality. Schupp and Wilson (1993) suggests that optimal phosphorus levels for lake trout, walleye, black crappie and white crappie are 10, 25, 70 and >100  $\mu$ g/L, respectively. A final factor is the fact that all of Missouri's reservoirs differ in terms of morphology, fish community composition, management, and fishing pressure. Given all of the variables, it is easy to see how a single "tipping point" is impossible to identify.

In Missouri's reservoirs there is a relationship between mean chlorophyll concentrations and maximum measured chlorophyll values (Figure 18). The slope of the relationship approaches 4:1, indicating that for each 1  $\mu$ g/L increase in mean chlorophyll there is approximately a 4  $\mu$ g/L increase in maximum chlorophyll (Knowlton and Jones 2003). A reservoir with a mean chlorophyll value of 20  $\mu$ g/L could be expected to have a maximum value of around 80  $\mu$ g/L, while increasing the mean value to 30  $\mu$ g/L would lead to a maximum of around 120  $\mu$ g/L.



Figure 18. The relationship between geometric mean chlorophyll and maximum chlorophyll values in Missouri reservoirs. Symbols represent data from individual reservoirs and the line represents the average relationship between the two parameters.  $[r^2 = 0.78]$ 

There is also a trend for the frequency of high chlorophyll measurements to increase with mean chlorophyll (Figure 19). About 4% of individual chlorophyll values were >75  $\mu$ g/L in reservoirs that average between 20 and 30  $\mu$ g/L chlorophyll. As mean chlorophyll increases so does the proportion of high values, with reservoirs that average between 30 - 40  $\mu$ g/L chlorophyll and 40 - 50  $\mu$ g/L chlorophyll having ~10% and 20% of individual measurements >75  $\mu$ g/L, respectively (Figure 19).

It is obvious that the risk of high chlorophyll concentrations, which are indicative of algal blooms, increases in both extremity and frequency as mean chlorophyll conditions increase. While we do not know definitively at which point the relation between algal biomass and fish production switches from being positive to negative, we can assume that some of the maximum values that have been measured in Missouri's reservoirs are extreme enough to pose a risk to aquatic life.





## Chlorophyll Rule

Regional chlorophyll - phosphorus ratio (CHL:P) factors were calculated to aid in the identification of reservoirs that have higher chlorophyll values than expected given phosphorus concentrations. These factors were calculated by taking the average CHL:P ratio for reservoirs that currently meet phosphorus criteria (Zones A and B) within each region and adding one standard deviation (calculated from the same data). The regional CHL:P factors are set at 0.44 for Plains Region reservoirs and 0.42 for both Ozark Border Region and Ozark Highland Region reservoirs.

## Zone A

**Water Quality Standard** - Reservoirs located in Zone A of the matrix (Figure 15) will have site-specific chlorophyll criteria that are set at the current long-term geometric mean chlorophyll value.

Action - No action taken.

**Rationale** - Reservoirs located in Zone A of the matrix are set at site-specific criteria to offer protection from loss of water clarity that would accompany increased algal chlorophyll (figure 7). Because these reservoirs have low levels of nutrients, they will not be targeted for further nutrient reductions even if geometric mean chlorophyll values are greater than the regional CHL:P factor multiplied by the geometric mean phosphorus.

## Zone B

**Water Quality Standard** - Reservoirs located in Zone B of the matrix (Figure 16) will have chlorophyll criteria set at either the regional CHL:P factor multiplied by the Reference phosphorus value (sub-zones B1 and B3) or the regional CHL:P factor multiplied by the Predicted phosphorus value (sub-zones B2 and B4).

**Action** - Reservoirs that have geometric mean chlorophyll values greater than either the regional CHL:P factor multiplied by the Reference phosphorus value (sub-zones B1 and B3) or the regional CHL:P factor multiplied by the Predicted phosphorus value (sub-zones B2 and B4) will be listed because of excess algal chlorophyll. If geometric mean chlorophyll value is less than the regional CHL:P factor multiplied by appropriate phosphorus value (Reference or Predicted) then no action taken.

**Rationale** - Reservoirs that have higher than normal CHL:P ratios will have, by definition, higher algal chlorophyll levels than expected given nutrient concentrations. Because of increased algal efficiency these reservoirs are more at risk of having extreme chlorophyll values than other reservoirs with similar nutrient concentrations. Target chlorophyll values will be calculated using Reference phosphorus or Predicted phosphorus values (depending on location in matrix) because reservoirs in Zone B of the matrix have these values as water quality standards. The scientific sub-committee felt that setting chlorophyll target values to existing geometric mean phosphorus values would be inappropriate given the potential for these values to fluctuate in the future.

## Zone C

**Water Quality Standard** - Reservoirs located in Zone C of the matrix (Figure 17) shall have chlorophyll criteria set at either the regional CHL:P factor multiplied by the Reference phosphorus value (sub-zone C1) or the regional CHL:P factor multiplied by the Predicted phosphorus value (sub-zone C2).

**Action** - Reservoirs with current long-term geometric mean chlorophyll values greater than the regional CHL:P factor multiplied by the Reference phosphorus value (sub-zone C1) or the regional CHL:P factor multiplied by the Predicted phosphorus value (sub-

zone C2) will be listed because of excess algal chlorophyll. If geometric mean chlorophyll value is less than the regional CHL:P factor multiplied by the appropriate phosphorus value (Reference or Predicted) then no action taken.

**Rationale** - Reservoirs located in Zone C are targeted for phosphorus reductions. If phosphorus concentrations are successfully brought down to target levels, but chlorophyll concentrations remain high; there would still be a risk of algal blooms that can impact aquatic life and recreational use.

## Literature Cited

American Public Health Association. 1995. Standard methods for the examination of water and wastewater. 19<sup>th</sup> ed. American Public Health Association, New York, NY.

Crumpton, W.G., T.M. Isehart and P.D. Mitchell. 1992. Nitrate and organic N analyses with second derivative spectroscopy. Limnol. Oceanogr. 37:907-913.

Dillion, P.J. and F.H. Rigler. 1974. The phosphorus-chlorophyll relationship in lakes. Limnol. Oceanogr. 19:767-772.

Dodds, W.K., E. Carney and R.T. Angelo. 2006. Determining ecoregional reference conditions for nutrients, Secchi depth and chlorophyll a in Kansas lakes and reservoirs. Lake and Reserv. Manage. 22:151-159.

Downing, J.A. and E. McCauley. 1992. The nitrogen : phosphorus relationship in lakes. Limnol. Oceanogr. 37:936-945.

Egertson, C.J. and J.A. Downing. 2004. Relationship of fish catch and composition to water quality in a suite of agriculturally eutrophic lakes. Can. J. Fish. Aquat. Sci. 61:1784-1796.

Elser, J.J., E.R. Marzolf and C.R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47:1468-1477.

Environmental Protection Agency. 1998. National strategy for the development of regional nutrient criteria. EPA-8221-R-98-002. Office if Water, Washington D.C.

Environmental Protection Agency . 2000. Nutrient criteria technical guidance manual – lakes and reservoirs. EPA-822-B00-001. Office of Water, Washington D.C.

Jones, J.R. and M.S. Kaiser. 1988. Limnological characteristics of Lake of the Ozarks, Missouri II: measurements following formation of a large reservoir upstream. Verh. Internat. Verwin. Limnol. 23:976-984.

Jones, J.R. and M.F. Knowlton. 1993. Limnology of Missouri reservoirs: an analysis of regional patterns. Lake and Reserv. Manage. 8:17-30.

Jones, J.R. and M.F. Knowlton. 2005. Suspended solids in Missouri reservoirs in relation to catchment features and internal processes. Water Research. 39:3629-3635.

Jones, J.R., M.F. Knowlton and M.S. Kaiser. 1998. Effects of aggregation on chlorophyll-phosphorus relations in Missouri reservoirs. Lake and Reserv. Manage. 14:1-9.

Jones, J.R., M.F. Knowlton and D.V. Obrecht. 2008. Role of land-cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management. Lake and Reserv. Manage. 24:1-9.

Jones, J.R., M.F. Knowlton, D.V. Obrecht and E.A. Cook. 2004. Importance of landscape variables and morphology on nutrients in Missouri reservoirs. Can. J. Fish. Aquat. Sci. 61:1503-1512.

Jones, J.R. and J.T. Novak. 1981. Limnological characteristics of Lake of the Ozarks, Missouri. Verh. Internat. Verein. Limnol. 21:919-925.

Kennedy, R.H. 2001. Considerations for establishing nutrient criteria for reservoirs. Lake and Reserv. Manage. 17:175-187.

Knowlton, M.F. 1984. Flow-through microcuvette for fluorometric determination of chlorophyll. Wat. Res. Bull. 20:795-799.

Knowlton, M.F., M.V. Hoyer and J.R. Jones. 1984. Sources of variability in phosphorus and chlorophyll and their effects on use of lake survey data. Water Resource Bull. 20:397-407.

Knowlton M.F. and J.R. Jones. 1995. Temporal and spatial dynamics of suspended sediment, nutrients, and algal biomass in Mark Twain Lake, Missouri. Arch. Hydrobiol. 135:145-178.

Knowlton M.F. and J.R. Jones. 1996. Nutrient addition experiments in a nitrogen-limited high plains reservoir where nitrogen-fixing algae seldom bloom. Journal of Freshwater Biology. 11:123-130.

Knowlton M.F. and J.R. Jones. 2003. Developing nutrient criteria for Missouri lakes. Report presented to Missouri Department of Natural Resources. 79p.

Knowlton M.F. and J.R. Jones. 2006a. Natural variability in lakes and reservoirs should be recognized in setting nutrient criteria. Lake and Reserv. Manage. 22:161-166.

Knowlton M.F. and J.R. Jones. 2006b. Temporal variation and assessment of trophic state indicators in Missouri reservoirs: implication for lake monitoring and management. Lake and Reserv. Manage. 22:261-271.

Levine, S.N. and D.W. Schindler. 1992. Modification of the N:P ratio in lakes by in situ processes. Limnol. Oceanogr. 37:917-935.

McCauley, E., J.A. Downing and S. Watson. 1989. Sigmoid relationship between nutrients and chlorophyll among lakes. Can. J. Fish. Aquat. Sci. 46:1171-1175.

Maceina, M.J. 1996. Compatibility between water clarity and quality black bass and crappie fisheries in Alabama. Multidimensional approaches to reservoir fisheries management. L.E. Miranda and D.R. DeVries ed. American Fisheries Society Symposium 16.

Missouri Department of Natural Resources (MDNR). 2005. Nutrient Criteria Plan. Jefferson City, MO 20p.

Naumann, E. 1929. The scope and chief problems of regional limnology. Int. Revue Ges Hydrobiol. 21:423.

Obrecht, D.V., A.P. Thorpe and J.R. Jones. 2005. Responses in the James River Arm of Table Rock Lake, Missouri to point-source phosphorus reduction. Verh. Internat. Verein. Limnol. 29:1043-1048.

Prairie, Y.T., C.M. Duarte and J. Kalff. 1989. Unifying nutrient-chlorophyll relationships in lakes. Can. J. Fish. Aquat. Sci. 46:1176-1182.

Sakamoto, M. 1966. Primary production by the phytoplankton community in some Japanese lakes and its dependence on lake depth. Arch. Hydrobiol. 62:1-28.

Satory, D.P. and J.U. Grobbelaar. 1986. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. Hydrobiolobia. 114:117-187.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science. 195:260-262.

Schupp, D. and B. Wilson. 1993. Developing lake goals for water quality and fisheries. Lake Line. 13:18-21.

Smith, V.H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. Limnol. Oceanogr. 27:1101-1112.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science. 221:669-671.

## Development of diatom indicators of ecological conditions for streams of the western US

## R. Jan Stevenson<sup>1,6</sup>, Yangdong Pan<sup>2,7</sup>, Kalina M. Manoylov<sup>3,8</sup>, Christian A. Parker<sup>2,9</sup>, David P. Larsen<sup>4,10</sup>, AND Alan T. Herlihy<sup>5,11</sup>

<sup>1</sup> Department of Zoology, Center for Water Sciences, Michigan State University,

East Lansing, Michigan 48824 USA

<sup>2</sup> Environmental Sciences and Management, Portland State University, Portland, Oregon 92707 USA

<sup>3</sup> Department of Zoology, Michigan State University, East Lansing, Michigan 48824 USA

<sup>4</sup> Pacific States Marine Fisheries Commission, c/o National Health and Environmental Effects Laboratory Western

Ecology Division, US Environmental Protection Agency, 200 SW 35<sup>th</sup> St., Corvallis, Oregon 97333 USA

<sup>5</sup> Department of Fisheries and Wildlife, Oregon State University, Nash Hall 104, Corvallis, Oregon 97331 USA

Abstract. The species composition of benthic diatoms was related to environmental conditions in streams throughout the western US to develop diatom traits, indicators for assessment of biological condition and indicators for diagnosing stressors. We hypothesized that indicators based on species traits determined for subsets of streams with similar natural landscape features would be more precisely related to environmental conditions than would be indicators calculated based on species traits for all streams in the data set. The ranges of many environmental conditions were wide among western streams, and these conditions covaried greatly along a major environmental gradient characterized by positive correlations among % watershed disturbed by agricultural and urban land uses (% WD), conductivity, total N, total P, and % fine sediments. Species traits were calculated for 242 diatom taxa. Weighted average (WA) methods were used to define species environmental optima, and regression approaches were used to determine whether species were sensitive or tolerant to environmental conditions indicated by % WD, total P, total N, a nutrient multivariate index, pH, conductivity, % fine sediments, % embeddedness, and a watershed disturbance multivariate index. Indicators based on WA optima and sensitive/tolerant traits were highly correlated with these environmental conditions. Natural and anthropogenic conditions varied greatly among classes of streams grouped by climate regions, but indicators developed for the entire western US were consistently more accurate than were regional indicators. Indicators for individual stressors, such as total P, conductivity, and % embeddedness, were highly correlated with values of respective stressors, but covariation among all indicators and stressors indicated that only 1 environmental gradient was reliably reflected by the indicators. Thus, robust indicators of the biological condition of diatom assemblages were developed for streams of the western US, but development of stressor-specific indicators will require application of additional analytical approaches.

Key words: diatoms, indicators, conductivity, stressors, nutrients, sediments, streams, western US.

<sup>8</sup> Present address: Department of Biological and Environmental Sciences, Georgia College and State University, 202 Herty Hall, Campus Box 81, Milledgeville, Georgia 31061 USA. E-mail: kalina.manoylov@gcsu.edu

<sup>9</sup> E-mail address: cparker@pdx.edu

<sup>10</sup> Present address: Pacific States Marine Fisheries Commission, US Environmental Protection Agency, 200 SW 35<sup>th</sup> St., Corvallis, Oregon 97333 USA. E-mail: larsen. phil@epa.gov

<sup>11</sup> E-mail address: herlihy.alan@epa.gov

The challenges of managing aquatic ecosystems will increase as use of those ecosystems and surrounding landscapes intensifies during the next century (Millennium Ecosystem Assessment 2005). Resource use will increase with human population and standard of living. Intensification of agriculture for food and fuel production conflicts with the demand for clean water for irrigation and drinking water (Postel 1998). This problem will be particularly great in regions, such as the western US, where demand for water far exceeds supply. Management of aquatic ecosystems will

<sup>&</sup>lt;sup>6</sup> Email addresses: rjstev@msu.edu

<sup>&</sup>lt;sup>7</sup> bwyp@odin.pdx.edu

require development of both policy and technical infrastructure to meet these challenges. Our paper describes development of diatom indicators of ecological condition that can support that infrastructure.

Diatoms have been used for aquatic ecosystem assessment around the world (Watanabe et al. 1986, Kelly et al. 1998, Wang et al. 2005, Chessman et al. 2007, Taylor et al. 2007, Porter et al. 2008). Diatoms most often have been used to diagnose levels of stressors, such as organic contamination, lake acidification, climate change, and nutrient concentrations (Slàdecek 1973, Dickman et al. 1984, Fritz et al. 1991, Potapova et al. 2004). We define stressors as the habitat alterations and contaminants that are managed to protect and restore valued ecological attributes (sensu Stevenson et al. 2004). Diatom indicators of stressors complement actual measurement of stressors by providing another perspective on stressor condition. For some highly variable stressors (e.g., nutrient concentrations), diatom indicators can be more precise than a 1-time measurement of water chemistry because they integrate stressor effects over time (Stevenson 2006).

In recent studies, the biological condition of diatoms has been related to nutrient concentrations to justify establishment of nutrient criteria (Wang et al. 2005, Stevenson et al. 2008). Biological condition is a measure that compares species composition, biomass, and function of organisms at the assessed site to natural or reference conditions (Davies and Jackson 2006, Stoddard et al. 2006). Thus, biological condition reflects valued natural capital and ecosystem services as broadly defined in the Millennium Ecosystem Assessment (2005). Some researchers (Karr 1991, Stevenson et al. 2004) would argue that biological condition is an ultimate management endpoint. Thus, diatom species composition and biomass can be used as indicators of biological condition because diatoms themselves are important elements of aquatic food webs and biogeochemical processes. Diatom diversity probably is important for supporting diatom functions in ecosystems (Cardinale et al. 2006). Diatoms also might provide a better estimate of the biological condition of other algae and heterotrophic microbes than other commonly used biological indicators because of their similarity to other algae and microbes with respect to their size, unicellular organization, metabolic rates, nutritional requirements, and sensitivities to abiotic and biotic factors.

The analytical distinction between diatom indicators that measure stressors and those that measure biological condition is small, but the difference in the meanings of the information for management is great (Stevenson and Smol 2002, Stevenson 2006). Both types of indicators require measures of the abundance and traits of taxa. Abundance measures can be presence/absence, abundance relative to other organisms in the habitat, or absolute density. Diatom traits could be calculated as weighted average (WA) optima of taxa on a continuous scale (ter Braak and van Dam 1989), assigned to ranks on an ordinal scale (van Dam et al. 1994), or simply characterized as sensitive or tolerant to changes associated with human alterations of watersheds (Fore and Grafe 2002) (see Diatom trait development and indicator evaluation in Methods for our rationale for using the terms indicators and traits). To infer stressors, WA indicators are calculated from the relative abundance and either WA or rank traits of all taxa in the assemblage (Zelinka and Marvan 1961, ter Braak and van Dam 1989). The number of taxa, percentage of taxa, or percentage of individuals within the sensitive or tolerant groups are more appropriate indicators for characterizing biological condition. When the sensitivity and tolerance is related to a human disturbance gradient, these groups of taxa are reference and nonreference or native and nonnative taxa. Changes in sensitive and tolerant taxa (or individuals) enable a more accurate (less ambiguous) indication of changes in biological condition, such as a loss of sensitive species or an increase in nonnative species (Davies and Jackson 2006), than do WA models inferring total P concentration or relative sediment impacts. The WA models infer stressor conditions, which is valuable, but they use all taxa, so it is not clear whether we have increases in sensitive taxa or decreases in tolerant taxa. However, indicators that use only a subset of species might be less precise than those that use all species because less information is used to calculate the indicator. Thus, slight differences in trait characterization and indicator calculation affect application of indicators. Moreover, tradeoffs might exist between accuracy (closeness in meaning) of indicators for characterizing biological condition and precision (repeatability) of those indicators.

Therefore, accurate characterizations of diatom traits are important for assessing biological conditions and diagnosing stressors in aquatic ecosystems. Characterizations of diatom species traits are available, but many of these are global- or continental-scale summaries and tests of traits (Lowe 1974, van Dam et al. 1994, Porter et al. 2008). Potapova and Charles (2002) observed regional variation in species traits within the US. Regional variation in species traits might arise from interpopulation divergence (Gallagher 1982), interactions with environmental conditions, or as perceived differences when calculations are based on relative abundances (because changes in abundances of some taxa affect relative abundances of all taxa) (Austin 2002). Diatom traits and indicators have not been evaluated widely in the western US.

The goal of our study was to characterize traits of diatoms that could be used to assess biological condition and to diagnose stressors of streams in the western US (West). First, we characterized major environmental gradients in the West to ensure that environmental variation was sufficient to affect diatom species composition and to characterize traits. Next, we characterized diatom traits and determined whether indicators based on them were sufficiently accurate to explain variation in biological condition among streams and to diagnose stressors. Last, we compared performances of indicators developed for the West and western climate regions to determine whether different diatom traits and indicators should be used in different types of streams. The West provided an excellent region to assess sources of variation in biological indicators because of the great variability in environmental conditions caused by both natural and anthropogenic processes.

#### Methods

#### Sampling and sample analysis

The sampling and sample analysis were conducted as part of the US Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program Western Pilot Survey (EMAP-West). Ecological conditions, water chemistry, in-stream habitat, riparian habitat, watershed land use, and geomorphic features were characterized for perennial wadeable streams and boatable rivers in the 12 western states of the US. Sites were selected throughout the study area using a spatially balanced probabilistic design (Stevens and Olsen 2004). A subset of 1203 of these streams and rivers in which benthic algae had been sampled was selected for the analyses in our paper. These sites spanned 1st-order streams to 8th-order rivers (Strahler 1952) and 3 climate regions, Mountain, Xeric, and Plains (Omernik 1987). Streams were sampled with wadeable-stream protocols (Peck et al. 2006), whereas rivers were sampled with rafts and boatable-river protocols (Peck et al., in press).

Watersheds were delineated for each site from US Geological Survey (USGS) 1:24,000 topographic maps. Stream order was determined with 1:100,000 USGS digital hydrography (Strahler 1952). Watershed conditions were characterized from the USGS 1992 National Land Cover Dataset, USGS runoff contour maps, and the 1994 parameter-elevation regressions on independent slopes model (PRISM) precipitation and air temperature database (http://www.prism. oregonstate.edu/docs/przfact.html). Conditions in-

cluded watershed area, stream order, mean slope, mean annual temperature and precipitation, elevation, latitude, longitude, and landuse attributes, such as % agricultural and urban land use, % forest, and road and population density. Percent watershed disturbed (% WD) was calculated as the percentage of land in some form of urban or agricultural land use based on Anderson level 1 designations (Anderson et al. 1976).

The sites were visited during extended summers (May-October) from 2000 to 2004. The length of the reach studied was defined as 40× the mean wetted width of the channel or a minimum length of 150 m. Channel depth and alterations, embeddedness, % sand and fines, current velocity, and substratum roughness were determined using methods described in Kaufmann et al. (1999) and Peck et al. (2006). Water samples were collected in one 4-L cubitainer and 2 sealed 60mL syringes near the middle of the stream in a flowing-water section for determination of waterchemistry attributes. Samples were kept on ice in the dark and shipped by overnight courier to a central processing laboratory where they were divided into aliquots and preserved within 72 h of collection. Base cations and anions were determined by atomic absorption and ion chromatography, respectively. Total N (TN) and total P (TP) were determined spectrophotometrically after persulfate digestion. pH was determined with a pH probe using closed headspace techniques and 1 sample from a sealed syringe. Details of water-chemistry analysis can be found in USEPA (1987).

Benthic algae were sampled at 1 of 3 locations along each of 11 evenly spaced transects in a study reach. The transects were limited to the wadeable shore area in nonwadeable rivers, but otherwise extended across wadeable streams. At each location, benthic algae were scraped from a 12-cm<sup>2</sup> area of substratum with a toothbrush if substrata were firm and large enough to hold. Otherwise, fine sediments were collected into a 60-mL syringe. All 11 benthic algal subsamples, whether from erosional or depositional habitats, were combined into 1 sample for the site.

Benthic algal samples were subsampled and acid cleaned for determination of diatom relative abundances at sites. Subsamples of cleaned diatoms were mounted on microscope slides using ZRAX<sup>®</sup> or NAPHRAX<sup>®</sup> (The Biology Shop, Hazelbrook, New South Wales, Australia; http://mywebsite.bigpond. com/thebiologyshop) as mounting medium. Six hundred diatom valves were identified and counted at 1000× with Leica DMLB microscopes and differential interference contrast optics (Leica Microsystems, Inc., Bannockburn, Illinois). Diatoms were identified primarily with keys provided in Krammer and LangeBertalot (1986, 1988, 1991a, b), Patrick and Reimer (1966, 1975), and more recent references. Consistency in diatom identification among several technicians was maintained by regular communications, exchange of digital images of specimens, and taxonomic workshops. Taxonomic composition and density of non-diatom algae were determined, but those results were not used in our paper.

#### Data analysis

Relationships among land use and environmental factors.-Principal components analysis (PCA) was done to determine which proximate environmental factors, i.e., those factors that directly affect diatom species composition (sensu Stevenson 1997), varied most among streams in the West. This analysis also was done to identify the land use and natural landscape factors that probably regulated the proximate environmental factors. Regional studies show that ionic factors, such as conductivity and pH and nutrient concentrations, affect diatom species composition in streams (Pan et al. 1996, Potapova and Charles 2002, Acs et al. 2004). We used 36 of the environmental factors characterized during the EMAP-West survey for each stream in the PCA because of their probable indirect or direct effect on diatom species composition (Stevenson 1997). Relationships between selected proximate environmental factors and % WD were established with linear regression to confirm that human activities were a likely determinant of these factors in streams of the West.

Diatom indicators were developed for 6 proximate factors that were selected because they are important determinants of diatom species composition and are common stressors in US streams. TP, TN, % fines, and % embeddedness were selected because sediments and nutrients are among the most common causes of impairment of biological condition in US streams (USEPA 2007). In the West, pH and conductivity levels vary over 3 orders of magnitude and are strongly determined by local geology. In addition, pH and conductivity can be affected by mining, agriculture, and urbanization. Diatom species composition in streams is highly correlated with these variables (Pan et al. 1996, Potapova and Charles 2002).

Two multivariate indicators (MVIs) of environmental conditions were calculated to provide more robust indicators of nutrients and watershed disturbance by humans. The MVIs were calculated by standardizing stressor or landuse variables (i.e., dividing the difference between observed and average stressor values by the standard deviation in stressor values); the MVI was calculated as the average of the standardized variables. TP and TN were included in the nutrient MVI because these variables tend to covary, and the limiting nutrient can be depleted when high algal biomasses accumulate. TN, TP, conductivity, and % WD were included in the watershed disturbance MVI (WD MVI). Use of both stressors and land use in a watershed disturbance index can correct for some agricultural land use classes that have relatively low impact.

Diatom trait development and indicator evaluation.—In our paper, the term trait refers to an attribute of individual species that reflects its fitness (performance, both absolute and relative to other species) in different environmental conditions. Traits could include environmental optima calculated by WA or generalized additive models (ter Braak and van Dam 1989, Yuan 2004) and sensitivity and tolerance to different environmental stressors determined by regression (our paper). Traits also could refer to possession of a keeled raphe (assumed to confer fitness in fine sediments), mucilaginous stalks, endosymbiotic cyanobacteria, quantitative measures of metabolic parameters, and size. In our paper, the term *indicator* refers to a measure of ecological condition that uses species traits and species abundances. The indicator reflects a shift in species composition that either is or is not correlated with some measure of human activities and includes all kinds of metrics, which then must be related to human activities (sensu Karr and Chu 1999) or WA inference models (ter Braak and van Dam 1989).

A variety of diatom indicators of ecological condition that were expected to vary in their accuracy for inferring stressors and biological condition, precision, and ease of explanation to public audiences (Table 1) were tested. Traits and indicators were calculated in 2 fundamentally different ways. Traits were calculated with either WAs to determine environmental optima or regression to determine sensitivity or tolerance to an environmental gradient. Indicators were calculated using WA models (ter Braak and van Dam 1989) or from the number of species, percentage of individuals, or percentage of species that were either sensitive or tolerant. Environmental gradients were defined by stressors, % WD, or MVIs of these variables. WA indicators were expected to be the most precise indicators of both stressors and environmental condition, but to be least accurate for characterizing biological condition because they use both relatively sensitive and relatively tolerant taxa (Table 1). Species indicators based on the presence and absence of sensitive and tolerant taxa were expected to be least precise of all indicators because fewer taxa in samples are used in the indicator calculation than are used in calculation of WA indicators.

TABLE 1. The expected precision and accuracy of diatom indicators calculated in this study. Sensitive (S) and tolerant (T) indicator species traits were determined by regression to be sensitive or tolerant to an environmental gradient. WA = weighted average, WAI = weighted average indicator, HA = highest accuracy,  $\approx$ HA = relatively high accuracy,  $\approx$ LA = relatively low accuracy, LA = lowest accuracy, HP = highest precision,  $\approx$ HP = relatively high precision,  $\approx$ LP = relatively low precision, and LP = lowest precision.

Indicator calculation and trait type	Stressors	Biological condition
Number of species either S or T	LA, LP	HA, LP
% species either S or T % individuals either S or T WAI using all taxon relative abundances weighted by WA optimum	≈LA, ≈LP ≈HA, ≈HP HA, HP	≈HA, ≈LP ≈LA, ≈HP LA, HP

WA optima (ter Braak and van Dam 1989) were calculated for taxa using an Access (Microsoft Office 2003, Redmond, Washington) database. WA optima were calculated for taxa that were observed at  $\geq$ 40 sites. The number of sites used was 1203 for all stressors except % embeddedness, for which 1061 sites were used because of missing data at large river sites where % embeddedness was not determined per protocol. Species optima were calculated for subsets of samples from climate regions if the species were observed in  $\geq$ 10 samples.

WA indicators were calculated with the optima and relative abundances of taxa in samples (ter Braak and van Dam 1989). WA indicators were tested by cross validation. Samples were randomly assigned to 2 groups, A and B. Indicators for samples in group A were calculated with optima derived from sample group B and vice versa. The correlation coefficients  $(r^2)$ for relationships between measured stressor conditions in streams and diatom-inferred stressor conditions were used to evaluate precision of WA indicators and to test for statistically significant relationships between indicators and measured stressor conditions. In addition, WA indicators were recalculated using the classical deshrinking method (Birks et al. 1990), plotted against measured values of stressors, % WD, and multivariate indices of stressors, and evaluated for bias in inferred condition.

The process of determining WA optima and testing WA indicators was repeated for each climate region to test the hypothesis that indicators based on species traits determined for subsets of streams with similar natural landscape features (climate regions) would be more precisely related to environmental conditions than would be indicators based on species traits determined for all streams in the data set. Climate region accounts for great variation in diatom species composition and environmental factors in streams of the West (YP, RJS, C. L. Weilhofer, Portland State University, CAP, ATH, P. K. Kaufmann, US EPA, and DPL, unpublished data). Covariance among indicators and all stressors was analyzed to determine their independence.

Sensitivity and tolerance (S/T) of taxa to different stressors were characterized by using linear regression to relate individual stressors to relative abundances of individual taxa. Simple linear regression was used rather than WA categories, indicator species analysis (Dufrene and Legendre 1997), or generalized additive models (Yuan 2004) because simple linear regression is easier to explain to the public and interpretation of results is straightforward. Future analyses should be conducted to determine whether other S/T trait calculation methods improve performance of indicators. Taxa that were significantly (p < 0.05) negatively or positively related to stressors were characterized as sensitive or tolerant, respectively, to that stressor. S/T traits were evaluated for all taxa observed in  $\geq 40$ samples. Six indicators based on S/T classification of taxa were calculated: the number of sensitive taxa, % sensitive taxa, % sensitive individuals, the number of tolerant taxa, % tolerant taxa, and % of tolerant individuals. These indicators were tested by cross validation with sample groups A and B, as for WA indicators.

#### Results

#### Relationships among land use and environmental factors

PCA of environmental variables indicated 1 relatively dominant gradient and a  $2^{nd}$  subdominant gradient that explained 32 and 10%, respectively, of the variation in the correlation matrix (Table 2). PCA axis 1 was strongly related to human activities in watersheds and associated stressors and, thus, represented a major environmental gradient. PCA axis 1 was positively correlated with % WD, % agricultural land use, conductivity, % embeddedness, and concentrations of TN, TP, and Cl<sup>-</sup> and negatively correlated with % forest cover, watershed slope, precipitation, and longitude. PCA axis 2 was strongly positively correlated with temperature and negatively correlated with latitude and elevation.

TP (Fig. 1A), TN (Fig. 1B), conductivity (Fig. 1C), pH (Fig. 1D), % fines (Fig. 1E), and % embeddedness (Fig. 1F) were significantly (p < 0.001) related to land use (Table 3). Upper and lower quartiles for these stressors for all sites in the study were 7 and 66 µg TP/L, 98 and 529 µg TN/L, pH 7.6 and 8.3, 83 and 584 µS/cm, 1 and

29% fines, and 38 and 77% embeddedness. The median TN:TP molar ratio was 19.6 (minimum = 0.7, quartiles = 10.1 and 49.8, maximum = 15,287). Variation in the stressor variables explained by land use was highest for TN ( $r^2 = 0.51$ ) and lowest for pH and % fines ( $r^2 = 0.10$  and 0.19, respectively).

Percent WD and environmental stressors varied greatly among climate regions (Kruskal–Wallis, p < 0.05). Percent WD averaged 1.0% (±4.8% SD, n = 699) in the Mountain climate region, 4.8% (±14.0%, n = 257) in the Xeric climate region, and 44.7% (±34.3%, n = 247) in the Plains climate region (Fig. 2). Environmental stressors also varied significantly among climate regions (Fig. 3A–F). In all cases, stressors were lower in the Mountain climate region than in the other climate regions. Among stressors, the magnitude of differences in pH among classes was less than the magnitude of differences for other stressors (Fig. 3D).

#### Diatom trait development and indicator evaluation

WA optima and S/T traits for 242 of the 1349 taxa were calculated for the 6 stressors and 2 MVIs of stressors (Appendix; available online from: http://dx. doi.org/10.1899/08-040.1.s). The precision of traits increased with average relative abundance of taxa in the data set, as illustrated by the negative relationship between taxon relative abundances and the standard deviation in the WA optima of taxa for the WD MVI between cross-validation data sets (Fig. 4). Fewer taxa were identified as sensitive than as tolerant for most environmental gradients. For example, 57 taxa were negatively related (sensitive) to the WD MVI, whereas 101 taxa were not significantly related to that WD MVI.

On average, diatom taxa that were sensitive to WD MVI had higher maximum abundances (Fig. 5A, B), were observed at more sites (Fig. 5C, D), and had higher relative abundances (Fig. 5E, F) than diatom taxa that were tolerant to WD MVI. Achnanthidium minutissimum (Kützing) Czarnecki, a taxon defined as WD MVI sensitive, was observed in more samples and with higher relative abundance than any other taxon. Cocconeis placentula and its varieties and Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot were the 2<sup>nd</sup> and 3<sup>rd</sup> most abundant sensitive species. Nitzschia inconspicua Grunow, Nitzschia frustulum (Kützing) Grunow, and Cocconeis pediculus Ehrenberg were the most commonly observed WD MVI tolerant taxa, occurring in >500 samples with 3 of the 4 highest average west-wide relative abundances. Nupela lapidosa (Krasske) Lange-Bertalot, Diatoma anceps (Ehrenberg) Kirchner, Gomphonema olivaceoides Hustedt, Karayevia suchlandtii (Hustedt) Bukhtiyarova, Ach-

TABLE 2. Loadings of environmental variables on ordination axes from principal components analysis. L and L1 indicate variables were  $\log_{10}(x)$  transformed or  $\log_{10}(x + 1)$ transformed, respectively, for analyses.

Variable	Axis 1	Axis 2
рH	0.427	0.138
Conductivity (L)	0.832	0.214
Acid neutralizing capacity (L)	0.745	0.265
Total suspended solids (L1)	0.663	-0.143
Total P (L1)	0.724	-0.063
Se (L1)	0.325	0.029
$NH_{4}^{+}$ (L1)	0.631	-0.084
$NO_{3}^{-}$ (L1)	0.349	0.028
Cl <sup>-</sup> (L1)	0.770	0.413
Total N (L)	0.839	-0.141
Zn (L)	0.024	-0.035
SiO <sub>2</sub> (L)	-0.011	0.283
$HCO_3^{-}(L)$	0.729	0.278
% embeddedness	0.703	-0.286
Channel slope (L)	-0.610	-0.125
Channel depth (L)	0.347	0.114
% fines (L1)	0.499	-0.432
% sand (L1)	0.229	-0.092
% slow-current habitat	0.559	0.144
% urban land use (L1)	0.498	0.154
% agricultural land use (L1)	0.812	-0.264
% forest	-0.615	0.357
% watershed disturbed (L1)	0.843	-0.215
Stream order	0.523	0.171
Road density (L1)	0.490	0.252
Population density (L1)	0.575	0.308
Elevation	-0.425	-0.552
Watershed slope	-0.794	0.230
Roughness	-0.172	0.528
Watershed area	0.278	0.084
Water temperature	0.545	0.365
Channel alteration	-0.420	-0.088
Mean annual air temperature	0.121	0.897
Mean annual precipitation (L)	-0.707	0.261
Latitude	0.140	-0.582
Longitude	-0.640	0.519

nanthes nodosa Cleve, and Diatoma mesodon (Ehrenberg) Kützing had the lowest optima for the WD MVI. Aulacoseira granulata (Ehrenberg) Simonsen, Stephanodiscus hantzschii Grunow, Cyclotella atomus Hustedt, Stephanodiscus medius Håkansson, Biremis circumtexta (Meister ex Hustedt) Lange-Bertalot et Witkowski, and Nitzschia desertorum Hustedt had the highest optima for the WD MVI.

WA indicators tested by cross validation were significantly related for all stressors and MVIs (Fig. 6A–I, Table 4). The WA indicators for conductivity (Fig. 6F) and % fines (Fig. 6H) were the most and least precise, respectively ( $r^2 = 0.687$  and 0.314), for single stressor measures. The WA indicator for pH (Fig. 6G) also was relatively imprecise ( $r^2 = 0.323$ ), compared to other indicators, which had  $r^2$  values ranging from



FIG. 1. Relationships among total P (TP) (A), total N (TN) (B), conductivity (C), pH (D), % fines (E), % embeddedness (F), and % watershed disturbed (% WD) by humans in watersheds of streams sampled in the western US. 1.0 was added to values of TP, TN, conductivity, and % fines so that all points could be plotted on a logarithmic scale.

TABLE 3. Correlations among stressors in streams of the western US. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

Stressor	TP	TN	Nutrient MVI	% WD	WD MVI	pН	Conductivity	% embeddedness
TN	0.497							
Nutrient MVI	0.852	0.852						
% WD	0.342	0.513	0.496					
WD MVI	0.748	0.835	0.927	0.755				
рH	0.062	0.075	0.080	0.096	0.099			
Conductivity	0.300	0.437	0.429	0.373	0.473	0.284		
% embeddedness	0.310	0.361	0.401	0.312	0.430	0.057	0.349	
% fines	0.233	0.253	0.285	0.190	0.288	0.013	0.247	0.555

0.492 to 0.545 (TP, TN, % embeddedness; Fig. 6A, B, F, respectively). The precision ( $r^2$ ) of WA indicators for the nutrient MVI (Fig. 6D) was higher, but not significantly higher, than WA indicators for TP or TN individually. Similarly, the WA indicator for the WD MVI (Fig. 6E) was more precise ( $r^2 = 0.667$ ) than all individual indicators except conductivity.

Bias was relatively low for WA diatom indicators for TP (Fig. 6A), % WD (Fig. 6C), % fines (Fig. 6H), and % embeddedness (Fig. 6I) compared to other indicators. In general, the relationships predicted by least squares regression between diatom-inferred conditions based on WA indicators and measured values followed a 1:1 relationship (Fig. 6A-I), but nonlinear bias was observed for some indicators. Diatom-inferred TN (Fig. 6B) and conductivity (Fig. 6F) were overestimated at high levels of measured TN and conductivity. This bias resulted in slight overestimation of the diatominferred nutrient and WD MVIs at high levels of measured condition. The diatom-inferred pH indicator (Fig. 6G) was biased at both ends of the pH range and underestimated measured pH at low levels and overestimated pH at high levels.

Stressor variables and WA indicators were highly interrelated (Tables 3, 5). All correlations among stressor variables and among WA indicators were highly significant (p < 0.001). Correlations involving pH and other stressors or the WA pH indicator and other indicators were weaker than correlations for other stressors or indicators. The median correlation coefficient for all correlations among stressors was 0.346, whereas the median correlation coefficient for all correlations was 0.880 (Table 5). Factor analysis indicated that 65% of variation in the 8 stressors was explained by the 1<sup>st</sup> ordination factor, whereas 91% of the variation among indicators was explained by the 1<sup>st</sup> ordination factor.

WA indicators often were most strongly correlated with a stressor that had not been used to develop it (Table 6). In the worst of these cases, the % fines WA indicator was more strongly correlated with 7 of the 8 stressors other than % fines. The % fines WA indicator was significantly correlated with measured % fines ( $r^2$ = 0.314) and with conductivity ( $r^2$  = 0.543) and the WD MVI ( $r^2$  = 0.585). The TP WA indicator was correlated with TP ( $r^2$  = 0.533), WD MVI ( $r^2$  =0.634), the nutrient MVI ( $r^2$  = 0.585), and conductivity ( $r^2$  = 0.533). The TN WA indicator was correlated with TN ( $r^2$  = 0.548), WD MVI ( $r^2$  = 0.663), the nutrient MVI ( $r^2$  = 0.575), and conductivity ( $r^2$  = 0.555). Only the WA indicators for the WD MVI and conductivity were best correlated with the stressor with which they had been developed.

WA indicators developed independently for each climate region were not more precise than indicators



FIG. 2. Box-and-whisker plots for % watershed disturbed (% WD) by humans in streams in Mountain (MT), Plains (PL), and Xeric (XE) climate regions in the western US. 1.0% was added to values of % WD to enable plotting 0.0% WD on a logarithmic scale. Lines in boxes show medians, boxes show interquartile ranges, and whiskers show  $2.5\times$  the interquartile range. Near and far outliers are indicated by asterisks and circles, respectively.

developed for all the sites throughout the West (Table 4). On average, precision of indicators decreased 22 percentage points from  $r^2 = 0.53$  to 0.41 when the climate region classification scheme was used rather than the west-wide scheme. Precision decreased most for % fines and % embeddedness indicators.

Indicators based on S/T traits of taxa were all significantly (p < 0.001) related to respective stressors (Table 7). The most precise S/T indicators were % taxa tolerant to conductivity ( $r^2 = 0.671$ ) and % taxa tolerant to the WD MVI ( $r^2 = 0.638$ ). The least precise indicator was the number of taxa sensitive to pH ( $r^2 = 0.209$ ). Indicators based on tolerant taxa were consistently more precise than were indicators based on sensitive taxa. Indicators based on % sensitive taxa were consistently more precise than were indicators based on % sensitive individuals, and both of those indicators were more precise than indicators based on number of sensitive taxa. Precision of the S/T indicators was seldom as high as precision of WA indicators for the same stressor.

#### Discussion

Nutrient concentrations, conductivity, and % fine sediments varied greatly among streams in the West. Nutrient concentrations and % fine sediments, 2 of the leading causes of biological impairment of US waters



FIG. 3. Box-and-whisker plots for total P (TP) (A), total N (TN) (B), conductivity (C), pH (D), % fines (E), and % embeddedness (F) streams in Mountain (MT), Plains (PL), and Xeric (XE) climate regions in the western US. 1.0 was added to values for TP, TN, conductivity, and % fines so all points could be plotted on a logarithmic scale. Lines in boxes show medians, boxes show interquartile ranges, and whiskers show 2.5× the interquartile range. Near and far outliers are indicated by asterisks and circles, respectively.

(USEPA 2007), were highly correlated with human alteration of watersheds in the West. Conductivity, a variable that commonly is correlated with soil disturbance (Herlihy et al. 1998), also was strongly correlated with nutrients, % fine sediments, and % WD. The high variability in levels of correlation between the suite of 6 proximate environmental factors and % WD indicated that the 6 proximate environmental indicators also were affected by nonanthropogenic factors. Many of these abiotic factors also varied among climate regions because they are regulated by precipitation, soils, geology, and stream hydrogeomorphology (Welch et al. 1998, YP, RJS, C. L. Weilhofer, Portland State University, CAP, ATH, P. K. Kaufmann, US EPA, and DPL, unpublished data). However, the extent of human land use in watersheds also varied among climate regions. Accurate distinction between natural and anthropogenic sources of stressors will be important for assessment of stream condition and diagnosis of stressors (Omernik 1987, Wright et al. 1993, Hawkins et al. 2000, Stevenson et al. 2004).

The ranges of many selected stressors in western US streams were sufficient to affect diatom species composition. Sufficient range is needed when developing indicators of stressors. We based this conclusion on comparisons of ranges of stressors that cause changes in species composition in experiments to ranges of stressors in western US streams. The ranges of both N and P concentrations that affect biomass and species composition of diatom assemblages in experiments (Bothwell 1989, Rier and Stevenson 2006, Manoylov and Stevenson 2006) are within the ranges observed in western streams. We know relatively little from experimental research about the ranges of conductivity, % fines, and % embeddedness needed to affect diatom species composition. However, the observed ranges of these variables were very wide (0-100% for % fines and % embeddedness) and most probably encompass the ranges within which diatom responses are expected. The range of conductivity values (2 to 12,000  $\mu$ S/cm) in western streams was greater than ranges in studies of lakes and other streams in which conductivity was implicated as a determinant of diatom species composition (Fritz et al. 1991, Pan et al. 1996). pH (6.1 to 9.9) is the least likely stressor to affect diatom species composition because its range did not span the acidic end of the scale (Lowe 1974, van Dam et al. 1994). Moreover, pH was not correlated well with other stressors. Therefore, we should be able to develop indicators of most stressors (expect pH), because ranges of stressor conditions were sufficient to affect diatom species composition in experiments in which cause-effect relationships were confirmed.

Diatom indicators were significantly and often strongly correlated with the stressors used to determine diatom traits in western US streams. However, indicators were often more highly correlated with other stressors (e.g., conductivity, WD MVI) than with the stressors from which they were developed, despite the fact that traits for diatoms were determined independently from WAs and regression models. This issue of covariation among multiple stressors and stressor indicators presents a problem for defining species traits and for diagnosing stressors with diatom indicators. Causal relationships should be evaluated thoroughly when developing biological indicators of stressor conditions (Yuan 2007). In addition, the high levels of covariation between indicators and multiple stressors prevented development of indicators that could have been used to diagnose specific stressors.

We expected that developing separate indicators for each climate region would minimize the confounding



FIG. 4. The relationship between the average relative abundances (RA) of 242 taxa at all stream sites and the standard deviations (SD) of the weighted average (WA) optima for taxa that were calculated for the watershed disturbance multivariate index (WD MVI) the 2 cross-validation data sets.

effects of covariation among stressors and produce more accurate and precise indicators for individual stressors (Potapova and Charles 2002). Species traits can be affected by direct interactions among environmental factors, by historic exposure to different conditions that produce intraspecific variation in physiologies among populations, or by the presence of other species that affect relative performance (Gallagher 1982, Austin 2002). Therefore, refinement of species traits for classes of streams, with classes defined by climate region or hydrogeomorphic attributes, should have improved indicator performance. However, indicators developed for individual climate regions were not more precise than those developed for all sites in the West. The relatively poor performance of climate region-specific indicators might have been the result of shorter environmental gradients within climate regions than across the West or of smaller sample sizes in climate region-specific data sets. However, sample sizes were large, even within individual climate regions, and they were held constant in comparisons. We think it more likely that the limited variation in % WD within climate regions compared to % WD in the West probably was the reason that  $r^2$  values for climate region–specific diatom indicators were lower than those for indicators based on all sites in the West.



FIG. 5. Distributions of counts of tolerant and sensitive taxa as a function of their maximum (max) relative abundances (RA) at all sites in the western US (A, B, respectively), the number (No.) of sites at which they were observed (C, D), and their average RA at a site (E, F).

Diatom indicators based on the WD MVI will be the most valuable of the indicators developed for assessing biological condition of diatoms in western streams. Therefore, only WA optima and S/T traits for the WD MVI are listed in the Appendix. This disturbance gradient is characterized by a shift from streams with low conductivity and low nutrient concentrations to streams with high conductivity and high nutrient conditions. Percent fine sediments also was strongly correlated with WD MVI. The species, such as *A. minutissimum*, that are sensitive to this gradient probably are adapted to low conductivity and are capable of sequestering nutrients when concentrations are low (Manoylov and Stevenson 2006). In contrast, the tolerant taxa probably are adapted to high conductivity and require high nutrient concentrations. The WD MVI is highly correlated with % agricultural and urban land use and with many stressors in the West. Therefore, it measures conditions along a dominant environmental gradient that is common across western US streams. Thus, the defined species traits and WD MVI indicator are more comparable to the general pollution indicators developed by Descy (1979) and Lange-Bertalot (1979) than to stressorspecific indicators. Application of the indicator in assessment will require establishment of appropriate reference conditions (e.g., Cao et al. 2007, Kelly et al. 2008) and appropriate comparison with reference



FIG. 6. Relationships between diatom-inferred condition for total P (TP) (A), total N (TN) (B), % watershed disturbance (% WD) (C), the nutrient multivariate index (MVI) (D), the watershed disturbance multivariate index (WD MVI) (E), conductivity (F), pH (G), % fines (H), and % embeddedness (I) and measured values of these conditions for streams throughout the western US. Lines show the 1:1 relationship between diatom-inferred and measured conditions.

conditions in climate regions with different extents of human activities (Davies and Jackson 2006, Stoddard et al. 2006).

Stressor-specific indicators developed from large data sets, such as EMAP-West data set, should be used with great caution. The EMAP-West TP indicator is strongly correlated with measured TP in Florida springs and in South Dakota streams, but so is the EMAP-West WD MVI indicator (Stevenson and Pinowska 2007, RJS, unpublished data). Large data sets offer much opportunity for harvesting information, but a new approach is needed for developing stressor-specific indicators from these data sets. Historically, correspondence analyses have been used to identify the water-chemistry variables most responsible for changes in diatom species composition and to limit development of indicators to only those variables that are most important (ter Braak 1995, Ponader et al. 2007). However, other approaches might enable development of indicators for subdominant factors. Multivariate maximum likelihood models might solve problems caused by covarying environmental factors (Yuan 2007). Our next steps will include stratifying the data set by stressors known to affect diatom species composition, randomly sampling streams from strata in which variation in nontarget stressors is controlled, characterizing taxon traits, and testing trait-based indicators in different settings.

The strong west-wide performance of diatom indicators should not be taken as an indication that

TABLE 4. Correlation coefficients ( $r^2$ ) between diatom weighted average (WA) indicators for environmental conditions and measured environmental conditions when traits were determined for all sites and sites by climate region. Correlation coefficients were determined after indicators had been corrected by classical deshrinking. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

WA indicator	All sites	Sites by climate region
TP	0.533	0.415
TN	0.548	0.314
% WD	0.587	0.425
Nutrient MVI	0.596	0.575
WD MVI	0.667	0.684
pН	0.323	0.289
Conductivity	0.687	0.678
% fines	0.314	0.040
% embeddedness	0.492	0.050

species traits do not vary among climate regions. That hypothesis was not tested directly. If species traits vary independently and without bias among stream types or biogeographically, then the mean indicator value across all species in a multispecies assemblage could remain the same when used in different regions. This central-limit-theorem property results from aggregating information from multiple sources (in this case, species). Thus, biological indicators using traits and abundance information for multiple species are "robust," "capable of performance under a wide range of conditions" (Merriam-Webster 2003). Robustness should be related to the number of species in the assemblages used in the multispecies indicator. Thus, the diatom indicators that commonly use information from  $\geq$ 20 species in a sample tend to be correlated well with environmental conditions even when species traits are derived from other regions (e.g., Fore and Grafe 2002).

S/T indicators are useful in assessments because they characterize valued ecological attributes more accurately than do indicators predicting stressors (Stevenson and Smol 2002, Stevenson 2006) or indicators that use all species to assess biological condition. S/T indicators unambiguously quantify the changes in taxa that are or are not characteristic of reference conditions vs indicators based on all species. However, S/T indicators use many fewer species than do indicators that include all species, so statistical

TABLE 5. Correlations among diatom weighted average (WA) indicators of stressors in streams of the western US. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

Indicator	TP	TN	Nutrient MVI	% WD	WD MVI	pН	Conductivity	% embeddedness
TN	0.891							
Nutrient MVI	0.970	0.972						
% WD	0.848	0.960	0.931					
WD MVI	0.941	0.984	0.992	0.970				
рH	0.524	0.473	0.513	0.461	0.503			
Conductivity	0.861	0.885	0.899	0.859	0.899	0.676		
% embeddedness	0.878	0.889	0.910	0.845	0.901	0.456	0.872	
% fines	0.867	0.872	0.895	0.819	0.882	0.361	0.830	0.931

TABLE 6. Correlations between diatom weighted average indicators (WAI) of environmental conditions and stressors. TP = total P, TN = total N, nutrient MVI = nutrient multivariate index, % WD = % watershed disturbed, WD MVI = watershed disturbance multivariate index.

	WAI								
Stressor	TP	TN	Nutrient MVI	% WD	WD MVI	pН	Conductivity	% embeddedness	% fines
TP	0.533	0.434	0.496	0.402	0.469	0.280	0.415	0.446	0.434
TN	0.465	0.548	0.521	0.513	0.527	0.268	0.466	0.493	0.465
Nutrient MVI	0.585	0.575	0.596	0.534	0.584	0.321	0.516	0.551	0.527
% WD	0.482	0.573	0.543	0.587	0.567	0.275	0.483	0.483	0.468
WD MVI	0.634	0.663	0.667	0.638	0.667	0.352	0.584	0.608	0.585
pН	0.123	0.110	0.120	0.102	0.115	0.323	0.177	0.104	0.088
Conductivity	0.533	0.555	0.560	0.518	0.554	0.507	0.687	0.566	0.543
% embeddedness	0.413	0.424	0.432	0.377	0.420	0.206	0.392	0.493	0.462
% fines	0.263	0.262	0.270	0.237	0.262	0.116	0.253	0.306	0.314

S/T indicator	TP	TN	Nutrient MVI	%  WD	WD MVI	pН	Conductivity	% fines	% embeddedness
Number of sensitive taxa Number of tolerant taxa % sensitive individuals % tolerant individuals % sensitive taxa % tolerant taxa	0.333 0.399 0.391 0.436 0.415 0.452	0.315 0.425 0.335 0.498 0.375 0.529	0.381 0.469 0.387 0.520 0.446 0.554	0.262 0.539 0.331 0.569 0.381 0.601	$\begin{array}{c} 0.404 \\ 0.558 \\ 0.397 \\ 0.604 \\ 0.469 \\ 0.638 \end{array}$	0.209 0.222 0.232 0.250 0.275 0.288	0.523 0.490 0.493 0.608 0.599 0.671	0.223 0.306 0.214 0.259 0.289 0.335	$\begin{array}{c} 0.305 \\ 0.425 \\ 0.341 \\ 0.416 \\ 0.436 \\ 0.437 \end{array}$

precision and robustness could be sacrificed for more refined information. In the West, some S/T diatom indicators did almost as well as the indicators based on all species in assemblages. Indicators based on % S/T taxa were more precise than indicators based on % S/T individuals or number of S/T taxa. Relative abundances vs presence/absence of species is almost always used in diatom indicator development. Percent S/T taxa is a valuable indicator because it quantifies changes in biodiversity at the species level more directly than does % S/T individuals, but any inference about changes in number of S/T taxa is suspect because of the gross underestimation of the total number of species in assemblages when counts of only 600 valves are used (Patrick et al. 1954, Stevenson 2006). However, % S/T individuals or number of S/T taxa are precise indicators of environmental change.

We also observed that S/T indicators based on tolerant species were more precisely related to water chemistry and watershed disturbance than were S/T indicators based on sensitive species. Higher precision of indicators based on tolerant taxa than of indicators based on sensitive taxa also was observed with indicators based on invertebrates along nutrient gradients in the Mid-Atlantic Highlands (Yuan and Norton 2003). Stevenson et al. (2008) argue that, as a group, tolerant taxa should be more responsive than sensitive taxa along nutrient gradients because low nutrient availability should constrain species membership in assemblages more than should high nutrient availability. This relationship might explain the wider distribution of sensitive than of tolerant diatom taxa in western US streams.

In conclusion, land use and water chemistry varied greatly in streams in different climate regions in the West. A dominant stressor gradient in western streams was defined by increases in conductivity, nutrient concentrations, and % fine sediments as % WD. This dominant gradient enabled development of diatom indicators of this generalized stressor gradient (sensu Davies and Jackson 2006), but it complicated development of diatom indicators for specific stressor conditions. The problem of developing diatom indicators for specific stressors could be solved by using different analytical approaches to calculate species traits in large data sets. Future work to refine definitions of reference condition and stressor indicators will enable more accurate assessments of these microbial communities and diagnosis of the stressors that threaten or impair their biodiversity.

#### Acknowledgements

Our research was supported by cooperative agreements from the US EPA with Portland State University (R-829026) (YP and RJS) and with Oregon State University (CR-831682) (ATH). We thank all the people involved with the EMAP-West project, especially Phil Kaufman, John Stoddard, and David Peck. Rosalina Hristova and Nadezhda Gillett helped analyze diatom samples. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

#### Literature Cited

- Acs, E., K. SZABO, B. TOTH, AND K. T. KISS. 2004. Investigation of benthic algal communities, especially diatoms of some Hungarian streams in connection with reference conditions of the water framework directives. Acta Botanica Hungarica 46:255–277.
- ANDERSON, J. R., E. E. HARDY, J. T. ROACH, AND R. E. WITMER. 1976. A land use and land cover classification system for use with remote sensor data. U.S. Geological Survey Professional Paper 964. US Geological Survey, Department of the Interior, Washington, DC. (Available from: http://landcover.usgs.gov/pdf/anderson.pdf)
- AUSTIN, M. P. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecological Modelling 157:101–118.
- BIRKS, H. J. B., J. M. LINE, S. JUGGINS, A. C. STEVENSON, AND C. J. F. TER BRAAK. 1990. Diatoms and pH reconstruction. Philosophical Transactions of the Royal Society of London B: Biological Sciences 327:263–278.
- BOTHWELL, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 46:1293–1301.
- CAO, Y., C. P. HAWKINS, J. OLSON, AND M. A. KOSTERMAN. 2007.

Modeling natural environmental gradients improves the accuracy and precision of diatom-based indicators. Journal of the North American Benthological Society 26:566–585.

- CARDINALE, B. J., D. S. SRIVASTAVA, J. E. DUFFY, J. P. WRIGHT, A. L. DOWNING, M. SANKARAN, AND C. JOUSEAU. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443:989–992.
- CHESSMAN, B. C., N. BATE, P. A. GELL, AND P. NEWALL. 2007. A diatom species index for bioassessment of Australian rivers. Marine and Freshwater Research 58:542–557.
- DAVIES, S. P., AND S. K. JACKSON. 2006. The biological condition gradient: a descriptive model for interpreting change in aquatic ecosystems. Ecological Applications 16:1251–1266.
- DESCY, J. P. 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia 64:305–323.
- DICKMAN, M., A. S. DIXIT, J. FORTESCUE, R. BARLOW, AND J. TERASMAE. 1984. Diatoms as indicators of the rate of lake acidification. Water, Air, and Soil Pollution 21:375–386.
- DUFRENE, M., AND P. LEGENDRE. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345–366.
- FORE, L. S., AND C. GRAFE. 2002. Using diatoms to assess the biological condition of large rivers in Idaho (USA). Freshwater Biology 47:2015–2037.
- FRITZ, S. C., S. JUGGINS, R. W. BATTARBEE, AND D. R. ENGSTRUM. 1991. Reconstruction of past changes in salinity and climate using a diatom-based transfer function. Nature 352:706–708.
- GALLAGHER, J. C. 1982. Physiological variation and electrophoretic banding patterns of genetically different seasonal populations of *Skeletonema costatum* (Bacillariophyceae). Journal of Phycology 18:148–162.
- HAWKINS, C. P., R. H. NORRIS, J. N. HOGUE, AND J. W. FEMINELLA. 2000. Development and evaluation of predictive models for measuring the biological integrity of streams. Ecological Applications 10:1456–1477.
- HERLIHY, A. T., J. L. STODDARD, AND C. B. JOHNSON. 1998. The relationship between stream chemistry and watershed land cover data in the mid-Atlantic region, U.S. Water, Air, and Soil Pollution 105:377–386.
- KARR, J. R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66–84.
- KARR, J. R., AND E. W. CHU. 1999. Restoring life in running waters. Island Press, Washington, DC.
- KAUFFMAN, P. R., P. LEVINE, E. G. ROBISON, C. SEELIGER, AND D. V. PECK. 1999. Quantifying physical habitat in wadeable streams. EPA 620-R-99-003. US Environmental Protection Agency, Corvallis, Oregon.
- KELLY, M. G., A. CAZAUBON, E. CORING, A. DELL'UOMO, L. ECTOR, B. GOLDSMITH, H. GUASCH, J. HÜRLIMANN, A. JARLMAN, B. KAWECKA, J. KWANDRANS, R. LAUGASTE, E.-A. LINDSTROM, M. LEITAO, P. MARVAN, J. PADISÁK, E. PIPP, J. PYRGIEL, E. ROTT, S. SABATER, H. VAN DAM, AND J. VIZNET. 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. Journal of Applied Phycology 10:215–224.

Kelly, M., S. Juggins, R. Guthrie, S. Pritchard, J. Jamieson, B.

RIPPLEY, H. HIRST, AND M. YALLOP. 2008. Assessment of ecological status in U.K. rivers using diatoms. Freshwater Biology 53:403–422.

- KRAMMER, K., AND H. LANGE-BERTALOT. 1986. Bacillariophyceae, Teil 1. Naviculaceae. Spektrum Akademischer Verlag, Heidelberg, Germany.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1988. Bacillariophyceae, Tiel 2. Bacillariophyceae, Epithemiaceae, Surirellaceae. Pages 1–876 in H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Süsswasserflora von Mitteleuropa. Spektrum Akademischer Verlag, Heidelberg, Germany.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1991a. Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotiaceae, Achnanthaceae. Spektrum Akademischer Verlag, Heidelberg, Germany.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1991b. Bacillariophyceae, Teil 4. Achnanthaceae, kritsche Erganzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis, Tiel 1–4. Spektrum Akademischer Verlag, Heidelberg, Germany.
- LANGE-BERTALOT, H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64:285–304.
- LOWE, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. EPA-670/4-74-005. US Environmental Protection Agency, Cincinnati, Ohio.
- MANOYLOV, K. M., AND R. J. STEVENSON. 2006. Densitydependent algal growth along N and P nutrient gradients in artificial streams. Pages 333–352 *in* N. Ognjanova-Rumenova and K. Manoylov (editors). Advances in phycological studies. Pensoft Publishers, Moscow, Russia.
- MERRIAM-WEBSTER. 2003. Merriam-Webster's collegiate dictionary. 11<sup>th</sup> edition. Merriam-Webster, Inc., Springfield, Massachusetts.
- MILLENNIUM ECOSYSTEM ASSESSMENT. 2005. Ecosystems and human well-being: synthesis. Island Press, Washington, DC.
- OMERNIK, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118–125.
- PAN, Y. D., R. J. STEVENSON, B. H. HILL, A. T. HERLIHY, AND G. B. COLLINS. 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. Journal of the North American Benthological Society 15:481–495.
- PATRICK, R., M. H. HOHN, AND J. H. WALLACE. 1954. A new method for determining the pattern of the diatom flora. Notulae Nature 259:1–12.
- PATRICK, R., AND C. W. REIMER. 1966. The diatoms of the United States, exclusive of Alaska and Hawaii. Volume 1. Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.
- PATRICK, R., AND C. W. REIMER. 1975. The diatoms of the United States, exclusive of Alaska and Hawaii. Volume 2, Part 1. Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.
- PECK, D. V., D. K. AVERILL, A. T. HERLIHY, R. M. HUGHES, P. R. KAUFMANN, D. J. KLEMM, J. M. LAZORCHAK, F. H. MC-

CORMICK, S. A. PETERSON, M. R. CAPPAERT, T. MAGEE, AND P. A. MONACO. Environmental monitoring and assessment program—surface waters western pilot study: field operations manual for non-wadeable rivers and streams. Office of Research and Development, US Environmental Protection Agency, Washington, DC (in press).

- PECK, D. V., A. T. HERLIHY, B. H. HILL, R. M. HUGHES, P. R. KAUFMANN, D. KLEMM, J. M. LAZORCHAK, F. H. MCCORMICK, S. A. PETERSON, P. L. RINGOLD, T. MAGEE, AND M. CAPPAERT. 2006. Environmental monitoring and assessment program—surface waters western pilot study: field operations manual for wadeable streams. EPA/620/R-06/003. Office of Research and Development, US Environmental Protection Agency, Washington, D.C.
- PONADER, K. C., D. F. CHARLES, AND T. J. BELTON. 2007. Diatom based TP and TN inference models and indices for monitoring nutrient enrichment of New Jersey streams. Ecological Indicators 7:79–93.
- PORTER, S. D., D. K. MUELLER, N. E. SPAHR, M. D. MUNN, AND N. M. DUBROVSKY. 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biology 53:1036–1054.
- Postel, S. L. 1998. Water for food production: will there be enough in 2025? BioScience 48:629–637.
- POTAPOVA, M., AND D. CHARLES. 2002. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. Journal of Biogeography 29:167–187.
- POTAPOVA, M. G., D. F. CHARLES, K. C. PONADER, AND D. M. WINTER. 2004. Quantifying species indicator values for trophic diatom indices: a comparison of approaches. Hydrobiologia 517:25–41.
- RIER, S. T., AND R. J. STEVENSON. 2006. Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. Hydrobiologia 561:131–147.
- SLADECEK, V. 1973. System of water quality from the biological point of view. Archiv für Hydrobiologie und Ergebnisse Limnologie 7:1–218.
- STEVENS, D. L. J., AND A. R. OLSEN. 2004. Spatially balanced sampling of natural resources. Journal of the American Statistical Association 99:262–277.
- STEVENSON, R. J. 1997. Scale-dependent determinants and consequences of benthic algal heterogeneity. Journal of the North American Benthological Society 16:248–262.
- STEVENSON, R. J. 2006. Refining diatom indicators for valued ecological attributes and development of water quality criteria. Pages 365–383 *in* N. Ognjanova-Rumenova and K. Manoylov (editors). Advances in phycological studies. Pensoft Publishers, Moscow, Russia.
- STEVENSON, R. J., R. C. BAILEY, M. C. HARASS, C. P. HAWKINS, J. ALBA-TERCEDOR, C. COUCH, S. DYER, F. A. FULK, J. M. HARRINGTON, C. T. HUNSAKER, AND R. K. JOHNSON. 2004. Designing data collection for ecological assessments. Pages 55–84 *in* M. T. Barbour, S. B. Norton, H. R. Preston, and K. W. Thornton (editors). Ecological assessment of aquatic resources: linking science to decision-making. Society of Environmental Toxicology and Contamination Publication, Pensacola, Florida.
- STEVENSON, R. J., B. E. HILL, A. T. HERLIHY, L. L. YUAN, AND S. B. NORTON. 2008. Algae–P relationships, thresholds, and

frequency distributions guide nutrient criterion development. Journal of the North American Benthological Society 27:259–275.

- STEVENSON, R. J., AND A. PINOWSKA. 2007. Diatom indicators of ecological conditions in Florida springs. Report to Florida Department of Environmental Protection, Tallahassee, Florida.
- STEVENSON, R. J., AND J. P. SMOL. 2002. Use of algae in environmental assessments. Pages 775–804 *in* J. D. Wehr and R. G. Sheath (editors). Freshwater algae in North America: classification and ecology. Academic Press, San Diego, California.
- STODDARD, J. L., D. P. LARSEN, C. P. HAWKINS, R. K. JOHNSON, AND R. H. NORRIS. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–1276.
- STRAHLER, A. N. 1952. Hyposometric (area-altitude) analysis of erosional topography. Bulletin of the Geological Society of America 63:1117–1142.
- TAYLOR, J. C., J. PRYGIEL, A. VOSLOO, P. A. DE LA REY, AND L. VAN RENSBURG. 2007. Can diatom-based pollution indices be used for biomonitoring in South Africa? A case study of the Crocodile West and Marico water management area. Hydrobiologia 592:455–464.
- TER BRAAK, C. J. F. 1995. Ordination. Pages 91–169 in R. H. G. Jongman, C. J. F. ter Braak, and O. F. R. van Tongeren (editors). Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, UK.
- TER BRAAK, C. J. F., AND H. VAN DAM. 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia 178:209–223.
- USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 1987. Handbook of methods for acid deposition studies, laboratory analysis for surface water chemistry. EPA-600-4-87-026. US Environmental Protection Agency, Washington, DC.
- USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2007. National water quality inventory: report to Congress. 2002 reporting cycle. EPA 841-R-07-001. Office of Research and Development, US Environmental Protection Agency, Washington, DC.
- VAN DAM, H., A. MERTENES, AND J. SINKELDAM. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28:117–133.
- WANG, Y. K., R. J. STEVENSON, AND L. METZMEIER. 2005. Development and evaluation of a diatom-based index of biotic integrity for the Interior Plateau Ecoregion. Journal of the North American Benthological Society 24: 990–1008.
- WATANABE, T., K. ASAI, A. HOUKI, S. TANAKA, AND T. HIZUKA. 1986. Saprophilous and eurysaprobic diatom taxa to organic water pollution and diatom assemblage index (DAIpo). Diatom 2:23–73.
- WELCH, E. B., J. M. JACOBY, AND C. W. MAY. 1998. Stream quality. Pages 69–94 in R. J. Naiman and R. E. Bilby (editors). River ecology and management. Springer-Verlag, New York.
- WRIGHT, J. F., M. T. FURSE, AND P. D. ARMITAGE. 1993. RIVPACS: a technique for evaluating the biological quality of rivers in the UK. European Water Pollution Control 3:15–25.

YUAN, L. L. 2004. Assigning macroinvertebrate tolerance classifications using generalised additive models. Freshwater Biology 49:662–667.

YUAN, L. L. 2007. Using biological assemblage composition to infer the values of covarying environmental factors. Freshwater Biology 52:1159–1175.

YUAN, L. L., AND S. B. NORTON. 2003. Comparing responses of

macroinvertebrate metrics to increasing stress. Journal of the North American Benthological Society 22:308–322.

ZELINKA, M., AND P. MARVAN. 1961. Zur Prazisierung der biologischen Klassifikation des Reinheit fliessender Gewässer. Archiv für Hydrobiologie 57:389–407.

> Received: 6 March 2008 Accepted: 1 September 2008



Using Diatoms as Indicators of Ecological Conditions in Lotic Systems: A Regional Assessment Author(s): Yangdong Pan, R. Jan Stevenson, Brian H. Hill, Alan T. Herlihy and Gary B. Collins Source: *Journal of the North American Benthological Society*, Vol. 15, No. 4 (Dec., 1996), pp. 481-495 Published by: Society for Freshwater Science

Stable URL: http://www.jstor.org/stable/1467800 Accessed: 19/12/2013 19:20

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.



Society for Freshwater Science is collaborating with JSTOR to digitize, preserve and extend access to Journal of the North American Benthological Society.

http://www.jstor.org

# Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment

YANGDONG PAN<sup>1,4</sup>, R. JAN STEVENSON<sup>1</sup>, BRIAN H. HILL<sup>2</sup>, ALAN T. HERLIHY<sup>3</sup>, AND GARY B. COLLINS<sup>2</sup>

<sup>1</sup> Department of Biology, University of Louisville, Kentucky 40292 USA

<sup>2</sup> National Exposure Research Laboratory, US Environmental Protection Agency, 3411 Church Street, Cincinnati, Ohio 45244 USA

<sup>3</sup> Department of Fisheries and Wildlife, Oregon State University, c/o US Environmental Protection Agency, 200 SW 35th Street, Corvallis, Oregon 97333 USA

*Abstract.* Benthic diatoms and water chemistry were sampled from 49 stream sites in the Mid-Atlantic Highlands region of the United States to evaluate the use of diatoms as indicators of environmental conditions in streams across varying geographic and ecoregional areas. Diatom samples were collected from depositional and erosional habitats in a randomly selected reach in each stream site. Patterns of diatom species distributions in relation to environmental variables were determined using canonical correspondence analysis. Diatom species in both habitats were highly correlated with a pH gradient. A second gradient was correlated with variables that were commonly associated with agricultural runoff such as turbidity and total phosphorus.

The relationship between diatoms and major environmental variables was quantified with regression and calibration models. The correlation between diatom-inferred and observed pH was high ( $r^2 = 0.90$ ). Cross-validation with jackknifing showed that pH models were reasonably robust ( $r^2 = 0.69$  for depositional habitats,  $r^2 = 0.67$  for erosional habitats). The regression and calibration models for the depositional habitats had only slightly higher predictive powers than those of erosional habitats.

The relationship between diatoms and important environmental variables was robust and quantifiable, and the sensitivity of diatom assemblages to environmental conditions did not differ between erosional and depositional habitats. Therefore we concluded that diatoms can be used as quantitative indicators of environmental conditions in lotic systems.

*Key words*: benthic diatoms, biomonitoring, canonical correspondence analysis (CCA), depositional and erosional habitats, Mid-Atlantic Highlands streams, pH, regression and calibration model.

Characterizing dynamic environmental conditions in heterogeneous systems such as streams requires innovative approaches. Water chemistry, especially limiting nutrients in streams, can vary temporally and spatially (Munn and Prepas 1986, Chambers et al. 1992, France and Peters 1992, Cattaneo and Prairie 1994). Benthic diatom assemblages are regulated by environmental conditions (e.g., limiting nutrients) (Pan and Lowe 1994). Species-rich diatom assemblages can integrate temporal variability of environmental conditions and the aggregate influence of different environmental factors (Plafkin et al. 1989). The key to use of diatoms as reliable bioindicators is deciphering the integrated environmental information in species-rich assemblages. Numerical approaches (canonical correspondence analysis and weight-

<sup>4</sup> Present address: Environmental Sciences and Resources, Portland State Univesity, Portland, Oregon 97207 USA. ed average regression) (ter Braak 1986, ter Braak and van Dam 1989, Birks et al. 1990) are effective in extracting interpretable information from species-rich, but often noisy, species data. These techniques have enabled development of accurate diatom indicators of pH (Birks et al. 1990), trophic status (Christie and Smol 1993), metal concentrations (Kingston et al. 1992), and other environmental conditions in lakes (Fritz 1990, Fritz et al. 1993).

Despite the success in lentic systems, diatom species distributions in streams in relation to environmental conditions have not been well established. One reason is that the accumulation of the benthic diatom assemblage in streams is much less temporally integrated than diatom assemblages on the surface of lake sediments, which often accumulate over several years (Fritz et al. 1993). In fact, benthic diatom assemblages in lotic systems may be as young as the most recent flood (Stevenson 1990). Because of high temporal variation of both environmental conditions and diatom assemblage characteristics, the use of diatoms as effective environmental indicators in streams has not been rigorously examined.

In this study, we used numerical techniques to examine the relationship between diatom species distribution in streams and environmental conditions across varying geographic and ecoregional areas. The main objective of this research was to determine if benthic diatoms can be used as effective and reliable indicators of wide ranges of physical and chemical conditions found in streams. We collected benthic diatoms and physical and chemical data from erosional and depositional habitats in 49 stream sites within the Mid-Atlantic Highlands region, as a part of the Mid-Atlantic Highlands Assessment component of the Environmental Monitoring and Assessment Program (EMAP) of the US Environmental Protection Agency (EPA). Our specific objectives were 1) to determine if variation in benthic diatom assemblages can reflect major environmental gradients across large geographic areas and ecoregions, 2) to quantify the relationship between diatoms and important environmental variables, and 3) to compare the sensitivity of diatoms in erosional and depositional habitats to changes in environmental conditions.

#### **Study Sites**

The Mid-Atlantic Highlands Assessment Project (MAHA) was conducted over a 211,300-km<sup>2</sup> area in the Appalachian Mountain portion of 4 EPA Region III states (Pennsylvania, Maryland, West Virginia, and Virginia; Fig. 1). The Mid-Atlantic Highlands (MAH) region consists of 3 somewhat linear physiographic provinces oriented from southwest to northeast. From east to west, they are the Blue Ridge Mountains, Valley and Ridge, and Appalachian Plateau (Fig. 1). The Blue Ridge Mountains make up a narrow forested ridge with high elevation. Streams are usually high-gradient with cool and clear water. Bedrock typically is composed of Cambrian and Precambrian metamorphosed sedimentary and complex gneissic and plutonic rocks. The Valley and Ridge Province is composed of a series of folded Paleozoic sedimentary rock layers, resulting in a sequence of valleys separated by narrow, parallel, linear ridges. The ridges typically are composed of more resistant sandstones, whereas limestone and shale formations are common in the valley bottoms. The streams often show a trellis drainage pattern due to the alternating valley and ridge structure. High-gradient streams are common in forested ridges while low-gradient streams occur in valleys. The Appalachian Plateau Province is separated from the Valley and Ridge Province to the east by a steep outfacing escarpment (e.g., Allegheny Front). The rocks in the Appalachian Plateau Province are younger than those in the other provinces, and clastic conglomerates, sandstones, and shales are predominant. The mountains of the Appalachian Plateau are the result of severe plateau dissection. For our current analyses we further subdivided the Appalachian Plateau into Western, Northern, and Central regions based on the ecoregion map of Omernik (1987) (Fig. 1). Located in a low plateau, the Western Appalachians ecoregion contains more human development; industrial activities including mining, have significant impact on ecosystems in this area. The landscape is a mosaic of urban-industrial activities, fields, pastures, forests, and mines. The Central Appalachians ecoregion is higher in elevation and therefore cooler in temperature than the Western Appalachians; it is further characterized by extensive forest cover, infertile soils, high precipitation, and short growing seasons. The Northern Appalachians ecoregion is a plateau which is lower and less forested than the Western Appalachians; it is a mosaic of cropland, pasture, and woodland.

As a part of MAHA and EMAP pilot survey, 65 stream sample sites in the MAH region were selected using a systematic, randomized sampling design. The stream network on digitized versions of 1:100,000 scale topographic maps (US Geological Survey) was used for sampling. The survey was restricted to wadeable streams defined as 1st, 2nd, or 3rd order (Strahler 1957) on the 1:100,000 scale map. Sample probabilities were set so that roughly equal numbers of 1st, 2nd, and 3rd order streams would appear in the sample. Each sample site has an expansion factor (weight) so that inference to the entire population of streams in the region can be made using the sample data. Streams were sampled in late spring (26 April-8 July) 1993. Of the 65 randomly selected sites, 13 were not sampled due to the absence of a stream channel, being too deep to safely wade, or access refusal by the landowner. Ten percent of the sites were sampled twice to begin compiling informa-



FIG. 1. A map of the Mid-Atlantic Highlands area showing sampling locations. The numbers correspond to the sample site codes in Table 2. The solid lines are ecoregional boundaries. The dashed lines are state boundaries.

tion on sampling variability. In addition to the random probability sites, an additional 31 "reference" (good condition) and 10 "test" (poor condition) sites were hand-picked to provide "end members" of stream ecological conditions in the region. The "reference" and "test" sites were selected based on experts' opinions. We used part of this large data set (see below) for our analyses.

#### Methods

#### Field sampling

The field crews laid out a study reach around each of the selected sample sites with a total length equal to 40 times the average wetted channel width (150 m minimum, 500 m maxi-

Sample volume	Filtration and preservation	Variables measured
Cubitainer		
250 mL	Filtered, $pH < 2$ with $HNO_3$	Ca, Mg, K, Na, total dissolved Al
125 mL	Filtered, $pH < 2$ with $H_2SO_4$	Dissolved organic carbon, NH <sub>4</sub>
250 mL	Filtered, no preservative	$Cl_{1}$ NO <sub>3</sub> SO <sub>4</sub> SiO <sub>2</sub>
500 mL	Unfiltered, no preservative	Acid neutralizing capacity, color, air equilibrated pH, turbidity, specific conductance, total sus- pended solids
250 mL	Unfiltered, $pH < 2$ with $H_2SO_4$	Total P, total N
Syringe		
60 mL	Unfiltered, no preservative, sealed from atmosphere	pH, dissolved inorganic carbon, monomeric Al

TABLE 1. Aliquots and processing of water chemistry samples. Cubitainer water samples from each stream were partitioned into 5 aliquots and processed for different chemical analyses. One syringe sample was used for each analysis.

mum). Eleven cross-section transects were set up in each study reach by dividing the reach into 10 equal length intervals (includes a transect at the start and end of each reach). At each stream, a periphyton sample was collected from each of the 11 transects and combined into either a depositional or erosional habitat composite sample. Transects with no visible water movement were defined as depositional habitat, those with visible water movement were considered erosional habitat. At each transect, periphyton was collected from a 12-cm<sup>2</sup> area of stream bed using a 1.5-cm-long piece of 3.9-cmdiameter PVC pipe as a template. For fine substrate, periphyton was sucked into a 60-mL syringe; in coarser substrate, periphyton was scraped off with a toothbrush and rinsed off with stream water (Lazorchak and Klemm 1993). Composite periphyton samples were then preserved with 37% formalin for transport to the laboratory. The end result was 1 composite periphyton sample for erosional habitats and 1 composite sample for depositional habitats for each stream site. For water chemistry, a 4-L cubitainer and four 60-mL syringes of stream water were collected at the randomly selected stream sample site at a flowing portion near the middle of the stream. The syringes were sealed with a Luer-lock valve to prevent gas exchange. All water samples were placed on ice and sent by overnight courier to the analytical laboratory.

#### Chemical analyses

Within 48–72 h of collection, water from the syringe samples was analyzed for closed head-

space measurements of pH, dissolved inorganic carbon (DIC), and monomeric aluminum, and the cubitainer sample was split into aliquots and preserved (Table 1). Detailed information on the analytical procedures used for each of the analyses can be found in US EPA (1987). In brief, base cations were determined by atomic absorption, anions by ion chromatography, dissolved organic carbon (DOC)/DIC by a carbon analyzer, and total N, P by persulfate oxidation.

#### Diatom analysis

A subsample of the preserved periphyton suspension was acid-cleaned and mounted in HYRAX<sup>®</sup> to enumerate diatom species (Patrick and Reimer 1966). At least 500 diatom valves were counted at 1000  $\times$  magnification. Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b) were used as primary references for diatom taxonomy.

#### Data analysis

Correspondence analysis (CA) was used to determine the major patterns of diatom species distribution (Hill 1973). These patterns were then related to measured environmental variables using canonical correspondence analysis (CCA) (ter Braak 1986). Diatom species composition can be affected by several processes of community development (e.g., reproduction, immigration, and grazing) and other factors such as geography and history. If there are such dominant ecological processes, we would expect that a considerable amount of variance in diatom species data would be distributed in a systematic way in association with these regulatory factors. Since the importance of CA axes sequentially decrease ( $\lambda_1 > \lambda_2 > \lambda_3 \dots$ ) and each axis is statistically independent, the percentage of variance in diatom species distribution captured by the 1st several CA axes often represents a large amount of interpretable variance in diatom species distribution. Because CCA axes are constructed to best explain variance in diatom species distribution under the restriction that the CCA axis must also be a linear combination of environmental variables, comparison between the percentage of species variance captured by CA axes and its corresponding CCA axes can suggest how effectively measured environmental variables explain interpretable variance in diatom species distribution.

Both CA and CCA were performed on diatom species with relative abundances greater than 1% at 5 or more sites using CANOCO v.3.0 (ter Braak 1987, 1990). All environmental variables except pH were log-transformed due to their skewed distributions. Eleven of 24 environmental variables were used for CCA, selection being based on correlations, variance inflation factors (VIF) in CCA (ter Braak 1987, 1990), and our ecological interests. The variables with high correlation coefficient (r > 0.85) and VIF (>10) with other variables were eliminated. The variables used in final analyses were selected to reflect water quality (e.g., total phosphorus, total nitrogen), land-use in watersheds (e.g., turbidity, total suspended solids, C1, A1), and unique characteristics of regional geology (e.g., pH). The significance of the 1st 2 CCA axes was tested using unrestricted Monte Carlo permutation tests (999 permutations, p = 0.05) (ter Braak 1987, 1990).

Diatom-based regression and calibration models were developed to quantify relationships between diatoms and important environmental variables using weighted averaging regression and calibration (Birks et al. 1990). Species optima and tolerance were calculated with weighted averaging methods. A weighted average estimate of a taxon's optimum and tolerance for an environmental variable is just the mean of the measured environmental variable weighted by species abundance for this taxon in all sites and its weighted standard deviation, respectively (Birks et al. 1990). Tolerance values were corrected for bias by taking account of the effective number of occurrences (Hill's N2) (Hill 1973).

The estimated optima of diatom species were used to infer the environmental variables of interest, a method termed "WA calibration" (Birks et al. 1990). The inferred value of the environmental variable for a site is simply the mean of species optima weighted by species abundance for all taxa in this site. WA calibration can be done as WA with downweighting by tolerance (WA<sub>(tol)</sub>) (Birks et al. 1990). In WA<sub>(tol)</sub>, one downweights the taxa's optima inversely with their squared tolerance (weighted standard deviation of the optima).

The performance of the models was measured as the coefficient of determination  $(r^2)$  between diatom-inferred and observed values for environmental variables of interest and the rootmean-squared error of prediction (RMSE). Models that produced high  $r^2$  and low RMSE were selected as the superior models. The diatom-inferred value of the environmental variable for a site was derived from an entire data set including the inferred site. The  $r^2$  calculated from the regression was termed as apparent  $r^2$ . Because apparent  $r^2$  was estimated from the entire dataset including inferred site, this measure sometimes may not be realistic for assessing the predictive power of the models (Cumming et al. 1995, Reavie et al. 1995). Cross-validation with leave-one-out jackknifing is required to validate apparent  $r^2$  (ter Braak and Juggins 1993). With leave-one-out jackknifing, the diatom-inferred value for a site was predicted by the weighted averaging species optima estimated from all sites except the inferred site. Thus jackknifingderived  $r^2$  and RMSE avoid the possible circularity with apparent measures and are most appropriate for assessing the predictive power of the models. Regression and calibration models were developed and validated using the computer program WACALIB v.3.3 (Line et al. 1994) and CALIBRATE v. 0.6 (Juggins and ter Braak 1992).

#### Results

#### Study site characteristics

For the analyses in this paper, we eliminated streams that did not have both an erosional and
TABLE 2. Stream/watershed physical characteristics for the 49 sites (2 of the 47 EMAP sites were visited twice). A dash shows data were unavailable.

					%		
		Land-			Sand/		
	Water-	use	-	Mean	fine	Slope	
C11 -	shed	ın	Eleva-	wetted	sub-	(reach	Classic
Site	area	water-	tion (m)	(m)	stra-	mean %	order
110.		stieu	(111)	(111)		70)	oruer
1	2.4	26	646	3.0	25	3.1	1
2	10.5	96	155	2.9	75	0.6	3
3	26.3	15	200	3.3	24	0.2	2
4	27.0	12	453	7.7	0	2.2	3
5	31.8	21	753	6.3	9		
6	16.9	72	732	3.1	67		
7	34.7	1	349	8.0	2	2.5	3
8	4.9	0	502	2.8	13	0.7	1
9	3.7	2	371	2.0	24	1.8	1
10*	28.0	62	182	4.1	53	2.0	3
10	28.0	62	182	4.1	47	1.9	3
11*	47.7	22	457	6.5	18	1.0	3
11	47.7	22	457	5.0	15	0.3	3
12	48.3	18	463	8.5	22	0.1	3
13	1.3	46	463	1.4	78	0.1	1
14	1.1	14	414	1.5	24	6.5	1
15	6.8	42	432	3.3	38	1.6	2
16	45.7	31	426	7.2	33	0.1	3
17	73.5	68	274	8.3	29	0.1	3
18	21.8	54	293	6.2	44	0.1	2
19	2.6	47	359	1.4	98	0.1	1
20	5.0	17	247	2.4	11	1.8	2
21	46.2	0	317	9.7	20	1.7	2
22	14.0	50	320	4.4	25		
23	90.9	34	177	8.8	13		
24	0.4	0	707	0.3	16	10.0	1
25	18.2	15	231	6.4	44	2.5	2
26	133.5	37	371	15.8	45	1.0	3
27	0.2	80	786	0.7	56	2.7	1
28	2.1	67	621	1.4	36	1.9	1
29	104.8	43	377	11.8	24	1.4	3
30	1.8	0	505	3.4	2	9.1	1
31	7.0	65	493	2.5	62	1.1	2
32	47.8	59	694	5.0	38	1.0	2
33	34.4	67	829	4.0	24	1.3	2
34	7.4	2	427	2.7	18	1.0	2
35	59.8	6	645	7.9	7	1.2	2
36	62.7	0	748	7.4	25	2.0	3
37	67.2	41	610	9.8	20	1.7	3
38	29.0	6	707	5.7	16	1.0	3
39	15.8	0	497	4.5	5	2.0	2
40	0.5	4	219	0.8	11	1.5	1
41	37.1	16	237	8.7	38	0.1	3
42	2.1	0	243	2.0	15	1.0	1
43	0.6	0	316	0.5	4	3.5	1
44	11.6	37	195	4.0	7	0.8	2
45	47.1	2	909	8.4	13	0.0	2

TABLE 2. Continued.

Site no.	Water- shed area (km²)	Land- use in water- shedª	Eleva- tion (m)	Mean wetted width <sup>b</sup> (m)	% Sand/ fine sub- stra- te <sup>c</sup>	Slope (reach mean %)	Stream order
46	177.0	7	845	11.1	2		
47	5.1	3	588	2.9	9		

<sup>a</sup> Percent watershed area in agriculture, urban, or mining landuse based on 1:250,000 scale USGS Land Use Data Analysis data

<sup>b</sup> Wetted width calculated as mean of 100 width measurements taken at equal intervals along the study reach

<sup>c</sup> Percent of stream area with sand or smaller sized substrate; based on 5 point observations of substrate at each of the 11 study transects.

\* Sites visited twice, with collections made at different reaches

a depositional habitat composite sample. The resulting database consisted of 47 sites from the MAHA and EMAP survey. Two of these sites were visited twice, the 2nd sample being taken from a different reach, giving a total of 49 sites for our analyses: 29 randomly selected sites, 14 reference sites, and 6 test sites. These sampling sites are distributed in 5 ecoregions: 19 in Valley and Ridge, 10 in Western Appalachians, 10 in Central Appalations, 6 in Northern Appalachians, and 4 in Blue Ridge.

The study sites span a wide range of physical characteristics (Table 2). Elevations range from 155 to 909 m. Average stream widths range from 0.3 to 16 m. Mean reach slopes vary from 0.0 to 10.0. Watershed areas range from 0.5 to 177 km<sup>2</sup>. Based on the Land Use Data Analysis of the US Geological Survey, human-related land-use such as agriculture, urban development, and mining in watersheds varies from 0% (no disturbance) to 80% (Table 2). Of 43 sites with stream order data, the proportions of 1st, 2nd, and 3rd order streams are 30%, 33%, and 37%, respectively.

#### Water chemistry

We excluded 13 of the original 24 environmental variables from the analyses, because they exhibited high correlation coefficients with the other variables (11). For example, cations such as Ca and Mg, alkalinity, and acid neu1996]

	Ordination		Ordination results				
Habitats	Method	Axis	λ	S	r		
Depositional	CA	Ι	0.70	15.30			
•		II	0.34	7.50			
	CCA	Ι	0.59	12.90	pH (-0.79)		
		II	0.25	5.50	turbidity (0.65)		
Erosional	CA	Ι	0.64	14.10			
		II	0.35	7.60			
	CCA	Ι	0.53	11.60	pH (-0.77)		
		п	0.24	5.30	TP (0.59), turbidity (0.56)		

TABLE 3. Summary of correspondence analysis (CA) and canonical correspondence analysis (CCA) results for the 1st 2 ordination axes.  $\lambda$ : eigenvalue, S: percentage of variance explained by the corresponding ordination axis, *r*: correlation coefficients between the ordination axis and environmental variables. *n* = 49.

tralizing capacity were all highly correlated with conductivity (r > 0.90). Ammonium and nitrate were correlated with total nitrogen (r >0.90). CCA performed with and without these 13 variables also indicated that elimination of these variables did not significantly lower the eigenvalues (from 0.63 to 0.59) or the percentage of species variances captured by the 1st CCA axis (from 13.9% to 12.9%) (Table 3).

A wide range of water chemistry was observed among sampled stream sites (Table 4). pH varied from 4.2 to 8.7 with a median of 8.05. The nutrient conditions such as total phosphorus (TP) and nitrogen (TN) ranged from 3 to 472  $\mu$ g P/L and from 0.14 to 6.09 mg N/L, respectively.

## Diatom species distribution and environmental gradients

The 1st 2 CA axes explained 22.8% (depositional habitats) and 21.7% (erosional habitats) of

variance in diatom species distribution among sites (Table 3). The eigenvalues of CA and CCA of the 1st 2 axes were similar. Comparison between CA and CCA indicated that the measured environmental variables accounted for most diatom species variance. CCA axis 1 and 2 collectively accounted for 18.4% (depositional habitats) and 16.9% (erosional habitats) of total diatom species variance (Table 3). The measured environmental variables collectively explained 50.3% (depositional habitats) and 48.4% (erosional habitats) of variance in diatom species distribution captured by the 1st 2 CCA axes. The species-environmental correlations were high for the 1st 2 axes (r = 0.92, 0.83 for CCA axis 1 and 2, respectively).

Monte Carlo permutation tests indicated that the 1st 2 CCA axes for both depositional and erosional habitats were statistically significant (p< 0.05). The 1st axis of CCA was strongly correlated with pH for both depositional (r =

TABLE 4. Summary of descriptive statistics of 11 measured environmental variables used for CCA from 49 stream sites in the Mid-Atlantic Highlands area. PCU: platinum-cobalt units, NTU: nephelometric turbidity units.

Variable	Median	Maximum	Minimum	Skewness (G1)	Kurtosis (G2)
Al (mg/L)	0.10	9.50	0.00	6.78	43.96
Cl (mg/L)	0.84	2.35	0.14	3.57	13.32
Color (PCU)	6.00	93.00	1.00	4.58	23.68
Conductivity (µS/cm)	130.00	1860.00	17.70	3.79	17.31
DOC (mg/L)	2.23	8.44	0.61	1.91	3.95
TN $(mg/L)$	0.54	6.09	0.14	2.54	6.08
pH	8.05	8.68	4.21	-2.46	7.29
$TP (\mu g/L)$	14.00	472.00	3.00	6.05	37.38
$SiO_2 (mg/L)$	5.87	15.66	1.63	1.34	2.85
TSS (mg/L)	5.50	61.00	0.40	2.48	5.87
Turbidity (NTU)	1.80	26.00	0.20	2.37	5.06

-0.79) and erosional habitats (r = -0.77) (Fig. 2, Table 3). Of our 49 sampling sites, only 3 had pH less than 6.5. To assess if these sites were responsible for significant correlations, a second CCA run excluding the 3 low pH sites, still yielded a strong correlation between 1st CCA axis and pH.

The 2nd CCA axis was correlated with variables that are commonly associated with surface runoff and land-use (Fig. 2, Table 3). For example, turbidity was correlated with the second axis in the depositional habitats (r = 0.65). In erosional habitats, TP (r = 0.59), turbidity (r = 0.56), chloride (r = 0.53), and DOC (r = 0.52) were all correlated with axis 2.

#### Diatom species distribution and indicator species

Diatom species composition shifted along the pH gradient (Table 5). For example, Eunotia incisa, E. minor, E. intermedia, Frustulia rhomboides, and Tabellaria flocculosa were most abundant at low pH sites. As pH increased, Navicula gregarica, N. minima, N. cryptotenella, and Nitszchia sociabilis became dominant.

Species distribution also shifted along the land-use axis (2nd CCA axis) (Table 5). For example, relative abundance of highly motile diatom taxa such as *Surirella*, *Nitzschia*, and *Navicula* were strongly associated with sites where turbidity was high.

Some species had a strong statistical relationship with environmental variables. CCA using a single environmental variable each time showed that greater than 10% of some individual species variance could be explained by the corresponding environmental variable (these species were listed in Table 5). Therefore these species can be used as indicators for these environmental variables.

#### Diatom-based calibration models

pH models provided the highest predictability of all regression and calibration models (Table 6), yielding strong correlations between diatom-inferred and observed pH for both depositional ( $r^2 = 0.90$ ) and erosional habitats ( $r^2 = 0.79$ ) (Fig. 3, Table 6). The cross-validation with leave-one-out jackknifing indicated the predictive ability of the pH models was reasonably good ( $r^2_{jack} = 0.69$  for depositional habitats,  $r^2_{jack} = 0.67$  for erosional habitats).



FIG. 2. CCA ordination diagram of 49 stream sites with environmental variables represented by arrows. Sampled sites from different ecoregions are indicated by different symbols. TSS: total suspended solids, TP: total phosphorus, DOC: dissolved organic carbon W App: Western Appalachians, V&R: Valley and Ridge, N App: Northern Appalachians, C App: Central Appalachians, and B1 Rg: Blue Ridge. A.—Depositional habitats B.—Erosional habitats.

TP models also yielded relatively high predictability with  $r^2$  of 0.65 and 0.63 for depositional and erosional habitats, respectively (Fig. 4, Table 6). These  $r^2$  values were significantly reduced from 0.65 to 0.27 after the cross-validation with jackknifing. TP models were developed excluding 3 low-pH sites, which may possibly interfere with TP models. Performance of the TP models without the low-pH sites was not improved.

#### Erosional habitats vs. depositional habitats

The apparent  $r^2$  and RMSE indicated that regression and calibration models developed from depositional habitats had slightly better predictability than those of erosional habitats. The TABLE 5. Indicator species for selected environmental variables. Weighted-averaged optima and tolerances were present only for the diatom species that had greater than 10% of the species variance explained by CCA with a single corresponding environmental variable. NTU: nephelometric turbidity units.

Diatom species	Optima	Tolerances
pH		
Eunotia intermedia (Kras.) Norp. & L–B.	5.95	0.79
Fragilaria exigua Grun.	5.98	0.91
Eunotia incisa W. Sm. ex Greg.	6.13	0.91
Eunotia paludosa Grun.	6.14	0.79
Eunotia exigua (Breb. ex Kutz.)	6.14	1.19
Tabellaria flocculosa (Roth) Kutz.	6.15	0.89
Achnanthes childanos Hohn & Hell.	6.54	1.02
Eunotia muscicola v. tridentula Norp. & L-B.	6.56	1.63
Achnanthes marginulata Grun.	6.63	0.55
Eunotia minor (Kutz.) Grun.	6.72	1.07
Fragilaria virescens Ralfs	6.88	1.06
Frustulia rhomboides (Ehr.) De Toni	6.90	1.28
Achnanthes pseudoswazi Cart.	6.95	1.15
Navicula gregarica Donk.	8.29	0.25
Navicula minima Grun.	8.30	0.29
Nitzschia sociabilis Hust.	8.35	0.22
Navicula cryptotenella L–B.	8.49	0.33
Amphora Pediculus (Kutz.) Grun. sense K.& L–B. 1986.	8.52	0.18
Turbidity (NTU)		
Achnanthes deflexa v. alpestris Lowe & Kocio.	2.16	2.30
Nitzschia dissipata (Kutz.) Grun.	6.72	6.11
Navicula capitata Ehr.	8.41	6.27
Nitzschia linearis (C.A. Ag. ex W. Sm.) W. Sm.	8.95	17.75
Navicula menisculus Schum.	9.04	6.10
Nitzschia sociabilis Hust.	9.53	9.06
Achnanthes lanceolata (Breb.) Grun.	9.64	8.18
Navicula minima Grun.	9.78	10.74
Amphipleura pellucida Kutz.	13.28	4.85
Navicula mutica Kutz.	13.51	10.70
Navicula desussis Ostr.	14.12	5.14
Navicula tantula Hust.	14.35	8.96
Navicula trivalis L–B.	15.37	11.17
Gomphonema angustatum (Kutz.) Rabh.	15.48	27.38
Nitzschia sociabilis Hust.	15.91	22.01
Surirella minuta Breb.	16.25	4.42
Surirella brebisonii K. & L–B.	19.79	31.80
Total phosphorus (µg/L)		
Synedra rumpens Kutz. V. rumpens	11.02	11.18
Achnanthes minutissima Kutz.	17.26	26.92
Navicula arvensis Hust.	42.68	22.02
Nitzschia sociabilis Hust.	43.29	55.67
Navicula veneta Kutz.	45.31	23.96
Achnanthes lanceolata (Breb.) Grun.	47.20	86.05
Navicula minima Grun.	51.16	65.97
Navicula capitata Ehr.	59.26	24.17
Navicula cryptotenella L–B.	60.50	65.55
Nitzschia palea (Kutz.) W. Sm.	61.51	100.75
Navicula viridula (Kutz.) Kutz. emend. V. H.	110.67	141.67
Nitzschia perminuta (Grun.) Perag.	127.96	184.37
Cymbella tumida (Breb. ex Kutz.) V.H.	142.55	185.25
Achnanthes clevel Grun.	185.30	213.64
Gyrosigma scalproides (Kabh.) Cl.	364.92	183.63

Y. PAN ET AL.

TABLE 6. Comparison of the predictive powers of diatom-based calibration models for depositional and
erosional habitats. The $r^2$ is coefficient of determination of regression between diatom-inferred and measured
pH or TP. RMSE is the root mean squared error. The r <sup>2</sup> and RMSE in parentheses were derived from jackknifing.
WA: simple weighted averaging; WA(tol): wieghted averaging with tolerance downweighed option.

	Depositional habitats $(n = 49)$				Erosional habitats $(n = 49)$			
	٧	٧A	WA	(tol)	W	VΑ	WA	(tol)
Parameter	<b>r</b> <sup>2</sup>	RMSE	r <sup>2</sup>	RMSE	r <sup>2</sup>	RMSE	<b>r</b> <sup>2</sup>	RMSE
pН	0.81 (0.67)	0.37 (0.49)	0.90 (0.64)	0.27 (0.55)	0.79 (0.66)	0.39 (0.49)	0.71 (0.57)	0.45 (0.60)
TP	0.63 (0.27)	0.23 (0.32)	0.65 (0.25)	0.22 (0.32)	0.63 (0.27)	0.23 (0.32)	0.62 (0.21)	0.23 (0.33)





FIG. 3. Observed pH values plotted against diatom-inferred (apparent and jackknifed) pH values. A.—Depositional habitats B.—Erosional habitats. The straight line was drawn as 1:1 ratio.

FIG. 4. Observed TP values plotted against diatom-inferred (apparent and jackknifed) TP values. A.—Depositional habitats B.—Erosional habitats. The straight line was drawn as 1:1 ratio.

 $r^2$  for pH ( $r^2 = 0.90$ ) in depositional habitats was 19% higher than that in erosional habitats ( $r^2 = 0.71$ ) (Table 5). However, the difference in model performance between the 2 habitats was diminished to negligible levels after cross-validation ( $r^2_{jack} = 0.67$  depositional habitats,  $r^2_{jack} = 0.66$  erosional habitats). The best models for pH performed equally well for the 2 habitats (Table 6).

#### Discussion

#### Using diatoms as indicators in streams

Despite wide ranges of physical and chemical conditions among sampled stream sites, diatom species distribution was strongly associated with major environmental gradients across the Mid-Atlantic Highlands. The results of CA showed that a large proportion of variance in diatom species distribution ( $\geq 20\%$ ) can be explained by the 1st 2 ordination axis, suggesting that diatom species distributed in a systematic way. CCA indicated that diatom species distribution was highly correlated with our measured environmental variables. Our data showed that the relationship between diatom species distribution and environmental gradients in streams was as strong, or stronger than those reported from lake studies. For example, the percentage of variance in diatom species distribution captured by the 1st 2 CCA axes has ranged from 11 to 16% in lake studies (Dixit et al. 1991, 1993, Hall and Smol 1992, Fritz et al. 1993) and 16.9-18.4% in MAH streams.

Of the variables we measured, pH was the most important environmental factor affecting diatom species composition in streams in this region. Unlike many lentic pH studies which have focused on lake acidification, the pH gradient in our study was skewed toward the alkaline side of the pH spectrum (6.4-8.7). High pH in these systems stemmed mainly from watershed geology and pH elevation due to landuse such as liming and agriculture-induced nutrient enrichment (Downey et al. 1994, Ivahnenko et al. 1988). The performance of our pH recalibration models gression and was comparable to those developed in lentic systems with more acidic waters. pH models developed for lentic systems have produced apparent  $r^2$  of 0.91 for 62 Adirondack lakes (Dixit et al. 1993), of 0.78 for 72 Sudbury lakes in Canada (Dixit et al. 1991), and of 0.82-0.85 for European lakes studied by Birks et al. (1990). Since cross-validation was not available when some of these studies were published, a direct comparison of jackknifing-derived  $r^2$  cannot be made. Using bootstrapping, a similar cross-validation procedure as jackknifing, RMSE<sub>boot</sub> did not significantly differ from apparent RMSE (Dixit et al. 1993). However, a large discrepancy between apparent RMSE (0.27) and jackknifing-derived RMSE (0.55) was observed in our study. The discrepancy in our data was likely due to poor predictability of low pH condition (Fig. 3). Accuracy and robustness of estimated species optima may rely heavily on sample sizes, ranges of environmental conditions, and particularly evenness of sample distribution along the environmental gradient. Nevertheless, diatom-based pH models in streams were reasonably robust and probably reflect long-term environmental selection of the species pool in streams.

In contrast to pH models, our TP models performed poorly. Poor performance of TP models was much more evident after cross-validation. Hall and Smol (1992) reported apparent  $r^2$  of 0.78 with 37 lakes. The  $r^2$  was dropped to 0.28 after bootstrapping (Cumming et al. 1995). A similar reduction of  $r^2$  was observed in our data (from 0.65 to 0.27). Poor performance of TP models may be related to the nature of nutrient variables in streams. Nutrients, especially limiting nutrients, can vary in time and space (Meyer and Likens 1979, Munn and Prepas 1986, Chambers et al. 1992, France and Peters 1992). For example, temporal variabilities were much higher for nutrients than for pH (Cattaneo and Prairie 1994). As a resident biotic component in streams, diatoms can register and integrate transient or episodic changes of nutrient conditions. Diatom assemblage characteristic at one time can be a result of, for example, the cumulative effects of environmental conditions from the previous 2 or 3 weeks (Allen et al. 1977). It is not surprising that our models using a snapshot of nutrient measurement (e.g., 1-time sampling of nutrient data in our study) have only limited success in predicting nutrient conditions in streams. Reliability and accuracy of using diatoms as indicators of trophic status in streams would be greatly enhanced if we could more accurately and adequately characterize the dynamics of limiting nutrients in streams. Such characterization may require high-frequency sampling of nutrients.

Development of diatom indicators of environmental conditions might be more successful if study sites were selected based on their representation of specific conditions along environmental gradients. Despite the fact that numerous studies reported that nutrients, especially phosphorus, are important for algal growth (Bothwell 1989, Stevenson et al. 1991), development of a TP regression and calibration model has been less successful, especially when pH and other physical variables were important (Hall and Smol 1992, Anderson et al. 1993, Reavie et al. 1995, Pan and Stevenson 1996). Sub-optimal pH can exert severe physiological stress on biotic components of aquatic ecosystems (Baker and Christensen 1990, Fairchild and Sherman 1993). pH can also mediate physical and chemical changes such as ionization of toxic metals (Schindler 1990, Kingston et al. 1992). Low pH can often eliminate pH-sensitive species at different trophic levels and constrain the food web (Schindler 1990, Locke and Sprules 1994). For example, in acidic conditions, only acidobiontic and acidophilic diatom species are abundant (Charles 1985, Davis and Anderson 1985, this study). Invertebrates respond to pH in a similar way (Locke and Sprules 1994). Regardless of the mechanisms involved, the interference of pH on developing TP models may force the selection of sites where pH is not a major factor. Such selective TP models may have only region-specific applications.

Complete turnover in diatom composition or membership along phosphorus gradients may not occur as dramatically as along pH gradients. It is likely that peak algal biomass and growth rates may be more sensitive to changes in TP than algal species representation, but assessing these variables in large-scale surveys may be impractical. Input of excessive phosphate often causes algal blooms and shifts algal assemblage composition from dominance by diatoms to dominance by bluegreen algae (Schindler 1974, 1990). Most TP regression and calibration models, however, were based only on diatoms, and thus may not be adequate to predict among-divisional shifts. Shifts of diatom species in response to P loading may represent only an initial phase of the response curve between algae and P. Reavie et al. (1995) reported that increased numbers of sample sites with TP values greater than 85 µg/L did not improve the performance of their TP calibration models; their residual plot of the TP model showed increased deviation as TP increased, perhaps because diatoms are unable to reflect TP conditions at high TP level. Application of a diatom-based regression and calibration model for TP may be limited to low and medium ranges of TP conditions, and success in developing such a model may still depend on a sufficiently large number of low-TP sites. Further research including all groups of algae may be needed to develop better indices of trophic status in streams.

#### Erosional habitats vs. depositional habitats

We expected that the diatom-based regression and calibration models based on data from depositional habitats might have a higher predictive power than those from erosional habitats. Broad-scaled spatial sources for diatoms can be integrated in benthic diatom assemblages in depositional habitats. Current slows settling, so diatom assemblages in erosional habitats are probably affected more by local conditions, whereas assemblages in depositional habitats are probably natural composites of many upstream assemblages (Stevenson 1984). However, our data showed that the best pH models for depositional habitats had only slightly higher predictive power ( $r_{jack}^2 = 0.67$ ) than those of erosional habitats ( $r_{jack}^2 = 0.66$ ) (Table 5). Integration of broad-scaled variation in depositional habitats, if it occurs, did not yield superior models.

In conclusion, benthic diatom species can reflect the major environmental gradients in streams across broad-scaled areas. Our diatombased models have demonstrated that the relationship between diatoms and environmental variables was robust and quantifiable. Therefore diatoms can be used as quantitative indicators of ecological conditions.

#### Acknowledgements

This research is a part of the Mid-Atlantic Highlands Assessment component of the US EPA's Environmental Monitoring and Assessment Program (EMAP). Patti Grace-Jarret counted periphyton samples. Patti Haggerty made the map. Discussion with Drs. John Birks and Stephen Juggins helped the data analyses. We thank Victoria Rogers for assistance with the EMAP database. We thank Drs. R. J. Mackay, C. G. Peterson, H. J. B. Birks, and an anonymous reviewer for their comments on earlier versions of this paper. This research was supported by a contract from US EPA to R. Jan Stevenson and a grant from NSF water and watershed (R 824783-01-0). Financial support was also provided by cooperative agreement CR821738 between Oregon State University and the EPA's National Health and Environmental Effects Laboratory in Corvallis, Oregon.

#### Literature Cited

- ALLEN, T. F. H., S. M. BARTELL, AND J. F. KOONCE. 1977. Multiple stable configurations in ordination of phytoplankton community change rates. Ecology 58:1076–1084.
- ANDERSON, N. J., B. RIPPEY, AND C. E. GIBSON. 1993. A comparison of sedimentary and diatom-inferred phosphorus profiles: implications for defining pre-disturbance nutrient conditions. Hydrobiologia 253:357–366.
- BAKER, J. P., AND S. W. CHRISTENSEN. 1990. Effects of acidification in biological communities in aquatic ecosystems. Pages 83–106 in D. F. Charles (editor). Acid deposition and aquatic ecosystems: regional case studies. Springer-Verlag, New York.
- BIRKS, H. J. B., J. M. LINE, S. JUGGINS, A. C. STEVENSON, AND C. J. F. TER BRAAK. 1990. Diatoms and pH reconstruction. Philosophical Transactions of the Royal Society of London. B. Series Biological Sciences 327:263–278.
- BOTHWELL, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 46:1293–1301.
- CATTANEO, A., AND Y. T. PRAIRIE. 1994. Temporal variability in the chemical characteristics along the Riviere de L'Achigan: how many samples are necessary to describe stream chemistry? Canadian Journal of Fisheries and Aquatic Sciences 52: 828–835.
- CHAMBERS, P. A., E. E. PREPAS, AND K. GIBSON. 1992. Temporal and spatial dynamics in riverbed chemistry: the influence of flow and sediment composition. Canadian Journal of Fisheries and Aquatic Sciences 49:2128–2140.
- CHARLES, D. F. 1985. Relationships between surface sediment diatom assemblages and lake water characteristics in Adirondack lakes. Ecology 66: 994–1011.
- CHRISTIE, C. E., AND J. P. SMOL. 1993. Diatom assemblages as indicators of lake trophic status in southeastern Ontario lakes. Journal of Phycology 29:575–586.
- CUMMING, B. F., S. E. WILSON, R. I. HALL, AND J. P.

SMOL. 1995. Diatoms from British Columbia (Canada) lakes and their relationship to salinity, nutrients and other limnological variables. Pages 1–207 *in* H. Lange-Bertalot (editor). Bibliotheca diatomologica, Band 31. J. Cramer, Berlin.

- DAVIS, R. B., AND D. S. ANDERSON. 1985. Methods of pH calibration of sedimentary diatom remains for reconstructing history of pH in lakes. Hydrobiologia 120:69–87.
- DIXIT, S. S., A. S. DIXIT, AND J. P. SMOL. 1991. Multivariable environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. Freshwater Biology 26:251–265.
- DIXIT, S. S., B. F. CUMMING, H. J. B. BIRKS, J. P. SMOL, J. C. KINGSTON, A. J. UUTALA, D. F. CHARLES, AND K. CAMBURN. 1993. Diatom assemblages from Adirondack lakes (New York, USA) and the development of inference models for retrospective environmental assessment. Journal of Paleolimnology 8:27–47.
- DOWNEY, D. M., C. R. FRENCH, AND M. ODOM. 1994. Low cost limestone treatment of acid sensitive trout streams in the Appalachian Mountains of Virginia. Water, Air, and Soil Pollution 77:49–77.
- FAIRCHILD, G. W., AND J. W. SHERMAN. 1993. Algal periphyton response to acidity and nutrients in softwater lakes: lake comparisons vs nutrient enrichment approach. Journal of the North American Benthological Society 12:157–167.
- FRANCE, R. L. AND R. H. PETERS. 1992. Temporal variance function for total phosphorus concentration. Canadian Journal of Fisheries and Aquatic Sciences 49:975–977.
- FRITZ, S. C. 1990. Twentieth-century salinity and water-level fluctuations in Devils Lake, North Dakota: test of a diatom transfer function. Limnology and Oceanography 35:1771–1781.
- FRITZ, S. C., S. JUGGINS, AND R. W. BATTARBEE. 1993. Diatom assemblages and ionic characterization of lakes of the northern Great Plains, N.A.: a tool for reconstructing past salinity and climate fluctuations. Canadian Journal of Fisheries and Aquatic Sciences 50:1844–1856.
- HALL, R. I., AND J. P. SMOL. 1992. A weighted-averaging regression and calibration model for inferring total phosphorus concentration from diatoms in British Columbia (Canada) lakes. Freshwater Biology 27:417–434.
- HILL, M. O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54:427–32.
- IVAHNENKO, T. I., J. J. RENTON, AND H. W. RAUCH. 1988. Effects of liming on water quality of two streams in West Virginia. Water, Air, and Soil Pollution 41:331–357.
- JUGGINS, S., AND C. J. F. TER BRAAK. 1992. CALI-BRATE—a program for species-environment calibration by [weighted-averaging] partial least squares regression. Unpublished computer pro-

gram, Environmental change research center, University College London.

- KINGSTON, J. C., H. J. B. BIRKS, A. J. UUTALA, B. F. CUMMING, AND J. P. SMOL. 1992. Assessing trends in fishery resources and lake water aluminum for paleolimnological analyses of siliceous algae. Canadian Journal of Fisheries and Aquatical Sciences 49:116–127.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1986. Bacillariophyceae. 1. Teil: Naviculaceae. VEB Gustav Fisher Verlag Jena.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1988. Bacillariophyceae. 2. Teil: Epithemiaceae, Bacillariaceae, Surirellaceae. VEB Gustav Fisher Verlag, Jena.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1991a. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae, Achnanthaceae. VEB Gustav Fisher Verlag, Jena.
- KRAMMER, K., AND H. LANGE-BERTALOT. 1991b. Bacillariophyceae. 4. Teil: Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gomphonema. VEB Gustav Fisher Verlag, Jena.
- LAZORCHAK, J. M., AND D. L. KLEMM. 1993. Environmental monitoring and assessment program: Surface waters and region 3 regional environmental monitoring and assessment program. 1993 Pilot field operations and methods manual. Stream. US Environmental Protection Agency, Environmental monitoring systems laboratory, Cincinnati.
- LINE, J. M., C. J. F. TER BRAAK, AND H. J. B. BIRKS. 1994. WACALIB version 3.3—a computer program to reconstruct environmental variables from fossil assemblages by weighted averaging and to derive sample-specific errors of prediction. Journal of Paleolimnology 10:147–152.
- LOCKE, A., AND W. G. SPRULES. 1994. Effects of lake acidification and recovery on the stability of zooplankton food webs. Ecology 75:498–506.
- MEYER, J. L., AND G. E. LIKENS. 1979. Transport and transformation of phosphorus in a forest stream ecosystem. Ecology 60:1255–1269.
- MUNN, N. L., AND E. N. PREPAS. 1986. Seasonal dynamics of phosphorus partitioning and export in two streams in Alberta, Canada. Canadian Journal of Fisheries and Aquatic Sciences 43:2464– 2471.
- OMERNIK, J. M. 1987. Aquatic ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118–125.
- PAN, Y., AND R. L. LOWE. 1994. Independent and interactive effects of nutrients and grazers on benthic algal community structure. Hydrobiologia 291:201–209.
- PAN, Y., AND R. J. STEVENSON. 1996. Gradient analysis of diatom communities in western Kentucky wetlands. Journal of Phycology 32:201–212.

PATRICK, R., AND C. W. REIMER. 1966. The diatoms of

the United States. Volume 1. Monographs of the Academy of Natural Sciences of Philadelphia. No. 13.

- PATRICK, R. AND C. W. REIMER. 1975. The diatoms of the United States. Volume 2. Part 1. Monographs of the Academy of Natural Sciences of Philadelphia. No. 13.
- PLAFKIN, J. L., M. T. BARBOUR, K. D. PORTER, S. K. GROSS, AND R. M. HUGHES. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. U.S. Environmental Protection Agency, Washington, D.C. EPA/440/4-89-001.
- REAVIE, E. D., R. I. HALL, AND J. P. SMOL. 1995. An expanded weighted-averaging model for inferring past total phosphorus concentrations from diatom assemblages in eutrophic British Columbia (Canada) lakes. Journal of Paleolimnology 14: 49–67.
- SCHINDLER, D. W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science 184:897–899.
- SCHINDLER, D. W. 1990. Experimental perturbations of whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos 57:25– 41.
- STEVENSON, R. J. 1984. How currents on different sides of substrates in streams affect mechanisms of benthic algal accumulation. Internationale Revue der gesamten Hydrobiologie 69:241–262.
- STEVENSON, R. J. 1990. Benthic algal community dynamics in a stream during and after a spate. Journal of the North American Benthological Society 9:277–288.
- STEVENSON, R. J., C. G. PETERSON, D. B. KIRSCHTEL, C. C. KING, AND N. C. TUCHMAN. 1991. Densitydependent growth, ecological strategies, and effects of nutrients and shading on benthic diatom succession in streams. Journal of Phycology 27: 59–69.
- STRAHLER, A. N. 1957. Quantitative analysis of watershed: geomorphology. American Geophysical Union Transactions 38:913–920.
- TER BRAAK, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1667– 1679.
- TER BRAAK, C. J. F. 1987. CANOCO—a FORTRAN Program for Canonical Community Ordination by [partial] [detrended] [Canonica] correspondence Analysis, Principal Components Analysis and Redundancy Analysis (version 2.0). TNO Institute of Applied Computer Science, Wageningen, The Netherlands.
- TER BRAAK, C. J. F. 1990. Update Notes: CANOCO v.3.1. Agricultural Mathematics Group, Wageningen, The Netherlands.
- TER BRAAK, C. J. F., AND S. JUGGINS. 1993. Weighted

averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. Hydrobiologia 269/270:485–502.

- TER BRAAK, C. J. F., AND H. VAN DAM. 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia 178: 209-223.
- US EPA. 1987. Handbook of methods for acid deposition studies, laboratory analysis for surface water chemistry. EPA 600/4-87/026. US Environmental Protection Agency, Office of Research and Development, Washington DC.

Received: 29 April 1996 Accepted: 26 September 1996



# Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment

Yangdong Pan, Alan Herlihy, Philip Kaufmann, Jim Wigington, John van Sickle & Tom Moser Environmental Science and Resources, Room 218A, Science Building II, Portland State University, 1719 SW 10th Ave., Portland, OR 97207, U.S.A. Tel.: 1-503-725-4981. Fax number: 1-503-725-3888. E-mail: pany@pdx.edu

Received 25 June 2003; in revised form 26 August 2003; accepted 1 September 2003

*Key words:* partial canonical correspondence analysis, gradient, periphyton, physical habitat, spatial scales, land cover/land use, Willamette River basin

#### Abstract

We assessed the importance of spatial scales (catchment, stream network, and sample reach) on the effects of agricultural land-use on lotic diatom assemblages along a land-use gradient in the agricultural Willamette Valley Ecoregion of Oregon. Periphyton, water chemistry, and physical habitat conditions were characterized for 25 wadeable streams during a dry season (July to September, 1997). Additional water chemistry samples were collected in the following wet season (February 1998) to assess seasonal effects of land-use on stream water chemistry. Percent agricultural land-use in the study catchments ranged from 10% to 89% with an average of 52%. Partial canonical correspondence analysis (CCA) with the first axis constrained by % agricultural land-use showed that % agricultural land-use at 3 spatial scales explained between 3.7%–6.3% of variability in the diatom species dataset. Monte Carlo Permutation tests indicated that the variance explained by % agricultural land-use was only significant at the spatial scale of the stream network with 10- and 30-m band width (p < 0.05, 999 permutations). In addition to the effects of % agricultural land-use, partial CCAs with a forward selection option showed that water chemistry (e.g., SiO<sub>2</sub>), reach-scale stream channel dimensions (e.g., width, depth, and slope), reach-scale in-stream habitats (substrates and filamentous algal cover in stream beds), and riparian vegetative buffer were all important with relation to diatom species assemblages. Percent of obligately nitrogen-heterotrophic taxa was the only diatom autecological metric that showed a significant but weak correlation with % agricultural land-use along the stream network (r = 0.50), but not at catchment or sample reach scale. Correlation between % agricultural land-use and water chemistry variables varied among the spatial scales and between seasons. Physical habitat variables ( $\log_{10}$ erodible substrate diameters and stream reach slope) were significantly correlated with % agricultural land-use along the stream network but not at catchment or sample reach scale. Our data suggest that spatial scales are important in assessing effects of land-use on stream conditions but the spatial scale effects may vary between seasons. Direct linkages between agricultural land-use and lotic diatom assemblages were weak during summer base-flow time regardless of the spatial scales. Summer sampling may underestimate the effects of catchment land-use on stream conditions in areas where seasonal patterns are so distinctive as in the Willamette Valley.

#### Introduction

One of the major challenges in water resource management is to identify environmental stressors and understand how these stressors affect aquatic ecosystems. Catchment land-use is a major stressor on stream ecosystems, especially in agricultural regions. For example, agricultural land-use in the catchment can significantly modify both water chemistry (Johnson et al., 1997) and physical habitat conditions (Roth et al., 1996), which eventually decrease biological integrity in these streams (Karr & Chu, 1999). Despite the large amount of literature on the effects of landuse on stream ecosystems, it is still challenging to quantify the relationships between land-use and biological integrity of stream ecosystems. One of the major problems is to identify comparable spatial and temporal scales for assessing such relationships (Allan et al., 1997; Harding et al., 1998; Arheimer & Liden, 2000).

Differences in lotic biotic assemblages with different evolutionary history and life spans may reflect environmental conditions of hierarchically organized stream habitats and associated human influences (e.g., channel unit, reach, and catchment) (Frissel et al., 1986). For example, Roth et al. (1996) reported that fish assemblages in Michigan streams were better related to catchment land-use. In contrast, others have found that macroinvertebrates and periphyton exhibit much stronger relationships to stream reach environmental conditions than to catchment variables (Richards et al., 1997; Pan et al., 2000). Regional climate/geology may set up ultimate constrains on overall regional periphyton species diversity. Stream reach-scaled determinants such as resources and abiotic stresses may largely control actual species membership in a stream reach biotic assemblage (Stevenson, 1997). Pan et al. (1999, 2000) reported that the regional/catchment determinants such as climate and geology alone poorly predicted stream reach periphyton assemblages. Approximately 64% of the variability in periphyton species richness among 12 New Zealand streams was explained by flood disturbance and nutrients (Biggs & Smith, 2002). Relative importance of the regional/catchment-level and stream reach-level determinants on periphyton assemblages is, however, not well assessed. Meanwhile, most of stream bioassessment programs aim to assess catchment-level stressors such as land-use on stream ecosystems. Better understanding of the relative importance of both regional/catchment and stream reach scale determinants on periphyton species assemblages is essential for interpreting changes in periphyton with relation to environmental stressors at different scales, which will eventually increase accuracy and precision of periphyton-based bioassessment.

This study was aimed at assessing the effects of agricultural land-use at multiple spatial scales (catchment, stream network, and sample reach) on stream diatom assemblages along a land-use gradient in an agricultural region. We sampled 25 wadeable streams in the Willamette Valley Ecoregion of western Oregon during summer base-flow. Major types of land cover/land use such as agriculture, forest, rural builtup, and riparian vegetative buffer were delineated based on recent aerial photographs at catchment, along the entire stream network, and at sample reach scales (Fig. 1). In addition, water chemistry and physical habitat conditions were also characterized at each sample reach. We wanted to relate changes in diatom species assemblages to the major types of land-use, particularly % agriculture at the 3 spatial scales. We also wanted to assess the relative importance of the effects of agricultural land-use and other environmental variables such as water chemistry and physical habitat conditions on diatom assemblages at each spatial scale. In addition, we collected water chemistry samples from the same sites in the following wet season to see if the effects of agricultural land-use on stream water chemistry vary between dry and wet seasons.

#### Materials and methods

#### Study sites

The study was conducted in the Willamette Valley Ecoregion, a lowland area dominated by agriculture with some forest (foothills) and some urban areas (Clarke et al., 1991). The lowland, approximately 170 km long and 70 km wide, is a structural trough downfolded between the Coastal Range in the west and the Cascade Mountains in the east with modest relief (Uhrich & Wentz, 1999). Climate in the Pacific Northwest is characterized by distinct dry and wet seasons. Most of the annual precipitation ( $\cong$ 75%) occurs from October through March with <5% occurring during July and August (Uhrich & Wentz, 1999). Stream flow patterns are highly correlated with the annual precipitation patterns. We used the blue-line network on 1:100 000 scale USGS maps to identify every stream in the Willamette Valley with catchment areas between 10–100 km<sup>2</sup>. We used this size range as our intent was to study streams big enough to support fish but small enough to be wadeable. As the objective of the study was to examine an agricultural land-use gradient, we dropped from consideration any streams that flowed through urban areas, had most of its catchment in the adjacent mountainous ecoregions or contained large impoundments. From the 49 streams remaining on the candidate list after this screening, 25 streams were selected at random for field sampling and aerial photo analysis (Fig. 2).

In the field, a sample reach was selected near the downstream end of each stream at a site where



*Figure 1.* A map of Cozine Creek watershed showing 3 spatial scales used for characterizing land cover/land use (e.g. catchment, stream network showing only one bandwidth, and sampling reach) and major types of land cover/land use.

we were able to obtain access permission from local landowners. Sample reaches were each 40 times as long as the mean wetted channel width at the site (with a 150 m minimum) and ranged from 150 to 320 m. Eleven cross-section transects were set up on each sample reach by dividing the reach into 10 equal length intervals (includes a transect at the start and end of each reach). All samples were collected between July and early September 1997. Additional water chemistry samples were collected in Feb., 1998 from the same sites. However, periphyton samples were not collected due to field sampling difficulties (e.g., high flow).

#### Land-use characterization

Aerial photographs and a geographic information system (GIS) were used to determine land cover/land-use (LCLU) at 3 spatial scales (catchment, stream network, and sample reach) in each study stream (Schuft et al., 1999). For the network scale, LCLU was determined for band widths of 10, 30, and 150 m on both sides of the stream for the entire upstream stream network. The sample reach scale included band widths of 10 and 30 m on both sides of the 150–320 m stream reach sampled in the field. Color-infrared aerial photographs (1: 24000 scale) of study catchments were taken in mid-July, 1997, and these were scanned, spliced and georeferenced to make digital orthophotos with a minimum resolvable unit of about one meter. Catchment area boundaries, and the perennial and intermittent stream networks, as interpreted from 1:24000 scale USGS topographic maps, were delineated on the digital orthophotos and digitized to create GIS coverages. The LCLU classification we used was a modification of that of Anderson et al. (1976) (for more details see Schuft et al., 1999; Moser et al., 2000). The LCLU categories include forest, shrub/scrub, grass/forb, agriculture, built-up (residential, industrial/commercial, transportation corridors or other built-up lands), barren land, water, and other. Agricultural land-use was further classified into 7 subcategories (cropland, Christmas tree farm, etc.). Due to many zero values, redundancy among the variables, and the study objectives, 5 variables (% forest, % agriculture, % riparian vegetative buffer, % built-up, and % row crop) were used for our analyses. Percent of riparian vegetative buffer was a summation of % forest, shrub/scrub, and grass/forb within each defined



*Figure 2.* A map of the State of Oregon showing the Willamette Valley and a site map of the Willamette Valley showing Willamette Valley ecoregion and portions of Coastal Range and Cascades ecoregions, and sampling stream locations.

band width. Accuracy of the classification was verified by ground-truthing (Schuft et al., 1999). GIS analyses were used to calculate the area in each LCLU category at each spatial scale/band width. LCLU areas were converted to proportions for use in further data analyses.

#### Water chemistry

A container (4 l) and 2 syringe (60 ml) water samples were collected from the reach start point in the middle of the stream. Water from the syringe samples was analyzed for closed headspace measurements of pH and dissolved inorganic carbon (DIC), and the container samples were analyzed for major anions/cations and nutrients. Base cations were determined by atomic absorption. Anions were measured by ion chromatography. Dissolved organic carbon (DOC)/DIC was determined using a carbon analyzer. Total nitrogen (TN) and total phosphorus (TP) were estimated by persulfate oxidation and colorimetry. Detailed information on the analytical procedures used for each of the analyses can be found in US EPA (1987).

#### Physical habitat

Vegetative cover over the stream was measured in 4 directions at each of the 11 cross section transects using a convex spherical densiometer. At each transect, the presence and the proximity of 11 categories of human activities in the riparian zone were estimated (i.e., row crops, pasture, dams and revetments, buildings, pavement, roadways, pipes, landfill or trash, parks/lawns, logging operations, and mining activities). Proximity-weighted riparian disturbance indices were calculated by tallying the number of riparian transects at which a particular type of disturbance was observed, weighting by its proximity to the stream, and then averaging over all transects on the reach (Kaufmann et al., 1999). Stream habitat characterization included thalweg depth measurements, mean wetted width, bank angle, slope, % of substrate embeddedness, and a systematic pebble count to characterize surficial substrates. Several metrics (e.g., log<sub>10</sub> erodible substrate diameters) were calculated. The erodible substrate diameter is defined as the critical mean diameter at bankfull flow. It was calculated from an estimate of shear stress during bankfull conditions. It is the mean value, within the reach, of the maximum size of substrate particle that is expected to be mobilized or 'eroded' and moved during bankfull flood conditions. Field methods and metric calculation, respectively, are described in more detail by Kaufmann & Robison (1998) and Kaufmann et al. (1999).

#### Periphyton

Periphyton samples were collected from each of the 11 transects and combined into a single composite sample. Periphyton was scraped off coarse substrates from a defined area of stream bed (12 cm<sup>2</sup>) with a toothbrush and an area delimiter. For fine substrates, periphyton was suctioned into a 60-ml syringe. The samples were then preserved with 37% formalin. Detailed sampling and collection methods are described by Hill (1998). An aliquot of algal suspension from each sample was acid-cleaned and mounted in NAPHRAX<sup>®</sup> after repeated rinse with distilled water to identify and enumerate diatom species. A minimum

of 500 diatom valves was counted at  $1000 \times$  magnification using a Nikon E600 Eclipse microscope with a phase contrast. Diatom taxonomy mainly followed Krammer & Lange-Bertalot (1986–91) and Patrick & Reimer (1966, 1975).

#### Data analysis

We used multiple endpoints (species assemblages, autecological metrics, and diatom indices) to relate diatom assemblages with land-use patterns characterized at multiple spatial scales. Canonical correspondence analysis (CCA) was used to summarize diatom species assemblages and their relationships with environmental variables (ter Braak, 1995). A series of CCAs with different options were performed.

To identify a subset of environmental variables which were significant with relation to diatom species distribution patterns, CCA with a forward selection option was run on all environmental variables, which included all major land-use types (5 types) characterized at 3 spatial scales, water chemistry variables (17), and physical habitat variables (17). A subset of environmental variables was selected using a Monte Carlo Permutation test (999 permutations, p < 0.05). Detailed procedure and explanation for the Monte Carlo Permutation test can be found in ter Braak & Smilauer (1998). To better illustrate diatom species distribution patterns captured by the first 4 CCA ordination axes, the site scores of the first 4 CCA axes, surrogates of the species assemblages with recognizable distribution patterns, were correlated with diatom autecological metrics and indices. Diatom autecological metrics were calculated based on Lange-Bertalot (1979), Balhs (1993), and van Dam et al. (1994). The Trophic Diatom Index (TDI) was calculated following Kelly & Whitton (1995).

A total of 6 separate partial CCAs were performed to assess effects of agricultural land-use at each spatial scale on the diatom species assemblages. For instance, to assess the effects of agricultural land-use on diatom assemblages at the sample reach scale with 10-m band width, a partial CCA was performed with the first CCA axis constrained to % agricultural land-use characterized at this particular spatial scale. Significance of each agricultural land-use variable on the diatom species data was tested using the Monte Carlo Permutation test (999 permutations, p < 0.05). To assess relative importance of effects of agricultural land-use and other environmental variables such as water chemistry and physical habitat conditions on the diatom







*Figure 3.* Changes in total nitrogen concentrations and conductivity along a % agricultural land-use gradient during a dry and a wet season. A. Total nitrogen,  $r_{dry} = 0.22$  (n = 23, remove two sites with 2 highest values),  $r_{wet} = 0.73$  (= 25). B. Conductivity, n = 25,  $r_{dry} = 0.36$ ,  $r_{wet} = 0.73$ .

species data, a total of 6 partial CCAs with a forward selection option were performed after the effects of agricultural land-use were 'removed' from the analyses (the agricultural land-use variables were treated as covariables). A subset of environmental variables was selected. Significance of each selected environmental variable was tested using the Monte Carlo Permutation test (999 permutations, p < 0.05) at each spatial scale with different band widths.

Environmental variables were log-transformed except for pH and percentage variables. Percentage variables were square root transformed followed by arcsine transformation. Diatom species data were squared root transformed. Rare species were down weighted in all CCAs. CCA was performed using CANOCO for Windows (version 4.0) (ter Braak & Smilauer, 1998).

#### Results

#### Land-use

Land-use showed considerable variability among and within catchments. Overall, catchment land-use was dominated by agriculture (mean = 52%, range = 10-89%), followed by forest (22%), among the 25 catchments. The land-use closest to the streams was most commonly dominated by forest with an average of 43% of the stream network within the 10-m

*Table 1.* Pearson correlation coefficients between % agricultural land-use in catchments, % agricultural land-use, forest and riparian vegetative buffer (e.g., forest, grass, shrub) along stream networks and sample reach. Bold number indicates a significant correlation at p < 0.05 (n = 25).

Spatial scales	Band width (m)	% agriculture	% forest	% riparian vegetation
Catchment			-0.88	
Stream network	10	0.54	-0.74	-0.69
	30	0.50	-0.63	-0.54
Sample reach	150	<b>0.84</b>	- <b>0.77</b>	- <b>0.82</b>
	10	-0.19	-0.10	-0.10
	30	-0.11	-0.06	0.07

band width being forested. Percent agricultural landuse increased as the band width increased at both the network and reach scale. Percent agriculture in the catchment was highly correlated with % agriculture at the stream network scale, especially the 150 m band width. However, % agriculture at the sample reach scale was not significantly correlated with % agriculture at the catchment scale (Table 1).

#### Water chemistry

Water chemistry was characterized by high spatial variability in nutrients and ionic strength among the 25 sites in both dry and wet seasons (Table 2). For example, total phosphorus (TP) concentrations varied from  $12 \ \mu g \ l^{-1}$  to  $6720 \ \mu g \ l^{-1}$  in the dry season and from 3 to  $368 \ \mu g \ l^{-1}$  in the wet season. The median concentrations of the variables associated with ionic strength in the dry season were in general higher than those in the wet season while TN showed an opposite trend. The median concentration of TN in the wet season was 2 times higher than that in the dry season. Overall, higher winter TN was largely contributed by increases in NO<sub>3</sub>-N. Mean % organic N in the wet season (50%).

Correlation between % agricultural land-use and water chemistry variables varied among the spatial scales and between seasons (Fig. 3, Table 3). Catchment agricultural land-use was significantly correlated with ionic strength variables, chloride (Cl<sup>-</sup>), and nutrients in the wet season (Table 3). The correlation was only significant with TP (r = 0.40) in the dry season. Percent of agricultural land along the stream network

was correlated significantly with ANC, conductivity,  $Cl^-$ , and DOC but not with nutrients in the dry season. The strength of the association also increased in the wet season (Table 3).

#### Physical habitat

Stream channel morphology and habitat conditions also varied considerably among the sampled sites. Wetted stream channel width ranged from 1.1 m to 10.5 m with a median of 4.4 m. Thalweg depth varied from 0.2 m to 0.9 m with a median of 0.4 m. Both wetted stream channel width and thalweg depth were significantly correlated with catchment area ( $r_w =$ 0.55,  $r_d = 0.74$ , n = 25). Stream substrates were dominated by fines (31%) and hardpan (29%). Percent of substrates as hardpan was significantly and negatively correlated with stream slope (r = -0.51). Midchannel riparian canopy density varied from nearly absent (2.5%) to dense (98.8%). Riparian disturbance index values ranged from 0.4 to 3.3.

Correlation between % agricultural land-use and stream physical habitat variables varied among the spatial scales (Table 4).  $Log_{10}$  erodible substrate diameters were significantly correlated with % agriculture along the stream network (Fig. 4). The variable was not, however, significantly correlated with % agriculture, either at reach or catchment scales. Stream reach slope showed a similar pattern (Table 4). Both total riparian disturbance and riparian agricultural disturbance indices were significantly correlated with % agriculture at the reach-scale but not at catchment or stream network scales.

#### Diatom assemblages

A total of 159 diatom species and varieties were identified from the 25 sites. Species richness varied from 12 to 50 with an average of 35. Diatom assemblages were dominated by Gomphonema parvulum (Kütz.) Kütz., Rhoicosphenia curvata (Kütz.) Grun. ex Rabh., Achnanthes lanceolata (Bréb.) Grun., A. deflexa Reim., Nitszchia palea (Kütz.) W. Sm., N. amphibia Grun., and Melosira varians Ag.. The diatom assemblages were characterized by a high proportion of halophilous diatom species (mean = 72%), eutrophic diatom species (mean = 56%), and alkaliphilous diatom species (mean = 51%). Approximately 41% of the diatoms identified were characterized as taxa tolerant of elevated nitrogen (N) levels and 25% were facultative N-heterotrophic species. Percent of pollution sensitive species ranged from 5% to 93%.

Table 2. Summary of selected water chemistry variables (median, range) in both dry and wet season (n=25) and Pearson correlation coefficients between two seasons.

Variables	Dry season		Wet season		r
рН	7.4 (7.0–8.8)		7.1 (6.4–7.5)		0.33
ANC ( $\mu eq l^{-1}$ )	1660	(299–5433)	692	(142–1448)	0.56
Conductivity ( $\mu$ S cm <sup>-1</sup> )	249	(41–716)	129	(31–269)	0.39
$SO_4^{2-}$ (µeq l <sup>-1</sup> )	124	(7–338)	157	(13-326)	0.41
$Cl^{-}$ (µeq $l^{-1}$ )	384	(47-4039)	187	(46–553)	0.49
Total phosphorus ( $\mu g l^{-1}$ )	115	(12-6720)	120	(3–368)	0.26
Total nitrogen ( $\mu g l^{-1}$ )	864	(176–84438)	1899	(515–9731)	-0.02

*Table 3.* Pearson correlation coefficients between % agricultural land-use at different spatial scales and selected water chemistry variables measured in a dry and wet season. Bold number indicates a significant correlation at p < 0.05 (n = 25). ANC: acid neutralizing capacity, DOC: dissolved organic carbon, TN: total nitrogen, TP: total phosphorus.

Variables	Season	% agricultural land-use in						
		Reach		Networ	'k	Catchment		
		10 m	30 m	10 m	30 m	150 m		
ANC	Dry	-0.43	-0.25	0.39	0.39	0.48	0.34	
	Wet	-0.39	-0.23	0.35	0.33	0.55	0.45	
Cl-	Dry	-0.34	-0.14	0.52	0.53	0.34	0.01	
	Wet	-0.44	-0.32	0.45	0.45	0.60	0.43	
Conductivity	Dry	-0.38	-0.21	0.53	0.55	0.55	0.29	
	Wet	-0.43	-0.32	0.39	0.37	0.66	0.59	
DOC	Dry	-0.14	-0.02	0.14	0.56	0.45	0.25	
	Wet	-0.15	0.22	0.63	0.62	0.66	0.27	
TN	Dry	-0.13	-0.21	0.02	0.02	0.22	0.28	
	Wet	-0.39	-0.43	0.13	0.07	0.51	0.76	
TP	Dry	-0.39	-0.35	0.26	0.22	0.39	0.40	
	Wet	-0.41	-0.20	0.46	0.43	0.55	0.42	

*Table 4.* Pearson correlation coefficients between % agricultural land-use at different spatial scales and selected physical habitat variables. Bold number indicates a significant correlation at p < 0.05 (n = 25).

Variables	% agricultural land-use in							
	Reach		Network	Network				
	10 m	30 m	10 m	30 m	150 m			
% filamentous algal cover	-0.11	0.21	0.35	0.40	0.30	0.14		
% fine substrate	0.15	0.15	0.40	0.40	0.35	0.12		
Erodible substrate diameter <sup>a</sup>	0.05	-0.20	-0.71	-0.73	-0.60	-0.30		
Riparian disturbance <sup>b</sup>	0.70	0.70	0.05	0.07	-0.08	-0.11		
Riparian ag. disturbance	0.51	0.68	0.21	0.25	0.04	-0.14		
Stream reach slope (%)	-0.02	-0.21	-0.65	-0.67	-0.54	-0.17		

<sup>a</sup>Erodible substrate diameter was  $\log_{10}$  transformed.

<sup>b</sup>Riparian disturbance measures are indices calculated from observations made on-site at both banks on 11 transects.

*Table 5.* Summary of Canonic Correspondence Analysis (CCA) with a forward selection option. Variables were selected from all environmental variables (land-use, water chemistry, physical habitats) using the Monte Carlo permutation test (999 permutations, p < 0.05). The numbers in parenthesis next to eigenvalues are % of variability in diatom species dataset explained by the each CCA ordination axis. The other numbers are correlations of environmental variables with CCA ordination axes. Correlation between dominant diatom species, diatom metrics and CCA ordination axes were calculated only for showing and interpreting species patterns captured by each CCA axis. Bold number indicates a significant correlation at p < 0.05 (n = 25).

	Ordination axis						
	1	2	3	4			
Eigenvalues $(\lambda)$	0.21 (10.5%)	0.18 (8.7%)	0.14 (6.8%)	0.12 (5.8%)			
Diatom metrics							
Taxa richness	-0.64	-0.37	-0.29	0.23			
% alkaliphilous taxa	-0.44	0.70	0.53	-0.09			
% eutrophic taxa	-0.41	0.70	0.59	-0.23			
% facultative N-heterotrophic taxa	-0.39	0.43	0.57	0.19			
% halophilous taxa	-0.70	0.36	0.36	-0.35			
% hypereutrophic taxa	-0.48	-0.62	-0.07	-0.07			
% obligately N-heterotrophic taxa	-0.36	-0.65	0.09	-0.03			
% pollution sensitive taxa	0.87	0.08	-0.13	-0.08			
% pollution less tolerant taxa	-0.50	-0.23	-0.07	0.06			
% polysaprobous taxa	-0.47	-0.62	-0.08	-0.09			
Diatom species							
Achnanthes deflexa	0.93	-0.08	-0.28	-0.08			
A. lanceolata	-0.15	0.02	-0.36	-0.25			
A. minutissima	0.18	-0.18	-0.21	-0.09			
Cymbella minuta	-0.09	-0.18	-0.23	-0.07			
Fragilaria capucina	-0.20	-0.22	-0.20	-0.26			
Gomphonema parvulum	-0.14	-0.15	-0.32	-0.15			
Melosira varians	-0.12	-0.40	0.11	-0.02			
Navicula minima	-0.39	0.23	0.07	0.01			
N. crptotenella	-0.26	0.16	0.07	0.01			
Nitszchia amphibia	-0.25	0.37	0.76	0.72			
N. palea	-0.20	-0.16	-0.03	-0.27			
Rhoicosphenia curvata	-0.10	0.32	-0.07	0.03			
Synedra ulna	-0.29	-0.54	-0.02	0.31			
Physical habitat							
Stream wetted width	0.56	-0.12	0.01	0.03			
Thalweg depth	-0.06	-0.01	-0.27	-0.10			
% filamentous algal cover	-0.06	0.07	0.67	0.41			
% sand & fine substrates (<2 mm)	-0.41	0.05	-0.40	0.16			
Water chemistry							
ANC	-0.57	0.12	0.34	0.30			
pH	0.06	0.17	0.20	0.89			
SiO <sub>2</sub>	-0.15	0.83	-0.15	-0.08			

Percent silt tolerant species varied from 1% to 66% with an average of 35%.

CCA with a forward selection on all environmental variables identified 7 environmental variables that explained significant variance in the diatom species assemblages (Monte Carlo Permutation test, p < 0.05)

(Fig. 5, Table 5). The first two CCA axes explained 19% of variance among sites in the diatom assemblage data (Table 5). The first CCA axis was positively correlated with % of pollution sensitive taxa (r = 0.87), but negatively with diatom taxa richness (r = -0.64) and % halophilous taxa (r = -0.70) (Table 5). This

*Table 6.* Summary of partial Canonic Correspondence Analysis (CCA) results. In the partial CCA analyses, the first ordination axis was constrained by % of agriculture (3 different spatial scales with different buffer widths). The numbers in parenthesis are % variability in diatom species dataset explained by the first two CCA axes. P-values are the results of the Monte Carlo Permutation test (999 permutations) of the first CCA axis constrained by % of agriculture. Listed variables are selected in the order using a forward selection option with the Monte Carlo permutation test (999 permutations, p < 0.05).

Buffer	Spatial scale	Partial CCA			Variables selected by forward	
width (m)		λ1	$\lambda_2$	<i>p</i> -value	selection $(p < 0.05)$	
10	Sample reach	0.08 (3.7%)	0.27 (13.4%)	0.76	SiO <sub>2</sub> , NH <sub>4</sub> , ANC, pH, % filamentous	
					algal cover, % riparian buffer within 10-m band	
30	Sample reach	0.10 (5.0%)	0.27 (13.5%)	0.19	SiO <sub>2</sub> , NH <sub>4</sub> , ANC, pH, % filamentous algal	
				,	cover, % riparian buffer within 30-m band	
					width around sampling reach	
10	Whole stream	0.12 (6.0%)	0.26 (13.1%)	0.05	SiO <sub>2</sub> , stream wetted width, thalweg depth,	
	network				% riparian buffer within 30-m band width	
					along the stream network	
30	Whole stream	0.13 (6.3%)	0.27 (13.2%)	0.02	$SiO_2$ , stream wetted width, channel slope,	
	network				% sand & fine substrates ( $<2$ mm),	
					% filamentous algal cover	
150	Whole stream	0.11 (5.5%)	0.26 (13.0%)	0.11	SiO <sub>2</sub> , TN, channel slope, % fine gravel	
	network				substrates (<16 mm), % filamentous algal	
					cover, % riparian buffer with 30-m band	
					width along the stream network	
	Whole	0.11 (5.5%)	0.27 (13.4%)	0.10	$SiO_2$ , stream wetted width, % sand & fine	
	catchment				substrates (<2 mm), % filamentous algal	
					cover, % riparian buffer within 10-m and	
					30-m band width along the stream network	

axis was significantly, but weakly correlated with water chemistry variables (e.g., acid neutralizing capacity (r = -0.57)) and physical habitat variables (e.g., stream wetted width (r = 0.56)). The second CCA axis was positively correlated with % alkaliphilous taxa (r = 0.70) and % eutrophic taxa (r = 0.70), but negatively with % obligately N-heterotrophic taxa (r = -0.65) and % polysaprobous taxa (r = -0.62). This axis was positively correlated with SiO<sub>2</sub> (r =0.83). None of the first 4 CCA axes were significantly correlated with % agricultural land-use or other major types of land-use regardless of the spatial scales.

Partial CCAs showed that % agricultural land-use at different spatial scales explained between 3.7%– 6.3% of variability in the diatom species dataset (Table 6). Monte Carlo Permutation tests indicated that the variance explained by % agricultural land use was only significant at the spatial scale of the stream network with 10- and 30-m band width. However, actual differences among the variability explained by %agricultural land use at different spatial scales were small. In contrast, the % variability in diatoms explained by the second CCA axis was higher, ranging from 13.0% to 13.5% (Table 6). In addition to the effects of % agricultural land use, the partial CCAs showed that water chemistry (e.g., SiO<sub>2</sub>), reach-scale stream channel dimensions (e.g., width, depth, and slope), reach-scale in-stream habitats (substrates and filamentous algal cover in stream beds), and riparian vegetative buffer were important with relation to diatom species assemblages (Table 6).

Percent obligately N-heterotrophic taxa was the only autecological metric that showed a significant correlation with % agricultural land-use and water chemistry (Cl<sup>-</sup>, r = 0.46). The correlation between this metric and % agriculture varied with spatial scales. A significant correlation (r = 0.50) was observed at the stream network scale (both 10- and 30-m band width), but not at the catchment or reach scales. The Trophic Diatom Index (TDI) was significantly and positively correlated with ANC (r = 0.62), conductivity (r = 0.63), and TP (r = 0.51).



% agricultural land-use in catchments

*Figure 4.* Comparison of the relationships between  $\log_{10}$  erodible substrate diameter and % of agricultural land use at 3 spatial scales. A. % agricultural land-use at the reach scale with 30-m band width. B. % agricultural land-use along the stream network with 30-m band width. C. % agricultural land-use at the catchment scale. A correlation coefficient >0.40 is significant at p = 0.05, n = 25.

#### Discussion

## Effects of land-use on lotic diatoms and importance of spatial scales

Catchment determinants such as land-use may have direct and indirect effects on lotic diatom assemblages. Diatom assemblages clearly reflected agriculturerelated impacts on sampled streams. For example, 72% of diatoms were halophilous taxa which may suggest that sampled streams may be enriched by minerals. However, our data showed that the direct linkage between agricultural land-use and lotic diatom assemblages was weak in the Willamette Valley streams during the base flow period. CCA with a forward selection option on all environmental variables selected 7 variables of water chemistry and habitat conditions as being significantly related to diatom assemblages. None of land cover/land use variables was selected. Lack of a strong direct relationship between agricultural land-use and diatom assemblages during summer base flows may be attributed to our inability to measure the diatom/habitat and habitat/land-use relationships precisely so that noise overwhelms any diatom/land-use signal in our data. In this study, we sampled along a land-use gradient within the same ecoregion and river basin to minimize coarse-level confounding factors such as climate (Omernik, 1987). Percent agriculture in the catchment ranged from 10 to 89% with an average of 52% so the sites we selected covered a good range of the land-use gradient in the basin. However, the stream sites along this land-use gradient may still differ in site-specific factors such as hydrogeology. McFarland (1983) divided the Willamette Basin into 6 major hydrogeological units. The majority of our study sites were located in the basinfill and alluvial aquifer while 3-5 sites were located in the Columbia River Basalt aquifer. The basin-fill and alluvial aquifers are a mixture of unconsolidated clay, sand, and gravel and are sensitive to contamination in areas with a shallow water table (Gonthier, 1985). The Columbia River Basalt aquifer is more permeable to water flow and with more dilute water chemistry.

An aggregated measure of land-use such as % agriculture in catchments may only represent the potential of land-use effects on streams. Spatial configuration of land-use such as agricultural patch shape and size, flow path between agricultural patches and streams, and localized activities may vary within and among catchments. Patch density in the catchment, for example, was one of the important factors in relating catchment land-use to stream water chemistry (Johnson et al., 1997). Kehmeier (2001) found that % agricultural land-use explained a significantly higher amount of variability in native fish biomass in the Willamette River basin streams when % agricultural land was weighted by flow path distance to streams.

## Effects of water chemistry and physical habitat conditions on diatom assemblages

The potential effects of agricultural land-use on diatom assemblages may depend on variables such as water chemistry and physical habitat conditions measured at the sample reach scale. Changes in diatom



*Figure 5*. Canonical correspondence analysis (CCA) ordination diagram with 25 stream sites. Solid circles: sites with >70% agricultural land-use in catchments, open squares: sites with <30 agriculture in catchments, open circles: sites with % agricultural land-use in catchments between 30% and 70%.

assemblages were related to some water chemistry and physical habitat variables in our sampled Willamette Valley ecoregion streams. CCA showed that diatom taxa richness, % halophilous taxa, % pollution less tolerant taxa, % eutrophic taxa, % hypereutrophic taxa, % polysaprobous taxa, and % alkaliphilous taxa all decreased while % of pollution sensitive taxa decreased along the first CCA axis (Table 5). The changes of the diatom taxa and metrics along the first CCA axis were associated with ionic strength (ANC) and habitat conditions (stream wetted width, % sand & fine substrate). Effects of water chemistry and physical habitat conditions on diatom assemblages become more evident after variability in diatom species explained by agricultural land-use is removed. Partial CCAs with a forward selection option showed that water chemistry (e.g., SiO<sub>2</sub>), reach-scale stream channel dimensions (e.g., width, depth, and slope), reach-scale in-stream habitats (substrates and filamentous algal

cover in stream beds), and riparian vegetative buffer were important to diatom species assemblages. Several researchers have found that changes in periphyton species were often related to ionic strength in the Pacific Northwest streams. Carpenter & Waite (2000) reported that agricultural stream sites and forested stream sites were separated along a conductivity gradient in the Willamette Valley. Conductivity was one of the key explanatory variables for lotic periphyton species assemblages in the Columbia Plateau, Washington, an agriculturally dominated region (Leland, 1995; Munn et al., 2002). Streams in this region are typically characterized by low ionic strength (conductivity, alkalinity) (Welch et al., 1998). Increases in ionic strength in streams may reflect changes in landuse and increases surface runoff. However, changes in diatom species assemblages in relation to stream habitat conditions have received less attention. Several studies have examined the effects of substrate types on periphyton assemblages (see review by Cattaneo & Amireault, 1992; Burkholder, 1996). Most of these studies assessed substrate-specific effects, not substrate at a reach-level, on periphyton. But Kutka & Richards (1996) reported that changes in diatom assemblages were related to stream reach habitat conditions (% bank erosion, % canopy coverage, and bankfull width) in a Minnesota agricultural basin.

## *Effects of land-use on stream conditions and importance of temporal scale*

We expected that high and frequent precipitation in the Pacific Northwest during the wet season may increase direct linkages between catchment land-use and stream conditions, and the stream conditions may better reflect cumulative effects of catchment landuse because the relative importance of land-use and other factors such as geology on stream water chemistry may be determined by hydrological connectivity between the catchment and streams (Blanchard & Lerch, 2000). Indeed, the Willamette River basin is characterized by a distinct change in climate and associated hydrological regimes between the dry and wet seasons. About 75% of annual precipitation occurs between October and March with <5% occurring during July and August (Uhrich & Wentz, 1999) and flows in the Willamette Valley streams are tightly coupled with precipitation. Bonn et al. (1995) estimated that low flow in the Willamette River basin streams during August and September only accounts for <2% of total annual streamflow. In their study on temporal changes of nutrients in 6 Willamette Valley streams, Bonn et al. (1995) reported that NO<sub>3</sub>-N concentrations showed a relatively strong correlation with streamflow except at a site covered by 100% forest. Seasonal trends of nutrients became less pronounced for flowadjusted nutrient data. Johnson et al. (1997) reported that surficial geology, but not land-use, was strongly correlated with stream water chemistry in the summer in Michigan catchments, whereas effects of land-use on stream water chemistry became more evident when the catchments and streams were more hydrologically connected in the fall. Arheimer & Liden (2000) also reported that catchment characteristics were better predictors of winter median nutrient concentrations. Bolstad & Swank (1997) reported that changes in water quality in relation to land-use were much more evident during a storm flow than a base flow period. Importance of the spatial scales in assessing effects of land-use on stream conditions becomes more evident in the wet season in the Willamette Valley. Percent agricultural land explained more variance at larger spatial scales (e.g., the catchment and stream network with 150-m band width) and then decreased at the local scale (e.g., sample reach), indicating stream water chemistry during wet seasons is more a function of catchment-wide biogeochemical processes and land-use patterns.

#### Implication to stream bioassessment

Present periphyton diversity in streams may result from long-term cumulative effects of climate, catchment geology, and land-use (Stevenson, 1997). Harding et al. (1998) reported that catchment land-use in 1950s was a better predictor of present macroinvertebrate assemblages than present land-use patterns (1990s), suggesting the macroinvertebrate species pool has not fully recovered from previous land-use impacts despite recent changes in land-use patterns. To better relate biotic assemblages collected from a stream reach to environmental stressors such as catchment land-use in stream assessments, we need to consider both spatial and temporal scales in designing field studies and interpreting data (Allan et al., 1997; Lammert & Allan, 1999). Most stream bioassessment programs recommend sampling stream biota during the summer base flow time because of stable hydrological conditions and diverse biotic assemblages (Karr & Chu, 1999). Summer sampling may underestimate the effects of land-use on stream conditions in areas where seasonal patterns are so distinctive as in the Willamette Valley.

Systematic changes in diatom assemblages such as species replacement may reflect integrated changes of environmental conditions over time. Direct linkages between systematic changes in diatom assemblages and environmental changes may not be always evident on short-term time scales (e.g., weekly) until the two reach equilibrium. Allen et al. (1977) suggest that changes in species assemblages in a system may correspond to environmental changes at an annual scale. Catchments and stream water may be more tightly coupled during the wet seasons. However, high flow, low temperature, and high turbidity (low light) may not be favorable for periphyton growth in streams during the wet seasons. It is unclear how the wet season events (e.g., abundant nutrient supplies but with unfavorable habitat conditions) are linked to biotic assemblages and their distribution patterns during the following dry season. Our temporal data were very limited in frequency and biotic responses. An integrated study between catchment hydrology and stream ecology over time may be needed to assess if such a linkage is important and to extract more information on changes in stream environmental conditions from biotic assemblages.

#### Acknowledgements

This research was funded by the USEPA including a cooperative agreement with Oregon State University (#CR824682) and a contract with Dynamac Corporation. Periphyton analysis was funded by the Portland State University Faculty Enhancement Award to the senior author. It has been subjected to the USEPA agency's peer and administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. We thank William Gerth for leading the field sampling and his detailed and welldocumented site-specific information, Greg Coleman and Dianna Lysgaard-Rutz for field work, Mike Schuft and Dale Lindeman for air photo interpretation, and Sue Pierson for making the site map. Critical reviews by Christine Weilhoefer, Brian Hill, Chauncey Anderson, and two anonymous reviewers improved the quality of the manuscript.

#### References

- Allan, J. D., D. L. Erickson & J. Fay, 1997. Influence of catchment land-use on stream integrity across multiple spatial scales. Freshwater Biology 37: 149–161.
- Allen, T. F. H., S. M. Bartell & J. F. Koonce, 1977. Multiple stable configurations in ordination of phytoplankton community change rates. Ecology 58: 1076–1084.
- Anderson, J. R., E. E. Hardy, J. T. Roach & R. E. Witmer, 1976. A land-use and land cover classification system for use with remote sensor data. Professional Paper 964, U.S. Geological Survey, 28 p.
- Arheimer, B. & R. Liden, 2000. Nitrogen and phosphorus concentrations from agricultural catchments – influence of spatial and temporal variables. Journal of Hydrology 227: 140–159.
- Bahls, L. L., 1993. Periphyton Bioassessment Methods for Montana Streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana. (Available from: Water Quality Bureau, Department of Health and Environmental Sciences, Room A-206 Cogswell Building, 1400 Broadway, Helena, Montana 59620, U.S.A.)
- Biggs, B. J. F. & R. A. Smith, 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnology and Oceanography 47: 1175–1186.
- Blanchard, P. E. & R. N. Lerch, 2000. Watershed vulnerability to losses of agricultural chemicals: interactions of chemistry, hy-

drology, and land-use. Environmental Science and Technology 34: 3315–3322.

- Bolstad, P. V. & W. T. Swank, 1997. Cumulative impacts of landuse on water quality in a southern Appalachian watershed. Journal of the American Water Resources Association 33: 519–533.
- Bonn, B. A., S. R. Hinkle, D. A. Wentz & M. A. Uhrich, 1995. Analysis of nutrient and ancillary water quality data for surface and ground water of the Willamette Basin, Oregon, 1980–90. U.S. Geological Survey, Water-Resources Investigations Report 95–4036. 88 pp.
- Burkholder, J. M., 1996. Interactions of benthic algae with their substrata. In Stevenson, R. J., M. Bothwell & R. Lowe (eds.), Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, California: 253–297.
- Carpenter, K. D. & I. R. Waite, 2000. Relations of habitatspecific algal assemblages to land use and water chemistry in the Willamette Basin, Oregon. Environmental Monitoring and Assessment 64: 247–257.
- Cattaneo, A. & M.C. Amireault, 1992. How artificial are artificial substrata for periphyton? Journal of North American Benthological Society 11: 244–256.
- Clarke, S. E., D. White & A. L. Schaedel, 1991. Oregon, USA, ecological regions and subregions for water quality management. Environmental Management 15: 847–856.
- Frissell, C. A., W. J. Liss, C. E. Warren & M. D. Hurley, 1986. A hierarchical framework for stream habitat classification: viewing streams in a watershed context. Environmental Management 10: 199–214.
- Gonthier, J. B., 1985. Oregon ground-water resources. In U.S. Geological Survey, National Water Summary 1984 – Hydrological Events, Selected Water-Quality Trends, and Ground-water Resources. U.S. Geological Survey, Water-Supply Paper 2275: 355–360.
- Harding, J. S., E. F. Benfield, P. V. Bolstad, G. S. Helfman & E. B. D. Jones III, 1998. Stream biodiversity: the ghost of landuse past. Proceedings of National Academy Sciences U.S.A. 95: 14843–14847.
- Hill, B. H., 1998. Periphyton. In Lazorchak, J. M., D. J. Klemm & D. V. Peck (eds.), Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams. EPA/620/R-94/004F. US Environmental Protection Agency, Washington, D.C.: 119–132.
- Johnson, L. B., C. Richards, G. E. Host & J. W. Arthur, 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshwater Biology 37: 193–208.
- Karr, J.R. & E.W. Chu, 1999. Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington, D.C.
- Kaufmann, P. R. & E. G. Robison, 1998. Physical habitat characterization. In Lazorchak, J. M., D. J. Klemm & D. V. Peck (eds), Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams. EPA/620/R-94/004F. US Environmental Protection Agency, Office of Research and Development, Washington, D.C.: 77–118.
- Kaufmann, P. R., P. R. Levine, E. G. Robison, C. Seeliger & D. V. Peck, 1999. Quantifying Physical Habitat in Wadeable Streams. EPA/620/R-99/003. U.S. Environmental Protection Agency, Washington, D.C.
- Kehmeier, J. W., 2001. A Spatially Explicit Method for Determining the Effects of Watershed Scale Land Use on Stream Conditions. MS thesis, Oregon State University.

- Kelly, M. G. & B. A. Whitton, 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. Journal of Applied Phycology 7: 433–444.
- Krammer, K. & H. Lange-Bertalot, 1986. Bacillariophyceae, Teil 1. Naviculaceae. VEB Gustav Fisher Verlag, Jena.
- Krammer, K. & H. Lange-Bertalot, 1988. Bacillariophyceae, Teil 2. Epithemiaceae, Bacillariaceae, Surirellaceae. VEB Gustav Fisher Verlag, Jena.
- Krammer, K. & H. Lange-Bertalot, 1991a. Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotiaceae, Achnanthaceae. VEB Gustav Fisher Verlag, Jena.
- Krammer, K. & H. Lange-Bertalot, 1991b. Bacillariophyceae, Teil 4. Achnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gomphonema. VEB Gustav Fisher Verlag, Jena.
- Kutka, F. J. & C. Richards, 1996. Relating diatom assemblage structure to stream habitat quality. Journal of North American Benthological Society 15: 469–480.
- Lammert, M. & J. D. Allan, 1999. Assessing biotic integrity of streams: effects of scale in measuring the influence of landuse/cover and habitat structure on fish and macroinvertebrates. Environmental Management 23: 257–270.
- Lange-Bertalot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64: 285–304.
- Leland, H.V., 1995. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 52: 1108–1129.
- McFarland, W. D., 1983. A Description of Aquifer Units in Western Oregon. U.S. Geological Survey Open-File Report 82–165, 35 pp.
- Moser, T. J., D. R. Lindeman, P. J. Wigington, Jr., M. J. Schuft & J. van Sickle, 2000. Methods for multi-spatial scale characterization of riparian corridors. In Wigington Jr., P. J. & R. L. Beschta (eds), Proceedings AWRA's 2000 Summer Specialty Conference: Riparian Ecology and Management in Multi-land-use Watersheds, Portland, OR.: 511–516.
- Munn, M. D., R. W. Black & A. J. Gruber, 2002. Response of benthic algae to environmental gradients in an agriculturally dominated landscape. Journal of North American Benthological Society 21: 221–237.
- Omernik, J. M., 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118–125.
- Pan, Y., R. J. Stevenson, B. Hill, P. Kaufmann & A. Herlihy, 1999. Spatial patterns and ecological determinants of benthic algal assemblages in the Mid-Atlantic streams. Journal of Phycology 35: 460–468.

- Pan, Y., R. J. Stevenson, B. Hill & A. Herlihy, 2000. Ecoregions and benthic diatom assemblages in Mid-Atlantic Highlands streams, USA. Journal of North American Benthological Society 19: 518– 540.
- Patrick, R. & C. W. Reimer, 1966. The Diatoms of the United States. Vol. 1. Monographs of the Academy of Natural Sciences of Philadelphia, No. 13.
- Patrick, R. & C. W. Reimer, 1975. The Diatoms of the United States. Vol. 2, Part 1. Monographs of the Academy of Natural Sciences of Philadelphia. No. 13.
- Richards, C., R. J. Haro, L. B. Johnson & G. E. Host, 1997. Catchment and reach-scale properties as indicators of macroinvertebrate species traits. Freshwater Biology 37: 219–230.
- Roth, N. E., J. D. Allan & D. L. Erickson, 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology 11: 141–156.
- Schuft, M. J., T. J. Moser, P. J. Wigington, Jr., D. L. Stevens, Jr., L. S. McAlllster, S. S. Chapman & T. L. Ernst, 1999. Development of landscape metrics for characterizing riparian-stream networks. Photogrammetric Engineer & Remote Sensing 65: 1157–1167.
- Stevenson, R. J., 1997. Scale-dependent determinants and consequences of benthic algal heterogeneity. Journal of North American Benthological Society 16: 248–62.
- ter Braak, C. J. F., 1995. Ordination. In Jongman, R. H. G., C. J. F. ter Braak & O. F. R. van Tongeren (eds.), Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, U.K.: 91–173.
- ter Braak, C. J. F. & P. Smilauer, 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Microcomputer Power, Ithaca, NY, 351 pp.
- Uhrich, M. A. & D. A. Wentz, 1999. Environmental setting of the Willamette Basin, Oregon. U.S. Geological Survey, Water-Resources Investigations Report 97-4082-A, 20 pp.
- USEPA (US Environmental Protection Agency), 1987. Handbook of methods for acid deposition studies, laboratory analysis for surface water chemistry. EPA 600/4-87/026. U. S. Environmental Protection Agency, Office of Research and Development, Washington D.C.
- van Dam, H., A. Mertens & J. Sinkeldam, 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28: 117– 133.
- Welch, E. B., J. M. Jacoby & C. W. May, 1998. Stream quality. In Naiman, R. J. & R. E. Bilby (eds), River Ecology and Management. Springer-Verlag, New York: 69–94.

## Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales)

Ann St. Amand<sup>1</sup>, Juli Dyble<sup>\*</sup>, Mark Aubel<sup>+</sup>, Andrew Chapman<sup>+</sup> and Joseph Eilers<sup>#</sup> PhycoTech, Inc.

620 Broad St., Suite 100, St. Joseph, Michigan

\*NOAA, Great Lakes Environmental Research Laboratory Ann Arbor, Michigan

> <sup>+</sup>GreenWater Laboratories 205 Zeagler Dr., Suite 302, Palatka, Florida

#MaxDepth Aquatics, Inc. 1900 NE 3rd St., Suite 106-10, Bend, Oregon

#### Abstract

St. Amand, A., J. Dyble, M. Aubel, A. Chapman and J. Eilers. 2007. Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales). Lake and Reserv. Manage. 23:193-202.

Algal samples were analyzed from 3 lakes, Crane Prairie Reservoir and Odell Lake in Oregon and an Anonymous North East System, using both standard taxonomic criteria for identification and DNA sequencing techniques. Two toxin-producing Anabaena populations, one with consistent akinete structure and another with variable akinete structure, were investigated. Samples were characterized based on several genetic markers (nifH, cpcBA-IGS, ITS1), toxins (anatoxin-a, saxitoxin, and microcystin) and morphological variation. Taxonomy within the Nostacales is based on vegetative and terminal cell structure, filament type and aggregation, and position and structure of heterocysts and akinetes. Many taxonomists rely heavily on akinete structure for microscopic identification. Identification from material preserved with Lugol's solution is challenging due to the breakup of colonies, cell distortion, and masking of pigment color. Based on morphological variation, the Crane Prairie and Odell populations were identified as A. flos-aquae, A. circinalis, or A. lemmermannii, and toxin analysis detected the presence of microcystin. These populations were most similar to A. lemmermannii (cpcBA-IGS) or Anabaena sp. (ITS1) by DNA sequence analysis. The Anonymous North East System population was identified as A. flos-aquae, A. circinalis or A. spiroides based on morphological variation, and both microcystin and anatoxin-a were detected in these samples. Sequences most similar to A. cylindrica (nifH), A. planktonica (cpcBA-IGS), A. spiroides or Aphanizomenon flos-aquae (ITS1) were identified in the Anonymous North East System samples, but there were no definitive matches. Although molecular methods can be useful tools for confirming identification based on field material, their ability to resolve issues of taxonomic identification are dependent on the comprehensiveness of the sequence database. Taxonomic keys based on cell morphology and identification based on current DNA sequence databases are subject to similar levels of variation and uncertainty.

Key words: akinete, Anabaena, anatoxin-a, Aphanizomenon, cpcBA-IGS, DNA, ITS1, microcystin, nifH

Corresponding author: Phone: 269-983-3654, Fax: 269-983-3654, E-mail: astamand@phycotech.com Increasingly, toxigenic cyanobacterial blooms are a problem for lake managers. Frequent monitoring is often considered the best approach for assessing ecological impacts and human health risks (Chorus 2005), but the value of monitoring is contingent upon high quality data. Counts and identifications based on microscope visualization remain the primary and lowest cost method for assessing algal communities (Falconer 2005). However, there is considerable taxonomic uncertainty among common toxin-producing cyanobacteria, especially within the genus Anabaena (Order Nostocales). Several closely related Anabaena species have overlapping diacritical (taxonomic characteristics that define a species) features, and there is evidence that certain characteristics, such as akinete structure, that have been heavily relied upon in the past to confirm identification are not as consistent as previously thought (Komarek and Anagnostidis 1989). Differences in how species identifications are interpreted among taxonomists adds to the difficulty in confirming identifications within the Nostocales.

One particularly difficult species complex includes *Anabaena flos-aquae*, *Anabaena circinalis*, *Anabaena lemmermannii* and peripherally *Anabaena spiroides*, all of which can produce toxins (microcystin, anatoxin, saxitoxin, depending on species; Table 1). Taxonomists use as many features as possible to confirm identification, but there are often one or more missing features and there may be considerable morphological variation within a population. Molecular techniques based on DNA sequence analysis (Gugger *et al.* 2002) appear to provide an opportunity to confirm troublesome identifications, thus improving the accuracy of field data.

Crane Prairie Reservoir and Odell Lake, both in Oregon, and an Anonymous North East System, contained morphologically variable Anabaena populations belonging to the A. flos-aquae complex. Samples from these 3 locations were characterized in 2004 and 2005 based on morphological diacritical features, DNA sequence analysis, and toxin production in an effort to confirm uncertain identifications. The DNA sequence analysis was based on 3 genes with different levels of variability. The most conserved gene is nifH, a functional gene involved in nitrogen fixation. The ITS1 is the internal transcribed spacer between the 16S and 23S genes, which are involved in ribosome synthesis. Another intergenic spacer region is cpcBA-IGS, which lies between the phycobilisome beta and alpha subunits in the phycocyanin operon. Both ITS1 and cpcBA-IGS are non-coding regions and thus would be expected to accumulate mutations at a higher rate and be more variable among species.

### Methods

#### Site description and sample collection

Crane Prairie Reservoir, Deschutes County, Oregon, is 1384 ha and has a maximum depth of 7 m. Odell Lake, Klamath County, Oregon, is 1383 ha and has a maximum depth of 89

Table 1.-Summary of Anabaena diacritical taxonomic features based on John *et al.* (2002), Desikachary (1959), Geitler (1932) and Hindak (2001).

Species	Vegetative Cells	Heterocyst Structure	Akinete Structure	Akinete Location	Trichome
A. flos-aquae	Spherical or subcylindrical, 3.2-8.0 µm long	Spherical	Ovate, Cylindrical, reniform 20-50 µm long	Remote or adjacent from heterocysts, multiple akinetes, spread throughout colony	Solitary, aggregated, twisted, entangled
A. circinalis	Spherical, barrel shaped, 3.8-14 µm long	Spherical	Ovate, cylindrical, slightly curved 24- 34 µm long	Remote from heterocysts, multiple akinetes spread throughout colony	Circinate, flexuous, open coils
A. lemmermannii	Subcylindrical, spherical, 3.0-8.0 µm long	Spherical	Cylindrical, curved, reniform 20-50 µm long	Remote or adjacent from heterocysts, multiple akinetes clustered in center of colony	Solitary, aggregated, twisted, entangled
A. spiroides	Spherical, 5.0- 8.5 µm long	Spherical	Spherical, ovate 13-33 µm long	Adjacent or remote to heterocysts, 1- several throughout filament	Circinate, open coils

m. The third sample was from an Anonymous North East System without site data. All 3 systems are highly eutrophic.

Samples (n = 5) were selected after preliminary examination of live material and were based on dominance ( $\geq 90\%$  by biomass of the species of interest) by potentially toxigenic Anabaena within the A. flos-aquae species complex with morphologically confusing diacritical features. Whole water samples (0.5-1.0 L) from the Oregon Lakes were collected by MaxDepth Aquatics, Inc. (MDA) from bloom material near shore. Crane Prairie Reservoir was sampled 31 August 2004, 29 June 2005 and 19 July 2005; Odell Lake was sampled 19 July 2005. The Anonymous North East System was sampled 25 August 2004. All samples were live and shipped overnight to PhycoTech, Inc., where they were immediately screened for the presence of potentially toxigenic Anabaena. The live samples were then split, packed on ice, and shipped overnight to National Oceanic and Atmospheric Administration (NOAA) for DNA analysis and GreenWater Laboratories for toxin analysis and morphological confirmation. Toxin data were also provided for microystin and anatoxin-a using similar methods to GreenWater Laboratories for the Oregon 2005 samples by the Deschutes National Forest. Algal material retained for taxonomic analysis by PhycoTech was split into a live component and one preserved to a final concentration of 0.25% glutaraldehyde.

### Morphological analysis and algal counts

Taxonomic analysis and algal counts were conducted using an Olympus BX60, research-grade compound microscope equipped with Nomarski optics (40x, 100x, 200x, 400x, and 1000×), Phase Optics (400×, 1000×), a 1.25-2× multiplier, epifluorescence (blue, green and UV excitation), and a trinocular head for photography, with a Microfire digital camera attached. Identifications were conducted on live material. All preserved samples were permanently mounted for algal counts using a modified HPMA mounting method (St. Amand 1990) and were enumerated at 400×. Anabaena species were counted using a threshold of 400 natural units and a minimum of 10 fields (APHA 2005) spread over 3 slides, with results reported as cells/mL. Counting was completed when the threshold was reached and the standard error of the mean number of natural units per field was <10%. Cell dimensions (vegetative, heterocyst, and akinete) were measured on wet, preserved material using an ocular micrometer at 400x. Ten representative cells of each cell type were measured in each population. Taxonomic references used to confirm identifications included Geitler (1932), Desikachary (1959), Komarek and Anagnostidis (1989), Hindak (2001) and John et al. (2002).

#### Toxin analysis

To determine the total toxin content of a given water sample, algal cells must be lysed and intracellular toxin released into the extracellular aqueous environment. In general, 250-600 mL of water was sonicated (8 min/sample) via an ultrasonic homogenizer (BioLogics model 300 V/T, Gainesville, Va.) and lyophilized in fast-freeze flasks (Labconco) on a Lab-Conco FreeZone 4.5 freeze-dry system. Upon lyophilization, the residue was reconstituted and transferred from the freeze flask with 75% methanol and 0.05% glacial acetic acid into glass tubes (70 mL total volume), filtered through Whatman GFC filter paper, evaporated to dryness using a TurboVap II concentrator (Zymark Corporation, Hopkinton, Mass.) and resuspended in 2.5-6.0 mL of ultra-pure water (resulting in a 100× preconcentration). All samples were then filtered using 0.45 µm centrifuge filters (Millipore, Ultrafree MC) to eliminate micro-particulates and high molecular weight photopigments that interfere with absorbance when using a spectrophotometer (enzyme linked immunosorbent assay) and plug liquid chromatographic (LC) columns.

#### Microcystins

#### Enzyme linked immunosorbent assay (ELISA)

The ELISA assay is based on the polyclonal antibody method described by Chu et al. (1990) and adapted by An and Carmichael (1994). Antibody-coated plates, standards, and all reagents were supplied by Abraxis LLC (Product No. 520011). The level of sensitivity for microcystin(s) and nodularin using this method is approximately 0.15 µg/L. With the typical 100× preconcentration achieved via lyophilization, detection limits of 0.0015 µg/L are possible. The Abraxis ELISA kit is a competitive colorimetric assay that provides a quantitative and sensitive congener-independent detection of microcystins and nodularin. Microcystins were quantified using a Stat Fax 303+ spectrophotometer at a wavelength of 450 nm in conjunction with a reference wavelength of 630 nm. A final estimate for microcystin content was obtained and calculated as the mean of at least 2 replicates. Standard checks  $(1 \mu g/L)$  and spiked recoveries  $(1 \mu g/L)$  were utilized for QA/QC and determining appropriate correction factors if necessary.

#### Saxitoxin

#### ELISA

The RIDASCREEN<sup>®</sup> Fast saxitoxin ELISA kit is a competitive colorimetric assay for the quantitative analysis of saxitoxin. Antibody-coated plates, standards, and all reagents were supplied by R-Biopharm AG, Darmstadt, Germany (Art. No. R1902). The level of sensitivity for saxitoxin using this method is approximately 2.5  $\mu$ g/L. With the typical 100× preconcentration achieved via lyophilization, detection limits of 0.025  $\mu$ g/L are possible. Saxitoxin was quantified using a Stat Fax 303+ spectrophotometer at a wavelength of 450 nm in conjunction with a reference wavelength of 630 nm. A final estimate for saxitoxin content was obtained and calculated as the mean of at least 2 replicates. Standard checks (4  $\mu$ g/L) and spiked recoveries (4  $\mu$ g/L) were utilized for QA/QC and determining appropriate correction factors if necessary.

#### Anatoxin-a

#### Liquid chromatography-mass spectrometry (LC/MS/MS)

The analysis was performed on a ThermoFinnigan LCQ Advantage MS<sup>n</sup> ion-trap liquid chromatography-mass spectrometer (LC/MS/MS) using the combined methodology of Aversano *et al.* (2004) and Friday and Carmichael (2001). The [M+H]<sup>+</sup> ions for ANTX-A (m/z 166) and CYN (m/z 416) were fragmented, and the major product ions ANTX-A (m/z 149, 131, 107, and 91) and CYN (m/z 336, 318, 274, and 194) provided both specificity and sensitivity. Standard addition was utilized for the quantification of all samples and also provides additional confirmation.

#### DNA extraction, cloning and sequencing

Water samples for DNA analysis were filtered through 0.45  $\mu$ m Supor filters (Pall-Gelman, Ann Arbor, Mich.), placed in 2 mL microfuge tubes, immediately frozen in liquid N<sub>2</sub> and stored at -80°C. The cells on each filter were lysed by adding guanidine thiocyanate-based DNAzol (MRC, Cincinnati, Oh.) to the tube containing the filter and heating to 90°C for 3-4 hours with frequent vortexing. The filter was removed from the tube after all the filtered material had been released, and the lysate was subjected to 2 rounds of bead beating (3 min each time using 150-200  $\mu$ m glass beads). The DNA was recovered with a chloroform extraction and 70% ethanol precipitation and further purified using the DNeasy Plant kit (Qiagen, Valencia, Calif.). A negative control without added DNA was run with every extraction set to check for carryover DNA between samples.

Three different primer pairs were used to compare cyanobacterial communities, each designed to specifically amplify cyanobacterial sequences to the exclusion of other bacteria. The "cyano nif F/R" primers were used to amplify a 324 base pair fragment of the *nifH* gene (Olson *et al.* 1998), which is involved in the production of the dinitrogenase reductase enzyme necessary for nitrogen fixation. The "PC $\beta$ F/PC $\alpha$ R" primers were used to amplify a 685 base pair fragment of the intergenic spacer (IGS) region between the *cpcB* and *cpcA* genes which control production of the cyanobacterial photopigment phycocyanin (Neilan *et al.* 1995). An approximately 500 base pair region of the internal transcribed spacer (ITS) between the 16S-23S rRNA genes was amplified

ng each of the primers and Taq polymerase (Invitrogen<sup>TM</sup>, Carlsbad, Calif.) in a final volume of 50 μL. The amplification parameters were 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 52°C (PCβF/PCαR and 16CITS/ 23CTIS), or 54°C (cyano nif) for 1 min, 72°C for 1 min, followed by an extension at 72°C for 7 min. Negative controls with no DNA added to the PCR reaction mix were run with each set of PCR amplifications.
For DNA sequencing, the PCR product was extracted from the agarose gel, ligated into a plasmid vector (pCR vector 2.14 Levice - Calif.)

the agarose gel, ligated into a plasmid vector (pCR vector 2.1, Invitrogen, Carlsbad, Calif.) and transformed into *E. coli* INV $\alpha$ F' (ultracompetent cells, Invitrogen). For each sample, 3-5 clones from a single cloning reaction were sequenced. The resulting sequences were aligned and compared to other sequences in the GenBank database using the SeqLab program and checked manually. Phylogenetic trees were generated by distance methods and the neighbor-joining algorithm (Saitou and Nei 1987) with PHYLIP software (University of Wisconsin Genetic Computer Group; Felsenstein 1989).

using the 16CITS and 23CITS primers (Neilan et al. 1997).

Extracted DNA was added to the following PCR reaction:

1X reaction buffer, 2.5 mM MgCl<sub>2</sub>, dNTPs, 2% DMSO, 100

### Results

#### Morphological analysis

The Oregon systems had populations with centrally located, curved and ovate akinetes, but variable vegetative cell and trichome features, which suggested A. flos-aquae, A. lemmermannii, or A. circinalis, depending on which diacritical feature was applied (Table 2; Fig. 1). In the past, the Oregon populations have been identified from material preserved with Lugol's solution as A. flos-aquae (St. Amand, unpublished data). In contrast to the Oregon systems, the Anonymous North East System population had variable akinete structure but consistent vegetative and trichome features, which suggested either A. circinalis, A. flos-aquae, A. lemmermannii, A. planctonica, or A. spiroides (Table 2; Fig. 2). In all populations there were conflicting and/or variable diacritical features that made visual identification uncertain. Most complex was the Anonymous North East System population, which had highly variable mature akinete structures ranging from spherical to reniform shapes, often within the same trichome (Figs 2 and 3).

e

C

21

A

D

Po

mo

oth

dat

fro

#### Algal counts and toxin analysis

In all but one sample (Crane Prairie, 21 July 2005), cell concentrations exceeded the World Health Organization threshold of 20,000 cells/mL (Table 3) associated with potential risk of toxin exposure (Chorus *et al.* 2000). All strains in the samples produced at least one toxin (Table 3), with the

Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales)

System	Vegetative Cells	Heterocyst Structure	Akinete Structure	Akinete Location	Trichome
Crane Prairie 8/31/2004	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii	lemmermannii	flos-aquae lemmermannii
Crane Prairie 6/29/2005	rane Prairiecircinaliscircinaliscircinalis29/2005flos-aquaeflos-aquaeflos-aquaelemmermanniilemmermanniilemmermannii		circinalis flos-aquae lemmermannii	Uncertain	flos-aquae lemmermannii
Crane Prairie 7/19/2005	i <b>rie</b> circinalis circinalis circinalis flos-aquae flos-aquae flos-aquae lemmermannii lemmermannii lemmermannii		Uncertain	flos-aquae lemmermannii	
Odell Lake 7/19/2005	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii	Uncertain	flos-aquae lemmermannii
Anonymous North East System 8/25/2004	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii	circinalis flos-aquae lemmermannii planctonica spiroides	planctonica spiroides circinalis flos-aquae	flos-aquae lemmermannii planctonica spiroides

Table 2.-Summary of the morphological characteristics of the Anabaena populations.



Figure 1A.-Crane Prairie, 31 August 2004. Images taken at 400x, Scale bar is 10 µm.1A1-nomarski, 1A2-phase.

exception of the 21 July 2005 Crane Prairie Sample. Microcystin was measured in the Odell and Crane Prairie samples, and both microcystin and anatoxin-a were detected in the Anonymous North East System population (Table 3).

### DNA extraction, cloning and sequencing

Populations from Crane Prairie, Odell Lake and the Anonymous North East System were compared to one another and to other species within the Nostocales based on DNA sequence data for *nifH*, *cpcBA*-IGS and ITS1. The sequence analysis from all 3 genes was sufficient to differentiate the Oregon populations from the one originating in the Anonymous North East System. However, there was a greater amount of genetic distance between these populations based on *cpcBA*-IGS sequences. Based on the *cpcBA*-IGS sequence data available in GenBank, the population from Odell Lake was 98% similar to *Anabaena lemmermannii*, Crane Prairie (2004) was 97% similar to *A. lemmermannii* and Anonymous North East System was 96% similar to *Anabaena planctonica* (Fig. 4). The *cpcBA*-IGS sequence was also useful in assessing among-year variability in the 2004 and 2005 populations in Crane Prairie (90% similar), which were morphometrically different in terms of cell size and colony conformation (Table 2; Figs.



Figure 1B.-Crane Prairie, 19 July 2005. Images taken at 400×, 1B1-nomarski, 1B2- nomarski. Scale bar is 10 µm.



Figure 1C.-Odell Lake, 19 July 2005. Images taken at 400x, 1C1-nomarski, 1C2- bright field. Scale bar is 10 µm.

2 and 3). The Odell and the 2004 Crane Prairie populations were identical for *cpcBA*-IGS sequence and shared a 98% similarity for *nifH*.

Sequence analysis based on *nifH* did not provide matches with levels of similarity as great as *cpcBA*-IGS sequences. The *nifH* sequence characterized the Anonymous North East System population as 97% related to *Anabaena cylindrica*, but the Crane Prairie population from both years was only 92% similar (Fig. 5). The population in Odell Lake was also most similar to *A. cylindrica*, but only at 91% similarity. There is currently no *A. lemmermannii nifH* sequence in GenBank.

Based on ITS1 sequences, the 2004 Anonymous North East System population was 97% similar to *Anabaena spiroides* 

Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales)



Figure 2.-Anonymous North East System, 25 August 2004. Images taken at 400x, 2-1-bright field, 2-2phase. Scale bar is 10 µm.

 Table 3.-Anabaena cell densities and environmental toxin concentrations. CP - Crane Prairie Reservoir; OL - Odell Lake; ANES - Anonymous North East System. ND is non-detect, \*-not tested.

		Concentration			
System	Date	(Cells/mL)	Microcystin	Anatoxin-a	Saxitoxin
СР	8/31/2004	847,903	1.0 μg/L	ND	ND
СР	6/29/2005	210,106	0.019 µg/L	ND	*
СР	7/19/2005	9,773	ND	ND	*
OL	7/19/2005	41,096	10 µg/L	ND	*
ANES	8/25/2004	1,065,454	1.2 μg/L	0.05-1.0 μg/L	ND



#### **Cell Measurements**

**Figure 3.-**Average cell measurements of the different *Anabaena* populations. CP1 - Crane Prairie Reservoir 31 August 2004; CP2 - Crane Prairie Reservoir 29 June 2005; CP3 - Crane Prairie Reservoir 19 July 2005; OL - Odell Lake 19 July 2005; ANES - Anonymous North East System 26 August 2005.

and 96% similar to *Aphanizomenon flos-aquae*. The 2004 Crane Prairie population was 98% similar to an unidentified *Anabaena* species and 96% similar to *Anabaena spiroides*. Not all samples were sequenced for ITS1.

Considering the results from all the sequence analyses, the 2004 Crane Prairie population and 2005 Odell population are likely *A. lemmermannii*. The 2005 Crane Prairie population is most likely a second strain of *A. lemmermannii*, closely related but distinct from the 2004 material. The *cpcBA*-IGS sequence indicated that the Anonymous North East System population was more closely related to A. circinalis than to other *Anabaena* species, whereas there were few matching sequences in the *nifH* database to compare with the Anonymous North East System population. By comparison, the ITS1 sequence indicated that the Anonymous North East System population was most closely related to *A. abaena spiroides*. The Anonymous North East System population did not match as well with available sequences in GenBank







Figure 5.-nifH phylogenetic tree

Figure 4.-cpcBA-IGS phylogenetic tree.

as either the Crane Prairie or Odell populations. The genetic uncertainty agrees with the high degree of uncertainty based on microscopic examination.

### Discussion

Anabaena is a morphologically diverse genus (Komarek and Anagnostidis1989), and the species within this genus likely have multiple origins (Gugger et al. 2002). Adequately describing cell dimensions and colonial attributes to correctly diagnose species is challenging (Fjerdingstad 1969); distinguishing among closely related species becomes even more difficult when the diacritical features overlap, and the taxonomic keys provide differing descriptions (e.g., John et al. 2002 vs. Desikachary 1959). Relationships between 2 closely related genera in the Nostocales, Anabaena and Aphanizomenon, exemplify the similarities among seemingly unrelated species. Although Anabaena trichomes and akinetes tend to be consistently wider than Aphanizomenon (Rajaniemi et al. 2005), DNA sequencing suggests that the genera may actually belong to the same genus and that the current taxonomy describing Anabaena and Aphanizomenon requires revision (Gugger et al. 2002). This may help explain why the ITS1 sequence indicated a strong relationship of the Anonymous North East System population to Aph. flos-aquae.

flos-aquae despite morphometric identifications. There is discussion concerning the potential synonomy of A. flosaquae and A. lemmermannii (John et al. 2002), despite the apparent distinct nature of A. flos-aquae and A. lemmermannii sequences currently in GenBank. Correct taxonomic assignment becomes particularly troublesome when the primary akinete descriptions overlap. Although most taxonomic keys indicate that A. flos-aquae and A. lemmermannii akinetes are curved or reniform, and A. circinalis akinetes are more ovate to cylindrical, there is often a mix of both types in otherwise morphologically consistent populations. Distortions in cell structure caused by preservatives, especially Lugol's iodine, also confuse identification (Hawkins et al. 2005). For example, the primary distinguishing characteristic between A. flos-aquae and A. lemmermannii is the location and position of the akinetes within the trichome and colony. These distinctions can be obliterated by the use of Lugol's, which can cause cells within a trichome to disaggregate. Morphological features can also vary greatly among populations derived from different environments. For example, presence or absence of a colonial sheath is also used to distinguish species (John et al. 2002), yet the character of the colonial sheath can be environmentally influenced.

In the current study no environmental samples matched A.

Given the morphological variation of these common *Anabaena* species, it was hoped that the genetic sequences would help resolve questionable identifications. One of the difficulties with this approach, however, was the use of environmental samples versus cultured material, which has been historically used to determine gene sequences filed in

## Efficacy of molecular DNA methods for confirming species identifications on morphologically variable populations of toxin-producing *Anabaena* (Nostocales)

GenBank. Although each sample was >90% Anabaena sp., there was potential confusion with other species present in lower abundances. This issue most likely would have affected the Crane Prairie populations where the material was not as dense and there were competing species. Yet the greatest uncertainty was encountered with the Anonymous North East System population, which consisted of nearly 100% of the species of interest, and the material was extremely dense (>1 × 10<sup>6</sup> cells/mL). Confusion among competing species should have been minimal, yet the Anonymous North East System population was the hardest to match consistently among the different sequences.

The uncertainty in genetic identification is related to the lack of published sequences for Anabaena species in GenBank, especially among the ITS1 and *nifH* sequences. For example, the only Anabaena species in the nifH database at the time of analysis were A. variabilis, A. cylindrica, A. aphanizomenoides, A. oscillariodies, and A. azollae. The database did not contain the common species present in North America. There have been few sequences submitted for a large number of species and strains within one cyanobacterial genera, despite success using certain sequences such as the rpoC1 gene for differentiating A. circinalis in culture or environmental samples (Fergusson and Saint 2000). Successful attempts to determine limited species assemblages in environmental samples using multiple sequences have been conducted (Mes et al. 2006, Castiglioni et al. 2002, Castiglioni et al. 2004), but these efforts require considerable expertise not yet widely available to taxonomists. This approach also requires high cell densities of the taxon of interest, which are difficult to obtain from field samples. The most challenging issue with using DNA sequencing to confirm taxonomic identifications lies in the same inconsistencies that affect identifications using morphological characteristics.

Most GenBank and similar DNA database entries rely on sequences determined from cultured material. The material submitted to GenBank is not refereed (general review for correct form only), increasing the likelihood that some published sequences are associated with incorrectly identified strains (Komarek and Anagnostidis 1989). Incorrectly identified culture material creates critical problems in trying to match sequences from environmental populations with sequences in genetic databases (Rudi et al. 1997). If we assume there is also regional genetic variability among different cyanobacterial populations, the chances of matching sequences of environmental material with correctly identified cultured material is further reduced. An additional issue with use of cultured material for resolving taxonomic uncertainties is that the morphological attributes of many taxa exhibit increasing deviation from the original population as a function of culture duration. For example, Microcystis aeruginosa, which is a naturally colonial form, often grows as single cells in culture.

Of the 3 sequences we examined, *cpcBA*-IGS yielded the best separation among our populations and has the largest number of sequences from multiple strains submitted to GenBank. The Crane Prairie 2004 and Odell 2005 populations were well matched to *A. lemmermannii* sequences in GenBank. The Crane Prairie 2005 populations were distinct, but closely related to the 2004 Crane Prairie population and were likely a different strain of *A. lemmermannii*. The Anonymous North East System sample was most likely *A. circinalis*, but no sequence provided a definitive match, and the DNA sequencing was not successful in helping to define the potential taxonomic choices. Genetic analysis has been somewhat successful for identification of microcystin producing genes (Borner and Dittmann 2005) but has not yet been developed as fully for other gene sequences.

Akinete structure was variable both within and among all populations to varying degrees, yet the DNA sequences did not appreciably reduce the amount of taxonomic uncertainty in the most variable populations (Anonymous North East System 2004 and Crane Prairie 2005). This suggests using caution when relying solely on akinete structure as the definitive diacritical feature when trying to identify species of *Anabaena*. Accounts of *A. flos-aquae* in Oregon, and perhaps elsewhere in the western United States, are questionable considering none of the sequences matched an *A. flos-aquae* sequence. Although DNA and PCR techniques offer promise for identifying environmental cyanobacterial samples, the time-intensive methods and inconsistencies in the currently available cultured material limits their usefulness for this application.

## Acknowledgments

We want to acknowledge all the wonderful support we received from the state and federal resource management agencies for this work, including the Deschutes National Forest and the Oregon Department of Environmental Quality for the microcystin and anatoxin 2005 toxin data on Crane Prairie Reservoir and Odell Lake. We also wish to thank the reviewers for their valuable comments and suggestions in revising this manuscript.

### References

- [APHA] American Public Health Association. 2005. Standard methods for the examination of water and wastewater, 21st Edition. Washington, D.C.
- An, J. and W.W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme-linked immunosorbent assay for the study of microcystins and nodularin. Toxicon 32:1495-1507.

- Aversano, C.D., G.K. Eaglesham and M.A. Quilliam. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. J. Chrom. 1028:155 -164.
- Borner, T. and E. Dittmann. 2005. Molecular biology of cyanobacterial toxins. P. 25-40. *In* J. Huisman, H.C.P. Matthijs and P.M. Visser (eds). Harmful Cyanobacteria. Springer, Dordrecht, the Netherlands.
- Castiglioni, B., E. Rizzi, A. Frosini, M.A. Mugnai, S. Ventura, K. Sivonen, P. Rajaniemi, A. Rantala, A. Wilmotte, C. Boutte, C. Consollandi, R. Bordoni, A. Miezzelani, E. Busti, L. Rossi Bernardi, C. Battaglia and G. De Bellis. 2002. Application of a universal DNA microarray to cyanobacterial diversity assessment. Minerva Biotec. 14:253-257.
- Castiglioni, B., E. Rizzi, A. Frosini, K. Sivonen, P. Rajaniemi, A. Rantala, M.A. Mugnai, S. Ventura, A. Wilmotte, C. Boutte, S. Grubisic, P. Balthasart, C. Consollandi, R. Bordoni, A. Miezzelani, C. Battaglia and G. De Bellis. 2004. Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. Appl. Environ. Microbiol. 70:7161-7172.
- Chorus, I., I. Falconer, H. Salas and J. Bartram. 2000. Health risks caused by freshwater cyanobacteria in recreational waters. J. Toxicol. Environ. Health Part B Crit. Rev. 3:323-347.
- Chorus, I. 2005. Water safety plans: A better regulatory approach to prevent human exposure to harmful cyanobacteria. P. 201-227.
   *In* J. Huisman, H.C.P. Matthijs and P.M. Visser (eds). Harmful Cyanobacteria. Springer, Dordrecht, the Netherlands.
- Chu, F.S., X. Huang and R.D. Wei. 1990. Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. J. Assoc. Off. Anal. Chem. 73:451-415.
- Desikachary, T.V. 1959. Cyanophyta. P. 391-418. In M.S. Randhawa (ed.). ICAR Monographs on Algae. Indian Council of Agricultural Research. New Delhi.
- Falconer, I.R. 2005. Cyanobacterial toxins of drinking water supplies: Cylindrospermopsins and microcystins. CRC Press. New York.
- Felsenstein, J. 1989. PHYLIP: phylogeny inference package. Cladistics 5:258-266.
- Fergusson, K. and C. Saint. 2000. Molecular Phylogeny of *Anabaena circinalis* and its identification in environmental samples by PCR. Appl. Environ. Micro. 66:4145-4148.
- Fjerdingstad, E. 1969. Cell dimensions and taxonomy of Anabaena variabilis Kutz. Emend. (Cyanophycea). Aquat. Sci. 31:1015-1621.
- Friday, C.L. and W.W. Carmichael. 2001. Simultaneous analysis of cylindrospermopsin and anatoxin-a in water samples using LC/MS (abstract). 5th International Conference on Toxic Cyanobacteria. Noosa, Queensland, Australia. 16-20 July 2001.

- Geitler, L. 1932. Cyanophyceae. Rabenhorst's Kryptogamenflora von Deutschland, Osterreich und der Scheweiz 14:1-1196.
- Gugger, M., C. Lyra, P. Henriksen, A. Coute, J.F. Humbert and K. Sivonen. 2002. Phylogenetic comparison of the cyanobacterial genera Anabaena and Aphanizomenon. Int. J. Syst. Evol. Microbiol. 52:1867-1880.
- Hawkins, P.R., J. Holliday, A. Kathuria and L. Bowling. 2005. Change in cyanobacterial biovolume due to preservation by Lugol's Iodine. Harmful Algae 4:1033-1043.
- Hindak, F. 2001. Fotograficky atlas mikrospopickych sinic (Atlas of Freshwater Cyanophytes). In Slovak, with English introduction, Latin species index and Latin nomenclature. VEDA, Publishing House of the Slovak Academy of Sciences. Bratislava, Czech Republic. 347 col. Photographs, 128 pp.
- John, D.M., B.A. Whitton and A.J. Brook. 2002. The freshwater algal flora of the British Isles. Cambridge University Press, U.K.
- Mes, T., M. Doeleman, N. Lodders, U. Nübel and L.J. Stal. 2006. Selection on protein-coding genes of natural cyanobacterial populations. Environ. Microbiol. 8:1534-1543.
- Komarek, J. and K. Anagnostidis. 1989. Modern approach to the classification system of Cyanophytes 4 – Nostocales. Arch. Hydrobiol. 82:247-345.
- Neilan, B.A., D. Jacobs and A.E. Goodman. 1995. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. Appl. Environ. Microbiol. 61:3875-3883.
- Neilan, B.A., J.L. Stuart, A.E. Goodman, P.T. Cox and P.R. Hawkins. 1997. Specific amplification and restriction polymorphisms of the cyanobacterial rRNA operon spacer region. Syst. Appl. Microbiol. 20:612-621.
- Olson, J.B., T.F. Steppe, R.W. Litaker and H.W. Paerl. 1998. N<sub>2</sub>fixing microbial consortia associated with the ice cover of Lake Bonney, Antarctica. Microbial Ecology 36:231-238.
- Rajaniemi, P., P. Hrouzek, K. Kaštovská, R. Willame, A. Rantala, L. Hoffmann, J. Komárek and K. Sivonen. 2005. Phylogenetic and morphological evaluation of the genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria). Int. J. Syst. Evol. Microbiol. 55:11-26.
- Rudi, K., O. Skulberg, F. Larsen and K. Jakobsen. 1997. Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the variable regions V6, V7 and V8. Appl. Environ. Micro. 63:2593-2599.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4:406-425.
- St. Amand, A. 1990. Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. dissertation. University of Notre Dame, Ind.



Gary L. Kelterborn Project Coordinator

August 25, 2009

VIA EMAIL

Mr. Ross del Rosario U.S. EPA, Region 5 77 West Jackson Blvd. Chicago, IL 60604-3590

## RE: Administrative Order on Consent for Removal Action, Docket No. VW-05-C-810 Submittal of Baseline Ecological Investigation Report LTB CKD Release Site – August 2009

Dear Mr. del Rosario,

This letter transmits to EPA the Baseline Ecological Investigation Report for the Little Traverse Bay CKD Release Site. Due to the file size, only portions (text, figures, and tables) are being transmitted via e-mail. The entire document is being shipped to you and is posted on the Barr website at the following location:

01) Project Information Plans and Reports Baseline Eco Investigation Rpt

If you have any questions, please contact me at 517-788-2482.

Sincerely,

Stab

Gary L. Kelterborn Project Coordinator

Cc: Ralph Dollhoph, EPA Chris Liszewski, EPA Counsel Rachel Schwarz, Odawa Robert Wagner, MDEQ Scott Kendzierski, Health Agency
# Baseline Ecological Investigation for Benthic Organisms and Periphyton Community for Reference and CKD Leachate Release Areas and Mercury Bioaccumulation Assessment by Sampling and Analysis of Biomass of Dreissenid Mussels

Prepared for:

CMS Land Company Jackson, MI

Prepared by:

lsh, Inc. Raleigh, NC

EA Engineering, Science, & Technology, Inc Deerfield, IL

> PhycoTech, Inc. St. Joseph, MI

> > August 2009

### TABLE OF CONTENTS

## <u>Page</u>

LIST OF FIGURES								
LIST OF TABLES								
LIST OF ACRONYMS AND SYMBOLSiv								
1.	Introd	Introduction1						
2.	. Methods							
	2.1	c Community	2					
		2.1.1	General Field Approach	2				
		2.1.2	Benthos Sample Collection Processing	4				
		2.1.3	Periphyton Sample Processing	5				
		2.1.4	Data Analysis	7				
	2.2 Mussel Tissue							
		2.2.1	Tissue Sample Processing	9				
		2.2.2	Data Analysis	9				
3. Results and Discussion								
	3.1 Aquatic Community							
		3.1.1	Benthic Macroinvertebrate Community	10				
		3.1.2	Periphyton Community	13				
		3.1.3	Physicochemical Measurements	14				
3.2 Mussel Tissue								
4.	Summ	ary and	Conclusions	16				
	4.1	Community	16					
		4.1.1	Benthic Macroinvertebrate Community	16				
		4.1.2	Periphyton Community	17				
		4.1.3	Physicochemical Data	17				
	4.2	Mussel	Tissue	17				
5.	Recommendations							
6.	References Cited							

Figures Tables

#### **TABLE OF CONTENTS (Continued)**

- Appendix A Baseline Ecological Characterization Workplan
- Appendix B USEPA Approval of Workplan Correspondence
- Appendix C Preliminary Ecological Characterization Field Study
- Appendix D Preliminary Dreissenid Mussel Survey
- Appendix E USEPA Method 1631
- Appendix F Raw Benthic Data
- Appendix G Raw Periphyton Data
- Appendix H Raw Physicochemical Data
- Appendix I Field Data Sheets
- Appendix J Columbia Analytical Services Mussel Tissue Lab Report

#### LIST OF FIGURES

#### Number

- 1 Rock Basket Sampling Locations In The WREF And WCKD Areas.
- 2 Rock Basket Sampling Locations In The SEEP2 And SEEP1 Areas.
- 3 Rock Basket Sampling Locations In The ECKD And EREF Areas.
- 4 Periphyton Average Total Concentration Nearshore.
- 5 Periphyton Average Total Biovolume Nearshore.
- 6 Periphyton Average Total Taxa Richness Nearshore.
- 7 Periphyton Average Total Concentration Offshore.
- 8 Periphyton Average Total Biovolume Offshore.
- 9 Periphyton Average Total Taxa Richness Offshore.
- 10 Periphyton Average Total Concentration June 2006.
- 11 Periphyton Average Total Concentration October 2006.
- 12 Periphyton Average Total Biovolume June 2006.
- 13 Periphyton Average Total Biovolume October 2006.
- 14 Periphyton Average Total Taxa Richness June 2006.
- 15 Periphyton Average Total Taxa Richness October 2006.
- 16 Periphyton Offshore Replicate Total Concentrations June 2006.
- 17 Periphyton Nearshore Replicate Total Concentrations October 2006.
- 18 Periphyton Offshore Replicate Total Concentrations October 2006.
- 19 Mussel Tissue Mercury Mean and +2/-2 Standard Deviation.

#### LIST OF TABLES

#### Number

- List of Benthic Macroinvertebrate Taxa Observed in Rock Basket and Qualitative Samples.
  The Composition, Number, and Relative Abundance of Macroinvertebrates in Rock Basket Samples from Nearshore Locations, June.
  The Composition, Number, and Relative Abundance of Macroinvertebrates in
- 3 The Composition, Number, and Relative Abundance of Macroinvertebrates in Rock Basket Samples from Offshore Locations, June.
- 4 The Composition, Number, and Relative Abundance of Macroinvertebrates in Rock Basket Samples from Nearshore Locations, October.
- 5 The Composition, Number, and Relative Abundance of Macroinvertebrates in Rock Basket Samples from Offshore Locations, October.
- 6 The Composition and Assigned Abundance of Benthic Macroinvertebrates Collected Qualitative Samples, October.
- 7 Results of Statistical Comparisons of Mean Parameters Between Nearshore and Offshore Locations Using Pooled Benthic Macroinvertebrate Data.
- 8 Results of Statistical Comparisons of Mean Parameters Among Study Areas Using Pooled Benthic Macroinvertebrate Data.
- 9 List of Algae Taxa Collected From Rock Basket Samplers and Natural Substrates.
- 10 Periphyton Nearshore June 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness; Includes Standard Deviation and CV%.
- 11 Periphyton Offshore June 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness; Includes Standard Deviation and CV%.
- 12 Periphyton Nearshore October 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness; Includes Standard Deviation and CV%.
- 13 Periphyton Offshore October 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness; Includes Standard Deviation and CV%.
- Mean (And Actual Range) Water Temperature (°C), Specific Conductance (μs/cm), Dissolved Oxygen (mg/l), pH, and Turbidity (NTU) at Basket Sampler Locations Near Bay Harbor.
- 15 Little Traverse Bay Zebra/Quagga Mussels Mercury Concentrations Data from Fall 2006 Sampling.

## LIST OF ACRONYMS AND SYMBOLS

°C	-	degrees centigrade
CKD	-	cement kiln dust
cm	-	centimeter
cm <sup>2</sup>	-	square centimeter
CMC-10	-	commercial slide mounting media
EA	-	EA Engineering, Science, Technology, Inc.
ed	-	editor
EPT	-	combined number of Ephemeroptera, Plecoptera, and Trichoptera taxa
et al.	-	et alii, Latin for "and others"
ft	-	feet
>	-	greater than
gal	-	gallon
GALD	-	glutaraldehyde
GPS	-	Global Positioning System
HPMA	-	2-hydroxypropyl methacrylate
i.e.,	-	exempli gratia, Latin for "for the sake of example"
IL	-	Illinois
in	-	inch
INHS	-	Illinois Natural History Survey
KOH	-	potassium hydroxide
L	-	liter
lb(s)	-	pound(s)
Lg	-	large
LSD	-	least significant difference
m	-	meter
mg/L	-	milligram per liter
mL	-	milliliter
mm	-	millimeter
μm	-	micrometer
µS/cm	-	microsiemens per centimeter
#	-	number
No.	-	number
NTU	-	nephelometric turbidity units
OEPA	-	Ohio Environmental Protection Agency
%	-	percent

# LIST OF ACRONYMS AND SYMBOLS (CONTINUED)

PA	-	Pennsylvania
pН	-	activity of hydrogen ions
PhD	-	doctor of philosophy
рр	-	pages
PVC	-	polyvinyl chloride
QA/QC	-	quality assurance/quality control
Ret.	-	retrieve
RPM	-	revolutions per minute
SEM	-	scanning electron microscopy
Sm	-	small
STL	-	Severn-Trent Laboratories
USEPA	-	United States Environmental Protection Agency
Vol.	-	volume

This Baseline Ecological Investigation has been prepared on behalf of CMS Land Company and CMS Capital, LLC to describe aquatic community and mercury bioassessment surveys conducted at the Little Traverse Bay Cement Kiln Dust (CKD) Release Site. The LTB CKD Release Site is located along five miles of shoreline on Little Traverse Bay of Lake Michigan, approximately five miles west of the City of Petoskey, Michigan.

The site contains four cement kiln dust (CKD) piles that are releasing leachate into Little Traverse Bay. These CKD piles are identified as East CKD (ECKD), Seep 1 (SEEP1), Seep 2 (SEEP2), and West CKD (WCKD). Remedial investigations have been conducted to examine the subsurface geological, hydrological, and chemical conditions. In addition, removal actions have been implemented to intercept, collect, and treat leachate from the affected areas.

As part of the overall CKD leachate environmental characterization work in this area, a baseline ecological assessment focused on benthic macroinvertebrates and periphytic algae (periphyton) was conducted from May through October of 2006. In addition to the baseline ecological characterization, zebra and quagga mussel tissue was collected and analyzed to assess the potential uptake of mercury associated with the CKD leachate in Little Travese Bay. The objectives of the baseline ecological evaluation were:

- 1) Provide a general ecological profile of the study area,
- 2) Evaluate effects, if any, related to the CKD leachate, and
- 3) Document the progress of the site remediation.

Six study areas were selected: the four affected areas described above and two reference areas east (EREF) and west (WREF) of the leachate release areas (Figures 1 - 3). Sampling sites within the CKD affected areas were selected based on historic elevated pH readings. The reference sampling sites were selected in close proximity of the affected areas; however, historic pH readings indicate that these areas are outside of the CKD affected area. In each study area, benthic macroinvertebrates and periphytic algae (periphyton) were collected at on-shore and off-shore sampling locations. Quantitative sampling was conducted in both spring and fall while qualitative collections were only made in the fall of 2006. Likewise, mussel tissue samples were collected from the same six study areas. However, as with the qualitative ecological sampling, the tissue collections were restricted to the fall when mussel biomass and density were likely highest. All field work and analyses were conducted in accordance with work plans approved by the US Environmental Protection Agency (USEPA) (Appendix A and Appendix B).

# 2.1 Aquatic Community

The baseline ecological characterization was carried out in two phases. In January 2006, a survey of the natural substrate was conducted in the affected and reference areas to determine an appropriate approach to sampling benthic macroinvertebrates and periphyton (Appendix C). The survey included a visual inspection of all target areas and qualitative sampling the benthic macroinvertebrate community. As expected for a nearshore lacustrine environment, the study area is turbulent and lacks unidirectional flow. This factor alone renders most active sampling devices such as kick nets, sweep nets, drift nets, Surber samplers, and Hess samplers relatively ineffective at collecting accurate quantitative data. In addition, the reconnaissance survey revealed that the study area substrate largely consists of cobble, boulder, and bedrock, which limits the usefulness of dredge devices for quantitative collections. Given the dominant substrate types and lack of flow, artificial substrate samplers that approximated the native substrate were recommended as the primary sampling method.

There are a number of review papers in the literature discussing the use of artificial substrate materials for the sampling and enumeration of benthic invertebrates from habitats difficult to sample by other means (Rosenberg and Resh, 1982; Golder Associates, Ltd., undated). Although Rosenberg and Resh (1982) listed cost, colonization selectivity, and sampler loss as possible limitations, they also listed several advantages to using artificial substrates in benthic macroinvertebrate sampling.

After consideration of the various sampler designs available, basket samplers filled with manufactured material rather than native rock were proposed. The advantages of using manufactured materials as the colonizing substrate included:

- Manufactured materials provide a standardized size and shape with a surface area that is easily quantified and replicated (Rosenberg and Resh 1982).
- In this particular instance, not only are the substrate pieces standardized, they approximate the natural cobble substrate in the study area.
- Manufactured materials eliminate error and effort associated with cleaning native substrates.
- Artificial materials such as those described above are readily available from manufacturers and can be used repeatedly, if necessary.

#### 2.1.1 General Field Approach

The second phase of the ecological characterization involved the deployment and collection of samplers from affected and reference areas. The basket samplers that were used for the study were essentially modified barbeque baskets. Each basket sampler had a diameter of 7-in and was 10-in long constructed with 0.75-in<sup>2</sup>, 14 gauge, PVC coated, galvanized wire mesh. Each

sampler was filled with 12, 3-in porcelain spheres to provide a consistent amount of surface area among all of the samplers. The 12 spheres completely filled the basket, leaving little room for movement of the substrate material.

In each of the six sampling areas, the basket samplers were deployed at two depths: near-shore at shallow depth and off-shore at deeper depth. Therefore, sampling was conducted at two locations in each of the six areas for a total of 12 sampling locations. Four benthos and four periphyton samplers were deployed at each location (total of 96 basket samplers). However, only three of the four samplers of each type were fully processed at each location. Two extra basket samplers were deployed as a contingency for those samplers potentially lost to vandalism, storms, etc. Therefore, a total of 72 basket samplers were processed during each sampling event; 36 benthic macroinvertebrate samples and 36 periphyton samples.

The samplers were deployed perpendicular to shore, along each depth contour, and on the lake bottom. The off-shore baskets were set in the water no more than 3-ft deep while the near-shore samplers were deployed at a depth of approximately 18-in. Two galvanized unistrut rails were set approximately 4-ft apart. Each end of both rails was bolted to a 50-lb concrete block and spreader bars to prevent the rails from moving together, increase the overall weight of the assembly, and enhance sampler stability. Eight baskets were placed between the rails, spaced approximately 4-6-in apart. Each basket was attached to the rails on opposite ends using 1/8-in diameter stainless steel braided cable. In addition, to maintain tension on the cable and minimize basket to the opposing rail. The photograph below illustrates a sampler assembly after being deployed at the EREF nearshore location:



For each assembly, the samplers were numbered one through eight from left to right when facing shore. During each sampling event, the samplers remained in place for at least a six-week colonization period. During each colonization period, debris collecting on the basket mesh was removed as necessary so as not to block sunlight from reaching the artificial substrates.

Since no method completely prevents the loss of organisms, sampler retrieval was conducted as carefully as possible to minimize the loss of organisms. Starting at one end of the assembly, each basket was handled separately. The first basket was stabilized by one person using a pair of man-hole cover hooks while the second person disconnected the shock cord and cable clips that were attached to each end of the basket. Once the basket was no longer tethered to the assembly rails, the second person placed a long-handled, box-type kick net with a 500-µm mesh bag parallel to the sampler. The person holding the sampler steady then gently placed the sampler in the net bag and in one consistent motion, the sampler was brought to the surface, out of the water, and then to the shore where it was processed. Each basket was retrieved from the assemblies in the same manner. Depending on the condition of each sampler in a given assembly, three samplers were individually processed as replicates for periphyton and three samplers were individually processed as replicates for benthic macroinvertebrates. If all eight samplers were in good condition, the two extra samplers were cleaned for the next sampling event but not preserved.

In addition to the benthic macroinvertebrate and periphyton sampling, physicochemical parameters were measured using a daily calibrated YSI 556 at each of the 12 sampling locations. The measured parameters included temperature (°C), specific conductance ( $\mu$ S/cm), dissolved oxygen (mg/L), pH, and turbidity (NTU). All parameters were measured at each sampling location during the set and retrieval events as well as, if possible, weekly during each colonization period.

Field studies for the baseline ecological characterization were completed in 2006. The quantitative (basket sampler) collections were conducted in June and October. However, the qualitative collections were only conducted in the fall.

## 2.1.2 Benthos Sample Collection Processing

Field sample processing of the retrieved basket samplers was conducted differently for the benthic macroinvertebrate and periphyton samplers. The benthic macroinvertebrate samplers were brought to shore and the spheres from each basket were transferred to individual 5-gallon buckets half-full of ambient water. Using a coarse cleaning pad and forceps, each of the spheres from each sampler were individually cleaned, rinsed, and placed back in the sampler. After all the spheres from each sampler were processed, the cleaning utensils were rinsed into the sample and inspected. The sample liquid and debris were then passed through a 500-µm sieve, transferred to a 1-L jar, and preserved with 10% formalin. Internal and external labels were placed in and on each jar identifying the project, location, sample replicate, date, and time. All samples remained in the custody of EA Engineering, Science, & Technology, Inc. (EA) and were directly transported to EA's laboratory in Deerfield, Illinois.

In October, along with the basket sample collections, a qualitative sample was collected from each of the six sampling areas. The qualitative collections consisted of sweep netting and handpicking natural substrates throughout each area until all habitat types were effectively sampled and no new taxa were observed. Qualitative sample handling and preservation was the same as the basket samplers.

In the laboratory, benthos sample handling and processing followed generally accepted methods described in Klemm et al. (1990). Upon arrival at the laboratory, the samples were logged and assigned an alpha-numeric tracking number. Prior to sorting, each sample was rinsed with water through a 500-µm mesh sieve to remove the preservative. The sample was then placed in a grided, white, photo-developing pan for sorting the organisms from the debris. All samples were examined for large and obviously rare taxa prior to sorting. For samples containing fewer than 500 organisms, the entire sample was "picked" and all benthic macroinvertebrates were removed. If the number of organisms in a given sample was excessive (>500 individuals), sub-sampling was conducted to achieve a more manageable number for analysis. Sub-sampling was conducted by randomly choosing grids and removing the entire contents of the selected grids until a minimum sample of 500 organisms was achieved (Klemm et al. 1990). All sample material that was not analyzed as part of the subsample was preserved using 70% ethyl alcohol and archived. Prior to identification, chironomids were cleared in 10% warm KOH and whole mounted on glass slides using CMC-10. The slides were allowed to set for a minimum of four to five days prior to handling.

Identifications were made to the lowest practical taxon (usually genus or species) using a 10-70X Bausch & Lomb Stereo-Zoom dissecting scope and 800-2000X Zeiss compound microscope. All identifications were made using established literature resources. Identifications and counts for each taxon were recorded on a standard laboratory data sheet. The total number of organisms for each taxon and for the sample as a whole was reported as number per sample.

### 2.1.3 Periphyton Sample Processing

Like the benthic macroinvertebrate samples, upon reaching shore, the periphyton samplers were opened and the spheres from each sampler were transferred into individual shallow wash basins filled with a minimum amount of water (i.e., < 1-L). A soft bristle brush and sponge were used to gently clean each of the 12 spheres in a sample before they were returned to the sampler basket. After all the spheres were processed, the cleaning utensils were rinsed into the sample and inspected. For each sample, approximately 10% was drawn off separately. This portion of the sample was preserved with 10% formalin for potential future microinvertebrate analysis. The remaining sample liquid and debris was then transferred to one or more amber 1-L jars and preserved with a 0.25% glutaraldehyde solution. Using glutaraldehyde instead of Lugol's solution as a preservative allowed for the use of fluorescence during microscopic examination of the sample. In turn, fluorescence allowed for faster and more accurate enumeration. An external label was placed on each jar identifying the project, location, replicate, date, and time. While in the field, the samples were stored in coolers to maintain dark and cool conditions until they were processed in the laboratory. If necessary, ice was used to keep the samples cool during collections. All samples remained in EA's custody during the field collections and were transported directly to Phycotech's laboratory in St. Joseph, Michigan.

As with the benthos collections, periphyton was sampled from the natural substrates in each of the six sampling areas during October. However, unlike the benthos, periphyton scrapes were collected in known quantities and thus were quantitative. The natural substrate periphyton collections consisted of collecting 10, 4-in<sup>2</sup> scrapes from the native substrate throughout each area. Natural substrate sample handling and preservation was the same as the basket samplers.

Upon arrival at the laboratory, each sample received was assigned an individual tracking number. The sample bottle, chain-of-custody, and sample log sheet, which accompany each sample sent, were then used in conjunction with one another, to track the individual samples. Rock basket and natural substrate samples were processed in an identical manner. The samples were permanently mounted in a multi-step process using both 2-hydroxypropyl methacrylate (HPMA) mounts for the whole sample analysis and Naphrax, acid cleaned mounts, for diatom analysis. The HPMA method for producing algal sample slides provides an optically clear background while permanently infiltrating and preserving the sample for archival purposes. Mounting distortion is minimal and the method provides the advantage of being able to go 100x to 1000x on the same specimen. Wet sample is always maintained in case clarification of identification is necessary. It offers minimal distortion and allows the use of epifluorescence on algal samples while counting, which can dramatically improve the final results. Periphyton samples in the Bay Harbor study were preserved in 0.25% glutaraldehyde final concentration, which is the preservative of choice for this method.

#### 2.1.3.1 Slide Preparation

The general procedure for slide preparation was based on Crumpton (1987) and St. Amand (1990). The actual details of slide preparation are proprietary; however, the general sample preparation procedures are as follows:

- 1. The sample was sonicated for two minutes and then shaken well (200 times). Millipore 6-place stainless steel manifold and 15 mL Millipore Filtration Towers were used to mount samples. Because these samples were dominated during certain times of year by macroalgae, we stratified the mounting and counting approach to include a separate macroalgae mount to better estimate macroalgae densities. On average, microalgae samples were mounted at 0.05 mL and macroalgae samples were mounted at 1.0 mL, although test mounts for each sample were completed prior to final mounting.
- 2. The membrane filters were put onto filtration bases and wet with distilled water. Excess water was drained through filter. Filter towers were assembled.
- 3. The periphyton sample was measured using a micropipetor or macropipetor. For microalgae, the periphyton sample was removed with micropipetor (usually from 0.05-0.5 mL), diluted to 10 mL in a graduated cylinder with distilled water, and agitated to mix. A Sample volume was chosen so that each field at 1000x contains approximately 20-30 cells.
- 4. The sample was added to the tower and the valve was opened. The graduated cylinder was rinsed into the tower. The sample was filtered until water just cleared the filter surface. The valve was closed and the filtration tower was removed just after the water disappeared from the inner edge of the tower.

- 5. The filter was placed face down, on a cover slip (# 1.5), being careful to avoid bubbles under the filter.
- 6. One to two drops of <u>clear resin</u> was added to the back of the filter, and the cover slip was rotated until the resin covered the back of the filter.
- 7. The cover slips were placed on the drying rack in a drying oven for 12 to 24 hours.
- 8. The cover slips were then removed from oven. One drop of resin was added to the filter side of the cover slip and attached to a labeled slide. As little resin as possible was added to cover the filter surface.
- 9. The slides were put back in the oven and allowed to polymerize for approximately 24 hours.
- 10. The slides were labeled with computer generated labels. All slides were labeled with a Tracking ID. The slide labels appear on the left side of the slide, and an additional tracking code label was embedded under the cover slip with the filter.

#### 2.1.3.2 Counting Procedure

The primary microscope was an Olympus BX51, research-grade compound microscope equipped with Brightfield optics( 40x, 100x, 200x, 400x, 1000x), Nomarski optics (100x, 200x, 400x, and 1000x), Phase Optics (200x, 400x, 1000x), a 1.25-2X multiplier, epifluorescence (blue, green and UV Excitation), and a trinocular head for photography, with a SpotFlex digital camera attached

Samples were enumerated within ASA directly, a proprietary database. All calculations were completed within ASA, including concentrations, biovolumes, biomasses and diversity indices with output generated by ASA and saved in Excel format.

Periphyton samples were dominated by diatoms, therefore a minimum of 15 fields was counted at 1000x, and a minimum of 400 natural units total (when possible, maximum of 100 fields). In addition, macroalgae were counted according to size distribution for a minimum of 45 fields at either 100x and up to 30 filed at 400x. The number of fields counted was spread evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields per slide). Counting was completed when the standard error of the mean of the total number of natural units per field was less than 10%. For species identifications of diatoms, acid cleaned mounts in Naphrax were also prepared, but counts were made off of the HPMA slides.

## 2.1.4 Data Analysis

The benthic macroinvertebrate basket sampler data was summarized as number (per sample) and relative abundance of each taxon, total density (number per sample), total taxa richness, and Ephemeroptera, Plecoptera, Trichoptera (EPT) richness for each replicate and location. Like the

benthic data, the periphyton basket sampler results were summarized as density and relative abundance of each taxon, total density, and total richness.

Individual taxa results from the qualitative benthos collections were summarized as assigned abundance; 1 for taxa represented by  $\leq$  3 individuals, 3 for taxa represented by 3-9 individuals, and 10 for taxa represented by  $\geq$  10 individuals in a given sample. In addition, total taxa richness and EPT richness were summarized for each area.

The periphyton data were summarized as total concentration (NU/sq<sup>2</sup>), total biovolume ( $um^{3}/sq^{2}$ ) and taxa richness data for the periphyton community characterization both in June and in October 2006 samples.

These data were summarized to characterize the general composition of the benthic and periphyton communities at the Little Traverse Bay CKD leachate release sites and the nearby reference areas.

Statistically, for each sampling location, the benthic basket sampler data were used to compute the arithmetic mean values for various response variables measured for each basket. Since the data are nonparametric, a two-factor (study area and location) Fisher's Least Significant Difference (LSD) test was performed on rankits as per the Conover-Inman procedure (Conover 1998) to compare means among the sample areas (e.g., East Reference vs. Seep 1) and between sample locations (i.e., near-shore versus off-shore). All treatment comparisons were conducted with a Type I error rate of 5% (i.e.,  $\alpha = 0.05$ ).

The periphyton data were analyzed using Primer 6 to determine if there were any assemblage differences among stations as determined by multi-dimensional scaling (MDS).

# 2.2 Mussel Tissue

In September 2006, CMS Land submitted a work plan to the USEPA for conducting a baseline ecological monitoring program for the potential effects of mercury discharge from the CKD leachate into the Little Traverse Bay. After several discussions and clarifications, USEPA approved the work plan in October (Appendix B) for collecting and analyzing dreissenid mussels from the Little Traverse Bay area for determining mercury concentrations in the mussel tissues.

Dreissenid mussels (Zebra and Quagga) are invasive aquatic species that easily attach to natural and artificial hard substrates in freshwater. They are relatively fast growing to moderate sizes, remain stationary in the water body where exposure and potential bioaccumulation of mercury (Hg) can occur, and are typically widespread and abundant in the Great Lakes. These characteristics make dreissends a suitable test organism for Hg uptake potential.

As with the baseline ecological characterization, the mussel tissue collections were conducted in two phases. The initial phase was conducted prior to submittal of the work plan to the USEPA in September 2006. Due to concerns raised by the USEPA regarding the presence and abundance of zebra mussels in the Little Traverse Bay to assess bioaccumulation of mercury, CMS asked the Ish Inc. team to conduct a field survey of the six areas sampled as part of the baseline

ecological characterization to determine if a sufficient quantity of zebra mussel biomass was available to perform the mercury bioaccumulation study. Marty Sneen of EA Engineering, Dan Staub of Pescador, and Ish Murarka of Ish Inc. carried out a qualitative assessment of the presence/absence of mussels in the six areas of Little Traverse Bay on 9 and 10 August 2006. Observations from this survey resulted in the conclusion that replicate samples of more than 10-g of zebra mussels biomass will be feasible from at least five of the six study areas (Appendix D).

The second phase of this study involved the collection of dreissenid mussels in conjunction with the fall benthic macroinvertebrate and periphyton sampling event to assess the occurrence and extent of mercury bioaccumulation associated with the CKD leachate.

## 2.2.1 Tissue Sample Processing

Sample processing and analysis generally followed *USEPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1, Fish Sampling and Analysis, Third Edition* (USEPA 2000). Dreissenid mussel samples were collected from the same six areas as the periphyton and benthic macroinvertibrate samples. Two replicates were collected from each sampling area. Each sampling area was divided in half (east to west) and one replicate was collected in each section of each sampling area. At ECKD, along with the replicate samples, mussels that had been originally collected from EREF and transplanted in the ECKD area during early August were collected. Finally, a field duplicate sample was collected from the WREF area. The mussels were collected whole and unshucked from the natural substrate with a preference for more mature (i.e., larger and older) specimens. Approximately 20-g of tissue was targeted for each replicate based on the relationship between whole-body and soft tissue mass and the desire to collect a minimum of 10-g of soft tissue. For each sample, the specimens were transferred to labeled bags, sealed, and placed in a cooler on ice.

Fourteen mussel tissue samples were shipped to Columbia Analytical Services in Kelso, WA for analysis of mercury in whole-body samples using USEPA method 1631 (Appendix E). The samples arrived at the laboratory on 1 November 2006 in good condition and consistent with the enclosed chain of custody form. The samples were stored by the laboratory at -20 °C immediately after they were received. Each sample was individually homogenized using a blender in the laboratory. After homogenization of the whole-body tissue, a representative aliquot was removed from the sample for freeze drying. Additional milling of the freeze dried material was necessary to homogenize the meal prior to analysis and the mercury analysis was performed on freeze dried material from each sample.

## 2.2.2 Data Analysis

The samples were analyzed for total mercury concentrations and were reported on a dry weight basis. The resulting mercury concentration data from each area was compared to the reference areas using a single-tailed t-test at the 95% confidence level.

## 3.1 Aquatic Community

The basket samplers were deployed and retrieved during two separate sampling events in 2006. For the spring/summer event, the samplers were deployed on 16-17 May and retrieved on 26-29 June. For the summer/fall sampling event, the samplers were deployed on 6-7 September and retrieved on 24-26 October. In addition to retrieving the basket samplers in October, the benthic macroinvertebrate and periphyton communities were sampled qualitatively in each of the six areas concurrent with the basket retrieval.

## 3.1.1 Benthic Macroinvertebrate Community

The combined quantitative and qualitative sample collections yielded 69 total benthic macroinvertebrate taxa (Table 1). Total taxa richness was similar between the two sample types. The rock basket samples yielded 50 total taxa while 54 taxa were observed in the qualitative samples. There were 36 taxa common to both types. Regardless of sample type, the most taxa rich major group was clearly Chironomidae with 33 taxa followed by Ephemeroptera with 11 taxa and Trichoptera with seven taxa. Ephemeroptera and Trichoptera, together with Plecoptera are collectively known as EPT taxa. EPT taxa are generally considered more environmentally intolerant than most other benthic macroinvertebrate groups. Overall, 19 EPT taxa were observed in the Little Traverse Bay collections with 16 EPT taxa each being observed in both the quantitative and qualitative samples. Raw data by area, location, and sampling event are presented in Appendix F.

#### 3.1.1.1 June Results

In June, based on the combined replicates by location, total richness ranged from 25 taxa at SEEP2 nearshore (Table 2) to 10 taxa at ECKD offshore (Table 3). Total abundance was also lowest at ECKD offshore in June but was highest at SEEP1 offshore. EPT richness was similarly low at all locations ranging from zero at EREF offshore to five taxa at the nearshore location of SEEP1 and WCKD.

Among the nearshore samples, total abundance, total richness, and EPT richness were similarly higher at the adjacent areas SEEP1 and SEEP2 (Table 2). In contrast, total abundance and richness were lowest at ECKD while EPT richness was lowest at EREF. Two chironomid taxa, *Cricotopus bicinctus* grp. and *C. tremulus* grp. represented the first or second most abundant taxon in each of the six nearshore sampling areas. Both taxa are common to North American lakes and streams, including Lake Michigan (Winnell and White 1985). Barton (1986) found that multiple *Cricotopus* taxa were most abundant in the rocky shallows of Lake Ontario similar to the Little Traverse Bay study area. In terms of environmental tolerance, *C. tremulus* grp. is considered facultative while *C. bicinctus* is generally viewed as extremely tolerant to a variety of impact forms (Simpson and Bode 1980). The more pollution intolerant taxa such as EPT and Tanytarsini chironomids (OEPA 1987) were observed in relatively low numbers among the nearshore areas. The only exceptions to this were the chironomids *Paratanytarsus* and

*Rheotanytarsus*, which were collected in all nearshore samples and were particularly more abundant at EREF. Additionally, the mayfly *Caenis* and caddisfly *Hydroptila* were observed in samples from the four western areas and in abundances greater than one percent of the total at some locations.

The offshore results were similar in many respects to nearshore. For example, offshore total abundance and total richness followed a pattern identical to nearshore; these parameters were highest at SEEP1 and SEEP2, respectively, and lowest at ECKD. In addition, as was observed in the nearshore samples, EPT richness was lowest at EREF (Table 3). Like nearshore, *C. bicinctus* was fairly common offshore and was the most abundant taxon at four of the six offshore sampling areas while the facultative aquatic worm Naidinae was the most abundant taxon at SEEP1 and SEEP2. *Rheotanytarsus* was roughly as abundant offshore as nearshore and was represented by greater than one percent of the total abundance at all offshore sampling areas. *Hydroptila* was the only EPT taxon offshore that achieved greater than one percent relative abundance and that occurred at WCKD.

#### 3.1.1.2 October Results

Overall, total abundance and total richness were lower in October compared to June while EPT richness in October was equal to or greater than in June. Although these seasonal differences were apparent over the entire study area, they were not always consistent by individual areas or locations. In October, based on the combined replicates by location, total richness ranged from 22 taxa at EREF nearshore (Table 4) to three taxa at SEEP2 offshore (Table 5). Total abundance was also lowest at SEEP2 offshore but was highest at EREF nearshore. EPT richness ranged from three to five taxa at most locations but was as high as eight taxa at WCKD offshore and as low as one taxon at SEEP2 offshore.

Among the nearshore samples, total abundance, and to a lesser extent total richness, were more or less higher in the three eastern areas compared to the three western areas (Table 4). Contrary to June when abundance and richness were highest at SEEP1 and SEEP2 and lowest at ECKD, in October, abundance, total richness, and EPT richness were all highest at EREF and second highest at ECKD (Table 4) while SEEP2 was among the lowest for all three parameters in October. Two of the most abundant taxa in June were again the most abundant taxa in October. In the EREF and ECKD areas, Naidinae was the most abundant organism while in the remaining four areas, *C. tremulus* grp. was the most abundant taxon. The more pollution intolerant EPT taxa represented a greater percentage of the total abundance during October compared to June, particularly at ECKD, WCKD, and WREF. In contrast, the abundance of Tanytarsini specifically and Chironomidae in general were noticeably lower in October compared to June.

As in June, the October offshore results were similar in many respects to nearshore. Again, total abundance, total richness, and EPT richness were highest at ECKD and EREF, respectively, and lowest at SEEP2 (Table 5). Like nearshore, Naidinae was the most abundant taxon at EREF and *C. tremulus* grp. was the dominant taxon among three of the four western areas (Table 5). Extremely low abundance and richness at SEEP2 limited the meaningful interpretation of the data from that location. As with the nearshore results, EPT abundance increased and Chironomidae abundance decreased in October compared to June. These patterns were consistent with those observed nearshore in October. The increase of EPT at ECKD both

nearshore and offshore was largely due to higher numbers of filter feeding Hydropsychidae caddisflies.

As described in the methods, qualitative samples were only collected during the October sampling event (see Section 2.1.2). Qualitative total and EPT richness values were generally much higher when compared to equivalent nearshore and offshore rock basket values. Nonetheless, taxa richness trends were similar among the two sample types. As with the October nearshore basket collections, total richness was highest at EREF and lowest at SEEP2 among the qualitative samples (Table 6). The total taxa richness values observed in the remaining four areas were relatively similar. EPT richness was also somewhat similar among the areas ranging from 10 taxa at ECKD, SEEP1, and SEEP2 to 15 taxa at WCKD, which also had the highest EPT richness among the October offshore basket collections.

## 3.1.1.3 Statistical Comparisons

The benthic macroinvertebrate data from both rock basket sampling events were pooled to develop more powerful comparisons. Statistical comparisons were made between locations (nearshore vs. offshore) and among areas (reference vs. CKD leachate release areas). These comparisons included total abundance, total richness, and EPT richness. These parameters were selected primarily due to their common use in benthic community studies and their utility in characterizing community production, diversity, and environmental tolerance. In addition, the total abundance of Ephemeroptera, Trichoptera, Chironomidae, and Oligochaeta were compared. As described above, Ephemeroptera and Trichoptera are generally considered relatively intolerant of environmental disturbance. In contrast, Chironomidae and Oligochaeta are generally considered more tolerant of environmental disturbance.

During both sampling events, similarities were common and differences were few between the nearshore and offshore locations in terms of composition and trends related to abundance and richness parameters. As such, it is not surprising that no significant differences were observed between the nearshore and offshore results for any of the parameters compared (Table 7).

Comparisons among the study areas revealed a few similarities. For example, mean total and mean EPT richness values were statistically similar among all six areas (Table 8). Likewise, mean Chironomidae abundance, with the similar dominant taxa and trends between sampling events, showed no significant difference among the six areas. Nonetheless, some differences were apparent.

In terms of total abundance, the samplers at EREF produced significantly higher mean numbers of organisms than SEEP2, WCKD, and WREF (Table 8). The higher abundance at EREF was primarily due to elevated density of Naidinae worms, particularly on the October nearshore samplers. Overall, mean Oligochaeta (i.e., Naidinae) abundance was significantly higher at both EREF and SEEP1 compared to WCKD and WREF. Although the significantly higher abundance at EREF was primarily due to densities observed in October, the highest Naidinae densities at SEEP1 occurred in June. As a group, worms are generally considered environmentally tolerant. However, Naidinae abundance is not necessarily related to environmental tolerance and may be a function of habitat. Naidinae prefer coarse, well

oxygenated, substrate with abundant periphyton (Learner et al. 1978), which describes the Bay Harbor study area, particularly EREF.

Despite the lack of difference in terms of EPT richness, both Ephemeroptera and Trichoptera abundance varied significantly among the areas. Ephemeroptera abundance was significantly lower at ECKD compared to WCKD, though both areas were statistically similar to the remaining four areas (Table 8). The higher abundance of Ephemeroptera at WCKD was largely due to higher numbers of *Caenis* in October when mayflies accounted for between 12 and 28 percent of the total abundance in that area. In contrast, Ephemeroptera accounted for slightly more than one percent of the total abundance at ECKD in June and slightly less than one percent in October.

Contrary to the low abundance of Ephemeroptera, Trichoptera abundance was significantly higher at ECKD compared to EREF, SEEP2, and WREF (Table 8). As described above, Hydropsychidae caddisflies were noticeably more abundant in October, particularly at ECKD. These are filter feeders that construct nets on coarse substrate in areas of flow or turbulence to capture small particles that make up their diet. Although this type of habitat exists throughout the study area, the reason for the elevated abundance at ECKD is not entirely clear.

## 3.1.2 Periphyton Community

Total concentration (NU/sq<sup>2</sup>), total biovolume ( $um^3/ sq^2$ ) and taxa richness data were collected for the periphyton community characterization both in June and in October 2006 samples. The resulting raw periphyton data for both sampling events are provided in Appendix G.

Several taxa common to the Great Lakes were observed during the study (Table 9). There was relatively high variation among replicates (Figures 4-15) which is not uncommon among periphyton samples due to normal differences in surface colonization and in this system, disturbance physically and potentially chemically. The samples analyzed yielded a total of 45 taxa for periphyton community, with relatively low variability. In general, the natural substrate samples had a higher biomass of green algae. This was likely due to the more complex substrate for attachment compared to the rock basket samples. Taxa richness between the two sample types in October was very similar and diatom taxa were nearly identical. Overall, based on concentration and taxa richness, the natural substrate samples were comparable to the rock baskets in October. This suggests that the rock basket samples were representative of the natural conditions.

Overall, mean total concentrations were higher in June compared to October (Tables 10-13). Although these seasonal differences were apparent over the entire study area, they were not always consistent by individual areas or locations. Multi-dimensional scaling (MDS) was used to determine if there were any assemblage differences among stations. MDS analysis did not indicate a clear difference among stations within sampling season. However, MDS did indicate 2 important trends: 1) There were distinct seasonal differences between June and October, not only in density but also assemblage structure, and 2) there was an environmental gradient from EREF towards WREF.

The gradient from EREF towards WREF was more pronounced in the offshore samples than in the nearshore samples, especially in the October sampling event with a decreasing trend in density from EREF towards WREF (Figures 16-18). In the June sampling event, this was most obvious in offshore samples (Figure 16), but in October, both nearshore and offshore showed distinct decreasing densities going toward the WREF station (Figures 17 and 18). MDS did not show as strong a pattern in biovolume, where only October samples indicated the same decreasing trend (Table 13). Taxa richness showed no significant patterns in the near or offshore samples or among seasons, except for a slight trend in the October samples (Tables 12 and 13). These patterns may reflect less of an impact of leachate in offshore vs. nearshore stations or may reflect a lack of disturbance and more established communities at the offshore sites where wave action is less of an issue. The gradient from EREF towards WREF is simply an environmental gradient which must be superimposed on any ecological analysis of the system and possibly is related more to location within Little Traverse Bay than to leachate concentrations.

Taxa differences among sites, stations and seasons appeared mostly as seasonal effects. In the June samples, diatoms and green algae dominated, with several small blue-green taxa. There were a host of general diatom taxa (Table 9, several *Cymbella* spp, *Navicula* spp, *Nitzschia* spp., Gomphonema spp. Synedra spp, and *Fragilaria* spp.) as well as several large green algae (*Cladophora fracta, Ulothrix zonata* and *U. aequalis, Oedogonium* sp. and *Spirogyra* sp.) and multiple species of micro-green algae (*Pediastrum* spp., *Chlorococcum* spp., *Scenedesmus* spp., and *Oocystis* spp.). There was a seasonal trend for density of mostly diatoms and green algae, with a significant component of small blue-green algae, but biovolume was almost exclusively equal biovolume of diatoms and green algae. The blue-green species present were small unicellular, colonial and filamentous taxa. In October, diatoms and blue-green algae co-dominated the assemblages (mostly small unicellular blue-greens), but diatoms dominated the biovolume. Greens represented only a small percentage of the density and almost no biovolume. Later in the season, the larger filamentous green algae (*Cladophora fracta, Ulothrix zonata* and *U. aequalis, Oedogonium* sp. and *Spirogyra* sp. and *Spirogyra* sp. drop out of the assemblage.

#### 3.1.3 Physicochemical Measurements

During the 2006 ecological surveys, over 850 water quality measurements were recorded between May and October (Appendix H and Appendix I). Heavy wave action or meter malfunction occasionally prevented the measurement of some or all parameters. Water temperature measurements during the May-June colonization period ranged from 7.88 °C to 20.76 °C while those from the September-October period were slightly warmer ranging from 8.98 °C to 24.67 °C (Table 14). The observed temperatures varied greatly but largely followed natural diel and seasonal trends. During May-June, mean temperatures were generally warmer nearshore compared to offshore with the reverse being true during September-October.

Specific conductance exhibited a similar range during both colonization periods from 248  $\mu$ S/cm to 356  $\mu$ S/cm and mean values were fairly consistent among areas and locations as well as over time (Table 14). The observed measurements are comparable with values observed throughout Lake Michigan (Vogel et al. 1976; Bartone and Schelske 1982).

Like specific conductance, the range of dissolved oxygen (DO) was fairly consistent between the two colonization periods ranging from 8.20 mg/L to 15.55 mg/L (Table 14). However, mean values were slightly higher during May-June compared to September-October. Nonetheless, these values are similar to other Lake Michigan observations and do not appear to be a limiting factor (Vogel et al. 1976).

Given the nature of CKD leachate, pH can be a fairly reliable indicator of its presence. Measurements taken prior to remediation efforts consistently produced pH values >9.0 and as high as 12.82 in some areas affected by the CKD leachate (Barr Engineering 2009). During the surveys, no pH measurements were observed above 9.0 in any of the study areas. Measured pH ranged from 7.25 during May-June to 8.91 during September-October (Table 14). Again the range was similar between colonization periods though slightly higher during the fall. However, all measurements were below and, in some cases, well below historic high values and similar to other observations on Lake Michigan (Bartone and Schelske 1982).

Turbidity values varied widely during the study. May-June turbidity readings ranged from 0.20 NTU to 6.46 NTU while those from September-October ranged from 0.19 NTU to 3.17 NTU (Table 14). In general, turbidity was slightly higher during May-June compared to September-October and, as would be expected, with rare exception, turbidity was higher nearshore compared to offshore. These differences as well as the overall variability are likely a function of algal bloom density and wave action severity.

# 3.2 Mussel Tissue

Except for ECKD, dreissenid mussels were fairly common throughout the study area. The target wet biomass of 20 g was easily surpassed for 12 of the 14 tissue samples collected (Table 15). The mussels ranged in size from approximately 4 mm to 27 mm with most in the 8-12 mm range. The mussels collected from the ECKD locations were not only sparse in abundance but also of small size (4-5 mm). In EREF and ECKD, there was a mixture of zebra and quagga mussels while in the remaining areas quagga mussels were clearly the dominant species.

The complete laboratory report from Columbia Analytical Services is presented in Appendix J. Dry weight of the tissue samples was approximately 50% of the field measured wet weight (Table 15). Dry weight Hg concentration ranged from 3.3 ng/g in the ECKD transplant sample to 7.7 ng/g in the ECKD Replicate A sample (Table 15). Overall, the mean Hg concentrations were similar among most of the sampling areas and well below the mean concentration of 78 ng/g reported for Lake Michigan surficial sediments (Rossmann 2002). Between the reference areas (EREF and WREF) mean concentrations were very similar and the standard deviation of replicate values was identical. Although the mean Hg concentrations from WCKD were slightly lower than the reference areas and mean concentrations from WCKD were slightly higher than the reference areas, these Hg concentrations were not significantly different from the reference areas at the 95% confidence interval (Figure 19). In contrast, the samples collected from the ECKD area showed a mean Hg concentration of 6.4 ng/g, which was significantly higher than the mean concentrations observed in the reference areas at the 95% confidence level.

## 4.1 Aquatic Community

Leachate collection systems have been installed and operated in the SEEP1 and SEEP2 areas since November 2005; at least six months prior to when the 2006 biological sampling activities were initiated. This assuredly resulted in a significant decrease in CKD leachate being released into Little Traverse Bay. Therefore, the result of these remedial actions may be that the 2006 ecological surveys were less a description of baseline conditions and more a characterization of the conditions in transition. If so, the results of the 2006 sampling effort suggest that the benthos and periphyton communities have made substantial initial progress toward natural conditions, as represented by results from the reference areas EREF and WREF.

#### 4.1.1 Benthic Macroinvertebrate Community

Based on the 2006 collections, the benthic macroinvertebrate community in the vicinity of Bay Harbor contains several components and taxa common to Lake Michigan and the other Great Lakes, though it is far from being described as an especially diverse or, in some ways, productive benthic assemblage. This is likely due to natural factors such as low nutrients, lack of diversity in substrate, and maximum exposure to the prevailing winds and waves that collectively limit the fauna in this area.

The rock basket results demonstrated that there were no meaningful differences between the nearshore locations and offshore locations. These results suggest that proximity to shoreline CKD leachate source points is currently inconsequential or was not a factor affecting the benthic macroinvertebrate community. This may be a result of rapid attenuation of the leachate in Little Traverse Bay or it may be an artifact associated with remediation and reduction of leachate entering the study area.

Comparisons among the study areas exposed some differences in the benthic community during the study. However, these differences do not necessarily appear to be related to impacts associated with CKD leachate. Among the comparisons that revealed significant differences among the areas, no consistent pattern emerged that would suggest that the quality of the benthic community in the reference areas was significantly better than areas exposed to CKD leachate. In terms of richness measures, which typically decrease as the result of impairment, no meaningful difference was observed among the areas. Despite the fact that abundance was significantly higher at EREF, the total abundances observed at SEEP1 and ECKD were statistically similar while the other reference area, WREF was significantly lower.

Although there were notable differences among areas within each sampling event, particularly ECKD in June and SEEP2 in October, consistent evidence suggesting that these results were related to impacts associated with the leachate is limited.

### 4.1.2 Periphyton Community

Based on the 2006 collections, the periphyton community in the vicinity of Bay Harbor contains several components and taxa common to Lake Michigan and the other Great Lakes. The rock basket results demonstrated that there were no meaningful differences between the near shore locations and offshore locations. These results suggest that proximity to shoreline CKD leachate discharge was inconsequential or was not a factor affecting the periphyton community during the 2006 time period. This may be a result of the rapid attenuation of leachate in Little Traverse Bay or it may be associated with remediation via leachate collection thereby minimizing discharge of leachate in the study area. There was a strong seasonal component to the data with a shift away from macroalgae in the fall, and a strong location based gradient from EREF to WREF; however, no discernable relationship between leachate exposure and community quality was observed.

#### 4.1.3 Physicochemical Data

No consistent trends were observed in the physicochemical data that indicate CKD leachate was a potential limiting factor in the sampling areas. In particular, pH measurements from previously affected areas were all within normal levels for Lake Michigan (Bartone and Schelske 1982).

## 4.2 Mussel Tissue

Based on the mussel tissue results, Hg concentrations were statistically similar among the SEEP1, SEEP2, WCKD, WREF and EREF areas. This suggests that there is no additional Hg bioaccumulation associated with the leachate relative to background and reference conditions. Therefore, it appears that further study of these areas is unnecessary.

In contrast, mussel tissue Hg concentrations were significantly higher in the ECKD area compared to the EREF and WREF areas. This suggests that Hg was continuing to bioaccumulate at the time samples were collected from ECKD in October 2006. However, installation of the leachate collection system for ECKD was completed in November 2006. Given the similar concentrations observed from the reference areas, lower levels of Hg bioaccumulation observed at SEEP1, SEEP2, and WCKD, where remediation activities were in operation one year prior to sampling, suggests that Hg bioaccumulation levels are returning to background levels.

Results of the baseline ecological characterization revealed no meaningful patterns related to CKD leachate. No significant differences among the affected sites and reference sites were observed in the benthos results. Likewise, no consistent pattern emerged from the periphyton results that suggest impairment due to CKD leachate. Physicochemical measurements were consistently within the normal range for Lake Michigan and pH was never observed above 8.91 during the study.

The lack of discernable difference among the affected and reference sites is possibly due to turbulence combined with the rapid attenuation of potential leachate impacts and/or remediation activities largely reducing the potential volume of leachate. Regardless, these results do not suggest that further study of this nature is warranted.

In contrast, the mercury concentrations observed from the ECKD tissue samples were significantly elevated at 95% confidence level compared to the EREF and WREF Areas. Therefore, in order to determine if mercury uptake by mussels in ECKD Area is statistically similar to the EREF Area, it is recommended that the collection of mussel tissues and analysis of mercury concentrations be repeated with three replicates for each of the ECKD and EREF Areas now that the collection system is operational. Three replicates are recommended due to the large variability in mercury concentrations observed in the ECKD replicate samples during 2006.

- Barr Engineering Co., 2009. *Removal Action Investigation/Remedial Investigation Report, East CKD Area.* Revision 2.0 June 4, 2008 as amended. Little Traverse Bay CKD Release Site. Emmet County, Michigan
- Barton, D.R. 1986. Nearshore benthic invertebrates of the Ontario waters of Lake Ontario. Journal of Great Lakes Research, 12(4): 270-280.
- Bartone, C.R. and C.L. Schelske. 1982. Lake-wide seasonal changes in limnological conditions in Lake Michigan in 1976. Journal of Great Lake Research, 8(3): 413-427.
- Conover, W. J. 1998. Practical Nonparametric Statistics. John Wiley & Sons, Inc. New York, New York.
- Golder Associates Ltd, Calgary. Undated. Review of artificial substrates for benthos sample collection. Canadian Centre for Mineral and Energy Technology, Aquatic Effects Technology Evaluation Program. 62 pp.
- Klemm, D.J, P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. USEPA, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA/600/4-90/030.
- Learner, M.A., G. Lochhead, and B.D. Hughes. 1978. A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. Freshwater Biology 8(4): 357-375.
- Rosenberg, D.M. and V.H. Resh. 1982. Chapter 6: The use of artificial substrates in the study of freshwater benthic macroinvertebrates *in* J. Cairns, Jr., ed., Artificial Substrates. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.
- Rossmann, R. 2002. Lake Michigan 1994-1996 surficial sediment mercury. Journal of Great Lakes Research, 28(1): 65-76.
- Simpson, K. W., and R. W. Bode. 1980. Common Larvae of Chironomidae (Diptera) from New York State Streams and Rivers with Particular Reference to the Fauna of Artificial Substrates. New York State Museum Bulletin No.439.
- USEPA. 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Volume 1. Fish Sampling and Analysis, Third Edition. Office of Science and Technology, Office of Water, Washington, DC. EPA 823-B-00-007.
- Vogel, A.H., J.J. Sygo, T.M. Kelly, R.P. Canale, H.E. Allen, and E.D. Rothman. 1976. Shortterm transient variations of limnological parameters in Grand Traverse Bay, Lake Michigan. Journal of Great Lakes Research, 2(1): 193-205.
- Winnell, M.H. and D.S. White. 1985. Ecology of some Chironomidae (Diptera) from Southeastern Lake Michigan. Transactions of the American Entomological Society, 111: 279-359.

Figures

Barr Footer: Date: 8/20/2009 9:02:45AM File: 1:\Client\CMS\LTB\_CKD\_Release\_Ste\LTB\_BaseData\Users\JTC\Figure 1 Eco Rock Basket Locations West CKD Area.mxd User: jtc



Imagery: April 2005









Figure 1

WEST CKD AREA ROCK BASKET LOCATIONS - 2006 16-17 MAY THROUGH 26-28 JUNE AND 6-7 SEPTEMBER THROUGH 24-26 OCTOBER

> Little Traverse Bay CKD Release Site Emmet County, Michigan

Barr Footer: Date: 8/20/2009 9:02:27 AM File: 1:\Client\CMS\LTB\_CKD\_Rele ase\_Site\LTB\_BaseData\Users\JTC\Figure 2 Eco Rock Basket Locations Seep 1 Seep 2 CKD Area.mxd User: jtc



Imagery: April 2005

+ Rock Basket Location Approximate CKD Extent







Figure 2

SEEP 1 AND SEEP 2 CKD AREAS ROCK BASKET LOCATIONS - 2006 16-17 MAY THROUGH 26-28 JUNE AND 6-7 SEPTEMBER THROUGH 24-26 OCTOBER

Little Traverse Bay CKD Release Site Emmet County, Michigan



Imagery: April 2005





200

Figure 3

EAST CKD AREA ROCK BASKET LOCATIONS - 2006 16-17 MAY THROUGH 26-28 JUNE AND 6-7 SEPTEMBER THROUGH 24-26 OCTOBER

> Little Traverse Bay CKD Release Site Emmet County, Michigan



Figure 4. Average periphyton total concentrations data for nearshore study areas

#### Periphyton Average Total Concentration - Near Shore

Figure 5. Average periphyton total biovolume data for nearshore study areas







Figure 6. Average periphyton total taxa richness data for nearshore study areas

Periphyton Average Total Taxa Richness - Near Shore

Figure 7. Average periphyton total concentration data for offshore study areas



Periphyton Average Total Concentration - Off Shore



Figure 8. Average periphyton total biovolume data for offshore study areas



Figure 9. Average periphyton total taxa richness data for offshore study areas



Periphyton Average Total Taxa Richness - Off Shore



Figure 10. Average periphyton total concentration data for June 2006

Periphyton Average Total Concentration June 2006

Figure 11. Average periphyton total concentration data for October 2006



Periphyton Average Total Concentration October 2006



Figure 12. Average periphyton total biovolume data for June 2006

Periphyton Average Biovolume June 2006

Figure 13. Average periphyton total biovolume data for October 2006



Periphyton Average Biovolume October 2006







Figure 15. Average periphyton total taxa richness data for October 2006



Periphyton Average Total Taxa Richness October 2006





#### Off Shore Replicate Total Concentrations - June 2006


Figure 17. Periphyton total concentrations data for each nearshore replicate for October 2006

Near Shore Replicate Total Concentrations - October 2006

Figure 18. Periphyton total concentrations data for each offshore replicate for October 2006

7,000,000 Replicate 1 Replicate 2 Replicate 3 6,000,000 Total Concentration (NU/sq cm) 5,000,000 4,000,000 3,000,000 2,000,000 1,000,000 0 EREF ECKD SEEP1 SEEP2 WCKD WREF Study Area

Off Shore Replicate Total Concentrations - October 2006

Figure 19. Measured mean mercury concentrations and two standard deviations at the 95% confidence level for the six locations in Little Traverse Bay project area.



Hg Concentration Dry Weight

Tables

Table 1. List of benthic macroinvertebrate taxa observed in rock basket and qualitative samples from Little Traverse Bay, MI -- June and October 2006.

NEMERTEA (Probosics Worms)XLepidoptera (Aquatic Moths)ANNELLIDADiptera (True Flies)Oligochaeta (Aquatic Worms)CeratopogonidaeLumbriculidaeXHemerodromiaNaididaeXXTubificidaeXChironomidae (Midges)Hirudinea (Leeches)Tanypodinae <sup>1</sup> MooreobdellaXAblabesmyia mallochiCRUSTACEAThienemannimyia grp.Isopoda (Sow Bugs)YAmerica (Sow Bugs)Y	X X X X X X X X X X X X X X X X X X X	X X X X X X X X
ANNELLIDA       Diptera (True Flies)         Oligochaeta (Aquatic Worms)       Ceratopogonidae         Lumbriculidae       X       Hemerodromia         Naididae       X       X         Tubificidae       X       Antocha         Tubificidae       X       Chironomidae (Midges)         Hirudinea (Leeches)       Tanypodinae <sup>1</sup> Mooreobdella       X       Ablabesmyia mallochi         CRUSTACEA       Thienemannimyia grp.         Isopoda (Sow Bugs)       Y       Y	X X X X X X X X X X X X X X X X X	X X X X X X X
Oligochaeta (Aquatic Worms)CeratopogonidaeLumbriculidaeXHemerodromiaNaididaeXXAntochaTubificidaeTubificidaeXChironomidae (Midges)Hirudinea (Leeches)Tanypodinae <sup>1</sup> MooreobdellaXAblabesmyia mallochiCRUSTACEAThienemannimyia grp.Isopoda (Sow Bugs)Pagastia	X X X X X X X X X X X X X X X X	X X X X X X X
LumbriculidaeXHemerodromiaNaididaeXXAntochaTubificidaeXHirudinea (Leeches)Tanypodinae <sup>1</sup> MooreobdellaXCRUSTACEAThienemannimyia grp.Isopoda (Sow Bugs)PagastiaOracidationX	X X X X X X X X X X X X X X X	X X X X X X
NaididaeXXAntochaTubificidaeXXChironomidae (Midges)Hirudinea (Leeches)Tanypodinae <sup>1</sup> MooreobdellaXAblabesmyia mallochiCRUSTACEAThienemannimyia grp.Isopoda (Sow Bugs)Pagastia	X X X X X X X X X X X X X	X X X X X
TubificidaeXChironomidae (Midges)Hirudinea (Leeches)Tanypodinae1MooreobdellaXAblabesmyia mallochiCRUSTACEAThienemannimyia grp.Isopoda (Sow Bugs)Pagastia	X X X X X X X X X X X	X X X X
Hirudinea (Leeches)     Tanypodinae <sup>1</sup> Mooreobdella     X     Ablabesmyia mallochi       CRUSTACEA     Thienemannimyia grp.       Isopoda (Sow Bugs)     Pagastia	X X X X X X X X X X X	X X X
Mooreobdella     X     Ablabesmyia mallochi       CRUSTACEA     Thienemannimyia grp.       Isopoda (Sow Bugs)     Pagastia	X X X X X X X X X	X X X
CRUSTACEA     Thienemannimyia grp.       Isopoda (Sow Bugs)     Pagastia	X X X X X X X	X X X
Isopoda (Sow Bugs) Pagastia	X X X X X X	X X
	X X X X X	Х
Caecidotea X X Potthastia	X X X	
Amphipoda (Side Swimmers) Corynoneura lobata	X	
Hyalella azteca X X Cricotopus bicinctus grp.	X	Х
Gammarus X Cricotopus sylvestris grp.	~	
Decapoda (Crayfish) Cricotopus tremulus grp.	Х	Х
Orconectes X Cricotopus trifascia grp.	Х	Х
	Х	
Hydracarina (Water Mites) X X Eukiefferiella devonica grp.	Х	Х
INSECTA Eukiefferiella gracei grp.	Х	
Ephemeroptera (Mavflies) Nanocladius <sup>1</sup>	Х	
Baetis flavistriga X X Nanocladius spiniplenus	X	
Acerpenna pygmaea X X Orthocladius	X	Х
Heptageniidae <sup>1</sup> X Parakiefferiella		х
Heptagenia X Parametriocnemus		X
Leucrocuta X Psectrocladius	X	~~~
Nixe X X Psilometriocnemus	X	
Stenacron X X Thienemanniella xena	X	
Maccaffertium <sup>1</sup> X Cryptochironomus		X
Maccaffertium vicarium X X Dicrotendines fumidus	X	X
Stenonema femoratum X X Glyntotendines	X	~
Tricorythodes X Microtendines	X	X
Leptophlebiidae X Nilothauma	X	X
Eurylophella bicolor grp. X X Polypedilum flavum	X	X
Caenis X X Pseudochironomus	X	X
Plecontera (Stoneflies) <sup>1</sup> X Stictochironomus		X
Acroneuria X Stictochironomus caffrarius arr	)	X
Trichoptera (Caddisflies)	<i>,</i> .	X
Polycentropus X Paratanytarsus	X	X
Hydronsychidae <sup>1</sup> X Rheotanytarsus	X	X
Cheumatonsyche X X Tanytarsus	~	X
Ceretonsyche morose arp X X Tenytarsus alebrescens arp	X	X
Ceratopsyche morosa X X Tanutarsus quallescens gip.	~	X
Hydronsyche X PFI FCYPODA (Mussels and Clame)		~
Helicopsyche borealis X Dreissena polymorpha		X
Hydroptila X X Dreissena rostriformis	V	~

<sup>1</sup> Taxon unidentifiable. Not counted as a discreet taxon for all samples combined. May be counted as a discreet taxon for individual samples or locations if it is the only representative of that family, order, or genus.

Table 2. The composition, number, and relative abundance of macroinvertebrates in rock basket samples from nearshore locations, Little Traverse Bay, MI -- June 2006.

Таха	EF	REF	EC	KD	SE	EP1	SE	EP2	WCKD		WREF	
Taxa	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Nemertea					1	0.19						
Naidinae	28	5.61	19	8.52	143	26.93	123	23.88	80	19.05	59	13.05
Caecidotea							1	0.19				
Hyalella azteca	1	0.20	3	1.35	26	4.90	33	6.41	11	2.62	6	1.33
Gammarus	2	0.40										
Hydracarina			1	0.45	1	0.19	7	1.36	6	1.43	4	0.88
Baetis flavistriga			1	0.45					1	0.24		
Acerpenna pygmaea	1	0.20			2	0.38						
Eurylophella bicolor grp.					2	0.38	3	0.58	1	0.24	1	0.22
Tricorythodes					1	0.19	1	0.19	1	0.24	1	0.22
Caenis					1	0.19	11	2.14	1	0.24	1	0.22
Hydropsychidae			4	1.79								
Hydropsyche			1	0.45								
Hydroptila					10	1.88	10	1.94	6	1.43	2	0.44
Ceratopogonidae									2	0.48		
Tanypodinae							1	0.19				
Ablabesmyia mallochi							2	0.39				
Thienemannimyia grp.			4	1.79	11	2.07	11	2.14	8	1.90	3	0.66
Potthastia					1	0.19	1	0.19				
Corynoneura lobata	2	0.40	4	1.79	3	0.56	3	0.58				
Thienemanniella xena	3	0.60			10	1.88	5	0.97	16	3.81	18	3.98
Cricotopus tremulus grp.	166	33.27	24	10.76	40	7.53	58	11.26	50	11.90	45	9.96
Cricotopus bicinctus grp.	79	15.83	121	54.26	141	26.55	125	24.27	141	33.57	245	54.20
Cricotopus trifascia grp.									2	0.48		
Cricotopus sylvestris grp.							2	0.39				
Orthocladius	14	2.81	3	1.35	13	2.45	8	1.55				
Nanocladius											2	0.44
Nanocladius spiniplenus							1	0.19				
Psectrocladius	1	0.20			5	0.94	6	1.17				
Dicrotendipes fumidus	9	1.80	25	11.21	107	20.15	72	13.98	52	12.38	26	5.75
Glyptotendipes											1	0.22

_					(00)							
Таха	El	EREF		ECKD		EP1	SE	EP2	W	CKD	W	REF
Taxa	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Microtendipes	1	0.20	1	0.45	5	0.94	6	1.17	5	1.19		
Nilothauma							1	0.19				
Polypedilum flavum	1	0.20										
Paratanytarsus	67	13.43	3	1.35	1	0.19	6	1.17	6	1.43	4	0.88
Rheotanytarsus	124	24.85	9	4.04	6	1.13	16	3.11	31	7.38	34	7.52
Tanytarsus glabrescens grp.							2	0.39				
Dreissena rostriformis					1	0.19						
Total Number (reps combined)	499	100.00	223	100.00	531	100.00	515	100.00	420	100.00	452	100.00
Total Taxa Richness	15		14		22		25		18		16	
EPT Taxa Richness	1		2		5		4		5		4	

Table 2 (cont.)

Table 3. The composition, number, and relative abundance of macroinvertebrates in rock basket samples from offshore locations, Little Traverse Bay, MI -- June 2006.

Таха	EREF		EC	ECKD S		EP1	SE	EP2	WC	KD	WF	REF
Taxa	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Naidinae	55	18.71	14	11.11	239	44.67	140	36.65	29	14.01	89	31.12
Caecidotea							2	0.52	1	0.48	1	0.35
Hyalella azteca			2	1.59	37	6.92	23	6.02	12	5.80	6	2.10
Hydracarina									2	0.97	1	0.35
Baetis flavistriga			1	0.79					1	0.48	1	0.35
Acerpenna pygmaea					1	0.19	1	0.26				
Eurylophella bicolor grp.									1	0.48		
Tricorythodes											1	0.35
Caenis			-		2	0.37	3	0.79	-		1	0.35
Plecoptera							1	0.26	-			
Hydroptila					2	0.37	2	0.52	7	3.38	1	0.35
Ceratopogonidae											1	0.35
Ablabesmyia mallochi					2	0.37	2	0.52				
Thienemannimyia grp.			1	0.79	5	0.93			6	2.90	1	0.35
Pagastia	1	0.34			3	0.56	1	0.26	1	0.48		
Potthastia							5	1.31				
Corynoneura lobata	3	1.02			2	0.37	2	0.52	2	0.97		
Thienemanniella xena	3	1.02			1	0.19	3	0.79			4	1.40
Cricotopus tremulus grp.	48	16.33	8	6.35	39	7.29	17	4.45	21	10.14	28	9.79
Cricotopus bicinctus grp.	77	26.19	51	40.48	83	15.51	64	16.75	52	25.12	107	37.41
Cricotopus trifascia grp.							2	0.52				
Eukiefferiella					1	0.19						
Orthocladius	5	1.70	7	5.56	10	1.87	11	2.88				
Nanocladius							1	0.26			3	1.05
Nanocladius spiniplenus			1	0.79								
Psectrocladius					6	1.12	1	0.26	2	0.97		
Psilometriocnemus							1	0.26				
Dicrotendipes fumidus	30	10.20	39	30.95	86	16.07	84	21.99	41	19.81	19	6.64
Microtendipes	2	0.68					3	0.79				
Nilothauma											1	0.35
Polypedilum flavum							1	0.26				
Pseudochironomus											1	0.35
Paratanytarsus	18	6.12			1	0.19			3	1.45	1	0.35
Rheotanytarsus	52	17.69	2	1.59	14	2.62	8	2.09	26	12.56	19	6.64
Dreissena rostriformis					1	0.19	4	1.05				
Total Number (reps combined)	294	100.00	126	100.00	535	100.00	382	100.00	207	100.00	286	100.00
Total Taxa Richness	11		10		19		24		16		19	
EPT Taxa Richness	0		1		3		4		3		4	

Table 4. The composition, number, and relative abundance of macroinvertebrates in rock basket samples from nearshore locations, Little Traverse Bay, MI -- October 2006.

Таха	EF	REF	EC	ECKD		EP1	SE	EP2	W	CKD	W	REF
Taxa	No.	%										
Naidinae	214	60.97	70	37.43	11	9.91						
Caecidotea	5	1.42							1	1.23	1	3.45
Hyalella azteca	2	0.57										
Gammarus	7	1.99	1	0.53	1	0.90						
Hydracarina	1	0.28										
Baetis flavistriga	1	0.28					1	4.55				
Leucrocuta	1	0.28									1	3.45
Heptagenia									1	1.23		
Eurylophella bicolor grp.					1	0.90			2	2.47	2	6.90
Caenis	14	3.99	1	0.53	3	2.70	1	4.55	8	9.88		
Hydropsychidae			11	5.88								
Cheumatopsyche			2	1.07	2	1.80	3	13.64				
Ceratopsyche morosa grp.	3	0.85	12	6.42	5	4.50			4	4.94	1	3.45
Ceratopsyche morosa	5	1.42	8	4.28								
Lepidoptera											1	3.45
Thienemannimyia grp.	1	0.28	1	0.53					2	2.47	2	6.90
Potthastia							1	4.55				
Thienemanniella xena			1	0.53								
Cricotopus tremulus grp.	17	4.84	21	11.23	71	63.96	16	72.73	59	72.84	18	62.07
Cricotopus bicinctus grp.	32	9.12	5	2.67	4	3.60			1	1.23		
Eukiefferiella devonica grp.	1	0.28									2	6.90
Eukiefferiella gracei grp.					1	0.90			1	1.23		
Orthocladius	12	3.42	45	24.06	9	8.11						
Dicrotendipes fumidus	4	1.14	1	0.53							1	3.45
Microtendipes	8	2.28	3	1.60	2	1.80			1	1.23		
Polypedilum flavum	2	0.57										
Paratanytarsus	10	2.85							1	1.23		
Rheotanytarsus	8	2.28	4	2.14	1	0.90						
Antocha	2	0.57	1	0.53								
Hemerodromia	1	0.28										
Total Number (reps combined)	351	100.00	187	100.00	111	100.00	22	100.00	81	100.00	29	100.00
Total Taxa Richness	22		15		12		5		11		10	
EPT Taxa Richness	5		4		4		3		4		3	

Table 5. The composition, number, and relative abundance of macroinvertebrates in rock basket samples from offshore locations, Little Traverse Bay, MI -- October 2006.

Таха	E	REF	EC	CKD	KD SEEP1		SE	EP2	WCKD		WREF	
Taxa	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Naidinae	150	53.76	166	55.70	16	31.37						
Caecidotea	1	0.36	1	0.34	1	1.96	1	25.00				
Hyalella azteca	2	0.72			1	1.96			2	5.26		
Gammarus	3	1.08					2	50.00	1	2.63		
Heptageniidae	1	0.36										
Leucrocuta									1	2.63	1	3.70
Stenacron									1	2.63		
Stenonema femoratum	1	0.36										
Maccaffertium vicarium			1	0.34					1	2.63		
Eurylophella bicolor grp.					1	1.96			4	10.53		
Caenis	19	6.81	3	1.01	4	7.84	1	25.00	4	10.53		
Hydropsychidae			13	4.36								
Cheumatopsyche			7	2.35	2	3.92			1	2.63		
Ceratopsyche morosa grp.	9	3.23	16	5.37					1	2.63		
Ceratopsyche morosa			4	1.34	2	3.92					2	7.41
Hydroptila									1	2.63		
Ceratopogonidae	1	0.36										
Thienemannimyia grp.	3	1.08	1	0.34	1	1.96			1	2.63	2	7.41
Cricotopus tremulus grp.	10	3.58	37	12.42	19	37.25			17	44.74	19	70.37
Cricotopus bicinctus grp.	6	2.15	3	1.01							1	3.70
Eukiefferiella devonica grp.			3	1.01								
Eukiefferiella gracei grp.			12	4.03	1	1.96						
Orthocladius	12	4.30	4	1.34							1	3.70
Dicrotendipes fumidus	7	2.51	6	2.01	1	1.96					1	3.70
Microtendipes	14	5.02	6	2.01					2	5.26		
Polypedilum flavum	1	0.36	5	1.68								
Paratanytarsus	22	7.89	3	1.01								
Rheotanytarsus	16	5.73	6	2.01	2	3.92						
Hemerodromia									1	2.63		
Dreissena rostriformis	1	0.36	1	0.34								
Total Number (reps combined)	279	100.00	298	100.00	51	100.00	4	100.00	38	100.00	27	100.00
Total Taxa Richness	18		19		12		3		14		7	
EPT Taxa Richness	3		5		4		1		8		2	

Таха	EREF	ECKD	SEEP1	SEEP2	WCKD	WREF
Taxa	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.
Lumbriculidae	3			3	10	10
Naididae	10	10	10	3	10	10
Tubificidae	3		1			
Mooreobdella			1			
Caecidotea	10	3	10	10	10	10
Hyalella azteca	3					
Gammarus	10	3	10	10	10	10
Orconectes	1				1	
Hydracarina	3	3	3		3	
Baetis flavistriga				1		
Acerpenna pygmaea					3	1
Leucrocuta	10	10	10	10	10	10
Nixe	3	3		1	1	3
Stenacron	10	10	3	3	10	10
Maccaffertium			1			
Stenonema femoratum	10	10	10	1	10	10
Maccaffertium vicarium	10	10		3	10	10
			1			
Eurylophella bicolor grp	10	10	10	10	10	10
Caenis	10	10	10	10	10	10
Acroneuria					1	
Polycentropus	1		1		3	1
Cheumatopsyche	10	10	10	3	10	10
Ceratopsyche morosa grp	10	1			יס ג	1
Ceratopsyche morosa	1	3	1	1	ט א	1
Heliconsyche horealis	1				1	
Hydroptila	10				10	3
Ceratopogonidae			1		1	
	10	10	10	3	10	10
Pagastia	10		10		10	
Potthastia			3		3	3
Cricotopus tremulus grp	10	10	3	3	10	10
Cricotopus hicinctus grp.	10	10	1	5	10	10
Cricotopus trifascia grp.	3					
Eukiefferiella devonica grp.	10	10	10	1	10	10
Orthocladius	10	10	10	3	10	10
Parakiefferiella	10	10		5		
Parametricenomus		1				
Cryptophiropomus	3					
Digrotondinos fumidus	10				3 10	
Microtendipes fumidus	10	10		1	10	10
Niloth average	10	10	3	1	10	10
Nilotnauma			1			
	10	1			3	
Pseudocnironomus	3		1			
Stictocnironomus	3					
Stictochironomus cattrarius grp.			3			
Cladotanytarsus	10		1			
Paratanytarsus	10	10			3	1

 Table 6. The composition and assigned abundance<sup>1</sup> of benthic macroinvertebrates

 collected qualitative samples at six locations in Little Traverse Bay, MI -- October 2006.

 Table 6. The composition and assigned abundance<sup>1</sup> of benthic macroinvertebrates

 collected qualitative samples at six locations in Little Traverse Bay, MI -- October 2006.

Таха	EREF	ECKD	SEEP1	SEEP2	WCKD	WREF
Taxa	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.
Rheotanytarsus	3	3				1
Tanytarsus					10	
Tanytarsus glabrescens grp.					10	1
Tanytarsus guerlus grp.	10	10	10			
Antocha	3	3	3	1	10	10
Hemerodromia	10	3	1		10	10
Dreissena polymorpha						3
Dreissena rostriformis	1	3	10	10	1	10
Total Taxa Richness	41	28	33	22	36	30
EPT Taxa Richness	13	10	10	10	15	13

<sup>1</sup>Abundance assigned as 1=1-2 individuals, 3=3-9 individuals, and 10=210 individuals.

Table 7. Results of statistical comparisons of mean parameters betweennearshore (NS) and offshore (OS) locations using pooled benthicmacroinvertebrate data, June-October 2006.

Meric	Gear	Level	Ν	Median	Mean	t Grouping <sup>1</sup>
Abundance	RB	NS	36	72.00	94.94	A
Abundance	RB	OS	35	58.00	72.17	А
Taxa Richness	RB	NS	36	10.00	9.78	А
Taxa Richness	RB	OS	35	9.00	9.29	А
EPT Taxa Richness	RB	NS	36	2.00	1.92	A
EPT Taxa Richness	RB	OS	35	1.00	1.77	А
Ephemeroptera Abund	RB	NS	36	1.00	1.86	А
Ephemeroptera Abund	RB	OS	35	1.00	1.60	А
Trichoptera Abund	RB	NS	36	1.00	2.47	А
Trichoptera Abund	RB	OS	35	1.00	2.00	А
Oligochaeta Abund	RB	OS	35	16.00	25.66	А
Oligochaeta Abund	RB	NS	36	10.50	20.75	А
Chironomidae Abund	RB	NS	36	39.50	66.22	A
Chironomidae Abund	RB	OS	35	31.00	39.69	A

<sup>1</sup>=Comparisons with a common letter are not significantly different.

Meric	Gear	Level	Ν	Median	Mean	t Grouping
Abundance	RB	EREF	12	89.50	118.58	А
Abundance	RB	SEEP1	12	59.50	102.33	AB
Abundance	RB	SEEP2	11	58.00	83.55	В
Abundance	RB	ECKD	12	50.50	69.42	AB
Abundance	RB	WREF	12	45.00	66.25	В
Abundance	RB	WCKD	12	43.00	62.17	В
Taxa Richness	RB	SEEP2	11	11.00	10.18	А
Taxa Richness	RB	EREF	12	10.00	11.17	А
Taxa Richness	RB	SEEP1	12	10.00	10.08	А
Taxa Richness	RB	WCKD	12	9.50	9.00	А
Taxa Richness	RB	ECKD	12	9.00	9.50	А
Taxa Richness	RB	WREF	12	8.00	7.33	А
EPT Taxa Richness	RB	WCKD	12	2.00	2.25	А
EPT Taxa Richness	RB	SEEP1	12	2.00	2.17	А
EPT Taxa Richness	RB	SEEP2	11	2.00	1.64	А
EPT Taxa Richness	RB	ECKD	12	1.50	2.33	А
EPT Taxa Richness	RB	WREF	12	1.50	1.33	А
EPT Taxa Richness	RB	EREF	12	1.00	1.33	А
Ephemeroptera Abund	RB	WCKD	12	2.00	2.33	А
Ephemeroptera Abund	RB	SEEP1	12	1.50	1.50	AB
Ephemeroptera Abund	RB	SEEP2	11	1.00	2.00	AB
Ephemeroptera Abund	RB	WREF	12	1.00	0.83	AB
Ephemeroptera Abund	RB	EREF	12	0.50	3.17	AB
Ephemeroptera Abund	RB	ECKD	12	0.00	0.58	В
Trichoptera Abund	RB	ECKD	12	2.00	6.50	А
Trichoptera Abund	RB	WCKD	12	1.50	1.67	AB
Trichoptera Abund	RB	SEEP1	12	1.00	1.92	AB
Trichoptera Abund	RB	EREF	12	0.50	1.42	В
Trichoptera Abund	RB	WREF	12	0.50	0.50	В
Trichoptera Abund	RB	SEEP2	11	0.00	1.36	В
Oligochaeta Abund	RB	EREF	12	24.00	37.25	А
Oligochaeta Abund	RB	SEEP2	11	23.00	23.91	CAB
Oligochaeta Abund	RB	SEEP1	12	19.00	34.08	А
Oligochaeta Abund	RB	ECKD	12	9.50	22.42	AB
Oligochaeta Abund	RB	WREF	12	5.50	12.33	CB
Oligochaeta Abund	RB	WCKD	12	0.00	9.08	С
Chironomidae Abund	RB	EREF	12	69.00	74.33	A
Chironomidae Abund	RB	WCKD	12	36.50	45.83	A
Chironomidae Abund	RB	ECKD	12	35.00	39.08	A
Chironomidae Abund	RB	SEEP1	12	32.00	59.00	A
Chironomidae Abund	RB	SEEP2	11	31.00	49.55	A
Chironomidae Abund	RB	WREF	12	29.50	50.75	A

Table 8. Results of statistical comparisons of mean parameters among studyareas using pooled benthic macroinvertebrate data, June-October 2006.

<sup>1</sup>=Comparisons with a common letter are not significantly different.

#### Table 9. List of algae taxa collected from rock basket samplers and natural substrates, Little Traverse Bay, MI, June and October 2006.

**Division: Bacillariophyta** Genus **Species** Taxa ID **Subspecies** Variety Form Morph Structure Authority 1010 Achnanthes Vegetative (Greg.) Hust. spp 108135 Vegetative Achnanthes deflexa C. W. Reimer in Patrick & Reimer 10939 Achnanthes laevis Vegetative Ostrup 108169 Achnanthes lanceolata lanceolatoides Vegetative (Sovereign) Reimer 9338 Achnanthes lanceolata lanceolata (de Brebisson) Grunow Vegetative 1013 Achnanthes minutissima Vegetative Kutzing 9765 Achnanthes Grunow in Cleve & Grunow taeniata Vegetative . 1341 Amphora ovalis Vegetative (Kutzing) Kutzing Vegetative 1343 Amphora pediculus (Kutzing) Grunow . 1347 Amphora Vegetative Kutzing veneta . 108384 Anomoeneis vitrea Vegetative (Grunow) Ross . 9351 Bacillaria Gmelin paradoxa Vegetative . 1066 Ehrenb. Cocconeis pediculus Vegetative 9212 (Ehrenb.) Van Heurck Cocconeis placentula lineata Vegetative Vegetative 1000073 Cyclotella hakanssoniae Wendker 1085 Cyclotella meneghiniana Vegetative K tz. . pseudostelligera 9361 Cyclotella Hust. Vegetative . 9854 Cyclotella (small) Job 07 (Kutzing) de Brebisson sp. 1 Vegetative 1090 (Schmidt ) Cleve Cymbella spp Vegetative . 1862 Cymbella affinis Vegetative K tz. 1098 Cymbella caespitosa Vegetative (Kutzing) Brun . 9955 Cymbella cf cistula Vegetative (Ehrhenberg) Kirchner 1099 Cymbella cistula Vegetative (Ehrenberg in Hemprich & Ehrenberg) Kirchner in .

1111	Cymbella	delicatula				Vegetative	K tz.
1000572	Cymbella	excisa		•		Vegetative	Kutzing
9371	Cymbella	gracilis		•		Vegetative	(Ehrenberg) Kutzing
9379	Cymbella	helmckei				Vegetative	Kramer
1113	Cymbella	hustedtii		•		Vegetative	Krasske
1091	Cymbella	microcephala		•		Vegetative	Grunow
1115	Cymbella	minuta		•		Vegetative	Hilse
1094	Cymbella	naviculiformis		•		Vegetative	Auersw. ex Heib.
1095	Cymbella	silesiaca				Vegetative	Bleisch
1096	Cymbella	tumida		•		Vegetative	(Br,b.) Van Heurck
9952	Cymbella	tumidula		•		Vegetative	Grunow
109032	Cymbella	turgidula		•		Vegetative	Grunow
109036	Cymbella	ventricosa	ventricosa	•		Vegetative	C. A. Agardh
10400	Cymbellonitzschia	spp				Vegetative	Hust.
109046	Denticula	tenuis		•		Vegetative	K tz.
1109	Diatoma	tenuis				Vegetative	Agardh
4272	Diatoma	vulgaris		•	distorta	Vegetative	Grunow in Van Heurck
1108	Diatoma	vulgaris	vulgaris	•		Vegetative	Bory
1354	Epithemia	turgida		•		Vegetative	(Ehrenb.) K tz.
1355	Epithemia	turgida	westermannii	•		Vegetative	(Ehrenb.) Grunow
109195	Eucocconeis	flexella		•		Vegetative	(K tz.) Cleve
1140	Eunotia	spp		•		Vegetative	N"rpell-Schempp & Lange-Bert. in Lange-Bert.
9407	Fragilaria	berolinensis		•		Vegetative	(Lemmermann) Lange-Bertalot
9397	Fragilaria	capucina	vaucheriae			Vegetative	(Kutzing) Lange-Bertalot
10379	Fragilaria	capucina	mesolepta			Vegetative	Rabenh.
9395	Fragilaria	capucina	gracilis			Vegetative	(ostrup) Hustedt
10372	Fragilaria	capucina	distans			Vegetative	Sippe

1159	Fragilaria	construens			construens	Vegetative	(Ehrenberg) Hustedt
9045	Fragilaria	construens	•		venter	Vegetative	(Ehrenberg) Hustedt
1152	Fragilaria	crotonensis				Vegetative	Kitton
1155	Fragilaria	leptostauron				Vegetative	(Ehrenberg) Hustedt
109340	Fragilaria	radians				Vegetative	(K tz.) D. M. Williams & Round
9321	Gomphoneis	herculeana				Vegetative	(Ehrenb.) Cleve
1160	Gomphonema	spp				Vegetative	C. Agardh
1166	Gomphonema	affine				Vegetative	K tz.
9058	Gomphonema	augur				Vegetative	Ehrenb.
1169	Gomphonema	clavatum				Vegetative	Ehrenberg
1163	Gomphonema	clevei				Vegetative	Fricke
9053	Gomphonema	minutum				Vegetative	(Agardh) Agardh
9286	Gomphonema	olivaceum				Vegetative	(Lyngbye) Desmazieres
1161	Gomphonema	parvulum				Vegetative	(K tz.) K tz.
9055	Gomphonema	pumilum				Vegetative	(Grunow) Reichardt & Lange-Bertalot
9057	Gomphonema	truncatum				Vegetative	Ehrenb.
9411	Mastogloia	smithii				Vegetative	Thwaites
1210	Navicula	spp				Vegetative	Bory .
1214	Navicula	cryptocephala				Vegetative	K tz.
9072	Navicula	cryptotenella				Vegetative	Lange-Bert.
1000065	Navicula	cryptotenelloides				Vegetative	
9305	Navicula	decussis		decussis		Vegetative	Ostrup
9686	Navicula	erifuga				Vegetative	Lange-Bertalot
9076	Navicula	goeppertiana		goeppertiana		Vegetative	(Bleisch) H.L. Smith
1216	Navicula	halophila				Vegetative	(Grunow) Cleve
9086	Navicula	minuscula	•			Vegetative	Grunow
10356	Navicula	radiosafallax			•	Vegetative	Lange-Bert.

9101	Navicula	subminuscula					Vegetative	Manguin
9102	Navicula	tripunctata					Vegetative	(O. F. M ll.) Bory
9223	Navicula	viridula		linearis			Vegetative	Hust.
1220	Nitzschia	spp					Vegetative	Hassall
1221	Nitzschia	acicularis					Vegetative	(Kutzing) W. Smith
9236	Nitzschia	constricta		•		•	Vegetative	(Kutzing) Ralfs .
9113	Nitzschia	dissipata					Vegetative	(K tz.) Grunow
9114	Nitzschia	fonticola					Vegetative	Grunow
1222	Nitzschia	gracilis					Vegetative	Hantzsch
9117	Nitzschia	intermedia					Vegetative	Hantzsch
9123	Nitzschia	palea					Vegetative	(K tz.) W. Sm.
1223	Nitzschia	perminuta					Vegetative	(Grunow) M. Perag.
9124	Nitzschia	recta					Vegetative	Hantzsch
1271	Rhoicosphenia	curvata					Vegetative	(kutzing) Grunow
1752	Rhopalodia	brebissonii					Vegetative	Krammer
1754	Rhopalodia	gibba		minuta			Vegetative	Krammer
1314	Synedra	delicatissima					Vegetative	W. Smith
1477	Synedra	filiformis					Vegetative	Grunow
1316	Synedra	nana					Vegetative	F. Meister
1000481	Synedra	radians					Vegetative	Kutz
1000541	Synedra	radians					Vegetative	•
1000540	Synedra	tenera					Vegetative	•
9504	Synedra	tenera					Vegetative	W. Sm.
1315	Synedra	ulna					Vegetative	(Nitzsch) Ehrenb.
Division:	Chlorophy	ta	_					
Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Authority
129642	*Chlorococcaceae	spp		•	•	•	Vegetative	· · · · · · · · · · · · · · · · · · ·

2687	*Chlorococcaceae	spp		-		> 1 um ovoid	Vegetative	(Brandt) Beijerinck
8190	Bulbochaete	spp			•		Vegetative	Transeau and Brown In Tiff.
2100	Chlorococcum	spp			•		Vegetative	(Naegeli) Rabenhor
2101	Chlorococcum	humicola	•		•		Vegetative	(Naegeli) Rabenhor
2791	Cladophora	fracta					Vegetative	(Dillw.) Kuetzing
2180	Cosmarium	spp	•		•		Vegetative	Corda
2211	Dictyosphaerium	pulchellum					Vegetative	Wood
2961	Dispora	crucigenioides					Vegetative	Printz
2246	Euastrum	spp					Vegetative	Nordstedt
10170	Geminella	spp					Vegetative	Prescott
8041	Monoraphidium	capricornutum	•		•		Vegetative	(Printz) Nygaard
2340	Mougeotia	spp					Vegetative	Kisselew
2350	Oedogonium	spp					Vegetative	De Bary
2369	Oocystis	lacustris	•		•		Vegetative	Chodat
2363	Oocystis	parva	•		•		Vegetative	West & West
2380	Pediastrum	spp	•		•		Vegetative	(Ehrenberg) Meneg.
2382	Pediastrum	boryanum	•		•		Vegetative	(Turpin) Meneghini
2389	Pediastrum	boryanum	•	longicorne	•		Vegetative	Raciborski
1000013	Pediastrum	duplex					Vegetative	West & West
102560	Pediastrum	integrum	•		•		Vegetative	Naeg.
2387	Pediastrum	tetras		tetraodon			Vegetative	(Corda) Rabenhorst
8451	Protoderma	viride			•		Vegetative	Kuetzing
2471	Rhizoclonium	hieroglyphicum					Vegetative	(C. Agardh) Kuetzing
2484	Scenedesmus	abundans					Vegetative	(Kirchn.) Chodat
8399	Scenedesmus	acutus					Vegetative	Lagh. Chodat
2483	Scenedesmus	bijuga	•				Vegetative	(Turpin) Lagerh.
2488	Scenedesmus	denticulatus					Vegetative	Lagerhiem

8393	Scenedesmus	dispar			Vegetative	(Brebisson) Rabenhorst .
8303	Scenedesmus	opoliensis	carinatus	-	Vegetative	Lemmermann
2884	Scenedesmus	quadricauda	-	-	Vegetative	(Turpin) Breb.
8302	Scenedesmus	quadricauda	longispina	-	Vegetative	(Chodat) G. M. Smith
8221	Scenedesmus	quadricauda	quadrispina		Vegetative	(Chodat) Smith
2500	Selenastrum	spp		-	Vegetative	Lagerheim
2950	Spirogyra	spp	-		Vegetative	Skuja
2540	Stigeoclonium	spp	-	-	Vegetative	(Hazen) Collins Em Cox and Bold
2550	Tetraedron	spp	-	-	Vegetative	(Reinsch) De Toni
2554	Tetraedron	minimum	-	-	Vegetative	(Braun) Hansgirg
130069	Trentepohlia	spp	-	-	Vegetative	(Flotow) Hansgrig
103684	Ulothrix	aequalis	-	-	Vegetative	Kuetzing
103704	Ulothrix	variabilis	-	-	Vegetative	Kuetzing
103705	Ulothrix	zonata	-	-	Vegetative	(Weber and Mohr) Kuetzing
8211	Uronema	elongatum		-	Vegetative	Hodgetts
2740	Zygnema	spp			Vegetative	Czurda

# Division: Chrysophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Authority
1653	*.	spp			•		Cyst	N/A
1000035	*Chrysocapsaceae	spp		•	•	>1 um spherical	Vegetative	N/A
1611	Stichogloea	olivacea					Vegetative	Chodat

# Division: Cryptophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Authority
3015	Cryptomonas	erosa					Vegetative	Ehrenberg .
3040	Rhodomonas	spp					Vegetative	Karsten .

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Authority
4050	Aphanocapsa	spp		•	•	•	Vegetative	W. and G. S. West
4054	Aphanocapsa	delicatissima					Vegetative	West & West
4051	Aphanocapsa	elachista					Vegetative	West & West
4052	Aphanocapsa	koordersi					Vegetative	Strom
4053	Aphanocapsa	pulchra					Vegetative	(Kutz.) Rabenh.
4055	Aphanocapsa	rivularis					Vegetative	(Carm.) Rabenhorst
4062	Aphanothece	nidulans					Vegetative	P. Richter
4069	Aphanothece	stagnina					Vegetative	(Spreg.) A. Br
4310	Calothrix	spp					Vegetative	(Naeg.) Born. and Flah.
4080	Chroococcus	spp					Vegetative	(Breb.) Naegeli
4512	Chroococcus	cohaerens					Vegetative	(Breb.) Naegeli
4083	Chroococcus	minimus					Vegetative	(Keis.) Lemmermann
4086	Chroococcus	minutus					Vegetative	(Kuetzing) Naegeli
4085	Chroococcus	prescottii					Vegetative	Drouet and Daily
4511	Chroococcus	turgidus					Vegetative	(Kutzing) Nageli .
4720	Cyanosarcina	spp					Vegetative	Kovacik
4150	Lyngbya	spp					Vegetative	Agardh
1000544	Lyngbya	spp				sp. 4	Vegetative	
107564	Lyngbya	birgei					Vegetative	
4157	Lyngbya	diguetii					Vegetative	Lemmermann
4309	Lyngbya	perelegans				•	Vegetative	Lemmermann
4421	Lyngbya	subtilis				•	Vegetative	West & West .
107595	Lyngbya	subtillissima					Vegetative	
4169	Merismopedia	elegans	•	•			Vegetative	A. Br. In Kutz 1849
4168	Merismopedia	punctata					Vegetative	Meyer In Wiegmann

**Division:** 

Cyanophyta

4170	Oscillatoria	spp			Vegetative	Gomont
4617	Oscillatoria	angustissima			Vegetative	West and West
4252	Oscillatoria	hamelii			Vegetative	Fremy 1930
4174	Oscillatoria	tenuis			Vegetative	Agardh
4190	Phormidium	spp			Vegetative	Lemmermann
4460	Pseudanabaena	spp			Vegetative	Lauterborn
4463	Pseudanabaena	galeata			Vegetative	Bocher .
4321	Synechococcus	elongatus			Vegetative	Nageli
4323	Synechococcus	sp. 1		< 1um ovoid	Vegetative	Nageli
4660	Synechocystis	spp			Vegetative	C. Sauvageau 1892
4285	Synechocystis	spp		>1 um spherical	Vegetative	N/A

## **Division:** Pyrrhophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Authority
6034	Gymnodinium	sp. 3					Vegetative	Stein

Nearshore - June 2006											
		EREF	ECKD	SEEP1	SEEP2	WCKD	WREF				
TOTAL Conc (NU/sq cm)	Average	3,021,075	3,338,150	2,544,291	3,401,787	2,246,753	3,081,203				
	Std. Dev	900,431	440,240	445,485	1,301,703	1,350,654	690,367				
	CV(%)	30	13	18	38	60	22				
Total Biovolume (um cube/sq cm)	Average	200,901,692	310,924,043	378,548,085	320,352,570	234,304,658	503,826,585				
	Std. Dev	37,312,951	84,396,124	221,727,438	121,464,678	111,976,396	101,465,890				
	CV(%)	19	27	59	38	48	20				
Taxa Richness	Average	40	38	43	42	41	39				
	Std. Dev	6	2	3	3	4	4				
	CV(%)	14	6	6	6	8	9				

 Table 10. Nearshore – June 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness;

 Includes Standard Deviation and CV%

 Table 11. Offshore – June 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa Richness;

 Includes Standard Deviation and CV%

Offshore - June 2006												
	EREF ECKD SEEP1 SEEP2 WCKD WREF											
TOTAL Conc (NU/sq cm)	Average	4,573,481	2,624,324	4,263,329	3,241,900	2,060,600	2,284,116					
	Std. Dev	1,100,858	298,715	1,430,039	1,411,504	541,217	426,715					
	CV(%)	24	11	34	44	26	19					
Total Biovolume (um cube/sq cm)	Average	391,005,757	262,539,300	483,455,145	430,142,831	529,069,329	333,700,880					
	Std. Dev	192,235,719	52,473,170	44,837,753	115,362,764	418,595,109	50,095,907					
	CV(%)	49	20	9	27	79	15					
Taxa Richness	Average	41	36	41	40	41	38					
	Std. Dev	0	2	2	4	9	4					
	CV(%)	0	5	5	9	22	9					

 Table 12. Nearshore – October 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa

 Richness; Includes Standard Deviation and CV%

Nearshore - October 2006											
	EREF ECKD SEEP1 SEEP2 WCKD WREF										
TOTAL Conc (NU/sq cm)	Average	3,094,511	2,281,611	778,351	991,839	1,266,863	733,770				
	Std. Dev	155,015	371,233	524,327	539,211	363,966	217,725				
	CV(%)	5	16	67	54	29	30				
Total Biovolume (um cube/sq cm)	Average	558,299,571	999,078,423	287,508,140	407,149,878	397,353,856	187,064,176				
	Std. Dev	215,684,020	199,732,586	225,904,089	383,149,191	71,841,201	60,581,083				
	CV(%)	39	20	79	94	18	32				
Taxa Richness	Average	46	39	47	43	38	30				
	Std. Dev	2	5	5	6	7	4				
	CV(%)	5	12	11	15	18	12				

 Table 13. Offshore – October 2006 Average Total Concentration, Average Total Biovolume, and Average Total Taxa

 Richness; Includes Standard Deviation and CV%

Offshore - October 2006												
	EREF ECKD SEEP1 SEEP2 WCKD WREF											
TOTAL Conc (NU/sq cm)	Average	2,406,401	3,294,258	1,180,103	287,072	966,675	1,350,940					
	Std. Dev	493,340	1,153,880	274,313	68,623	160,185	216,537					
	CV(%)	21	35	23	24	17	16					
Total Biovolume (um cube/sq cm)	Average	1,027,492,173	1,617,902,233	474,073,318	41,918,889	228,032,265	585,156,157					
	Std. Dev	348,016,137	335,368,219	233,595,369	7,180,421	90,472,978	233,979,356					
	CV(%)	34	21	49	17	40	40					
Taxa Richness	Average	47	41	44	28	40	37					
	Std. Dev	3	7	7	4	3	1					
	CV(%)	7	17	15	13	7	3					

#### TABLE 14

#### MEAN (AND ACTUAL RANGE) WATER TEMPERATURE (C), SPECIFIC CONDUCTANCE (μS/cm), DISSOLVED OXYGEN (mg/L), pH, AND TURBIDITY (NTU) AT BASKET SAMPLER LOCATIONS NEAR BAY HARBOR, LITTLE TRAVERSE BAY, M MAY - JUNE AND SEPTEMBER - OCTOBER 2006

May - June									
Location	Temp.	Sp. Cond.	DO	рН	Turbidity				
EREF-NS	14.64	274	12.37	8.55	1.46				
EREF-OS	14.19	271	12.19	8.45	0.87				
ECKD-NS	13.84	272	11.90	8.45	1.05				
ECKD-OS	13.50	269	11.94	8.36	0.91				
SEEP1-NS	14.95	271	11.84	8.46	1.11				
SEEP1-OS	13.72	266	12.14	8.37	1.10				
SEEP2-NS	13.72	273	11.62	8.36	1.90				
SEEP2-OS	13.08	268	11.73	8.32	1.52				
WCKD-NS	13.82	282	12.00	8.31	1.19				
WCKD-OS	13.08	268	12.00	8.27	1.21				
WREF-NS	13.19	271	11.45	8.08	1.68				
WREF-OS	12.66	268	11.53	8.10	1.24				
Min	7.88	248	8.59	7.25	0.20				
Max	20.76	356	15.55	8.72	6.46				
September - October									
		September -	October						
Location	Temp.	September - Sp. Cond.	October DO	рН	Turbidity				
Location EREF-NS	<b>Temp.</b> 13.99	September - Sp. Cond. 282	October DO 12.05	<b>рН</b> 8.48	Turbidity 1.32				
Location EREF-NS EREF-OS	<b>Temp.</b> 13.99 14.26	September -           Sp. Cond.           282           284	October DO 12.05 11.97	рН 8.48 8.47	Turbidity 1.32 0.69				
Location EREF-NS EREF-OS ECKD-NS	Temp.           13.99           14.26           13.96	September -           Sp. Cond.           282           284           274	October DO 12.05 11.97 10.63	<b>pH</b> 8.48 8.47 8.29	Turbidity 1.32 0.69 1.19				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS	Temp.           13.99           14.26           13.96           13.92	September -           Sp. Cond.           282           284           274           270	October DO 12.05 11.97 10.63 10.46	<b>pH</b> 8.48 8.47 8.29 8.28	Turbidity           1.32           0.69           1.19           0.89				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS	Temp.           13.99           14.26           13.96           13.92           13.08	September -           Sp. Cond.           282           284           274           270           272	October DO 12.05 11.97 10.63 10.46 11.04	<b>pH</b> 8.48 8.47 8.29 8.28 8.35	Turbidity 1.32 0.69 1.19 0.89 0.81				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43	September -           Sp. Cond.           282           284           274           270           272           269	October DO 12.05 11.97 10.63 10.46 11.04 10.64	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.45	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12	September -           Sp. Cond.           282           284           274           270           272           269           276	October DO 12.05 11.97 10.63 10.46 11.04 10.64 10.75	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.35 8.45 8.31	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-OS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12           13.55	September -           Sp. Cond.           282           284           274           270           272           269           276           270	October DO 12.05 11.97 10.63 10.46 11.04 10.64 10.75 10.28	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.45 8.45 8.31 8.32	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-OS WCKD-NS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12           13.55           13.17	September -           Sp. Cond.           282           284           274           270           272           269           276           270           281	DO           12.05           11.97           10.63           10.46           11.04           10.64           10.75           10.28           10.00	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.35 8.45 8.31 8.31 8.32 8.22	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59           1.24				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-NS SEEP2-OS WCKD-NS WCKD-OS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12           13.55           13.17           13.20	September - 6           Sp. Cond.           282           284           274           270           272           269           276           270           281           279	DO           DO           12.05           11.97           10.63           10.46           11.04           10.64           10.75           10.28           10.00           10.15	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.45 8.31 8.32 8.32 8.22 8.24	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59           1.24           1.04				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-NS SEEP2-OS WCKD-NS WCKD-NS WCKD-NS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.55           13.17           13.20           13.21	September - 6           Sp. Cond.           282           284           274           270           272           269           276           270           271           269           276           270           281           279           276	DO           12.05           11.97           10.63           10.46           11.04           10.64           10.75           10.28           10.00           10.15           10.19	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.45 8.31 8.31 8.32 8.22 8.22 8.24 8.20	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59           1.24           1.04           0.61				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-NS SEEP2-OS WCKD-NS WCKD-OS WREF-NS WREF-NS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12           13.55           13.17           13.20           13.21           13.46	September - 6           Sp. Cond.           282           284           274           270           272           269           276           270           281           279           276           273	DO           12.05           11.97           10.63           10.46           11.04           10.64           10.75           10.28           10.00           10.15           10.19           10.10	<b>pH</b> 8.48 8.47 8.29 8.28 8.35 8.35 8.45 8.31 8.32 8.32 8.22 8.22 8.24 8.20 8.26	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59           1.24           1.04           0.58				
Location EREF-NS EREF-OS ECKD-NS ECKD-OS SEEP1-NS SEEP1-OS SEEP2-NS SEEP2-NS SEEP2-OS WCKD-NS WCKD-NS WREF-NS WREF-NS	Temp.           13.99           14.26           13.96           13.92           13.08           13.43           13.12           13.55           13.17           13.20           13.21           13.46	September - 6           Sp. Cond.           282           284           274           270           272           269           276           279           276           273	DO           DO           12.05           11.97           10.63           10.46           11.04           10.63           10.46           11.04           10.63           10.46           11.04           10.63           10.175           10.28           10.00           10.15           10.19           10.10           8.20	рН 8.48 8.47 8.29 8.28 8.35 8.45 8.31 8.32 8.22 8.22 8.24 8.20 8.26 7.67	Turbidity           1.32           0.69           1.19           0.89           0.81           0.79           0.85           0.59           1.24           1.04           0.61           0.58           0.19				

		Field Wet	Dry Wgt.	Dry Wgt. Hg	Mean	Std. Dev.
Location	Replicate	Wgt. (g)	(%)	Conc. (ng/g)	(ng/g)	(ng/g)
EREF A	1	32.7	48.3	5.5		
EREF B	2	31.8	53.8	3.5	4.5	1.4142
ECKD A	1	5.7	40.5	7.7		
ECKD B	2	4.0	69.4	5.1	6.4	1.8385
SEEP1 A	1	31.9	45.5	4.0		
SEEP1 B	2	32.4	49.7	3.8	3.9	0.1414
SEEP2 A	1	38.7	48.0	4.4		
SEEP2 B	2	35.2	50.0	3.6	4.0	0.5657
WCKD A	1	33.5	49.9	6.1		
WCKD B	2	33.8	52.7	3.4	4.8	1.9092
WREF A	1	31.9	55.6	3.4		
WREF B	2	32.3	49.5	5.4	4.4	1.4142
WREF Dup	Field Dup	32.4	47.9	4.0		
ECKD						
Transplant		32.7	52.9	3.3		
MEAN		29.2	51.0	4.5	4.7	

Table 15. Little Traverse Bay Zebra/Quagga Mussels Mercury Concentrations Data fromFall 2006 Sampling

Mean and Standard Deviations are calculated using the replicate values for each sampling area



620 Broad Street - Suite 100 - St. Joseph - MI 49085 - Phone: 269-983-3654 - Fax: 269-983-3653 info@phycotech.com - www.phycotech.com

> Algae Analysis Report and Data Set

Customer ID: 282

Tracking Code:	120001-282	<u>Sample ID:</u>	D2	<b><u>Replicate:</u></b>	
Customer ID:	282	<u>Sample Date:</u>	7/2/2012	Sample Level:	Benthic
Job ID:	1	Station:	LITR00.1	Sample Depth:	0
System Name:	Little River	<u>Site:</u>	Westfield	Preservative:	Lugols
<b><u>Report Notes:</u></b>					

<b>Division:</b>	Bacillariophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
9349	Achnanthes	biasolettiana					Vegetative	2.000	0.37
9013	Achnanthes	bioreti		·			Vegetative	2.000	0.37
9334	Achnanthes	delicatula		•	•	•	Vegetative	1.000	0.18
1013	Achnanthes	minutissima		•	•	·	Vegetative	400.000	73.66
1061	Cocconeis	neothumensis		•	•	·	Vegetative	1.000	0.18
9212	Cocconeis	placentula		lineata	•	·	Vegetative	4.000	0.74
9218	Cocconeis	placentula	•	pseudolineata	•	•	Vegetative	3.000	0.55
1095	Cymbella	silesiaca				•	Vegetative	17.000	3.13
1119	Cymbella	sinuata	•	·	•	•	Vegetative	2.000	0.37
1000646	Diatoma	moniliformis		•	•	·	Vegetative	2.000	0.37
1157	Fragilaria	brevistriata	•	•	•	•	Vegetative	2.000	0.37
9397	Fragilaria	capucina		vaucheriae		•	Vegetative	53.000	9.76
10379	Fragilaria	capucina		mesolepta	•	•	Vegetative	13.000	2.39
1160	Gomphonema	spp		•	•	•	Vegetative	2.000	0.37
1161	Gomphonema	parvulum	•	•	•	•	Vegetative	9.000	1.66

 $\square$  = Identification is Uncertain

**\*** = Family Level Identification

120001-282 Periphyton - Diatom Only Monday, May 06, 2013 Page 2 of 11

9055	Gomphonema	pumilum					Vegetative	6.000	1.10
9057	Gomphonema	truncatum	•				Vegetative	3.000	0.55
1193	Melosira	varians			·		Vegetative	4.000	0.74
9072	Navicula	cryptotenella			•		Vegetative	7.000	1.29
9093	Navicula	rhynchocephala				•	Vegetative	1.000	0.18
1222	Nitzschia	gracilis					Vegetative	1.000	0.18
9506	Synedra	ulna		ulna	•	•	Vegetative	6.000	1.10

Summary for Division ~ Bacillariophyta (22 detail records)

Sum Total Bacillariophyta

99.63

#### **Total Sample Concentration**

541.000

541.000

Frustules

Tracking Code:	120002-282	Sample ID:	D1	<b><u>Replicate:</u></b>	
Customer ID:	282	Sample Date:	7/2/2012	<u>Sample Level:</u>	Benthic
Job ID:	1	Station:	WSFR10.3	Sample Depth:	0
System Name:	Westfield River	<u>Site:</u>	Westfield	Preservative:	Lugols
<b><u>Report Notes:</u></b>					

<b>Division:</b>	Bacillariophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
☑ 1000755	Achnanthes	minutissima		robusta	-		Vegetative	236.000	55.79
1013	Achnanthes	minutissima		•	•		Vegetative	46.000	10.87
1343	Amphora	pediculus	•	•	•	•	Vegetative	1.000	0.24
9212	Cocconeis	placentula	•	lineata	•	•	Vegetative	39.000	9.22
1098	Cymbella	caespitosa	•	•	•	•	Vegetative	3.000	0.71
1095	Cymbella	silesiaca	•	•	•	•	Vegetative	4.000	0.95
1000646	Diatoma	moniliformis		•	•	•	Vegetative	2.000	0.47
1140	Eunotia	spp	•	•	•	•	Vegetative	2.000	0.47
9397	Fragilaria	capucina	•	vaucheriae	•	•	Vegetative	7.000	1.65
1152	Fragilaria	crotonensis		•	•	•	Vegetative	22.000	5.20
1161	Gomphonema	parvulum		•	•	•	Vegetative	13.000	3.07
9055	Gomphonema	pumilum	•	•	•	•	Vegetative	20.000	4.73
1193	Melosira	varians	•	•	•	•	Vegetative	2.000	0.47
1201	Meridion	circulare					Vegetative	1.000	0.24
9072	Navicula	cryptotenella					Vegetative	6.000	1.42

 $\square$  = Identification is Uncertain

**\*** = Family Level Identification

									423.000
								Total Sample Co	oncentration
Summary for	Division ~ Chryso	phyta (1 detail record)				Sum Total	Chrysophyta	1.000	0.24
1653	*.	spp	•				Cyst	1.000	0.24
Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
<b>Division:</b>	Chr	ysophyta							
Summary for	Division ~ Bacilla	riophyta (23 detail records)	)			Sum Total	Bacillariophyta	422.000	99.76
1331	Tabellaria	fenestrata			•		Vegetative	1.000	0.24
9506	Synedra	ulna	•	ulna			Vegetative	6.000	1.42
1477	Synedra	filiformis					Vegetative	1.000	0.24
9771	Synedra	arcus		arcus		·	Vegetative	1.000	0.24
9124	Nitzschia	recta		•	•	•	Vegetative	3.000	0.71
9482	Navicula	salinarum		•			Vegetative	2.000	0.47
1369	Navicula	pupula					Vegetative	1.000	0.24
1000065	Navicula	cryptotenelloides	•	•	•	•	Vegetative	3.000	0.71

Tracking Code:	120003-282	Sample ID:	D3	<b><u>Replicate:</u></b>	
Customer ID:	282	Sample Date:	7/11/2012	Sample Level:	Benthic
Job ID:	1	Station:	WSFR12.7	Sample Depth:	0
System Name:	Westfield River	<u>Site:</u>	Westfield	Preservative:	Lugols
<b><u>Report Notes:</u></b>					

<b>Division:</b>	Bacil	llariophyta							
Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
1014	Achnanthes	clevei					Vegetative	1.000	0.25
☑ 1000755	Achnanthes	minutissima		robusta			Vegetative	287.000	70.69
1013	Achnanthes	minutissima					Vegetative	88.000	21.67
1095	Cymbella	silesiaca					Vegetative	3.000	0.74
9397	Fragilaria	capucina		vaucheriae			Vegetative	14.000	3.45
1161	Gomphonema	parvulum		•	•	•	Vegetative	6.000	1.48
9072	Navicula	cryptotenella	•	•	•	•	Vegetative	4.000	0.99
9123	Nitzschia	palea		•	•	·	Vegetative	2.000	0.49
9124	Nitzschia	recta		•	•	•	Vegetative	1.000	0.25
Summary for I	Division ~ Bacillari	ophyta (9 detail records	)			Sum Total	Bacillariophyta	406.000	100.00

## **Total Sample Concentration**

406.000

Frustules

Tracking Code:	120004-282	Sample ID:	D4	<b><u>Replicate:</u></b>	
Customer ID:	282	Sample Date:	7/11/2012	Sample Level:	Benthic
Job ID:	1	Station:	WSFR17.2	Sample Depth:	0
System Name:	Westfield River	Site:	Westfield	Preservative:	Lugols
<b><u>Report Notes:</u></b>					

<b>Division:</b>	Bacil	lariophyta							
Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
1013	Achnanthes	minutissima				•	Vegetative	79.000	18.90
☑ 1000755	Achnanthes	minutissima		robusta	•	•	Vegetative	302.000	72.25
1343	Amphora	pediculus		•	•	•	Vegetative	1.000	0.24
108384	Anomoeoneis	vitrea					Vegetative	1.000	0.24
1095	Cymbella	silesiaca					Vegetative	2.000	0.48
9397	Fragilaria	capucina		vaucheriae			Vegetative	10.000	2.39
1161	Gomphonema	parvulum		•			Vegetative	2.000	0.48
9055	Gomphonema	pumilum	•	•		•	Vegetative	15.000	3.59
9072	Navicula	cryptotenella	•				Vegetative	6.000	1.44
Summary for I	Division ~ Bacillari	ophyta (9 detail records	)			Sum Total	Bacillariophyta	418.000	100.00

## **Total Sample Concentration**

418.000 Frustules

Tracking Code:	120005-282	Sample ID:	D5	<b><u>Replicate:</u></b>	
Customer ID:	282	<u>Sample Date:</u>	7/9/2012	Sample Level:	Benthic
Job ID:	1	Station:	WB01	Sample Depth:	0
System Name:	Westfield River	<u>Site:</u>	Huntington	Preservative:	Lugols
<b><u>Report Notes:</u></b>					

Division:	Bacillariophyta
	Ducinariophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	Morph	Structure	Count Frustules	Relative Count
4275	Achnanthes	flexella					Vegetative	2.000	0.50
1013	Achnanthes	minutissima		•	•	•	Vegetative	276.000	69.00
☑ 1000755	Achnanthes	minutissima		robusta			Vegetative	54.000	13.50
108384	Anomoeoneis	vitrea					Vegetative	12.000	3.00
9212	Cocconeis	placentula		lineata			Vegetative	1.000	0.25
1099	Cymbella	cistula					Vegetative	1.000	0.25
1095	Cymbella	silesiaca	•				Vegetative	2.000	0.50
1140	Eunotia	spp	•				Vegetative	2.000	0.50
9397	Fragilaria	capucina	•	vaucheriae			Vegetative	21.000	5.25
1152	Fragilaria	crotonensis	•	•			Vegetative	15.000	3.75
9055	Gomphonema	pumilum	·	•		·	Vegetative	6.000	1.50
9057	Gomphonema	truncatum					Vegetative	1.000	0.25
9072	Navicula	cryptotenella	·	•			Vegetative	6.000	1.50
9776	Synedra	ulna	•	acus			Vegetative	1.000	0.25
Summary for 1	Division ~ Bacillario	ophyta (14 detail record	s)			Sum Total	Bacillariophyta	400.000	100.00

\* = Family Level Identification

120005-282 Periphyton - Diatom Only Monday, May 06, 2013 Page 8 of 11

Total Sample Concentration 400.000 Frustules

# **Species List**

# Division: Bacillariophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	PhysiState	Structure	Authority
9349	Achnanthes	biasolettiana					Vegetative	(Ktz.) Grunow
9013	Achnanthes	bioreti					Vegetative	Germain
1014	Achnanthes	clevei					Vegetative	Grunow
9334	Achnanthes	delicatula					Vegetative	(Kutzing) Grunow
4275	Achnanthes	flexella					Vegetative	Brun
1013	Achnanthes	minutissima					Vegetative	Kutzing
1000755	Achnanthes	minutissima		robusta			Vegetative	Hustedt
1343	Amphora	pediculus					Vegetative	(Kutzing) Grunow
108384	Anomoeoneis	vitrea			•		Vegetative	(Grunow ) Ross
1061	Cocconeis	neothumensis			•		Vegetative	Krammer
9212	Cocconeis	placentula		lineata			Vegetative	(Ehrenb.) Van Heurck
9218	Cocconeis	placentula		pseudolineata			Vegetative	Geitler
1098	Cymbella	caespitosa			•		Vegetative	(Kutzing) Brun
1099	Cymbella	cistula					Vegetative	(Ehrenberg in Hemprich & Ehrenberg) Kirch
1095	Cymbella	silesiaca			•		Vegetative	Bleisch
1119	Cymbella	sinuata			•		Vegetative	Gregory
1000646	Diatoma	moniliformis			•		Vegetative	Kutzing 1833
1140	Eunotia	spp			•		Vegetative	N"rpell-Schempp & Lange-Bert. in Lange-B
1157	Fragilaria	brevistriata			•		Vegetative	Grunow in Van Heurck
10379	Fragilaria	capucina		mesolepta			Vegetative	Rabenh.
9397	Fragilaria	capucina		vaucheriae	•		Vegetative	(Kutzing) Lange-Bertalot
1152	Fragilaria	crotonensis			•		Vegetative	Kitton
1160	Gomphonema	spp					Vegetative	C. Agardh

1161	Gomphonema	parvulum				Vegetative	(Ktż.) Ktż.
9055	Gomphonema	pumilum				Vegetative	(Grunow) Reichardt & Lange-Bertalot
9057	Gomphonema	truncatum				Vegetative	Ehrenb.
1193	Melosira	varians				Vegetative	C. A. Agardh (for genus)
1201	Meridion	circulare				Vegetative	(Greville) C. Agardh
9072	Navicula	cryptotenella				Vegetative	Lange-Bert.
1000065	Navicula	cryptotenelloides				Vegetative	
1369	Navicula	pupula				Vegetative	Kutzing
9093	Navicula	rhynchocephala				Vegetative	Ktz.
9482	Navicula	salinarum				Vegetative	Grunow
1222	Nitzschia	gracilis				Vegetative	Hantzsch
9123	Nitzschia	palea				Vegetative	(Ktz.) W. Sm.
9124	Nitzschia	recta				Vegetative	Hantzsch
9771	Synedra	arcus		arcus		Vegetative	(Ehrenberg) Cleve
1477	Synedra	filiformis				Vegetative	Grunow
9776	Synedra	ulna		acus		Vegetative	(Nitzsch) Ehrenb.
9506	Synedra	ulna		ulna		Vegetative	(Nitzsch) Ehrenb.
1331	Tabellaria	fenestrata	•		•	Vegetative	(Lyngb.) Ktz.

# Division: Chrysophyta

Taxa ID	Genus	Species	Subspecies	Variety	Form	PhysiState	Structure	Authority				
1653	*.	spp					Cyst	N/A				
											shannon_	shannon
-------------	------	--------	--------	-----------------	------------	----------	-------------	-----------	----------	-----------	------------	------------
											diversity_	_diversity
											standard	_standar
	cust										_algal_co	d_algal_
	ome		sample							maximum_	ncentratio	cell_conc
tracking_id	r_id	job_id	_id	system_name	site	station	sample_date	replicate	richness	diversity	n	entration
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012		22	3.091	1.1743	1.2255
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012		24	3.1781	1.731	1.731
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012		9	2.1972	0.8926	0.8926
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012		9	2.1972	0.8993	0.8993
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012		14	2.6391	1.1748	1.1748

						shannon_	shannon_	shannon_	shannon_		shannon_	shannon_
			shannon_	shannon_	shannon_	diversity_s	diversity_s	diversity_s	diversity_s	shannon_	diversity_s	diversity_s
			diversity_s	diversity_s	diversity_s	tandard_al	tandard_a	mall_sam	mall_sam	diversity_s	mall_sam	mall_sam
	cust		tandard_al	tandard_al	tandard_al	gal_bioma	nimal_bio	ple_algal_	ple_algal_	mall_sam	ple_algal_t	ple_algal_t
	ome		gal_total_	gal_total_v	gal_total_	ss_concen	mass_con	concentrat	cell_conce	ple_algal_t	otal_volu	otal_biovol
tracking_id	r_id	job_id	area	olume	biovolume	tration	centration	ion	ntration	otal_area	me	ume
120001-282	282	1	2.2931	2.1716	2.1716	2.1717	0	1.0926	1.1457	2.2927	2.1712	2.1712
120002-282	282	1	2.3431	2.2402	2.2402	2.2402	0	1.6161	1.6161	2.3427	2.2398	2.2398
120003-282	282	1	1.1146	1.2059	1.2059	1.2056	0	0.8495	0.8495	1.1142	1.2051	1.2051
120004-282	282	1	1.1828	1.2704	1.2704	1.2698	0	0.8575	0.8575	1.1824	1.2698	1.2698
120005-282	282	1	2.132	2.3818	2.3818	2.3816	0	1.105	1.105	2.1313	2.3809	2.3809

			shannon_	shannon_									
			diversity_s	diversity_s	5								eveness_
			mall_sam	mall_sam							mcintosh_	mcintosh_	based_sh
			ple_algal_	ple_anima	n m	ncintosh_	mcintosh_		mcintosh_	mcintosh_	u_algal_bi	u_animal_	annon_sta
	cust		biomass_c	I_biomass	u,	_algal_co	u_algal_ce	mcintosh_	u_algal_to	u_algal_to	omass_co	biomass_c	ndard_alg
	ome		oncentrati	_concentr	n	centratio	Il_concent	u_algal_to	tal_volum	tal_biovolu	ncentratio	oncentrati	al_concen
tracking_id	r_id	job_id	on	ation	n	1	ration	tal_area	е	me	n	on	tration
120001-282	282	1	0.8678	C	)	163547	164054	1.33E+09	1.74E+09	1.74E+09	1737	0	0.3799
120002-282	282	1	0.1059	C	)	60573	60573	1.76E+09	2.28E+09	2.28E+09	2281	0	0.5447
120003-282	282	1	0.0867	C	)	90376	90376	7.96E+08	2.17E+08	2.17E+08	217	0	0.4062
120004-282	282	1	0.3931	C	)	97816	97816	1.13E+09	3.51E+08	3.51E+08	351	0	0.4093
120005-282	282	1	1.3168	C	)	79990	79990	3.25E+08	96124449	96124449	96	0	0.4452

			eveness_				eveness_	eveness_		eveness_		
			based_sh	eveness_	eveness_	eveness_	based_sh	based_sh	eveness_	based_sh	eveness_	eveness_
			annon_sta	based_sh	based_sh	based_sh	annon_sta	annon_sta	based_sh	annon_sm	based_sh	based_sh
			ndard_alg	annon_sta	annon_sta	annon_sta	ndard_alg	ndard_ani	annon_sm	all_sample	annon_sm	annon_sm
	cust		al_cell_co	ndard_alg	ndard_alg	ndard_alg	al_biomas	mal_biom	all_sample	_algal_cell	all_sample	all_sample
	ome		ncentratio	al_total_ar	al_total_vo	al_total_bi	s_concent	ass_conce	_algal_con	_concentr	_algal_tot	_algal_tot
tracking_id	r_id	job_id	n	ea	lume	ovolume	ration	ntration	centration	ation	al_area	al_volume
120001-282	282	1	0.3965	0.7419	0.7025	0.7025	0.7026	0	0.2887	0.3027	0.6059	0.5737
120002-282	282	1	0.5447	0.7373	0.7049	0.7049	0.7049	0	0.4175	0.4175	0.6052	0.5786
120003-282	282	1	0.4062	0.5073	0.5488	0.5488	0.5487	0	0.2939	0.2939	0.3855	0.4169
120004-282	282	1	0.4093	0.5383	0.5782	0.5782	0.5779	0	0.2967	0.2967	0.4091	0.4393
120005-282	282	1	0.4452	0.8078	0.9025	0.9025	0.9025	0	0.3316	0.3316	0.6396	0.7145

					eveness_		verietien				verietien	verietien
			eveness_	eveness_	based_sn		variation_				variation_	variation_
			based_sh	based_sh	annon_sm	variation_	based_sh	variation_	variation_	variation_	based_sh	based_sh
			annon_sm	annon_sm	all_sample	based_sh	annon_sta	based_sh	based_sh	based_sh	annon_sta	annon_sta
			all_sample	all_sample	_animal_b	annon_sta	ndard_alg	annon_sta	annon_sta	annon_sta	ndard_alg	ndard_ani
	cust		_algal_tot	_algal_bio	iomass_co	ndard_alg	al_cell_co	ndard_alg	ndard_alg	ndard_alg	al_biomas	mal_biom
	ome		al_biovolu	mass_con	ncentratio	al_concen	ncentratio	al_total_ar	al_total_vo	al_total_bi	s_concent	ass_conce
tracking_id	r_id	job_id	me	centration	n	tration	n	ea	lume	ovolume	ration	ntration
120001-282	282	1	0.5737	0.2293	0	3.9232	3.9949	6.3367	5.9554	5.9554	-4334.036	0
120002-282	282	1	0.5786	0.0273	0	5.1821	5.1821	6.6266	6.2258	6.2258	-3729.745	0
120003-282	282	1	0.4169	0.03	0	1.9498	1.9498	2.7558	3.0316	3.0316	-9923.72	0
120004-282	282	1	0.4393	0.136	0	2.0371	2.0371	2.6831	2.8513	2.8513	-9573.112	0
120005-282	282	1	0.7145	0.3952	0	3.1741	3.1741	5.5046	6.0481	6.0481	-16681.16	0

	•	•
100	10	100
		102
1110		100
-	-	

								berger_pa	berger_pa		margalef_	
			berger_pa	berger_pa	berger_pa	berger_pa	berger_pa	rker_algal	rker_anim	margalef_	diversity_a	margalef_
	cust		rker_algal	rker_algal	rker_algal	rker_algal	rker_algal	_biomass	al_biomas	diversity_a	lgal_cell_c	diversity_a
	ome		_concentr	_cell_conc	_total_are	_total_volu	_total_biov	_concentr	s_concent	lgal_conce	oncentrati	lgal_total_
tracking_id	r_id	job_id	ation	entration	а	me	olume	ation	ration	ntration	on	area
120001-282	282	1	1.3525	1.385	3.2479	2.7286	2.7286	2.7288	0	3.3368	3.3243	1.8336
120002-282	282	1	1.7924	1.7924	4.2377	3.1143	3.1143	3.1145	0	3.8033	3.8033	1.9778
120003-282	282	1	1.4146	1.4146	1.4412	1.4672	1.4672	1.4671	0	1.3319	1.3319	0.7556
120004-282	282	1	1.3841	1.3841	1.55	1.6468	1.6468	1.6465	0	1.3255	1.3255	0.7393
120005-282	282	1	1.4493	1.4493	2.7428	5.5566	5.5566	5.5595	0	2.1698	2.1698	1.2195

					margalef_	margalef_		simpson_				simpson_
			margalef_	margalef_	diversity_a	diversity_a	simpson_	diversity_a	simpson_	simpson_	simpson_	diversity_a
	cust		diversity_a	diversity_a	lgal_biom	nimal_bio	diversity_a	lgal_cell_c	diversity_a	diversity_a	diversity_a	lgal_biom
	ome		lgal_total_	lgal_total_	ass_conce	mass_con	lgal_conce	oncentrati	lgal_total_	lgal_total_	lgal_total_	ass_conce
tracking_id	r_id	job_id	volume	biovolume	ntration	centration	ntration	on	area	volume	biovolume	ntration
120001-282	282	1	1.8272	1.8272	4.58	0	1.7896	1.8708	6.6862	5.5305	5.5305	5.5312
120002-282	282	1	1.9683	1.9683	4.8141	0	2.9539	2.9539	7.1526	6.1882	6.1882	6.1883
120003-282	282	1	0.8031	0.8031	2.6195	0	1.8239	1.8239	1.9739	2.0672	2.0672	2.0669
120004-282	282	1	0.7782	0.7782	2.3719	0	1.7863	1.7863	2.2112	2.4226	2.4226	2.4218
120005-282	282	1	1.2614	1.2614	3.8256	0	2.0003	2.0003	5.5911	9.3069	9.3069	9.3055

					eveness_				eveness_	eveness_		
				eveness_	based_si	eveness_	eveness_	eveness_	based_si	based_si		
			simpson_	based_si	mpsons_d	based_si	based_si	based_si	mpsons_d	mpsons_d	palmer_w	
			diversity_a	mpsons_d	iversity_al	mpsons_d	mpsons_d	mpsons_d	iversity_al	iversity_an	ater_qualit	
	cust		nimal_bio	iversity_al	gal_cell_c	iversity_al	iversity_al	iversity_al	gal_bioma	imal_biom	y_index_b	alpha_alg
	ome		mass_con	gal_conce	oncentrati	gal_total_	gal_total_v	gal_total_	ss_concen	ass_conce	ased_on_	al_concen
tracking_id	r_id	job_id	centration	ntration	on	area	olume	biovolume	tration	ntration	algae	tration
120001-282	282	1	0	0.0813	0.085	0.3039	0.2514	0.2514	0.2514	0	6	4.6082
120002-282	282	1	0	0.1231	0.1231	0.298	0.2578	0.2578	0.2578	0	5	5.5132
120003-282	282	1	0	0.2027	0.2027	0.2193	0.2297	0.2297	0.2297	0	4	1.6299
120004-282	282	1	0	0.1985	0.1985	0.2457	0.2692	0.2692	0.2691	0	1	1.6195
120005-282	282	1	0	0.1429	0.1429	0.3994	0.6648	0.6648	0.6647	0	3	2.822

			alpha_alg				alpha_alg	alpha_ani
	cust		al_cell_co	alpha_alg	alpha_alg	alpha_alg	al_biomas	mal_biom
	ome		ncentratio	al_total_ar	al_total_vo	al_total_bi	s_concent	ass_conce
tracking_id	r_id	job_id	n	ea	lume	ovolume	ration	ntration
120001-282	282	1	4.5798	2.0493	2.0409	2.0409	8.8201	0
120002-282	282	1	5.5132	2.2153	2.2027	2.2027	9.0694	0
120003-282	282	1	1.6299	0.8359	0.8933	0.8933	5.9067	0
120004-282	282	1	1.6195	0.8164	0.8631	0.8631	4.4521	0
120005-282	282	1	2.822	1.3515	1.4048	1.4048	10.2543	0

											shannon_
	cust										div_stand
	ome		sample							maximum	algal_co
tracking_id	r_id	job_id	_id	system_name	site	station	sample_date	replicate	diatom_richness	div	nc
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012		22	3.09	1 1.1743
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012		23	3.135	5 1.7184
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012		9	2.197	2 0.8926
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012		9	2.197	2 0.8993
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012		14	2.639	1 1.1748

			shannon				shannon_		shannon_	shannon_	shannon_	shannon_	shannon_
			_div_sta	shannon_	shannon_	shannon_	div_stand_	shannon_	div_small_	div_small_	div_small_	div_small_	div_small_
	cust		nd_algal	div_stand_	div_stand_	div_stand_	algal_bio	div_small_	sample_al	sample_al	sample_al	sample_al	sample_al
	ome		_cell_con	algal_total	algal_total	algal_total	mass_con	sample_al	gal_cell_c	gal_total_	gal_total_v	gal_total_	gal_bioma
tracking_id	r_id	job_id	С	_area	_vol	_biovol	С	gal_conc	onc	area	ol	biovol	ss_conc
120001-282	282	1	1.2255	2.2931	2.1716	2.1716	2.1717	1.0926	1.1457	2.2927	2.1712	2.1712	0.8678
120002-282	282	1	1.7184	2.3403	2.2383	2.2383	2.2384	1.6083	1.6083	2.3399	2.2379	2.2379	0.9231
120003-282	282	1	0.8926	1.1146	1.2059	1.2059	1.2056	0.8495	0.8495	1.1142	1.2051	1.2051	0.0867
120004-282	282	1	0.8993	1.1828	1.2704	1.2704	1.2698	0.8575	0.8575	1.1824	1.2698	1.2698	0.3931
120005-282	282	1	1.1748	2.132	2.3818	2.3818	2.3816	1.105	1.105	2.1313	2.3809	2.3809	1.3168

								mcintosh_	even_sha	even_sha	even_sha	even_sha
	cust		mcintosh_	mcintosh_	mcintosh_	mcintosh_	mcintosh_	u_algal_bi	nnon_stan	nnon_stan	nnon_stan	nnon_stan
	ome		u_algal_co	u_algal_ce	u_algal_to	u_algal_to	u_algal_to	omass_co	d_algal_co	d_algal_ce	d_algal_to	d_algal_to
tracking_id	r_id	job_id	nc	ll_conc	tal_area	tal_vol	tal_biovol	nc	nc	ll_conc	tal_area	tal_vol
120001-282	282	1	163547	164054	1.33E+09	1.74E+09	1.74E+09	1737	0.3799	0.3965	0.7419	0.7025
120002-282	282	1	60572	60572	1.76E+09	2.28E+09	2.28E+09	2281	0.5481	0.5481	0.7464	0.7139
120003-282	282	1	90376	90376	7.96E+08	2.17E+08	2.17E+08	217	0.4062	0.4062	0.5073	0.5488
120004-282	282	1	97816	97816	1.13E+09	3.51E+08	3.51E+08	351	0.4093	0.4093	0.5383	0.5782
120005-282	282	1	79990	79990	3.25E+08	96124449	96124449	96	0.4452	0.4452	0.8078	0.9025

										even_sha		
				even_sha		even_sha	even_sha	even_sha	even_sha	nnon_sma		
			even_sha	nnon_stan	even_sha	nnon_sma	nnon_sma	nnon_sma	nnon_sma	Il_sample_	variation_s	variation_s
	cust		nnon_stan	d_algal_bi	nnon_sma	Il_sample_	Il_sample_	Il_sample_	Il_sample_	algal_bio	hannon_st	hannon_st
	ome		d_algal_to	omass_co	Il_sample_	algal_cell_	algal_total	algal_total	algal_total	mass_con	and_algal	and_algal
tracking_id	r_id	job_id	tal_biovol	nc	algal_conc	conc	_area	_vol	_biovol	с	_conc	_cell_conc
120001-282	282	1	0.7025	0.7026	0.2887	0.3027	0.6059	0.5737	0.5737	0.2293	3.9232	3.9949
120002-282	282	1	0.7139	0.7139	0.4201	0.4201	0.6112	0.5845	0.5845	0.2411	5.0996	5.0996
120003-282	282	1	0.5488	0.5487	0.2939	0.2939	0.3855	0.4169	0.4169	0.03	1.9498	1.9498
120004-282	282	1	0.5782	0.5779	0.2967	0.2967	0.4091	0.4393	0.4393	0.136	2.0371	2.0371
120005-282	282	1	0.9025	0.9025	0.3316	0.3316	0.6396	0.7145	0.7145	0.3952	3.1741	3.1741

			variation_s	variation_s	variation_s	variation_s						
			hannon_st	hannon_st	hannon_st	hannon_st			berger_pa	berger_pa	berger_pa	berger_pa
	cust		and_algal	and_algal	and_algal	and_algal	berger_pa	berger_pa	rker_algal	rker_algal	rker_algal	rker_algal
	ome		_total_are	_total_volu	_total_biov	_biomass	rker_algal	rker_algal	_total_are	_total_volu	_total_biov	_biomass
tracking_id	r_id	job_id	а	me	olume	_conc	_conc	_cell_conc	а	me	olume	_conc
120001-282	282	1	6.3367	5.9554	5.9554	-4334.036	1.3525	1.385	3.2479	2.7286	2.7286	2.7288
120002-282	282	1	6.6014	6.2079	6.2079	-3731.498	1.7881	1.7881	4.2359	3.1134	3.1134	3.1138
120003-282	282	1	2.7558	3.0316	3.0316	-9923.72	1.4146	1.4146	1.4412	1.4672	1.4672	1.4671
120004-282	282	1	2.6831	2.8513	2.8513	-9573.112	1.3841	1.3841	1.55	1.6468	1.6468	1.6465
120005-282	282	1	5.5046	6.0481	6.0481	-16681.16	1.4493	1.4493	2.7428	5.5566	5.5566	5.5595

						margalef_	margalef_	margalef_				simpson_
	cust		margalef_	margalef_	margalef_	div_algal_t	div_algal_t	div_algal_	simpson_	simpson_	simpson_	div_algal_t
	ome		div_algal_	div_algal_	div_algal_t	t otal_volu	otal_biovol	biomass_c	div_algal_	div_algal_	div_algal_t	otal_volu
tracking_id	r_id	job_id	conc	cell_conc	otal_area	me	ume	onc	conc	cell_conc	otal_area	me
120001-282	282	1	3.3368	3.3243	1.8336	6 <u>1.8272</u>	1.8272	4.58	1.7896	1.8708	6.6862	5.5305
120002-282	282	1	3.6394	3.6394	1.8919	1.8827	1.8827	4.6051	2.94	2.94	7.1464	6.1849
120003-282	282	1	1.3319	1.3319	0.7556	6 0.8031	0.8031	2.6195	1.8239	1.8239	1.9739	2.0672
120004-282	282	1	1.3255	1.3255	0.7393	3 0.7782	0.7782	2.3719	1.7863	1.7863	2.2112	2.4226
120005-282	282	1	2.1698	2.1698	1.2195	5 1.2614	1.2614	3.8256	2.0003	2.0003	5.5911	9.3069

									even_sim	even_sim	palmer_w	
			simpson_	simpson_	even_sim	even_sim	even_sim	even_sim	psons_div	psons_div	ater_qualit	
	cust		div_algal_t	div_algal_	psons_div	psons_div	psons_div	psons_div	_algal_tot	_algal_bio	y_index_b	pollution_t
	ome		otal_biovol	biomass_c	_algal_con	_algal_cell	_algal_tot	_algal_tot	al_biovolu	mass_con	ased_on_	olerance_
tracking_id	r_id	job_id	ume	onc	С	_conc	al_area	al_volume	me	С	algae	algal_conc
120001-282	282	1	5.5305	5.5312	0.0813	0.085	0.3039	0.2514	0.2514	0.2514	6	2.784
120002-282	282	1	6.1849	6.1852	0.1278	0.1278	0.3107	0.2689	0.2689	0.2689	5	2.6685
120003-282	282	1	2.0672	2.0669	0.2027	0.2027	0.2193	0.2297	0.2297	0.2297	4	2.6891
120004-282	282	1	2.4226	2.4218	0.1985	0.1985	0.2457	0.2692	0.2692	0.2691	1	2.8017
120005-282	282	1	9.3069	9.3055	0.1429	0.1429	0.3994	0.6648	0.6648	0.6647	3	2.8786

								relative_a	relative_a			
						pollution_t	pollution_t	bundance	bundance			
			pollution_t	pollution_t	pollution_t	olerance_	olerance_	_achnanth	_achnanth			siltation_st
	cust		olerance_	olerance_	olerance_	algal_total	algal_bio	es_minutis	es_minutis	siltation_st	siltation_st	and_algal
	ome		algal_cell_	algal_total	algal_total	_biovolum	mass_con	sima_algal	sima_algal	and_algal	and_algal	_total_are
tracking_id	r_id	job_id	conc	_area	_volume	е	С	_conc	_cell_conc	_conc	_cell_conc	а
120001-282	282	1	2.7655	2.4653	2.4029	2.4029	2.4031	0.739372	0.722022	0.016636	0.016245	0.03585
120002-282	282	1	2.6685	2.4771	2.3678	2.3678	2.3679	0.668246	0.668246	0.035545	0.035545	0.0478
120003-282	282	1	2.6891	2.3361	2.1787	2.1787	2.1807	0.923645	0.923645	0.017241	0.017241	0.053149
120004-282	282	1	2.8017	2.7064	2.6845	2.6845	2.6838	0.911483	0.911483	0.014354	0.014354	0.03578
120005-282	282	1	2.8786	2.773	2.7789	2.7789	2.7788	0.825	0.825	0.015	0.015	0.045857

			siltation_st	siltation_st	siltation_st		siltation_in	siltation_in	siltation_in	siltation_in	siltation_in	
	cust		and_algal	and_algal	and_algal	siltation_in	clusive_al	clusive_al	clusive_al	clusive_al	clusive_al	ra_sensitiv
	ome		_total_volu	_total_biov	_biomass	clusive_al	gal_cell_c	gal_total_	gal_total_v	gal_total_	gal_bioma	e_algal_co
tracking_id	r_id	job_id	me	olume	_conc	gal_conc	onc	area	olume	biovolume	ss_conc	nc
120001-282	282	1	0.027076	0.027076	0.027035	0.016636	0.016245	0.03585	0.027076	0.027076	0.027035	0.794824
120002-282	282	1	0.035253	0.035253	0.035188	0.035545	0.035545	0.0478	0.035253	0.035253	0.035188	0.327014
120003-282	282	1	0.070902	0.070902	0.070755	0.017241	0.017241	0.053149	0.070902	0.070902	0.070755	0.221675
120004-282	282	1	0.046171	0.046171	0.046296	0.014354	0.014354	0.03578	0.046171	0.046171	0.046296	0.227273
120005-282	282	1	0.049368	0.049368	0.049482	0.015	0.015	0.045857	0.049368	0.049368	0.049482	0.76

					ra_sensitiv	ra_sensitiv	ra_sensitiv				ra_aberra	ra_aberra
	cust		ra_sensitiv	ra_sensitiv	e_algal_to	e_algal_to	e_algal_bi	ra_aberra	ra_aberra	ra_aberra	nt_algal_t	nt_algal_t
	ome		e_algal_ce	e_algal_to	tal_volum	tal_biovolu	omass_co	nt_algal_c	nt_algal_c	nt_algal_t	otal_volu	otal_biovol
tracking_id	r_id	job_id	ll_conc	tal_area	е	me	nc	onc	ell_conc	otal_area	me	ume
120001-282	282	1	0.776173	0.458388	0.292579	0.292579	0.292695	0	0	0	0	0
120002-282	282	1	0.327014	0.390739	0.308688	0.308688	0.308696	0	0	0	0	0
120003-282	282	1	0.221675	0.156452	0.122647	0.122647	0.123113	0	0	0	0	0
120004-282	282	1	0.227273	0.262836	0.282771	0.282771	0.282579	0	0	0	0	0
120005-282	282	1	0.76	0.679149	0.708473	0.708473	0.708459	0	0	0	0	0

			ra_aberra		generic_a	generic_a	generic_a	generic_a	generic_a		centrales_	centrales_
	cust		nt_algal_bi	generic_a	cc_cmn_al	cc_cmn_al	cc_cmn_al	cc_cmn_al	cc_cmn_al	centrales_	pennales_	pennales_
	ome		omass_co	cc_cmn_al	gal_cell_c	gal_total_	gal_total_v	gal_total_	gal_bioma	pennales_	algal_cell_	algal_total
tracking_id	r_id	job_id	nc	gal_conc	onc	area	olume	biovolume	ss_conc	algal_conc	conc	_area
120001-282	282	1	0	86.4	86.4	3.9647	0.713	0.713	0.7134	0.0074	0.0072	0.1057
120002-282	282	1	0	65.6	65.6	9.4664	2.8928	2.8928	2.894	0.0047	0.0047	0.0358
120003-282	282	1	0	126.3333	126.3333	42.3205	33.2871	33.2871	33.2115	0	0	0
120004-282	282	1	0	0	0	0	0	0	0	0	0	0
120005-282	282	1	0	0	0	0	0	0	0	0	0	0

				centrales_	centrales_							
			centrales_	pennales_	pennales_							
	cust		pennales_	algal_total	algal_bio		alpha_alg	alpha_alg	alpha_alg	alpha_alg	alpha_alg	
	ome		algal_total	_biovolum	mass_con	alpha_alg	al_cell_co	al_total_ar	al_total_vo	al_total_bi	al_biomas	dominanc
tracking_id	r_id	job_id	_volume	е	С	al_conc	nc	ea	lume	ovolume	s_conc	e_conc
120001-282	282	1	0.3665	0.3665	0.3665	4.6082	4.5798	2.0493	2.0409	2.0409	8.8201	0.7394
120002-282	282	1	0.1083	0.1083	0.1083	5.222	5.222	2.114	2.1019	2.1019	8.4975	0.5592
120003-282	282	1	0	0	0	1.6299	1.6299	0.8359	0.8933	0.8933	5.9067	0.7069
120004-282	282	1	0	0	0	1.6195	1.6195	0.8164	0.8631	0.8631	4.4521	0.7225
120005-282	282	1	0	0	0	2.822	2.822	1.3515	1.4048	1.4048	10.2543	0.69

										acidobionti	acidobionti	acidobionti
	cust		dominanc	dominanc	dominanc	dominanc	dominanc	acidobionti	acidobionti	c_species	c_species	c_species
	ome		e_cell_con	e_total_ar	e_total_vol	e_total_bi	e_biomas	c_species	c_species	_total_are	_total_volu	_total_biov
tracking_id	r_id	job_id	С	ea	ume	ovolume	s_conc	_conc	_cell_conc	а	me	olume
120001-282	282	1	0.722	0.3079	0.3665	0.3665	0.3665	0	0	0	0	0
120002-282	282	1	0.5592	0.2361	0.3212	0.3212	0.3212	0	0	0	0	0
120003-282	282	1	0.7069	0.6939	0.6816	0.6816	0.6816	0	0	0	0	0
120004-282	282	1	0.7225	0.6452	0.6072	0.6072	0.6073	0	0	0	0	0
120005-282	282	1	0.69	0.3646	0.18	0.18	0.1799	0	0	0	0	0

			acidobionti			eutraphent	eutraphent	eutraphent	eutraphent	araphid_c	araphid_c	araphid_c
	cust		c_species	eutraphent	eutraphent	ic_species	ic_species	ic_species	ic_species	entrales_i	entrales_i	entrales_i
	ome		_biomass	ic_species	ic_species	_total_are	_total_volu	_total_biov	_biomass	ndex_con	ndex_cell_	ndex_total
tracking_id	r_id	job_id	_conc	_conc	_cell_conc	а	me	olume	_conc	С	conc	_area
120001-282	282	1	0	0.1885	0.2076	0.5778	0.7796	0.7796	0.7795	19	22.25	3.1321
120002-282	282	1	0	0.2441	0.2441	0.5979	0.7287	0.7287	0.7287	20	20	9.5196
120003-282	282	1	0	0.0665	0.0665	0.1374	0.1805	0.1805	0.1802	0	0	0
120004-282	282	1	0	0.0478	0.0478	0.0844	0.0987	0.0987	0.0988	0	0	0
120005-282	282	1	0	0.145	0.145	0.3795	0.5327	0.5327	0.5329	0	0	0

				araphid_c	araphid_c								
			araphid_c	entrales_i	entrales_i								
	cust		entrales_i	ndex_total	ndex_bio								
	ome		ndex_total	_biovolum	mass_con		dib	i_cell_c	dibi_total_	dibi_total_	dibi_total_	dibi_biom	diatom_m
tracking_id	r_id	job_id	_volume	е	С	dibi_con	c ond	C	area	volume	biovolume	ass_conc	etric_conc
120001-282	282	1	0.7046	0.7046	0.7043		0	0	0	0	0	0	) 1
120002-282	282	1	3.9682	3.9682	3.9674		0	0	0	0	0	0	0.9976
120003-282	282	1	0	0	0		0	0	C	0	0	0	) 1
120004-282	282	1	0	0	0		0	0	C	0	0	0	) 1
120005-282	282	1	0	0	0		0	0	0	0	0	0	) 1

						diatom_m						
	cust		diatom_m	diatom_m	diatom_m	etric_total	diatom_m		cyano_me	cyano_me	cyano_me	cyano_me
	ome		etric_cell_	etric_total	etric_total	_biovolum	etric_biom	cyano_me	tric_cell_c	tric_total_	tric_total_v	tric_total_
tracking_id	r_id	job_id	conc	_area	_volume	е	ass_conc	tric_conc	onc	area	olume	biovolume
120001-282	282	1	1	1	1	1	1	0	0	0	0	0
120002-282	282	1	0.9976	0.9996	0.9997	0.9997	0.9997	0.0024	0.0024	0.0004	0.0003	0.0003
120003-282	282	1	1	1	1	1	1	0	0	0	0	0
120004-282	282	1	1	1	1	1	1	0	0	0	0	0
120005-282	282	1	1	1	1	1	1	0	0	0	0	0

	cust		cyano_me			rhopalodia	rhopalodia		
	ome		tric_bioma	centrales_	centrales_	les_nfixer	les_nfixer	stability_c	stability_c
tracking_id	r_id	job_id	ss_conc	conc	cell_conc	_conc	_cell_conc	onc	ell_conc
120001-282	282	1	0	0.0074	0.0072	0	0	0.1405	0.1606
120002-282	282	1	0.0003	0.0047	0.0047	0	0	0.0948	0.0948
120003-282	282	1	0	0	0	0	0	0.0345	0.0345
120004-282	282	1	0	0	0	0	0	0.0239	0.0239
120005-282	282	1	0	0	0	0	0	0.0925	0.0925

# Nygaards

												nygaard_	nygaard_	-	
	cust										nygaard_c	bacillario	euglenop	nygaard_	_C
	ome		sample								hlorophyta	phyta_qu	hyta_quo	ompound	b
tracking_id	r_id	job_id	_id	system_name	site	station	sample_date	replicate	nygaard_cyanophyta_	_quotient	_quotient	otient	tient	_quotien <sup>®</sup>	t
120001-282	282	1	D2							C	) C	0.04545	0	)	0
120002-282	282	1	D1							C	) C	0.04348	0	)	0
120003-282	282	1	D3							C	) C	) 0	0	)	0
120004-282	282	1	D4							C	) C	) 0	0	)	0
120005-282	282	1	D5							C	) C	) 0	0	)	0

Totals Algae

	customer		sample				sample_d			
tracking_id	_id	job_id	_id	system_name	site	station	ate	calculation_type	level_	replicate_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	

								conc						
								entra					algal_cell_	algal_bioma
								tion_			total_biovolu	total_biom	concentrat	ss_concentr
								natur	total_area_sq	total_volume_	me_cubic_um	ass_per_s	ion_per_s	ation_mg_pe
	customer						report_no	al_u	uare_um_per	cubic_um_per	_per_square_	quare_cm	quare_cm	r_square_c
tracking_id	_id	job_id	depth	fraction	biovolume	taxa_level	te	nits_	_square_cm_	_square_cm_	cm_	_	_	m_
120001-282	282	1		0	Yes	Species		541	94165.9195	98019.7089	98019.7089	0	554	0.00009802
120002-282	282	1		0	Yes	Species		423	112304.4959	118822.5618	118822.5618	0	423	0.00011882
120003-282	282	1		0	Yes	Species		406	39627.0296	21200.1776	21200.1776	0	406	0.0000212
120004-282	282	1		0	Yes	Species		418	50069.0384	29159.1292	29159.1292	0	418	0.00002916
120005-282	282	1		0	Yes	Species		400	42618.1251	29910.2523	29910.2523	0	400	0.00002991

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

											concentrat ion_natura	
								repor		customer_r	I_units_pe	
	customer		replicate					t_not		equested_u	r_square_	relative_con
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	division	nits	cm_	centration
120001-282	282	1		0		Yes	Species		Bacillariophyta	NU/sq. cm	541	1
120002-282	282	1		0		Yes	Species		Bacillariophyta	NU/sq. cm	422	0.99763593
120002-282	282	1		0		Yes	Species		Chrysophyta	NU/sq. cm	1	0.00236407
120003-282	282	1		0		Yes	Species		Bacillariophyta	NU/sq. cm	406	1
120004-282	282	1		0		Yes	Species		Bacillariophyta	NU/sq. cm	418	1
120005-282	282	1		0		Yes	Species		Bacillariophyta	NU/sq. cm	400	1

							total_biovolum		total_biom	
			total_area_sq		total_volume_		e_cubic_um_		ass_per_s	relative_to
	customer		uare_um_per	relative_total	cubic_um_per	relative_total	per_square_c	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	_square_cm_	_area	_square_cm_	_volume	m_	_biovolume	_	SS
120001-282	282	1	94165.9195	1	98019.7089	1	98019.7089	1	0	0
120002-282	282	1	112255.5628	0.99956428	118790.3785	0.99972915	118790.3785	0.99972915	0	0
120002-282	282	1	48.9331	0.00043572	32.1833	0.00027085	32.1833	0.00027085	0	0
120003-282	282	1	39627.0296	1	21200.1776	1	21200.1776	1	0	0
120004-282	282	1	50069.0384	1	29159.1292	1	29159.1292	1	0	0
120005-282	282	1	42618.1251	1	29910.2523	1	29910.2523	1	0	0

			algal_cell_		algal_bioma	
			concentrat		ss_concentr	
			ion_per_s	relative_algal	ation_mg_pe	relative_algal
	customer		quare_cm	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_	tration	_	oncentration
120001-282	282	1	554	1	0.00009802	1
120002-282	282	1	422	0.99763593	0.00011879	0.99974752
120002-282	282	1	1	0.00236407	0.0000003	0.00025248
120003-282	282	1	406	1	0.0000212	1
120004-282	282	1	418	1	0.00002916	1
120005-282	282	1	400	1	0.00002991	1

## Class Algae

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

## Class Algae

	customer		replicate					repor t_not		customer_r equested_u	concentrat ion_natura I_units_pe r_square_
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	class_	nits	cm_
120001-282	282	1		0		Yes	Species		Bacillariophyceae	NU/sq. cm	461
120001-282	282	1		0		Yes	Species		Coscinodiscophyceae	NU/sq. cm	4
120001-282	282	1		0		Yes	Species		Fragilariophyceae	NU/sq. cm	76
120002-282	282	1		0		Yes	Species		Bacillariophyceae	NU/sq. cm	379
120002-282	282	1		0		Yes	Species		Chrysophyceae	NU/sq. cm	1
120002-282	282	1		0		Yes	Species		Coscinodiscophyceae	NU/sq. cm	2
120002-282	282	1		0		Yes	Species		Fragilariophyceae	NU/sq. cm	41
120003-282	282	1		0		Yes	Species		Bacillariophyceae	NU/sq. cm	392
120003-282	282	1		0		Yes	Species		Fragilariophyceae	NU/sq. cm	14
120004-282	282	1		0		Yes	Species		Bacillariophyceae	NU/sq. cm	408
120004-282	282	1		0		Yes	Species		Fragilariophyceae	NU/sq. cm	10
120005-282	282	1		0		Yes	Species		Bacillariophyceae	NU/sq. cm	363
120005-282	282	1		0		Yes	Species		Fragilariophyceae	NU/sq. cm	37

## Class Algae

				total_area_s		total_volume		total_biovolu		total_biom
				quare_um_p		_cubic_um_		me_cubic_u		ass_per_s
	customer		relative_con	er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm
tracking_id	_id	job_id	centration	m_	_area	cm_	_volume	re_cm_	_biovolume	_
120001-282	282	1	0.85212569	53040.7993	0.56326959	36786.5639	0.37529762	36786.5639	0.37529762	0
120001-282	282	1	0.00739372	9952.5656	0.1056918	35923.0648	0.36648818	35923.0648	0.36648818	0
120001-282	282	1	0.14048059	31172.5546	0.3310386	25310.0802	0.25821419	25310.0802	0.25821419	0
120002-282	282	1	0.89598109	69183.5098	0.61603509	54538.0668	0.45898747	54538.0668	0.45898747	0
120002-282	282	1	0.00236407	48.9331	0.00043572	32.1833	0.00027085	32.1833	0.00027085	0
120002-282	282	1	0.00472813	4021.2386	0.03580657	12867.9636	0.10829563	12867.9636	0.10829563	0
120002-282	282	1	0.09692671	39050.8144	0.34772263	51384.3481	0.43244606	51384.3481	0.43244606	0
120003-282	282	1	0.96551724	37843.8118	0.95499996	19971.2072	0.94203018	19971.2072	0.94203018	0
120003-282	282	1	0.03448276	1783.2178	0.04500004	1228.9704	0.05796982	1228.9704	0.05796982	0
120004-282	282	1	0.97607656	48711.2424	0.97288152	28395.0942	0.97379774	28395.0942	0.97379774	0
120004-282	282	1	0.02392344	1357.796	0.02711848	764.035	0.02620226	764.035	0.02620226	0
120005-282	282	1	0.9075	34858.4898	0.8179264	25270.6219	0.8448816	25270.6219	0.8448816	0
120005-282	282	1	0.0925	7759.6353	0.1820736	4639.6304	0.1551184	4639.6304	0.1551184	0
Class Algae

			algal_cell_			algal_bioma		
			concentrat				ss_concentr	
			relative_to	ion_per	s	relative_algal	ation_mg_pe	relative_algal
	customer		tal_bioma	quare_c	m.	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	SS	_	t	tration	_	oncentration
120001-282	282	1	0	2	161	0.83212996	0.0000368	0.37543358
120001-282	282	1	0		4	0.00722022	0.00003592	0.36645583
120001-282	282	1	0		89	0.16064982	0.0000253	0.25811059
120002-282	282	1	0	3	379	0.89598109	0.00005454	0.45901363
120002-282	282	1	0		1	0.00236407	0.0000003	0.00025248
120002-282	282	1	0		2	0.00472813	0.00001287	0.1083151
120002-282	282	1	0		41	0.09692671	0.00005138	0.43241878
120003-282	282	1	0	3	392	0.96551724	0.00001997	0.94198113
120003-282	282	1	0		14	0.03448276	0.00000123	0.05801887
120004-282	282	1	0	2	108	0.97607656	0.0000284	0.9739369
120004-282	282	1	0		10	0.02392344	0.00000076	0.0260631
120005-282	282	1	0	3	363	0.9075	0.00002527	0.84486794
120005-282	282	1	0		37	0.0925	0.00000464	0.15513206

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

											concentrat	
											ion_natura	
								repor		customer_r	l_units_pe	
	customer		replicate					t_not		equested_u	r_square_	relative_con
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	order_	nits	cm_	centration
120001-282	282	1		0		Yes	Species		Achnanthales	NU/sq. cm	413	0.76340111
120001-282	282	1		0		Yes	Species		Bacillarales	NU/sq. cm	1	0.00184843
120001-282	282	1		0		Yes	Species		Cymbellales	NU/sq. cm	39	0.07208872
120001-282	282	1		0		Yes	Species		Fragilariales	NU/sq. cm	76	0.14048059
120001-282	282	1		0		Yes	Species		Melosirales	NU/sq. cm	4	0.00739372
120001-282	282	1		0		Yes	Species		Naviculales	NU/sq. cm	8	0.01478743
120002-282	282	1		0		Yes	Species		Achnanthales	NU/sq. cm	321	0.76066351
120002-282	282	1		0		Yes	Species		Bacillarales	NU/sq. cm	3	0.007109
120002-282	282	1		0		Yes	Species		Cymbellales	NU/sq. cm	40	0.09478673
120002-282	282	1		0		Yes	Species		Eunotiales	NU/sq. cm	2	0.00473934
120002-282	282	1		0		Yes	Species		Fragilariales	NU/sq. cm	40	0.09478673
120002-282	282	1		0		Yes	Species		Melosirales	NU/sq. cm	2	0.00473934
120002-282	282	1		0		Yes	Species		Naviculales	NU/sq. cm	12	0.02843602
120002-282	282	1		0		Yes	Species		Tabellariales	NU/sq. cm	1	0.00236967
120002-282	282	1		0		Yes	Species		Thalassiophysales	NU/sq. cm	1	0.00236967
120003-282	282	1		0		Yes	Species		Achnanthales	NU/sq. cm	376	0.92610837
120003-282	282	1		0		Yes	Species		Bacillarales	NU/sq. cm	3	0.00738916
120003-282	282	1		0		Yes	Species		Cymbellales	NU/sq. cm	9	0.02216749
120003-282	282	1		0		Yes	Species		Fragilariales	NU/sq. cm	14	0.03448276
120003-282	282	1		0		Yes	Species		Naviculales	NU/sq. cm	4	0.00985222
120004-282	282	1		0		Yes	Species		Achnanthales	NU/sq. cm	381	0.91148325
120004-282	282	1		0		Yes	Species		Cymbellales	NU/sq. cm	19	0.04545455
120004-282	282	1		0		Yes	Species		Fragilariales	NU/sq. cm	10	0.02392344
120004-282	282	1		0		Yes	Species		Naviculales	NU/sq. cm	7	0.01674641
120004-282	282	1		0		Yes	Species		Thalassiophysales	NU/sq. cm	1	0.00239234
120005-282	282	1		0		Yes	Species		Achnanthales	NU/sq. cm	333	0.8325
120005-282	282	1		0		Yes	Species		Cymbellales	NU/sq. cm	10	0.025
120005-282	282	1		0		Yes	Species		Eunotiales	NU/sq. cm	2	0.005
120005-282	282	1		0		Yes	Species		Fragilariales	NU/sq. cm	37	0.0925
120005-282	282	1		0		Yes	Species		Naviculales	NU/sq. cm	18	0.045

			total_area_s		total_volume		total_biovolu		total_biom	
			quare_um_p		_cubic_um_		me_cubic_u		ass_per_s	relative_to
	customer		er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	m_	_area	cm_	_volume	re_cm_	_biovolume	_	SS
120001-282	282	1	36487.1	0.3874767	22419.0513	0.22871983	22419.0513	0.22871983	0	0
120001-282	282	1	309.6354	0.00328819	160.8495	0.00164099	160.8495	0.00164099	0	0
120001-282	282	1	13177.8695	0.13994309	11713.4949	0.11950143	11713.4949	0.11950143	0	0
120001-282	282	1	31172.5546	0.3310386	25310.0802	0.25821419	25310.0802	0.25821419	0	0
120001-282	282	1	9952.5656	0.1056918	35923.0648	0.36648818	35923.0648	0.36648818	0	0
120001-282	282	1	3066.1944	0.03256161	2493.1682	0.02543538	2493.1682	0.02543538	0	0
120002-282	282	1	47632.1277	0.42431864	34412.7579	0.28969314	34412.7579	0.28969314	0	0
120002-282	282	1	1394.8671	0.01242582	904.7787	0.0076166	904.7787	0.0076166	0	0
120002-282	282	1	14412.443	0.12838957	13539.9676	0.11398202	13539.9676	0.11398202	0	0
120002-282	282	1	1730.4394	0.01541518	2372.5308	0.01997242	2372.5308	0.01997242	0	0
120002-282	282	1	36758.3344	0.32745223	47444.5081	0.39939689	47444.5081	0.39939689	0	0
120002-282	282	1	4021.2386	0.03582218	12867.9636	0.10832497	12867.9636	0.10832497	0	0
120002-282	282	1	3970.9728	0.0353744	3282.9393	0.02763641	3282.9393	0.02763641	0	0
120002-282	282	1	2292.48	0.02042197	3939.84	0.03316632	3939.84	0.03316632	0	0
120002-282	282	1	42.6598	0.00038002	25.0925	0.00021123	25.0925	0.00021123	0	0
120003-282	282	1	33270.8167	0.83959906	16723.6823	0.78884633	16723.6823	0.78884633	0	0
120003-282	282	1	803.2424	0.02027006	518.7397	0.02446865	518.7397	0.02446865	0	0
120003-282	282	1	2466.8715	0.06225224	1744.386	0.08228167	1744.386	0.08228167	0	0
120003-282	282	1	1783.2178	0.04500004	1228.9704	0.05796982	1228.9704	0.05796982	0	0
120003-282	282	1	1302.8812	0.0328786	984.3992	0.04643354	984.3992	0.04643354	0	0
120004-282	282	1	38972.6509	0.77837826	20810.6798	0.71369346	20810.6798	0.71369346	0	0
120004-282	282	1	7481.9696	0.14943306	5876.4604	0.20153072	5876.4604	0.20153072	0	0
120004-282	282	1	1357.796	0.02711848	764.035	0.02620226	764.035	0.02620226	0	0
120004-282	282	1	2080.9912	0.04156244	1563.4575	0.05361811	1563.4575	0.05361811	0	0
120004-282	282	1	175.6307	0.00350777	144.4965	0.00495545	144.4965	0.00495545	0	0
120005-282	282	1	23046.4105	0.54076547	11798.4114	0.39446044	11798.4114	0.39446044	0	0
120005-282	282	1	5027.3447	0.1179626	5019.1651	0.16780751	5019.1651	0.16780751	0	0
120005-282	282	1	2136.1824	0.0501238	5016.093	0.1677048	5016.093	0.1677048	0	0
120005-282	282	1	7759.6353	0.1820736	4639.6304	0.1551184	4639.6304	0.1551184	0	0
120005-282	282	1	4648.5522	0.10907454	3436.9524	0.11490884	3436.9524	0.11490884	0	0

			algal_	cell_		algal_bioma	
			conce	entrat		ss_concentr	
			ion_p	er_s	relative_algal	ation_mg_pe	relative_algal
	customer		quare	_cm	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_		tration	_	oncentration
120001-282	282	1		413	0.74548736	0.00002243	0.22883085
120001-282	282	1		1	0.00180505	0.00000016	0.00163232
120001-282	282	1		39	0.07039711	0.00001172	0.11956744
120001-282	282	1		89	0.16064982	0.0000253	0.25811059
120001-282	282	1		4	0.00722022	0.00003592	0.36645583
120001-282	282	1		8	0.01444043	0.00000249	0.02540298
120002-282	282	1		321	0.76066351	0.00003442	0.28975503
120002-282	282	1		3	0.007109	0.0000009	0.0075764
120002-282	282	1		40	0.09478673	0.00001354	0.11398266
120002-282	282	1		2	0.00473934	0.00000237	0.01995117
120002-282	282	1		40	0.09478673	0.00004744	0.39936022
120002-282	282	1		2	0.00473934	0.00001287	0.10834245
120002-282	282	1		12	0.02843602	0.00000328	0.02761175
120002-282	282	1		1	0.00236967	0.00000394	0.03316778
120002-282	282	1		1	0.00236967	0.0000003	0.00025255
120003-282	282	1		376	0.92610837	0.00001673	0.78915094
120003-282	282	1		3	0.00738916	0.00000052	0.0245283
120003-282	282	1		9	0.02216749	0.00000174	0.08207547
120003-282	282	1		14	0.03448276	0.00000123	0.05801887
120003-282	282	1		4	0.00985222	0.0000098	0.04622642
120004-282	282	1		381	0.91148325	0.00002081	0.71364883
120004-282	282	1		19	0.04545455	0.00000588	0.20164609
120004-282	282	1		10	0.02392344	0.00000076	0.0260631
120004-282	282	1		7	0.01674641	0.00000157	0.05384088
120004-282	282	1		1	0.00239234	0.00000014	0.0048011
120005-282	282	1		333	0.8325	0.00001179	0.39418255
120005-282	282	1		10	0.025	0.00000502	0.16783684
120005-282	282	1		2	0.005	0.00000502	0.16783684
120005-282	282	1		37	0.0925	0.00000464	0.15513206
120005-282	282	1		18	0.045	0.00000344	0.1150117

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

											concentrat	
											ion_natura	
								repor		customer_r	l_units_pe	
	customer		replicate					t_not		equested_u	r_square_	relative_con
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	family	nits	cm_	centration
120001-282	282	1		0		Yes	Species		Achnanthaceae	NU/sq. cm	405	0.74861368
120001-282	282	1		0		Yes	Species		Bacillariaceae	NU/sq. cm	1	0.00184843
120001-282	282	1		0		Yes	Species		Cocconiedaceae	NU/sq. cm	8	0.01478743
120001-282	282	1		0		Yes	Species		Cymbellaceae	NU/sq. cm	19	0.03512015
120001-282	282	1		0		Yes	Species		Fragilariaceae	NU/sq. cm	76	0.14048059
120001-282	282	1		0		Yes	Species		Gomphonemataceae	NU/sq. cm	20	0.03696858
120001-282	282	1		0		Yes	Species		Melosiraceae	NU/sq. cm	4	0.00739372
120001-282	282	1		0		Yes	Species		Naviculaceae	NU/sq. cm	8	0.01478743
120002-282	282	1		0		Yes	Species		Achnanthaceae	NU/sq. cm	282	0.66824645
120002-282	282	1		0		Yes	Species		Bacillariaceae	NU/sq. cm	3	0.007109
120002-282	282	1		0		Yes	Species		Catenulaceae	NU/sq. cm	1	0.00236967
120002-282	282	1		0		Yes	Species		Cocconiedaceae	NU/sq. cm	39	0.09241706
120002-282	282	1		0		Yes	Species		Cymbellaceae	NU/sq. cm	7	0.01658768
120002-282	282	1		0		Yes	Species		Eunotiaceae	NU/sq. cm	2	0.00473934
120002-282	282	1		0		Yes	Species		Fragilariaceae	NU/sq. cm	40	0.09478673
120002-282	282	1		0		Yes	Species		Gomphonemataceae	NU/sq. cm	33	0.07819905
120002-282	282	1		0		Yes	Species		Melosiraceae	NU/sq. cm	2	0.00473934
120002-282	282	1		0		Yes	Species		Naviculaceae	NU/sq. cm	12	0.02843602
120002-282	282	1		0		Yes	Species		Tabellariaceae	NU/sq. cm	1	0.00236967
120003-282	282	1		0		Yes	Species		Achnanthaceae	NU/sq. cm	376	0.92610837
120003-282	282	1		0		Yes	Species		Bacillariaceae	NU/sq. cm	3	0.00738916
120003-282	282	1		0		Yes	Species		Cymbellaceae	NU/sq. cm	3	0.00738916
120003-282	282	1		0		Yes	Species		Fragilariaceae	NU/sq. cm	14	0.03448276
120003-282	282	1		0		Yes	Species		Gomphonemataceae	NU/sq. cm	6	0.01477833
120003-282	282	1		0		Yes	Species		Naviculaceae	NU/sq. cm	4	0.00985222
120004-282	282	1		0		Yes	Species		Achnanthaceae	NU/sq. cm	381	0.91148325
120004-282	282	1		0		Yes	Species		Catenulaceae	NU/sq. cm	1	0.00239234
120004-282	282	1		0		Yes	Species		Cymbellaceae	NU/sq. cm	2	0.00478469
120004-282	282	1		0		Yes	Species		Fragilariaceae	NU/sq. cm	10	0.02392344
120004-282	282	1		0		Yes	Species		Gomphonemataceae	NU/sq. cm	17	0.04066986
120004-282	282	1		0		Yes	Species		Naviculaceae	NU/sq. cm	7	0.01674641

	customer		replicate				repor t_not		customer_r equested_u	concentrat ion_natura I_units_pe r_square_	relative_con
tracking_id	_id	job_id	_	depth fraction	biovolume	taxa_level	е	family	nits	cm_	centration
120005-282	282	1		0	Yes	Species		Achnanthaceae	NU/sq. cm	332	0.83
120005-282	282	1		0	Yes	Species		Cocconiedaceae	NU/sq. cm	1	0.0025
120005-282	282	1		0	Yes	Species		Cymbellaceae	NU/sq. cm	3	0.0075
120005-282	282	1		0	Yes	Species		Eunotiaceae	NU/sq. cm	2	0.005
120005-282	282	1		0	Yes	Species		Fragilariaceae	NU/sq. cm	37	0.0925
120005-282	282	1		0	Yes	Species		Gomphonemataceae	NU/sq. cm	7	0.0175
120005-282	282	1		0	Yes	Species		Naviculaceae	NU/sq. cm	18	0.045

			total_area_s		total_volume		total_biovolu		total_biom	
			quare_um_p		_cubic_um_		me_cubic_u		ass_per_s	relative_to
	customer		er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	m_	_area	cm_	_volume	re_cm_	_biovolume	_	SS
120001-282	282	1	29875.7818	0.31726746	13162.3212	0.13428239	13162.3212	0.13428239	0	0
120001-282	282	1	309.6354	0.00328819	160.8495	0.00164099	160.8495	0.00164099	0	0
120001-282	282	1	6611.3182	0.07020925	9256.7301	0.09443744	9256.7301	0.09443744	0	0
120001-282	282	1	4199.5318	0.04459715	3309.8642	0.03376733	3309.8642	0.03376733	0	0
120001-282	282	1	31172.5546	0.3310386	25310.0802	0.25821419	25310.0802	0.25821419	0	0
120001-282	282	1	8978.3377	0.09534594	8403.6307	0.08573409	8403.6307	0.08573409	0	0
120001-282	282	1	9952.5656	0.1056918	35923.0648	0.36648818	35923.0648	0.36648818	0	0
120001-282	282	1	3066.1944	0.03256161	2493.1682	0.02543538	2493.1682	0.02543538	0	0
120002-282	282	1	31037.733	0.27649171	19103.7018	0.1608186	19103.7018	0.1608186	0	0
120002-282	282	1	1394.8671	0.01242582	904.7787	0.0076166	904.7787	0.0076166	0	0
120002-282	282	1	42.6598	0.00038002	25.0925	0.00021123	25.0925	0.00021123	0	0
120002-282	282	1	16594.3947	0.14782693	15309.0561	0.12887455	15309.0561	0.12887455	0	0
120002-282	282	1	3639.0391	0.03241745	5428.9408	0.04570186	5428.9408	0.04570186	0	0
120002-282	282	1	1730.4394	0.01541518	2372.5308	0.01997242	2372.5308	0.01997242	0	0
120002-282	282	1	36758.3344	0.32745223	47444.5081	0.39939689	47444.5081	0.39939689	0	0
120002-282	282	1	10773.4039	0.09597212	8111.0268	0.06828017	8111.0268	0.06828017	0	0
120002-282	282	1	4021.2386	0.03582218	12867.9636	0.10832497	12867.9636	0.10832497	0	0
120002-282	282	1	3970.9728	0.0353744	3282.9393	0.02763641	3282.9393	0.02763641	0	0
120002-282	282	1	2292.48	0.02042197	3939.84	0.03316632	3939.84	0.03316632	0	0
120003-282	282	1	33270.8167	0.83959906	16723.6823	0.78884633	16723.6823	0.78884633	0	0
120003-282	282	1	803.2424	0.02027006	518.7397	0.02446865	518.7397	0.02446865	0	0
120003-282	282	1	722.7837	0.01823966	543.6756	0.02564486	543.6756	0.02564486	0	0
120003-282	282	1	1783.2178	0.04500004	1228.9704	0.05796982	1228.9704	0.05796982	0	0
120003-282	282	1	1744.0878	0.04401258	1200.7104	0.05663681	1200.7104	0.05663681	0	0
120003-282	282	1	1302.8812	0.0328786	984.3992	0.04643354	984.3992	0.04643354	0	0
120004-282	282	1	38972.6509	0.77837826	20810.6798	0.71369346	20810.6798	0.71369346	0	0
120004-282	282	1	175.6307	0.00350777	144.4965	0.00495545	144.4965	0.00495545	0	0
120004-282	282	1	557.359	0.01113181	474.5898	0.01627586	474.5898	0.01627586	0	0
120004-282	282	1	1357.796	0.02711848	764.035	0.02620226	764.035	0.02620226	0	0
120004-282	282	1	6924.6106	0.13830125	5401.8706	0.18525487	5401.8706	0.18525487	0	0
120004-282	282	1	2080.9912	0.04156244	1563.4575	0.05361811	1563.4575	0.05361811	0	0

			total_area_s		total_volume		total_biovolu		total_biom	
			quare_um_p		_cubic_um_		me_cubic_u		ass_per_s	relative_to
	customer		er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	m_	_area	cm_	_volume	re_cm_	_biovolume	_	SS
120005-282	282	1	21475.6142	0.503908	8970.978	0.29992987	8970.978	0.29992987	0	0
120005-282	282	1	1570.7963	0.03685747	2827.4334	0.09453058	2827.4334	0.09453058	0	0
120005-282	282	1	991.1286	0.02325603	780.561	0.02609677	780.561	0.02609677	0	0
120005-282	282	1	2136.1824	0.0501238	5016.093	0.1677048	5016.093	0.1677048	0	0
120005-282	282	1	7759.6353	0.1820736	4639.6304	0.1551184	4639.6304	0.1551184	0	0
120005-282	282	1	4036.2161	0.09470656	4238.6041	0.14171074	4238.6041	0.14171074	0	0
120005-282	282	1	4648.5522	0.10907454	3436.9524	0.11490884	3436.9524	0.11490884	0	0

			algal_ce	ell_		algal_bioma	
			concent	rat		ss_concentr	
			ion_per_	S	relative_algal	ation_mg_pe	relative_algal
	customer		quare_c	m	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_		tration		oncentration
120001-282	282	1	4	05	0.73104693	0.00001316	0.13425831
120001-282	282	1		1	0.00180505	0.00000016	0.00163232
120001-282	282	1		8	0.01444043	0.00000927	0.09457254
120001-282	282	1		19	0.03429603	0.00000331	0.03376862
120001-282	282	1		89	0.16064982	0.0000253	0.25811059
120001-282	282	1		20	0.03610108	0.00000841	0.08579882
120001-282	282	1		4	0.00722022	0.00003592	0.36645583
120001-282	282	1		8	0.01444043	0.00000249	0.02540298
120002-282	282	1	2	82	0.66824645	0.00001911	0.16087213
120002-282	282	1		3	0.007109	0.0000009	0.0075764
120002-282	282	1		1	0.00236967	0.0000003	0.00025255
120002-282	282	1		39	0.09241706	0.00001531	0.1288829
120002-282	282	1		7	0.01658768	0.00000543	0.04571092
120002-282	282	1		2	0.00473934	0.00000237	0.01995117
120002-282	282	1		40	0.09478673	0.00004744	0.39936022
120002-282	282	1		33	0.07819905	0.00000811	0.06827174
120002-282	282	1		2	0.00473934	0.00001287	0.10834245
120002-282	282	1		12	0.02843602	0.00000328	0.02761175
120002-282	282	1		1	0.00236967	0.00000394	0.03316778
120003-282	282	1	3	76	0.92610837	0.00001673	0.78915094
120003-282	282	1		3	0.00738916	0.00000052	0.0245283
120003-282	282	1		3	0.00738916	0.00000054	0.0254717
120003-282	282	1		14	0.03448276	0.00000123	0.05801887
120003-282	282	1		6	0.01477833	0.0000012	0.05660377
120003-282	282	1		4	0.00985222	0.00000098	0.04622642
120004-282	282	1	3	81	0.91148325	0.00002081	0.71364883
120004-282	282	1		1	0.00239234	0.00000014	0.0048011
120004-282	282	1		2	0.00478469	0.00000047	0.01611797
120004-282	282	1		10	0.02392344	0.00000076	0.0260631
120004-282	282	1		17	0.04066986	0.00000541	0.18552812
120004-282	282	1		7	0.01674641	0.00000157	0.05384088

			algal_cell_		algal_bioma	
			concentrat		ss_concentr	
			ion_per_s	relative_algal	ation_mg_pe	relative_algal
	customer		quare_cm	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_	tration	_	oncentration
120005-282	282	1	332	0.83	0.00000896	0.29956536
120005-282	282	1	1	0.0025	0.00000283	0.09461718
120005-282	282	1	3	0.0075	0.00000078	0.02607823
120005-282	282	1	2	0.005	0.00000502	0.16783684
120005-282	282	1	37	0.0925	0.00000464	0.15513206
120005-282	282	1	7	0.0175	0.00000424	0.14175861
120005-282	282	1	18	0.045	0.00000344	0.1150117

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

											concentrat	
											ion_natura	
								repor		customer	_r I_units_pe	
	customer		replicate					t_not		equested	_u r_square_	relative_con
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	genus	nits	cm_	centration
120001-282	282	1		0		Yes	Species		Achnanthes	NU/sq. cr	n 405	0.74861368
120001-282	282	1		0		Yes	Species		Cocconeis	NU/sq. cr	n 8	0.01478743
120001-282	282	1		0		Yes	Species		Cymbella	NU/sq. cr	n 19	0.03512015
120001-282	282	1		0		Yes	Species		Diatoma	NU/sq. cr	n 2	0.00369686
120001-282	282	1		0		Yes	Species		Fragilaria	NU/sq. cr	n 68	0.12569316
120001-282	282	1		0		Yes	Species		Gomphonema	NU/sq. cr	n 20	0.03696858
120001-282	282	1		0		Yes	Species		Melosira	NU/sq. cr	n 4	0.00739372
120001-282	282	1		0		Yes	Species		Navicula	NU/sq. cr	n 8	0.01478743
120001-282	282	1		0		Yes	Species		Nitzschia	NU/sq. cr	n 1	0.00184843
120001-282	282	1		0		Yes	Species		Synedra	NU/sq. cr	n 6	0.01109057
120002-282	282	1		0		Yes	Species		Achnanthes	NU/sq. cr	n 282	0.66824645
120002-282	282	1		0		Yes	Species		Amphora	NU/sq. cr	n 1	0.00236967
120002-282	282	1		0		Yes	Species		Cocconeis	NU/sq. cr	n 39	0.09241706
120002-282	282	1		0		Yes	Species		Cymbella	NU/sq. cr	n 7	0.01658768
120002-282	282	1		0		Yes	Species		Diatoma	NU/sq. cr	n 2	0.00473934
120002-282	282	1		0		Yes	Species		Eunotia	NU/sq. cr	n 2	0.00473934
120002-282	282	1		0		Yes	Species		Fragilaria	NU/sq. cr	n 29	0.06872038
120002-282	282	1		0		Yes	Species		Gomphonema	NU/sq. cr	n 33	0.07819905
120002-282	282	1		0		Yes	Species		Melosira	NU/sq. cr	n 2	0.00473934
120002-282	282	1		0		Yes	Species		Meridion	NU/sq. cr	n 1	0.00236967
120002-282	282	1		0		Yes	Species		Navicula	NU/sq. cr	n 12	0.02843602
120002-282	282	1		0		Yes	Species		Nitzschia	NU/sq. cr	n 3	0.007109
120002-282	282	1		0		Yes	Species		Synedra	NU/sq. cr	n 8	0.01895735
120002-282	282	1		0		Yes	Species		Tabellaria	NU/sq. cr	n 1	0.00236967
120003-282	282	1		0		Yes	Species		Achnanthes	NU/sq. cr	n 376	0.92610837
120003-282	282	1		0		Yes	Species		Cymbella	NU/sq. cr	n 3	0.00738916
120003-282	282	1		0		Yes	Species		Fragilaria	NU/sq. cr	n 14	0.03448276
120003-282	282	1		0		Yes	Species		Gomphonema	NU/sq. cr	n 6	0.01477833
120003-282	282	1		0		Yes	Species		Navicula	NU/sq. cr	n 4	0.00985222
120003-282	282	1		0		Yes	Species		Nitzschia	NU/sq. cr	n 3	0.00738916
120004-282	282	1		0		Yes	Species		Achnanthes	NU/sq. cr	n 381	0.91148325

											concentrat	
											ion_natura	
								repor		customer_r	I_units_pe	
	customer		replicate					t_not		equested_u	ı r_square_	relative_con
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	genus	nits	cm_	centration
120004-282	282	1		0		Yes	Species		Amphora	NU/sq. cm	1	0.00239234
120004-282	282	1		0		Yes	Species		Anomoeoneis	NU/sq. cm	1	0.00239234
120004-282	282	1		0		Yes	Species		Cymbella	NU/sq. cm	2	0.00478469
120004-282	282	1		0		Yes	Species		Fragilaria	NU/sq. cm	10	0.02392344
120004-282	282	1		0		Yes	Species		Gomphonema	NU/sq. cm	17	0.04066986
120004-282	282	1		0		Yes	Species		Navicula	NU/sq. cm	6	0.01435407
120005-282	282	1		0		Yes	Species		Achnanthes	NU/sq. cm	332	0.83
120005-282	282	1		0		Yes	Species		Anomoeoneis	NU/sq. cm	12	0.03
120005-282	282	1		0		Yes	Species		Cocconeis	NU/sq. cm	1	0.0025
120005-282	282	1		0		Yes	Species		Cymbella	NU/sq. cm	3	0.0075
120005-282	282	1		0		Yes	Species		Eunotia	NU/sq. cm	2	0.005
120005-282	282	1		0		Yes	Species		Fragilaria	NU/sq. cm	36	0.09
120005-282	282	1		0		Yes	Species		Gomphonema	NU/sq. cm	7	0.0175
120005-282	282	1		0		Yes	Species		Navicula	NU/sq. cm	6	0.015
120005-282	282	1		0		Yes	Species		Synedra	NU/sq. cm	1	0.0025

			total_area_s		total_volume		total_biovolu		total_biom	
			quare_um_p		_cubic_um_		me_cubic_u		ass_per_s	relative_to
	customer		er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	m_	_area	cm_	_volume	re_cm_	_biovolume	_	SS
120001-282	282	1	29875.7818	0.31726746	13162.3212	0.13428239	13162.3212	0.13428239	0	0
120001-282	282	1	6611.3182	0.07020925	9256.7301	0.09443744	9256.7301	0.09443744	0	0
120001-282	282	1	4199.5318	0.04459715	3309.8642	0.03376733	3309.8642	0.03376733	0	0
120001-282	282	1	288.5238	0.00306399	190.2046	0.00194047	190.2046	0.00194047	0	0
120001-282	282	1	22488.2548	0.23881522	15228.0356	0.15535687	15228.0356	0.15535687	0	0
120001-282	282	1	8978.3377	0.09534594	8403.6307	0.08573409	8403.6307	0.08573409	0	0
120001-282	282	1	9952.5656	0.1056918	35923.0648	0.36648818	35923.0648	0.36648818	0	0
120001-282	282	1	3066.1944	0.03256161	2493.1682	0.02543538	2493.1682	0.02543538	0	0
120001-282	282	1	309.6354	0.00328819	160.8495	0.00164099	160.8495	0.00164099	0	0
120001-282	282	1	8395.776	0.08915939	9891.84	0.10091685	9891.84	0.10091685	0	0
120002-282	282	1	31037.733	0.27649171	19103.7018	0.1608186	19103.7018	0.1608186	0	0
120002-282	282	1	42.6598	0.00038002	25.0925	0.00021123	25.0925	0.00021123	0	0
120002-282	282	1	16594.3947	0.14782693	15309.0561	0.12887455	15309.0561	0.12887455	0	0
120002-282	282	1	3639.0391	0.03241745	5428.9408	0.04570186	5428.9408	0.04570186	0	0
120002-282	282	1	321.699	0.00286577	231.6234	0.00194985	231.6234	0.00194985	0	0
120002-282	282	1	1730.4394	0.01541518	2372.5308	0.01997242	2372.5308	0.01997242	0	0
120002-282	282	1	10456.8297	0.093152	7516.3376	0.06327396	7516.3376	0.06327396	0	0
120002-282	282	1	10773.4039	0.09597212	8111.0268	0.06828017	8111.0268	0.06828017	0	0
120002-282	282	1	4021.2386	0.03582218	12867.9636	0.10832497	12867.9636	0.10832497	0	0
120002-282	282	1	770.2057	0.00686118	321.6991	0.00270812	321.6991	0.00270812	0	0
120002-282	282	1	3970.9728	0.0353744	3282.9393	0.02763641	3282.9393	0.02763641	0	0
120002-282	282	1	1394.8671	0.01242582	904.7787	0.0076166	904.7787	0.0076166	0	0
120002-282	282	1	25209.6	0.22457328	39374.848	0.33146496	39374.848	0.33146496	0	0
120002-282	282	1	2292.48	0.02042197	3939.84	0.03316632	3939.84	0.03316632	0	0
120003-282	282	1	33270.8167	0.83959906	16723.6823	0.78884633	16723.6823	0.78884633	0	0
120003-282	282	1	722.7837	0.01823966	543.6756	0.02564486	543.6756	0.02564486	0	0
120003-282	282	1	1783.2178	0.04500004	1228.9704	0.05796982	1228.9704	0.05796982	0	0
120003-282	282	1	1744.0878	0.04401258	1200.7104	0.05663681	1200.7104	0.05663681	0	0
120003-282	282	1	1302.8812	0.0328786	984.3992	0.04643354	984.3992	0.04643354	0	0
120003-282	282	1	803.2424	0.02027006	518.7397	0.02446865	518.7397	0.02446865	0	0
120004-282	282	1	38972.6509	0.77837826	20810.6798	0.71369346	20810.6798	0.71369346	0	0

			total_area_s		total_volume		total_biovolu		total_biom	
			quare_um_p		_cubic_um_		me_cubic_u		ass_per_s	relative_to
	customer		er_square_c	relative_total	per_square_	relative_total	m_per_squa	relative_total	quare_cm	tal_bioma
tracking_id	_id	job_id	m_	_area	cm_	_volume	re_cm_	_biovolume	_	SS
120004-282	282	1	175.6307	0.00350777	144.4965	0.00495545	144.4965	0.00495545	0	0
120004-282	282	1	289.5292	0.0057826	217.1469	0.00744696	217.1469	0.00744696	0	0
120004-282	282	1	557.359	0.01113181	474.5898	0.01627586	474.5898	0.01627586	0	0
120004-282	282	1	1357.796	0.02711848	764.035	0.02620226	764.035	0.02620226	0	0
120004-282	282	1	6924.6106	0.13830125	5401.8706	0.18525487	5401.8706	0.18525487	0	0
120004-282	282	1	1791.462	0.03577984	1346.3106	0.04617115	1346.3106	0.04617115	0	0
120005-282	282	1	21475.6142	0.503908	8970.978	0.29992987	8970.978	0.29992987	0	0
120005-282	282	1	2694.2304	0.06321795	1960.3536	0.06554119	1960.3536	0.06554119	0	0
120005-282	282	1	1570.7963	0.03685747	2827.4334	0.09453058	2827.4334	0.09453058	0	0
120005-282	282	1	991.1286	0.02325603	780.561	0.02609677	780.561	0.02609677	0	0
120005-282	282	1	2136.1824	0.0501238	5016.093	0.1677048	5016.093	0.1677048	0	0
120005-282	282	1	7452.4353	0.1748654	4410.2544	0.14744959	4410.2544	0.14744959	0	0
120005-282	282	1	4036.2161	0.09470656	4238.6041	0.14171074	4238.6041	0.14171074	0	0
120005-282	282	1	1954.3218	0.04585659	1476.5988	0.04936765	1476.5988	0.04936765	0	0
120005-282	282	1	307.2	0.0072082	229.376	0.00766881	229.376	0.00766881	0	0

			algal_cell_		algal_bioma	
			concentrat		ss_concentr	
			ion_per_s	relative_algal	ation_mg_pe	relative_algal
	customer		quare_cm	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_	tration		oncentration
120001-282	282	1	405	0.73104693	0.00001316	0.13425831
120001-282	282	1	8	0.01444043	0.00000927	0.09457254
120001-282	282	1	19	0.03429603	0.00000331	0.03376862
120001-282	282	1	2	0.00361011	0.00000019	0.00193838
120001-282	282	1	81	0.14620939	0.00001522	0.15527443
120001-282	282	1	20	0.03610108	0.00000841	0.08579882
120001-282	282	1	4	0.00722022	0.00003592	0.36645583
120001-282	282	1	8	0.01444043	0.00000249	0.02540298
120001-282	282	1	1	0.00180505	0.00000016	0.00163232
120001-282	282	1	6	0.01083032	0.00000989	0.10089778
120002-282	282	1	282	0.66824645	0.00001911	0.16087213
120002-282	282	1	1	0.00236967	0.0000003	0.00025255
120002-282	282	1	39	0.09241706	0.00001531	0.1288829
120002-282	282	1	7	0.01658768	0.00000543	0.04571092
120002-282	282	1	2	0.00473934	0.0000023	0.00193619
120002-282	282	1	2	0.00473934	0.00000237	0.01995117
120002-282	282	1	29	0.06872038	0.00000752	0.06330499
120002-282	282	1	33	0.07819905	0.00000811	0.06827174
120002-282	282	1	2	0.00473934	0.00001287	0.10834245
120002-282	282	1	1	0.00236967	0.0000032	0.00269383
120002-282	282	1	12	0.02843602	0.00000328	0.02761175
120002-282	282	1	3	0.007109	0.0000009	0.0075764
120002-282	282	1	8	0.01895735	0.00003937	0.3314252
120002-282	282	1	1	0.00236967	0.00000394	0.03316778
120003-282	282	1	376	0.92610837	0.00001673	0.78915094
120003-282	282	1	3	0.00738916	0.00000054	0.0254717
120003-282	282	1	14	0.03448276	0.00000123	0.05801887
120003-282	282	1	6	0.01477833	0.0000012	0.05660377
120003-282	282	1	4	0.00985222	0.00000098	0.04622642
120003-282	282	1	3	0.00738916	0.00000052	0.0245283
120004-282	282	1	381	0.91148325	0.00002081	0.71364883

			algal_cell_ concentrat		algal_bioma ss concentr	
			ion_per_s	relative_algal	ation_mg_pe	relative_algal
	customer		quare_cm	_cell_concen	r_square_cm	_biomass_c
tracking_id	_id	job_id	_	tration	_	oncentration
120004-282	282	1	1	0.00239234	0.00000014	0.0048011
120004-282	282	1	1	0.00239234	0.00000022	0.00754458
120004-282	282	1	2	0.00478469	0.00000047	0.01611797
120004-282	282	1	10	0.02392344	0.00000076	0.0260631
120004-282	282	1	17	0.04066986	0.00000541	0.18552812
120004-282	282	1	6	0.01435407	0.00000135	0.0462963
120005-282	282	1	332	0.83	0.00000896	0.29956536
120005-282	282	1	12	0.03	0.00000196	0.06552992
120005-282	282	1	1	0.0025	0.00000283	0.09461718
120005-282	282	1	3	0.0075	0.0000078	0.02607823
120005-282	282	1	2	0.005	0.00000502	0.16783684
120005-282	282	1	36	0.09	0.00000441	0.14744233
120005-282	282	1	7	0.0175	0.00000424	0.14175861
120005-282	282	1	6	0.015	0.00000148	0.04948178
120005-282	282	1	1	0.0025	0.0000023	0.00768974

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic

	customer		sample				sample_d	receive_dat		
tracking_id	_id	job_id	_id	system_name	site	station	ate	е	calculation_type	level_
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012	Periphyton - Diatom Only	Benthic

								repor					
	customer		replicate					t_not		custom_t			
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	taxa_id	axa_id	organism	habitat	phylum
120001-282	282	1		0		Yes	Species		1013	i	Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1157		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9506		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1095		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9057		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9072		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9397		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9212		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1193		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1160		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9093	i	Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9334		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		10379		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9013		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1161		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1222		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1119		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1000646		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9055		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9218		Algae	Freshwater	
120001-282	282	1		0		Yes	Species		9349	i	Algae	Freshwater	
120001-282	282	1		0		Yes	Species		1061		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9212		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1000755		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1161		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1013		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9397		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9072		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1152		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1477		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9124		Algae	Freshwater	

								repor					
	customer		replicate					t_not		custom_t			
tracking_id	_id	job_	_id _	depth	fraction	biovolume	taxa_level	е	taxa_id	axa_id	organism	habitat	phylum
120002-282	282	1		0		Yes	Species		1331		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1000646		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9506		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1095		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1098		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1140		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1343		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9482		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1653		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1000065		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1201		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9055		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		9771		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1369		Algae	Freshwater	
120002-282	282	1		0		Yes	Species		1193		Algae	Freshwater	
120003-282	282	1		0		Yes	Species		1000755		Algae	Freshwater	
120003-282	282	1		0		Yes	Species		1013		Algae	Freshwater	
120003-282	282	1		0		Yes	Species		9397	i	Algae	Freshwater	
120003-282	282	1		0		Yes	Species		9072		Algae	Freshwater	
120003-282	282	1		0		Yes	Species		1095	i	Algae	Freshwater	
120003-282	282	1		0		Yes	Species		1161	i	Algae	Freshwater	
120003-282	282	1		0		Yes	Species		9123	i	Algae	Freshwater	
120003-282	282	1		0		Yes	Species		9124	i	Algae	Freshwater	
120003-282	282	1		0		Yes	Species		1014		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		9397	i	Algae	Freshwater	
120004-282	282	1		0		Yes	Species		1095		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		1013	i	Algae	Freshwater	
120004-282	282	1		0		Yes	Species		9055		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		1000755		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		9072		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		1161		Algae	Freshwater	
120004-282	282	1		0		Yes	Species		1343		Algae	Freshwater	

								repor					
	customer		replicate					t_not		custom_t			
tracking_id	_id	job_id	_	depth	fraction	biovolume	taxa_level	е	taxa_id	axa_id	organism	habitat	phylum
120004-282	282	1		0		Yes	Species		108384		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9055		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1013		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9397		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		108384		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1152		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9776		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1000755		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1099		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1140		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9057		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		4275		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9072		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		1095		Algae	Freshwater	
120005-282	282	1		0		Yes	Species		9212		Algae	Freshwater	

	customer				subclass			
tracking_id	_id	job_id	division	class_	_	order_	suborder	family
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Cocconiedaceae
120001-282	282	1	Bacillariophyta	Coscinodiscophyceae		Melosirales		Melosiraceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales		Bacillariaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Cocconiedaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Cocconiedaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Cocconiedaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales		Bacillariaceae

	customer				subclass			
tracking_id	_id	job_id	division	class_	_	order_	suborder	family
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Tabellariales		Tabellariaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Eunotiales		Eunotiaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Thalassiophysales		Catenulaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120002-282	282	1	Chrysophyta	Chrysophyceae				
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120002-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120002-282	282	1	Bacillariophyta	Coscinodiscophyceae		Melosirales		Melosiraceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120003-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales		Bacillariaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales		Bacillariaceae
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120004-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Thalassiophysales		Catenulaceae

	customer				subclass			
tracking_id	_id	job_id	division	class_	_	order_	suborder	family
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales		Fragilariaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Eunotiales		Eunotiaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Gomphonemataceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Achnanthaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales		Naviculaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales		Cymbellaceae
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales		Cocconiedaceae

	customer							subspeci		
tracking_id	_id	job_id	subfamily	tribe	genus	subgenus	species	es	variety	form_ morph
120001-282	282	1			Achnanthes		minutissima			
120001-282	282	1			Fragilaria		brevistriata			
120001-282	282	1			Synedra		ulna		ulna	
120001-282	282	1			Cymbella		silesiaca			
120001-282	282	1			Gomphonema		truncatum			
120001-282	282	1			Navicula		cryptotenella			
120001-282	282	1			Fragilaria		capucina		vaucheriae	
120001-282	282	1			Cocconeis		placentula		lineata	
120001-282	282	1			Melosira		varians			
120001-282	282	1			Gomphonema					
120001-282	282	1			Navicula		rhynchocephala			
120001-282	282	1			Achnanthes		delicatula			
120001-282	282	1			Fragilaria		capucina		mesolepta	
120001-282	282	1			Achnanthes		bioreti			
120001-282	282	1			Gomphonema		parvulum			
120001-282	282	1			Nitzschia		gracilis			
120001-282	282	1			Cymbella		sinuata			
120001-282	282	1			Diatoma		moniliformis			
120001-282	282	1			Gomphonema		pumilum			
120001-282	282	1			Cocconeis		placentula		pseudolineata	
120001-282	282	1			Achnanthes		biasolettiana			
120001-282	282	1			Cocconeis		neothumensis			
120002-282	282	1			Cocconeis		placentula		lineata	
120002-282	282	1			Achnanthes		minutissima		robusta	
120002-282	282	1			Gomphonema		parvulum			
120002-282	282	1			Achnanthes		minutissima			
120002-282	282	1			Fragilaria		capucina		vaucheriae	
120002-282	282	1			Navicula		cryptotenella			
120002-282	282	1			Fragilaria		crotonensis			
120002-282	282	1			Synedra		filiformis			
120002-282	282	1			Nitzschia		recta			

	customer							subspeci		
tracking_id	_id	job_id	subfamily	tribe	genus	subgenus	species	es	variety	form_ morph
120002-282	282	1			Tabellaria		fenestrata			
120002-282	282	1			Diatoma		moniliformis			
120002-282	282	1			Synedra		ulna		ulna	
120002-282	282	1			Cymbella		silesiaca			
120002-282	282	1			Cymbella		caespitosa			
120002-282	282	1			Eunotia					
120002-282	282	1			Amphora		pediculus			
120002-282	282	1			Navicula		salinarum			
120002-282	282	1								
120002-282	282	1			Navicula		cryptotenelloides			
120002-282	282	1			Meridion		circulare			
120002-282	282	1			Gomphonema		pumilum			
120002-282	282	1			Synedra		arcus		arcus	
120002-282	282	1			Navicula		pupula			
120002-282	282	1			Melosira		varians			
120003-282	282	1			Achnanthes		minutissima		robusta	
120003-282	282	1			Achnanthes		minutissima			
120003-282	282	1			Fragilaria		capucina		vaucheriae	
120003-282	282	1			Navicula		cryptotenella			
120003-282	282	1			Cymbella		silesiaca			
120003-282	282	1			Gomphonema		parvulum			
120003-282	282	1			Nitzschia		palea			
120003-282	282	1			Nitzschia		recta			
120003-282	282	1			Achnanthes		clevei			
120004-282	282	1			Fragilaria		capucina		vaucheriae	
120004-282	282	1			Cymbella		silesiaca			
120004-282	282	1			Achnanthes		minutissima			
120004-282	282	1			Gomphonema		pumilum			
120004-282	282	1			Achnanthes		minutissima		robusta	
120004-282	282	1			Navicula		cryptotenella			
120004-282	282	1			Gomphonema		parvulum			
120004-282	282	1			Amphora		pediculus			

	customer							subspeci			
tracking_id	_id	job_id	subfamily	tribe	genus	subgenus	species	es	variety	form_	morph
120004-282	282	1			Anomoeoneis		vitrea				
120005-282	282	1			Gomphonema		pumilum				
120005-282	282	1			Achnanthes		minutissima				
120005-282	282	1			Fragilaria		capucina		vaucheriae		
120005-282	282	1			Anomoeoneis		vitrea				
120005-282	282	1			Fragilaria		crotonensis				
120005-282	282	1			Synedra		ulna		acus		
120005-282	282	1			Achnanthes		minutissima		robusta		
120005-282	282	1			Cymbella		cistula				
120005-282	282	1			Eunotia						
120005-282	282	1			Gomphonema		truncatum				
120005-282	282	1			Achnanthes		flexella				
120005-282	282	1			Navicula		cryptotenella				
120005-282	282	1			Cymbella		silesiaca				
120005-282	282	1			Cocconeis		placentula		lineata		

						concentrat				
						ion_natura		total_area_s		total_volume
					customer_r	l_units_pe		quare_um_p		_cubic_um_
	customer				equested_u	r_square_	relative_con	er_square_c	relative_total	per_square_
tracking_id	_id	job_id	coloniality	structure_	nits	cm_	centration	m_	_area	cm_
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	400	0.73937153	28993.12	0.30789398	12457.8
120001-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	2	0.00369686	291.5398	0.00309602	241.2744
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	6	0.01109057	8395.776	0.08915939	9891.84
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	17	0.03142329	3629.9318	0.03854825	2808.1042
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	3	0.00554529	2363.3058	0.02509725	3009.1935
120001-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	7	0.012939	2406.7113	0.0255582	1824.0341
120001-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	53	0.09796673	12249.8794	0.13008825	8612.2138
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	4	0.00739372	3209.3504	0.03408187	4395.0528
120001-282	282	1	Filament	Vegetative	NU/sq. cm	4	0.00739372	9952.5656	0.1056918	35923.0648
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00369686	825.9478	0.0087712	677.9848
120001-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	1	0.00184843	659.4831	0.00700342	669.1341
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00184843	126.669	0.00134517	86.8588
120001-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	13	0.02402957	9946.8356	0.10563095	6374.5474
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00369686	546.8884	0.00580771	514.7186
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	9	0.01663586	3983.3613	0.04230152	3659.6484
120001-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	1	0.00184843	309.6354	0.00328819	160.8495
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00369686	569.6	0.0060489	501.76
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00369686	288.5238	0.00306399	190.2046
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	6	0.01109057	1805.7228	0.01917597	1056.804
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	3	0.00554529	3317.5218	0.0352306	4825.4862
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00369686	209.1044	0.0022206	102.9438
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00184843	84.446	0.00089678	36.1911
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	39	0.09219858	16594.3947	0.14776251	15309.0561
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	236	0.55791962	26501.1716	0.23597605	16996.8144
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	13	0.03073286	4104.1819	0.03654513	3383.6088
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	46	0.10874704	4536.5614	0.04039519	2106.8874
120002-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	7	0.01654846	1238.5415	0.01102842	900.7572
120002-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	6	0.0141844	1773.366	0.01579069	1331.8344
120002-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	22	0.05200946	9218.2882	0.08208298	6615.5804
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00236407	291.84	0.00259865	114.688
120002-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	3	0.0070922	1394.8671	0.0124204	904.7787

						concentrat				
						ion_natura		total_area_s		total_volume
					customer_r	l_units_pe		quare_um_p		_cubic_um_
	customer				equested_u	r_square_	relative_con	er_square_c	relative_total	per_square_
tracking_id	_id	job_id	coloniality	structure_	nits	cm_	centration	m_	_area	cm_
120002-282	282	1	Complex-Filament	Vegetative	NU/sq. cm	1	0.00236407	2292.48	0.02041307	3939.84
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00472813	321.699	0.00286452	231.6234
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	6	0.0141844	23950.08	0.21326021	38154.24
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	4	0.00945626	382.1692	0.00340297	165.0688
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	3	0.0070922	3256.8699	0.02900035	5263.872
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00472813	1730.4394	0.01540846	2372.5308
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00236407	42.6598	0.00037986	25.0925
120002-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	2	0.00472813	760.014	0.00676744	579.0584
120002-282	282	1	Cell-Nonmotile	Cyst	NU/sq. cm	1	0.00236407	48.9331	0.00043572	32.1833
120002-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	3	0.0070922	814.3008	0.00725083	608.0112
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00236407	770.2057	0.00685819	321.6991
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	20	0.04728132	6669.222	0.05938517	4727.418
120002-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00236407	967.68	0.00861657	1105.92
120002-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	1	0.00236407	623.292	0.00555002	764.0353
120002-282	282	1	Filament	Vegetative	NU/sq. cm	2	0.00472813	4021.2386	0.03580657	12867.9636
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	287	0.70689655	27496.322	0.69387795	14449.2733
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	88	0.21674877	5537.2416	0.13973396	2055.6536
120003-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	14	0.03448276	1783.2178	0.04500004	1228.9704
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	4	0.00985222	1302.8812	0.0328786	984.3992
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	3	0.00738916	722.7837	0.01823966	543.6756
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	6	0.01477833	1744.0878	0.04401258	1200.7104
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	2	0.00492611	377.9964	0.00953885	193.0194
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	1	0.00246305	425.246	0.01073121	325.7203
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00246305	237.2531	0.00598715	218.7554
120004-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	10	0.02392344	1357.796	0.02711848	764.035
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00478469	557.359	0.01113181	474.5898
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	79	0.18899522	6669.2511	0.1332011	3104.5104
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	15	0.03588517	6315.063	0.12612711	4996.335
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	302	0.72248804	32303.3998	0.64517716	17706.1694
120004-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	6	0.01435407	1791.462	0.03577984	1346.3106
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.00478469	609.5476	0.01217414	405.5356
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00239234	175.6307	0.00350777	144.4965

						concentrat				
						ion_natura		total_area_s		total_volume
					customer_r	l_units_pe		quare_um_p		_cubic_um_
	customer				equested_u	r_square_	relative_con	er_square_c	relative_total	per_square_
tracking_id	_id	job_id	coloniality	structure_	nits	cm_	centration	m_	_area	cm_
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.00239234	289.5292	0.0057826	217.1469
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	6	0.015	2268.9894	0.05324001	1640.6952
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	276	0.69	15538.0548	0.36458795	5382.828
120005-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	21	0.0525	2917.6098	0.06845937	1810.5234
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	12	0.03	2694.2304	0.06321795	1960.3536
120005-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	15	0.0375	4534.8255	0.10640603	2599.731
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.0025	307.2	0.0072082	229.376
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	54	0.135	5173.524	0.12139258	2703.4776
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.0025	363.955	0.00853991	241.2544
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.005	2136.1824	0.0501238	5016.093
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.0025	1767.2267	0.04146655	2597.9089
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.005	764.0354	0.01792748	884.6724
120005-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	6	0.015	1954.3218	0.04585659	1476.5988
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	2	0.005	627.1736	0.01471612	539.3066
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	1	0.0025	1570.7963	0.03685747	2827.4334
								algal_cell_		algal_bioma
-------------	----------	--------	----------------	---------------	----------------	------------	-------------	-------------	----------------	-------------
				total_biovolu		total_biom		concentrat		ss_concentr
				me_cubic_u		ass_per_s	relative_to	ion_per_s	relative_algal	ation_mg_pe
	customer		relative_total	m_per_squa	relative_total	quare_cm	tal_bioma	quare_cm	_cell_concen	r_square_cm
tracking_id	_id	job_id	_volume	re_cm_	_biovolume	_	SS	_	tration	_
120001-282	282	1	0.12709485	12457.8	0.12709485	0	0	400	0.72202166	0.00001246
120001-282	282	1	0.00246149	241.2744	0.00246149	0	0	2	0.00361011	0.00000024
120001-282	282	1	0.10091685	9891.84	0.10091685	0	0	6	0.01083032	0.00000989
120001-282	282	1	0.02864836	2808.1042	0.02864836	0	0	17	0.03068592	
120001-282	282	1	0.03069988	3009.1935	0.03069988	0	0	3	0.00541516	0.00000301
120001-282	282	1	0.01860885	1824.0341	0.01860885	0	0	7	0.01263538	0.00000182
120001-282	282	1	0.08786206	8612.2138	0.08786206	0	0	53	0.09566787	0.00000861
120001-282	282	1	0.04483846	4395.0528	0.04483846	0	0	4	0.00722022	0.0000044
120001-282	282	1	0.36648818	35923.0648	0.36648818	0	0	4	0.00722022	0.00003592
120001-282	282	1	0.00691682	677.9848	0.00691682	0	0	2	0.00361011	0.0000068
120001-282	282	1	0.00682653	669.1341	0.00682653	0	0	1	0.00180505	0.0000067
120001-282	282	1	0.00088614	86.8588	0.00088614	0	0	1	0.00180505	0.0000009
120001-282	282	1	0.06503332	6374.5474	0.06503332	0	0	26	0.04693141	0.00000637
120001-282	282	1	0.00525117	514.7186	0.00525117	0	0	2	0.00361011	0.00000051
120001-282	282	1	0.03733584	3659.6484	0.03733584	0	0	9	0.01624549	0.00000366
120001-282	282	1	0.00164099	160.8495	0.00164099	0	0	1	0.00180505	0.00000016
120001-282	282	1	0.00511897	501.76	0.00511897	0	0	2	0.00361011	0.0000005
120001-282	282	1	0.00194047	190.2046	0.00194047	0	0	2	0.00361011	0.00000019
120001-282	282	1	0.01078155	1056.804	0.01078155	0	0	6	0.01083032	0.00000106
120001-282	282	1	0.04922975	4825.4862	0.04922975	0	0	3	0.00541516	0.00000483
120001-282	282	1	0.00105024	102.9438	0.00105024	0	0	2	0.00361011	0.0000001
120001-282	282	1	0.00036922	36.1911	0.00036922	0	0	1	0.00180505	0.00000004
120002-282	282	1	0.12883964	15309.0561	0.12883964	0	0	39	0.09219858	0.00001531
120002-282	282	1	0.14304366	16996.8144	0.14304366	0	0	236	0.55791962	0.000017
120002-282	282	1	0.02847615	3383.6088	0.02847615	0	0	13	0.03073286	0.00000338
120002-282	282	1	0.01773137	2106.8874	0.01773137	0	0	46	0.10874704	0.00000211
120002-282	282	1	0.00758069	900.7572	0.00758069	0	0	7	0.01654846	0.0000009
120002-282	282	1	0.0112086	1331.8344	0.0112086	0	0	6	0.0141844	0.00000133
120002-282	282	1	0.05567613	6615.5804	0.05567613	0	0	22	0.05200946	0.00000662
120002-282	282	1	0.0009652	114.688	0.0009652	0	0	1	0.00236407	0.00000011
120002-282	282	1	0.00761454	904.7787	0.00761454	0	0	3	0.0070922	0.0000009

								algal_cell_		algal_bioma
				total_biovolu		total_biom		concentrat		ss_concentr
				me_cubic_u		ass_per_s	relative_to	ion_per_s	relative_algal	ation_mg_pe
	customer		relative_total	m_per_squa	relative_total	quare_cm	tal_bioma	quare_cm	_cell_concen	r_square_cm
tracking_id	_id	job_id	_volume	re_cm_	_biovolume	_	SS	_	tration	_
120002-282	282	1	0.03315734	3939.84	0.03315734	0	0	1	0.00236407	0.00000394
120002-282	282	1	0.00194932	231.6234	0.00194932	0	0	2	0.00472813	0.0000023
120002-282	282	1	0.32110265	38154.24	0.32110265	0	0	6	0.0141844	0.00003815
120002-282	282	1	0.0013892	165.0688	0.0013892	0	0	4	0.00945626	0.00000017
120002-282	282	1	0.04430027	5263.872	0.04430027	0	0	3	0.0070922	0.00000526
120002-282	282	1	0.01996701	2372.5308	0.01996701	0	0	2	0.00472813	0.00000237
120002-282	282	1	0.00021118	25.0925	0.00021118	0	0	1	0.00236407	0.0000003
120002-282	282	1	0.0048733	579.0584	0.0048733	0	0	2	0.00472813	0.00000058
120002-282	282	1	0.00027085	32.1833	0.00027085	0	0	1	0.00236407	0.0000003
120002-282	282	1	0.00511697	608.0112	0.00511697	0	0	3	0.0070922	0.00000061
120002-282	282	1	0.00270739	321.6991	0.00270739	0	0	1	0.00236407	0.00000032
120002-282	282	1	0.03978552	4727.418	0.03978552	0	0	20	0.04728132	0.00000473
120002-282	282	1	0.00930732	1105.92	0.00930732	0	0	1	0.00236407	0.00000111
120002-282	282	1	0.00643005	764.0353	0.00643005	0	0	1	0.00236407	0.00000076
120002-282	282	1	0.10829563	12867.9636	0.10829563	0	0	2	0.00472813	0.00001287
120003-282	282	1	0.68156379	14449.2733	0.68156379	0	0	287	0.70689655	0.00001445
120003-282	282	1	0.09696398	2055.6536	0.09696398	0	0	88	0.21674877	0.00000206
120003-282	282	1	0.05796982	1228.9704	0.05796982	0	0	14	0.03448276	0.00000123
120003-282	282	1	0.04643354	984.3992	0.04643354	0	0	4	0.00985222	0.00000098
120003-282	282	1	0.02564486	543.6756	0.02564486	0	0	3	0.00738916	0.00000054
120003-282	282	1	0.05663681	1200.7104	0.05663681	0	0	6	0.01477833	0.0000012
120003-282	282	1	0.00910461	193.0194	0.00910461	0	0	2	0.00492611	0.00000019
120003-282	282	1	0.01536404	325.7203	0.01536404	0	0	1	0.00246305	0.0000033
120003-282	282	1	0.01031856	218.7554	0.01031856	0	0	1	0.00246305	0.00000022
120004-282	282	1	0.02620226	764.035	0.02620226	0	0	10	0.02392344	0.00000076
120004-282	282	1	0.01627586	474.5898	0.01627586	0	0	2	0.00478469	0.00000047
120004-282	282	1	0.10646787	3104.5104	0.10646787	0	0	79	0.18899522	0.0000031
120004-282	282	1	0.1713472	4996.335	0.1713472	0	0	15	0.03588517	0.000005
120004-282	282	1	0.60722559	17706.1694	0.60722559	0	0	302	0.72248804	0.00001771
120004-282	282	1	0.04617115	1346.3106	0.04617115	0	0	6	0.01435407	0.00000135
120004-282	282	1	0.01390767	405.5356	0.01390767	0	0	2	0.00478469	0.00000041
120004-282	282	1	0.00495545	144.4965	0.00495545	0	0	1	0.00239234	0.00000014

								algal_cell_		algal_bioma
				total_biovolu		total_biom		concentrat		ss_concentr
				me_cubic_u		ass_per_s	relative_to	ion_per_s	relative_algal	ation_mg_pe
	customer		relative_total	m_per_squa	relative_total	quare_cm	tal_bioma	quare_cm	_cell_concen	r_square_cm
tracking_id	_id	job_id	_volume	re_cm_	_biovolume	_	SS	_	tration	_
120004-282	282	1	0.00744696	217.1469	0.00744696	0	0	1	0.00239234	0.00000022
120005-282	282	1	0.05485394	1640.6952	0.05485394	0	0	6	0.015	0.00000164
120005-282	282	1	0.17996598	5382.828	0.17996598	0	0	276	0.69	0.00000538
120005-282	282	1	0.06053187	1810.5234	0.06053187	0	0	21	0.0525	0.00000181
120005-282	282	1	0.06554119	1960.3536	0.06554119	0	0	12	0.03	0.00000196
120005-282	282	1	0.08691772	2599.731	0.08691772	0	0	15	0.0375	0.0000026
120005-282	282	1	0.00766881	229.376	0.00766881	0	0	1	0.0025	0.00000023
120005-282	282	1	0.09038632	2703.4776	0.09038632	0	0	54	0.135	0.0000027
120005-282	282	1	0.00806594	241.2544	0.00806594	0	0	1	0.0025	0.00000024
120005-282	282	1	0.1677048	5016.093	0.1677048	0	0	2	0.005	0.00000502
120005-282	282	1	0.0868568	2597.9089	0.0868568	0	0	1	0.0025	0.0000026
120005-282	282	1	0.02957756	884.6724	0.02957756	0	0	2	0.005	0.0000088
120005-282	282	1	0.04936765	1476.5988	0.04936765	0	0	6	0.015	0.00000148
120005-282	282	1	0.01803083	539.3066	0.01803083	0	0	2	0.005	0.00000054
120005-282	282	1	0.09453058	2827.4334	0.09453058	0	0	1	0.0025	0.00000283

	customer		relative_algal		ioh tallv n
tracking id	id	iob id	oncentration	alternate taxa name	otes
120001-282	282	1	0 12711691	Achnanthidium minutissimum	0100
120001-282	282	1	0.00244848	Pseudostaurosira brevistriata	
120001-282	282	1	0 10089778		
120001_282	202	1	0.02866762	Encyonoma silesiacum	
120001-202	202	1	0.02000702		
120001-202	202	1	0.03070802		
120001-202	202	1	0.01000704		
120001-282	202	1	0.00703922		
120001-202	202	1	0.0440000		
120001-282	202	1	0.30043363		
120001-282	202	1	0.00693736		
120001-282	282	1	0.00683534		
120001-282	282	1	0.00091818	Achnantnes nauckiana var. rostrata	
120001-282	282	1	0.06498674		
120001-282	282	1	0.00520302	Psammothidium bioretii	
120001-282	282	1	0.03733932		
120001-282	282	1	0.00163232		
120001-282	282	1	0.005101		
120001-282	282	1	0.00193838		
120001-282	282	1	0.01081412		
120001-282	282	1	0.04927566		
120001-282	282	1	0.0010202		
120001-282	282	1	0.00040808		
120002-282	282	1	0.12885036		
120002-282	282	1	0.14307356	Achnanthidium minutissimum v. robusta	
120002-282	282	1	0.02844639		
120002-282	282	1	0.01775795	Achnanthidium minutissimum	
120002-282	282	1	0.00757448		
120002-282	282	1	0.0111934		
120002-282	282	1	0.05571453		
120002-282	282	1	0.00092577		
120002-282	282	1	0.00757448		

			relative_algal			
	customer		_biomass_c			job_tally_n
tracking_id	_id	job_id	oncentration	alternate_taxa_name		otes
120002-282	282	1	0.0331594			
120002-282	282	1	0.0019357			
120002-282	282	1	0.32107389			
120002-282	282	1	0.00143074	Encyonema silesiacum		
120002-282	282	1	0.04426864			
120002-282	282	1	0.01994614			
120002-282	282	1	0.00025248	Amphora perpusilla		
120002-282	282	1	0.00488133			
120002-282	282	1	0.00025248			
120002-282	282	1	0.00513382			
120002-282	282	1	0.00269315			
120002-282	282	1	0.03980811			
120002-282	282	1	0.00934186			
120002-282	282	1	0.00639623	Sellaphora pupula		
120002-282	282	1	0.1083151			
120003-282	282	1	0.68160377	Achnanthidium minutissimum	v. robusta	
120003-282	282	1	0.09716981	Achnanthidium minutissimum		
120003-282	282	1	0.05801887			
120003-282	282	1	0.04622642			
120003-282	282	1	0.0254717	Encyonema silesiacum		
120003-282	282	1	0.05660377			
120003-282	282	1	0.00896226			
120003-282	282	1	0.01556604			
120003-282	282	1	0.01037736	Karayevia clevei		
120004-282	282	1	0.0260631			
120004-282	282	1	0.01611797	Encyonema silesiacum		
120004-282	282	1	0.10631001	Achnanthidium minutissimum		
120004-282	282	1	0.17146776			
120004-282	282	1	0.60733882	Achnanthidium minutissimum	v. robusta	
120004-282	282	1	0.0462963			
120004-282	282	1	0.01406036			
120004-282	282	1	0.0048011	Amphora perpusilla		

			relative_algal	
	customer		_biomass_c	job_tally_n
tracking_id	_id	job_id	oncentration alternate_taxa_name	otes
120004-282	282	1	0.00754458 Brachysira neoexilis	
120005-282	282	1	0.05483116	
120005-282	282	1	0.17987295 Achnanthidium minutissimum	
120005-282	282	1	0.06051488	
120005-282	282	1	0.06552992 Brachysira neoexilis	
120005-282	282	1	0.08692745	
120005-282	282	1	0.00768974	
120005-282	282	1	0.09027081 Achnanthidium minutissimum v. robusta	
120005-282	282	1	0.00802407	
120005-282	282	1	0.16783684	
120005-282	282	1	0.08692745	
120005-282	282	1	0.0294216 Eucocconeis flexella	
120005-282	282	1	0.04948178	
120005-282	282	1	0.01805416 Encyonema silesiacum	
120005-282	282	1	0.09461718	

	customer		sample				sample_d	receive_dat
tracking_id	_id	job_id	_id	system_name	site	station	ate	е
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012

	customer		sample				sample_d	receive_dat
tracking_id	_id	job_id	_id	system_name	site	station	ate	е
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012
120005-282	282	1	D5	Westfield River	Huntington	WB01	7/9/2012	11/27/2012

	customer				replicate			
tracking_id	_id	job_id	calculation_type	level_	_	depth	fraction	biovolume
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120001-202	282	1	Periphyton - Diatom Only	Benthic		0		Ves
120001 202	202	1	Periphyton - Diatom Only	Benthic		0		Ves
120001-202	202	1	Periphyton - Diatom Only	Benthic		0		Ves
120001-202	202	1	Periphyton - Diatom Only	Benthic		0		Vec
120001-202	202	1	Periphyton - Diatom Only	Bonthio		0		Voc
120001-202	202	1	Periphyton - Diatom Only	Bonthio		0		Voc
120001-202	202	1	Periphyton - Diatom Only	Denthio		0		Vee
120001-202	202	1	Periphyton - Diatom Only	Denthio		0		Yes
120001-202	202	1	Periphyton - Diatom Only	Denthio		0		Yes
120001-202	202	1	Periphyton - Diatom Only	Denthio		0		Yes
120001-282	202	1	Periphyton - Diatom Only	Denthio		0		Yes
120001-282	202	1	Periphyton - Diatom Only	Denthic		0		Yes
120002-282	202	1	Periphyton - Diatom Only	Denthic		0		Yes
120002-282	202	1	Periphyton - Diatom Only	Denthio		0		Yes
120002-282	202	1	Periphyton - Diatom Only	Denthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	202	1	Periphyton - Diatom Only	Denthia		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		res
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120002-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes

	customer				replicate			
tracking_id	_id	job_id	calculation_type	level_	_	depth	fraction	biovolume
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120003-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120004-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes
120005-282	282	1	Periphyton - Diatom Only	Benthic		0		Yes

				repor				
	customer			t_not				
tracking_id	_id	job_id	taxa_level	е	taxa_id	organism	habitat	phylum
120001-282	282	1	Species		1013	Algae	Freshwater	
120001-282	282	1	Species		1157	Algae	Freshwater	
120001-282	282	1	Species		9506	Algae	Freshwater	
120001-282	282	1	Species		1095	Algae	Freshwater	
120001-282	282	1	Species		9057	Algae	Freshwater	
120001-282	282	1	Species		9072	Algae	Freshwater	
120001-282	282	1	Species		9397	Algae	Freshwater	
120001-282	282	1	Species		9212	Algae	Freshwater	
120001-282	282	1	Species		1193	Algae	Freshwater	
120001-282	282	1	Species		1160	Algae	Freshwater	
120001-282	282	1	Species		9093	Algae	Freshwater	
120001-282	282	1	Species		9334	Algae	Freshwater	
120001-282	282	1	Species		10379	Algae	Freshwater	
120001-282	282	1	Species		9013	Algae	Freshwater	
120001-282	282	1	Species		1161	Algae	Freshwater	
120001-282	282	1	Species		1222	Algae	Freshwater	
120001-282	282	1	Species		1119	Algae	Freshwater	
120001-282	282	1	Species		1000646	Algae	Freshwater	
120001-282	282	1	Species		9055	Algae	Freshwater	
120001-282	282	1	Species		9218	Algae	Freshwater	
120001-282	282	1	Species		9349	Algae	Freshwater	
120001-282	282	1	Species		1061	Algae	Freshwater	
120002-282	282	1	Species		9212	Algae	Freshwater	
120002-282	282	1	Species		1000755	Algae	Freshwater	
120002-282	282	1	Species		1161	Algae	Freshwater	
120002-282	282	1	Species		1013	Algae	Freshwater	
120002-282	282	1	Species		9397	Algae	Freshwater	
120002-282	282	1	Species		9072	Algae	Freshwater	
120002-282	282	1	Species		1152	Algae	Freshwater	
120002-282	282	1	Species		1477	Algae	Freshwater	
120002-282	282	1	Species		9124	Algae	Freshwater	
120002-282	282	1	Species		1331	Algae	Freshwater	
120002-282	282	1	Species		1000646	Algae	Freshwater	
120002-282	282	1	Species		9506	Algae	Freshwater	
120002-282	282	1	Species		1095	Algae	Freshwater	
120002-282	282	1	Species		1098	Algae	Freshwater	
120002-282	282	1	Species		1140	Algae	Freshwater	
120002-282	282	1	Species		1343	Algae	Freshwater	
120002-282	282	1	Species		9482	Algae	Freshwater	
120002-282	282	1	Species		1653	Algae	Freshwater	
120002-282	282	1	Species		1000065	Algae	Freshwater	
120002-282	282	1	Species		1201	Algae	Freshwater	
120002-282	282	1	Species		9055	Algae	Freshwater	
120002-282	282	1	Species		9771	Algae	Freshwater	
120002-282	282	1	Species		1369	Algae	Freshwater	
120002-282	282	1	Species		1193	Algae	Freshwater	
120003-282	282	1	Species		1000755	Algae	Freshwater	
120003-282	282	1	Species		1013	Algae	Freshwater	
120003-282	282	1	Species		9397	Algae	Freshwater	
			-			-		

				repor				
	customer			t_not				
tracking_id	_id	job_id	taxa_level	е	taxa_id	organism	habitat	phylum
120003-282	282	1	Species		9072	Algae	Freshwater	
120003-282	282	1	Species		1095	Algae	Freshwater	
120003-282	282	1	Species		1161	Algae	Freshwater	
120003-282	282	1	Species		9123	Algae	Freshwater	
120003-282	282	1	Species		9124	Algae	Freshwater	
120003-282	282	1	Species		1014	Algae	Freshwater	
120004-282	282	1	Species		9397	Algae	Freshwater	
120004-282	282	1	Species		1095	Algae	Freshwater	
120004-282	282	1	Species		1013	Algae	Freshwater	
120004-282	282	1	Species		9055	Algae	Freshwater	
120004-282	282	1	Species		1000755	Algae	Freshwater	
120004-282	282	1	Species		9072	Algae	Freshwater	
120004-282	282	1	Species		1161	Algae	Freshwater	
120004-282	282	1	Species		1343	Algae	Freshwater	
120004-282	282	1	Species		108384	Algae	Freshwater	
120005-282	282	1	Species		9055	Algae	Freshwater	
120005-282	282	1	Species		1013	Algae	Freshwater	
120005-282	282	1	Species		9397	Algae	Freshwater	
120005-282	282	1	Species		108384	Algae	Freshwater	
120005-282	282	1	Species		1152	Algae	Freshwater	
120005-282	282	1	Species		9776	Algae	Freshwater	
120005-282	282	1	Species		1000755	Algae	Freshwater	
120005-282	282	1	Species		1099	Algae	Freshwater	
120005-282	282	1	Species		1140	Algae	Freshwater	
120005-282	282	1	Species		9057	Algae	Freshwater	
120005-282	282	1	Species		4275	Algae	Freshwater	
120005-282	282	1	Species		9072	Algae	Freshwater	
120005-282	282	1	Species		1095	Algae	Freshwater	
120005-282	282	1	Species		9212	Algae	Freshwater	

	customer				subclass	
tracking_id	_id	job_id	division	class_	_	order_
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Coscinodiscophyceae		Melosirales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120001-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120002-202	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120002-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120002-202	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120002 202	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120002-202	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120002 202	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120002-202	282	1	Bacillariophyta	Bacillariophyceae		Racillarales
120002-202	202	1	Bacillariophyta	Eragilariophyceae		Tabellariales
120002-202	202	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120002-202	202	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120002-202	202	1	Bacillariophyta	Praglianophyceae		
120002-282	202	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120002-282	202	1	Bacillariophyta	Bacillariophyceae		Eupotiolog
120002-202	202	1	Bacillariophyta	Bacillariophyceae		Thelession
120002-282	202	1	Bacillariophyta	Bacillariophyceae		Noviculator
120002-282	202	1	Chrycophyta	Chryconbycopo		INAVICUIAIES
120002-202	202	1	Decilloriente			Novioulalaa
120002-262	202	1	Bacillariophyta	Fragilarianbyceae		Fragilarialas
120002-202	202	1	Bacillarianbuta	Provillariophyseae		Cumballalas
120002-282	202	1	Bacillarianophyta			
120002-282	202	1	Dacillariophyta	r ragilariophyceae		riagliariales
120002-282	202	1	Bacillariophyta			Naviculaies
120002-282	282	1	Bacillariophyta			
120003-282	282	1	Bacillariophyta	Bacillariopnyceae		Acnnantnales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120003-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales

	customer				subclass	
tracking_id	_id	job_id	division	class_	_	order_
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Bacillarales
120003-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120004-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Thalassiophysales
120004-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120005-282	282	1	Bacillariophyta	Fragilariophyceae		Fragilariales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Eunotiales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Naviculales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Cymbellales
120005-282	282	1	Bacillariophyta	Bacillariophyceae		Achnanthales

tracking_id         job_id         suborder         family         subfamily         tribe         genus           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Gomphonemataceae         Gomphonemataceae           120001-282         282         1         Naviculaceae         Navicula           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Melosiraceae         Melosira           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Achnanthes           120001-282         282         1         Cocconiedaceae         Cocconeis		customer						
120001-282         282         1         Achnanthaceae         Achnanthese           120001-282         282         1         Fragilariaceae         Fragilariaceae           120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Cymbellaceae         Cymbella           120001-282         282         1         Naviculaceae         Navicula           120001-282         282         1         Naviculaceae         Navicula           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Mavicula           120001-282         282         1         Achnanthaceae         Achnanthaceae           120001-282         282         1         Achnanthaceae         Achnanthaceae           120001-282         282         1         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae           120001-282         282         1         Gomphonemataceae         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282 <td< td=""><td>tracking id</td><td>id</td><td>iob id</td><td>suborder</td><td>family</td><td>subfamilv</td><td>tribe</td><td>aenus</td></td<>	tracking id	id	iob id	suborder	family	subfamilv	tribe	aenus
120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Cymbellaceae         Cymbella           120001-282         282         1         Comphonemataceae         Comphonema           120001-282         282         1         Raviculaceae         Navicula           120001-282         282         1         Fragilariaceae         Fragilaria           120001-282         282         1         Melosiraceae         Melosira           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Gomphonemataceae         Gomphonemataceae         Diatoma           120001-282         282         1         Corconiedaceae         Corconeis           120001-282         282         1         Corconiedaceae         Corconeis           120001-282         282         1         Corconiedaceae	120001-282	282	1		Achnanthaceae	,		Achnanthes
120001-282         282         1         Fragilariaceae         Synedra           120001-282         282         1         Cymbellaceae         Cymbella           120001-282         282         1         Gomphonemateceae         Gomphonemateceae         Fragilaria           120001-282         282         1         Fragilariaceae         Fragilaria           120001-282         282         1         Cocconeidaceae         Cocconeis           120001-282         282         1         Gomphonemataceae         Gomphonemataceae           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Omphonemataceae         Gomphonemataceae           120001-282         282         1         Fragilariaceae         Diatoma           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         <	120001-282	282	1		Fragilariaceae			Fragilaria
120001-282         282         1         Cymbellaceae         Cymbella           120001-282         282         1         Gomphonemataceae         Gomphonema           120001-282         282         1         Fragilariaceae         Fragilaria           120001-282         282         1         Cocconiedaceae         Mavicula           120001-282         282         1         Cocconiedaceae         Melosira           120001-282         282         1         Maviculaceae         Mavicula           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Comphonemataceae         Gomphonema           120001-282         282         1         Corconiedaceae         Comphonemataceae           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis	120001-282	282	1		Fragilariaceae			Svnedra
International and the second	120001-282	282	1		Cymbellaceae			Cymbella
Loos Particulate         Complication         Complication           120001-1282         282         1         Fragilariaceae         Navicula           120001-282         282         1         Croconiedaceae         Cocconiedis           120001-282         282         1         Cocconiedaceae         Melosira           120001-282         282         1         Gomphonemataceae         Gomphonema           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Comphonemataceae         Gomphonema           120001-282         282         1         Corconiedaceae         Corconiedaceae           120001-282         282         1         Corconiedaceae         Corconiedaceae           120001-282         282         1         Corconiedaceae         Corconies           120001-282         282         1         Corconiedaceae         Corconies           120002-282         282         1         Corconiedaceae         Corconies	120001-282	282	1		Gomphonemataceae			Gomphonema
International         International         International           120001-1282         282         1         Fragilariaceae         Fragilaria           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Melosiraceae         Melosira           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Achnanthes           120001-282         282         1         Achnanthaceae         Gomphonema           120001-282         282         1         Comphonemataceae         Gomphonema           120001-282         282         1         Comphonemataceae         Comphonemataceae           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Cocconiedaceae         Cocconeis           12	120001 202	282	1		Naviculaceae			Navicula
Toron 122         Toron 122 <thtoron 122<="" th=""> <thtoror 122<="" th=""> <tht< td=""><td>120001 202</td><td>282</td><td>1</td><td></td><td>Franilariaceae</td><td></td><td></td><td>Fragilaria</td></tht<></thtoror></thtoron>	120001 202	282	1		Franilariaceae			Fragilaria
Totol 122         Statul         Concording to the construction         Construction           120001-1282         282         1         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Achnanthaceae         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Diatoma           120001-282         282         1         Concontegration         Gomphonemataceae         Gomphonemataceae         Gomphonemataceae         Diatoma           120001-282         282         1         Concontegration         Cocconied         Cocconies           120001-282         282         1         Cocconiedaceae         Cocconies         Cocconies           120001-282         282         1         Cocconiedaceae         Cocconies         Cocconies           120001-282         282         1         Cocconiedaceae         Cocconies         Cocconies           120002-282         282         1         Cocconiedaceae         Cocconies         Cocconies           120002-282         282         1         Achnanthaceae	120001-202	202	1		Cocconiedaceae			Cocconeis
Intersection         Intersection         Intersection           120001-1282         282         1         Gomphonemataceae         Gomphonemataceae           120001-282         282         1         Naviculaceae         Navicula           120001-282         282         1         Achnanthaceae         Achnanthaceae         Achnanthaceae           120001-282         282         1         Achnanthaceae         Achnanthaceae         Navicula           120001-282         282         1         Gomphonemataceae         Gomphonemataceae         Nitzschia           120001-282         282         1         Gomphonemataceae         Optimotamataceae         Cymbellaceae           120001-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Fragilaria         Propilariaceae         Fragilaria	120001 202	282	1		Melosiraceae			Melosira
TableComplete MatcaceComplete Matcace12001-12822821NaviculaceaeNavicula12001-2822821FragilariaceaeFragilaria12001-2822821AchnanthaceaeAchnanthes12001-2822821GomphonemataceaeGomphonema12001-2822821BacillariaceaeNitzschia12001-2822821GomphonemataceaeCymbellaceae12001-2822821GomphonemataceaeGomphonema12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821Catenula	120001 202	282	1		Gomphonemataceae			Gomphonema
12001-2822821AchnanthaceaeAchnanthes12001-2822821AchnanthaceaeAchnanthes12001-2822821AchnanthaceaeAchnanthes12001-2822821BacillariaceaeMitzschia12001-2822821BacillariaceaeDiatoma12001-2822821GomphonemataceaeGomphonema12001-2822821GomphonemataceaeDiatoma12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821CocconiedaceaeCocconeis12001-2822821AchnanthaceaeAchnanthes12001-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNaviculaceae120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821Fragi	120001-282	282	1		Naviculaceae			Navicula
TableTendinationTendination120011-2822821Fragilaria120001-2822821AchnanthaceaeAchnanthes120001-2822821GomphonemataceaeGomphonema120001-2822821CymbellaceaeNitzschia120001-2822821FragilariaceaeDiatoma120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella	120001-282	282	1		Achnanthaceae			Achnanthes
120001-2822821AchnanthaceaeAchnanthes120001-2822821GomphonemataceaeGomphonema120001-2822821BacillariaceaeNitzschia120001-2822821FragilariaceaeDiatoma120001-2822821GomphonemataceaeGomphonema120001-2822821GomphonemataceaeGomphonema120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821<	120001-282	282	1		Fragilariaceae			Fragilaria
120001-2822821GomphonemataceaeGomphonema120001-2822821BacillariaceaeNitzschia120001-2822821CymbellaceaeCymbella120001-2822821FragilariaceaeDiatoma120001-2822821CocconiedaceaeCocconeis120001-2822821AchnanthaceaeAchnanthes120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821TabellariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821CatenulaceaeCymbellaceae120002-2822821CatenulaceaeAmphora120002-2822821Ca	120001-282	282	1		Achnanthaceae			Achnanthes
120001-282         282         1         Bacillariaceae         Nitzschia           120001-282         282         1         Cymbellaceae         Cymbella           120001-282         282         1         Fragilariaceae         Diatoma           120001-282         282         1         Gomphonemataceae         Gomphonema           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120001-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Achnanthaceae         Achnanthes           120002-282         282         1         Achnanthaceae         Achnanthes           120002-282         282         1         Fragilariaceae         Fragilaria           120002-282         282         1         Fragilariaceae         Synedra           120002-282         282         1         Fragilariaceae         Synedra           120002-282         282         1         Fragilariaceae         Synedra <tr< td=""><td>120001-282</td><td>282</td><td>1</td><td></td><td>Gomphonemataceae</td><td></td><td></td><td>Gomphonema</td></tr<>	120001-282	282	1		Gomphonemataceae			Gomphonema
120001-2822821Demonstrate GomphonemataceaeCymbella120001-2822821GomphonemataceaeGomphonemataceae120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeGomphonemataceae120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-282 <td>120001-282</td> <td>282</td> <td>1</td> <td></td> <td>Bacillariaceae</td> <td></td> <td></td> <td>Nitzschia</td>	120001-282	282	1		Bacillariaceae			Nitzschia
120001-2822821Gymbolic GodGymbolic Gymbolic120001-2822821GomphonemataceaeGomphonema120001-2822821CocconiedaceaeCocconeis120001-2822821AchnanthaceaeAchnanthes120001-2822821CocconiedaceaeCocconeis120001-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821Na	120001-282	282	1		Cymbellaceae			Cymbella
120001-2822821GomphonemataceaeGomphonemataceae120001-2822821AchnanthaceaeAchnanthaceae120001-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeNitzschia120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeAmphora120002-2822821RaviculaceaeNavicula120002-2822821	120001-282	282	1		Fragilariaceae			Diatoma
12001-2822821CocconiedaceaeCocconeis120001-2822821AchnanthaceaeAchnanthes120002-2822821CocconiedaceaeCocconeis12002-2822821CocconiedaceaeCocconeis12002-2822821CocconiedaceaeAchnanthes12002-2822821AchnanthaceaeAchnanthes12002-2822821AchnanthaceaeAchnanthes12002-2822821AchnanthaceaeAchnanthes12002-2822821FragilariaceaeFragilaria12002-2822821FragilariaceaeFragilaria12002-2822821FragilariaceaeFragilaria12002-2822821FragilariaceaeNavicula12002-2822821FragilariaceaeNitzschia12002-2822821FragilariaceaeDiatoma12002-2822821FragilariaceaeDiatoma12002-2822821FragilariaceaeSynedra12002-2822821CymbellaceaeCymbella12002-2822821CymbellaceaeAmphora12002-2822821CathulaceaeAmphora12002-2822821CathulaceaeAmphora12002-2822821CathulaceaeAmphora12002-2822821NaviculaceaeAmphora12002-2822821CathulaceaeAmphora	120001-282	282	1		Gomphonemataceae			Gomphonema
12001-2822821AchnanthaceaeAchnanthes12001-2822821CocconiedaceaeCocconeis12002-2822821AchnanthaceaeAchnanthes12002-2822821AchnanthaceaeAchnanthes12002-2822821AchnanthaceaeGomphonemataceaeGomphonemataceae12002-2822821AchnanthaceaeAchnanthes12002-2822821AchnanthaceaeAchnanthes12002-2822821FragilariaceaeFragilaria12002-2822821FragilariaceaeRigilaria12002-2822821FragilariaceaeSynedra12002-2822821FragilariaceaeSynedra12002-2822821FragilariaceaeDiatoma12002-2822821FragilariaceaeSynedra12002-2822821FragilariaceaeSynedra12002-2822821FragilariaceaeSynedra12002-2822821CymbellaceaeCymbella12002-2822821CymbellaceaeMavicula12002-2822821CatenulaceaeNavicula12002-2822821CatenulaceaeNavicula12002-2822821RaviculaceaeNavicula12002-2822821RaviculaceaeNavicula12002-2822821RaviculaceaeNavicula12002-2822821Raviculacea	120001-282	282	1		Cocconiedaceae			Cocconeis
120001-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Cocconiedaceae         Cocconeis           120002-282         282         1         Achnanthaceae         Achnanthes           120002-282         282         1         Gomphonemataceae         Gomphonema           120002-282         282         1         Achnanthaceae         Achnanthes           120002-282         282         1         Achnanthaceae         Achnanthes           120002-282         282         1         Fragilariaceae         Fragilaria           120002-282         282         1         Fragilariaceae         Navicula           120002-282         282         1         Fragilariaceae         Synedra           120002-282         282         1         Tabellariaceae         Nitzschia           120002-282         282         1         Tabellariaceae         Diatoma           120002-282         282         1         Fragilariaceae         Synedra           120002-282         282         1         Cymbellaceae         Cymbella           120002-282         282         1         Cymbellaceae         Cymbella	120001-282	282	1		Achnanthaceae			Achnanthes
120002-2822821CocconiedaceaeCocconeis120002-2822821AchnanthaceaeAchnanthes120002-2822821GomphonemataceaeGomphonema120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeMavicula120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeMaphora120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeSynedra120002-2822821Fragilariaceae	120001-282	282	1		Cocconiedaceae			Cocconeis
12002-282       282       1       Achnanthaceae       Achnanthes         12002-282       282       1       Gomphonemataceae       Gomphonema         12002-282       282       1       Achnanthaceae       Achnanthes         12002-282       282       1       Fragilariaceae       Fragilaria         12002-282       282       1       Fragilariaceae       Fragilaria         12002-282       282       1       Fragilariaceae       Navicula         12002-282       282       1       Fragilariaceae       Navicula         12002-282       282       1       Fragilariaceae       Synedra         12002-282       282       1       Fragilariaceae       Synedra         12002-282       282       1       Fragilariaceae       Diatoma         12002-282       282       1       Fragilariaceae       Cymbella         12002-282       282       1       Cymbellaceae       Cymbella         12002-282       282       1       Cymbellaceae       Cymbella         12002-282       282       1       Cymbellaceae       Cymbella         120002-282       282       1       Catenulaceae       Amphora         120002	120002-282	282	1		Cocconiedaceae			Cocconeis
120002-2822821GomphonemataceaeGomphonema120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeNavicula120002-2822821FragilariaceaeSynedra120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeNitzschia120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeMavicula120002-2822821CatenulaceaeMavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821Naviculaceae <td>120002-282</td> <td>282</td> <td>1</td> <td></td> <td>Achnanthaceae</td> <td></td> <td></td> <td>Achnanthes</td>	120002-282	282	1		Achnanthaceae			Achnanthes
120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeSynedra120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeNitzschia120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeDiatoma120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeAmphora120002-2822821CatenulaceaeNavicula120002-2822821NaviculaceaeMavicula120002-2822821NaviculaceaeMavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821NaviculaceaeMeridion120002-2822821NaviculaceaeMe	120002-282	282	1		Gomphonemataceae			Gomphonema
120002-2822821FragilariaceaeFragilaria120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeFragilaria120002-2822821FragilariaceaeNitzschia120002-2822821BacillariaceaeNitzschia120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeMavicula120002-2822821CatenulaceaeMavicula120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNa	120002-282	282	1		Achnanthaceae			Achnanthes
120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeFragilaria120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeNitzschia120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821NaviculaceaeMeridion120002-2822821NaviculaceaeMeridion	120002-282	282	1		Fragilariaceae			Fragilaria
120002-2822821FragilariaceaeFragilaria120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeTabellaria120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821RomphonemataceaeGomphonemataceae120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821Achnanthacea	120002-282	282	1		Naviculaceae			Navicula
120002-2822821FragilariaceaeSynedra120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeDiatoma120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CatenulaceaeAmphora120002-2822821CatenulaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821ReiosiraSynedra120002-2822821MelosiraceaeMelosira120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes	120002-282	282	1		Fragilariaceae			Fragilaria
120002-2822821BacillariaceaeNitzschia120002-2822821TabellariaceaeTabellaria120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821RelosiraceaeMelosira120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821Achnanthaceae <t< td=""><td>120002-282</td><td>282</td><td>1</td><td></td><td>Fragilariaceae</td><td></td><td></td><td>Synedra</td></t<>	120002-282	282	1		Fragilariaceae			Synedra
120002-2822821TabellariaceaeTabellaria120002-2822821FragilariaceaeDiatoma120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CymbellaceaeCymbella120002-2822821CunotiaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821ReidionSynedra120002-2822821ReidionSynedra120002-2822821ReidionSynedra120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeFragilaria <td>120002-282</td> <td>282</td> <td>1</td> <td></td> <td>Bacillariaceae</td> <td></td> <td></td> <td>Nitzschia</td>	120002-282	282	1		Bacillariaceae			Nitzschia
120002-2822821FragilariaceaeDiatoma120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821EunotiaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeSynedra120002-2822821FragilariaceaeMeridion120002-2822821ReigilariaceaeSynedra120002-2822821NaviculaceaeMeridion120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria120003-2822821FragilariaceaeAchnanthes120003-2822821FragilariaceaeFragilaria120003-2822821FragilariaceaeFragilaria120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Tabellariaceae			Tabellaria
120002-2822821FragilariaceaeSynedra120002-2822821CymbellaceaeCymbella120002-2822821EunotiaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeAchnanthaceae120002-2822821AchnanthaceaeAchnanthes120002-2822821AchnanthaceaeAchnanthes120002-2822821FragilariaceaeFragilaria	120002-282	282	1		Fragilariaceae			Diatoma
120002-282       282       1       Cymbellaceae       Cymbella         120002-282       282       1       Cymbellaceae       Cymbella         120002-282       282       1       Eunotiaceae       Eunotia         120002-282       282       1       Catenulaceae       Amphora         120002-282       282       1       Catenulaceae       Amphora         120002-282       282       1       Naviculaceae       Navicula         120002-282       282       1       Naviculaceae       Navicula         120002-282       282       1       V       V       V         120002-282       282       1       Naviculaceae       Navicula       V         120002-282       282       1       Fragilariaceae       Meridion       V         120002-282       282       1       Gomphonemataceae       Gomphonema         120002-282       282       1       Naviculaceae       Navicula         120002-282       282       1       Naviculaceae       Melosira         120002-282       282       1       Naviculaceae       Achnanthes         120002-282       282       1       Achnanthaceae       Achnanthes <td>120002-282</td> <td>282</td> <td>1</td> <td></td> <td>Fragilariaceae</td> <td></td> <td></td> <td>Synedra</td>	120002-282	282	1		Fragilariaceae			Synedra
120002-2822821CymbellaceaeCymbella120002-2822821EunotiaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeMeridion120002-2822821RaviculaceaeSynedra120002-2822821NaviculaceaeMelosira120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Cymbellaceae			Cymbella
120002-2822821EunotiaceaeEunotia120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonemataceae120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMelosira120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Cymbellaceae			Cymbella
120002-2822821CatenulaceaeAmphora120002-2822821NaviculaceaeNavicula120002-2822821120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonemataceae120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeMelosira120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Eunotiaceae			Eunotia
120002-2822821NaviculaceaeNavicula120002-2822821120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Catenulaceae			Amphora
120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Naviculaceae			Navicula
120002-2822821NaviculaceaeNavicula120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonemataceae120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120002-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1					
120002-2822821FragilariaceaeMeridion120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeFragilaria120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Naviculaceae			Navicula
120002-2822821GomphonemataceaeGomphonema120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Fragilariaceae			Meridion
120002-2822821FragilariaceaeSynedra120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Gomphonemataceae			Gomphonema
120002-2822821NaviculaceaeNavicula120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Fragilariaceae			Synedra
120002-2822821MelosiraceaeMelosira120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Naviculaceae			Navicula
120003-2822821AchnanthaceaeAchnanthes120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120002-282	282	1		Melosiraceae			Melosira
120003-2822821AchnanthaceaeAchnanthes120003-2822821FragilariaceaeFragilaria	120003-282	282	1		Achnanthaceae			Achnanthes
120003-282 282 1 Fragilariaceae Fragilaria	120003-282	282	1		Achnanthaceae			Achnanthes
	120003-282	282	1		Fragilariaceae			Fragilaria

	customer						
tracking_id	_id	job_id	suborder	family	subfamily	tribe	genus
120003-282	282	1		Naviculaceae			Navicula
120003-282	282	1		Cymbellaceae			Cymbella
120003-282	282	1		Gomphonemataceae			Gomphonema
120003-282	282	1		Bacillariaceae			Nitzschia
120003-282	282	1		Bacillariaceae			Nitzschia
120003-282	282	1		Achnanthaceae			Achnanthes
120004-282	282	1		Fragilariaceae			Fragilaria
120004-282	282	1		Cymbellaceae			Cymbella
120004-282	282	1		Achnanthaceae			Achnanthes
120004-282	282	1		Gomphonemataceae			Gomphonema
120004-282	282	1		Achnanthaceae			Achnanthes
120004-282	282	1		Naviculaceae			Navicula
120004-282	282	1		Gomphonemataceae			Gomphonema
120004-282	282	1		Catenulaceae			Amphora
120004-282	282	1		Naviculaceae			Anomoeoneis
120005-282	282	1		Gomphonemataceae			Gomphonema
120005-282	282	1		Achnanthaceae			Achnanthes
120005-282	282	1		Fragilariaceae			Fragilaria
120005-282	282	1		Naviculaceae			Anomoeoneis
120005-282	282	1		Fragilariaceae			Fragilaria
120005-282	282	1		Fragilariaceae			Synedra
120005-282	282	1		Achnanthaceae			Achnanthes
120005-282	282	1		Cymbellaceae			Cymbella
120005-282	282	1		Eunotiaceae			Eunotia
120005-282	282	1		Gomphonemataceae			Gomphonema
120005-282	282	1		Achnanthaceae			Achnanthes
120005-282	282	1		Naviculaceae			Navicula
120005-282	282	1		Cymbellaceae			Cymbella
120005-282	282	1		Cocconiedaceae			Cocconeis

	customer				subspeci			
tracking_id	_id	job_id	subgenus	species	es	variety	form_	morph
120001-282	282	1		minutissima				
120001-282	282	1		brevistriata				
120001-282	282	1		ulna		ulna		
120001-282	282	1		silesiaca				
120001-282	282	1		truncatum				
120001-282	282	1		cryptotenella				
120001-282	282	1		capucina		vaucheriae		
120001-282	282	1		placentula		lineata		
120001-282	282	1		varians				
120001-282	282	1						
120001-282	282	1		rhvnchocephala				
120001-282	282	1		delicatula				
120001-282	282	1		capucina		mesolepta		
120001-282	282	1		bioreti				
120001-282	282	1		parvulum				
120001-282	282	1		oracilis				
120001-282	282	1		sinuata				
120001-282	282	1		moniliformis				
120001-282	282	1		numilum				
120001-282	282	1		placentula		pseudolineata		
120001-282	282	1		hiasolettiana		pooluointoulu		
120001-282	282	1		neothumensis				
120001 202	282	1		nlacentula		lineata		
120002 202	282	1		minutissima		robusta		
120002-202	282	1		narvulum		1000310		
120002-202	282	1		minutissima				
120002-202	282	1		capucina		vaucheriae		
120002-202	282	1		cryptotenella		vauenenae		
120002-282	282	1		crotonensis				
120002-202	282	1		filiformis				
120002-202	282	1		recta				
120002-202	282	1		fenestrata				
120002-202	282	1		moniliformis				
120002-282	282	1		ulna		ulna		
120002-202	282	1		silesiaca		ana		
120002-202	282	1		caespitosa				
120002-282	282	1		ouoophoou				
120002-282	282	1		nediculus				
120002-202	282	1		salinarum				
120002-282	282	1						
120002-282	282	1		cryptotenelloides				
120002-282	282	1		circulare				
120002-282	282	1		pumilum				
120002-282	282	1		arcus		arcus		
120002-282	282	1		pupula				
120002-282	282	1		varians				
120003-282	282	1		minutissima		robusta		
120003-282	282	1		minutissima				
120003-282	282	1		capucina		vaucheriae		
20000 202		-						

	customer				subspeci			
tracking_id	_id	job_id	subgenus	species	es	variety	form_	morph
120003-282	282	1		cryptotenella				
120003-282	282	1		silesiaca				
120003-282	282	1		parvulum				
120003-282	282	1		palea				
120003-282	282	1		recta				
120003-282	282	1		clevei				
120004-282	282	1		capucina		vaucheriae		
120004-282	282	1		silesiaca				
120004-282	282	1		minutissima				
120004-282	282	1		pumilum				
120004-282	282	1		minutissima		robusta		
120004-282	282	1		cryptotenella				
120004-282	282	1		parvulum				
120004-282	282	1		pediculus				
120004-282	282	1		vitrea				
120005-282	282	1		pumilum				
120005-282	282	1		minutissima				
120005-282	282	1		capucina		vaucheriae		
120005-282	282	1		vitrea				
120005-282	282	1		crotonensis				
120005-282	282	1		ulna		acus		
120005-282	282	1		minutissima		robusta		
120005-282	282	1		cistula				
120005-282	282	1						
120005-282	282	1		truncatum				
120005-282	282	1		flexella				
120005-282	282	1		cryptotenella				
120005-282	282	1		silesiaca				
120005-282	282	1		placentula		lineata		

					customer r		
	customer				equested u	nh snectr	nutrient s
tracking id	id	ioh id	coloniality	structure	nite		nectrum
120001-282	282	100_10	Cell-Nonmotile	Vegetative	NU/sa cm	P3	N6
120001-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	P4	N1
120001-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N1
120001 202	202	1		Vogotativo	NU/sq. cm		
120001-202	202	1		Vegetative	NU/sq. cm		NO
120001-202	202	1	Cell-Normoule	Vegetative	NU/sq. cm		
120001-202	202	1	Leteral Filement	Vegetative	NU/sq. cm		
120001-202	202	1		Vegetative	NU/sq. cm		NI NI
120001-202	202	1	Eilomont	Vegetative	NU/sq. cm	F4 D4	NI NI
120001-202	202	1		Vegetative	NU/sq. cm	Γ4	
120001-202	202	1		Vegetative	NU/sq. cm	D4	N11
120001-202	202	1		Vegetative	NU/sq. cm	F4 D5	
120001-202	202	1		Vegetative	NU/sq. cm	F0 D4	N1
120001-262	202	1		Vegetative	NU/Sq. cm	P4	
120001-202	202	1		Vegetative	NU/Sq. cm	F3 D2	
120001-282	202	1		Vegetative	NU/Sq. cm	гэ рэ	
120001-262	202	1		Vegetative	NU/Sq. cm	гэ рэ	
120001-282	202	1		Vegetative	NU/Sq. cm	го D4 D5	IN3
120001-262	202	1		Vegetative	NU/sq. cm	P4-P5	•
120001-202	202	1		Vegetative	NU/Sq. cm	D4	N4
120001-282	202	1		Vegetative	NU/Sq. cm	P4	
120001-262	202	1		Vegetative	NU/Sq. cm	P4	IN 3
120001-202	202	1		Vegetative	NU/Sq. cm	D4	N4
120002-202	202	1		Vegetative	NU/Sq. cm		IN I NG
120002-282	202	1		Vegetative	NU/sq. cm	гэ 12	
120002-202	202	1		Vegetative	NU/sq. cm	г.) D2	
120002-202	202	1		Vegetative	NU/sq. cm	FJ D4	
120002-282	202	1		Vegetative	NU/sq. cm	F4 D4	
120002-282	202	1	Latoral Filamont	Vegetative	NU/sq. cm	F4 D4	
120002-282	202	1		Vegetative	NU/sq. cm	F4 D2	NE NE
120002-282	202	1		Vegetative	NU/sq. cm		INJ
120002-282	202	1	Complex Filement	Vegetative	NU/sq. cm	F4 D2	NO NE
120002-282	202	1		Vegetative	NU/sq. cm		INU-INO
120002-282	202	1		Vegetative	NU/sq. cm	F4-F5 D4	N1
120002-282	202	1		Vegetative	NU/sq. cm	F4 D2	INT
120002-282	202	1		Vegetative	NU/sq. cm	гJ	•
120002-202	202	1		Vegetative	NU/sq. cm	D2 D2	NI1 NI5
120002-282	202	1		Vegetative	NU/sq. cm	FZ-F3 D4	NI1
120002-282	202	1		Vegetative	NU/sq. cm	F4 D3	N1
120002-282	202	1	Cell-Monmotilo	Cyct	NU/sq. cm	гJ	INT
120002-202	202	1		Vogototivo	NU/sq. cm	•	•
120002-202	202	1		Vegetative	NU/sq. cm	D4	N11
120002-202	202	1		Vegetative	NU/sq. cm	Γ4	
120002-202	202	1		Vegetative	NU/sq. cm	•	•
120002-282	202	1		Vegetative	NU/sq. cm	02	N4
120002-202	202 282	1 1	Filomont	Vegetative	NU/sq. cm	F3 D/	N1
120002-202	202 282	1 1	Coll-Nonmotilo	Vegetative	NU/Sq. CIII	D2	NG
120003-202	202 202	1		Vogototivo	NU/Sq. CIII	го D2	NG
120003-202	202 202	1		Vegetative		гง ⊓≀	
120003-282	282	I	Lateral-Filament	vegetative	NU/sq. cm	۲4	INT

					customer_r		
	customer				equested_u	ph_spectr	nutrient_s
tracking_id	_id	job_id	coloniality	structure_	nits	um	pectrum
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	P4	N0
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N1
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	P3	N1
120003-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	P4	
120003-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N1
120004-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	P4	N1
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N6
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm		
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N6
120004-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	P4	N0
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N1
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N1
120004-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3-P5	N1-N5
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm		
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N6
120005-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	P4	N1
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3-P5	N1-N5
120005-282	282	1	Lateral-Filament	Vegetative	NU/sq. cm	P4	N1-N3
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm		
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	N6
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N1
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P2-P3	N1-N5
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N2
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P2-P3	N5
120005-282	282	1	Cell-Motile	Vegetative	NU/sq. cm	P4	N0
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P3	
120005-282	282	1	Cell-Nonmotile	Vegetative	NU/sq. cm	P4	N1

								seasonal_	temperatu
	customer			saprobien			communit	distributio	re_spectru
tracking_id	_id	job_id	halobion	_spectrum	flow	location	У	n	m
120001-282	282	1	H8	S2-S5	F3		C3		Т6
120001-282	282	1	H8	S5	F3	L3-L4	C3		
120001-282	282	1	H8	S4-S5	F3		C1	D2-D4	T2-T3
120001-282	282	1	H8	S3					
120001-282	282	1	H8	S4					
120001-282	282	1	H8	S4					
120001-282	282	1	H8	S4	F3-F4	L6	C3		ТЗ
120001-282	282	1	H8	S4-S5	F3-F4		C3/C7	D4	
120001-282	282	1	H8	S3-S4	F3	L3/L4/L6	C3	D3	T6/T3-T5
120001-282	282	1							
120001-282	282	1	H7-H8	S4		L4	C3	D2/D4	
120001-282	282	1	H4						
120001-282	282	1	H8	S4	F3-F4	L6	C3		ТЗ
120001-282	282	1	H8	S5					
120001-282	282	1	H8	S2	F4		C3		T2/T4
120001-282	282	1	H6	S4					
120001-282	282	1	H8	S4	F1		C3		T2-T3
120001-282	282	1	H9	S7					T3-T4
120001-282	282	1	H8						
120001-282	282	1	H8	S5	F3-F4		C3	D4	
120001-282	282	1	H8						
120001-282	282	1	H8						
120002-282	282	1	H8	S4-S5	F3-F4		C3/C7	D4	
120002-282	282	1	H8	S2-S5	F3		C3		Т6
120002-282	282	1	H8	S2	F4		C3		T2/T4
120002-282	282	1	H8	S2-S5	F3		C3		T6
120002-282	282	1	H8	S4	F3-F4	L6	C3		T3
120002-282	282	1	H8	S4					
120002-282	282	1	H8	S4-S5	F3		C1		
120002-282	282	1	H8	S5	F4				
120002-282	282	1	H8	S4					
120002-282	282	1	H9	S4-S5/S7	F1	L3-L4	C3/C9	D2/D4	
120002-282	282	1	H9	S7					T3-T4
120002-282	282	1	H8	S4-S5	F3		C1	D2-D4	T2-T3
120002-282	282	1	H8	S3					
120002-282	282	1	H8	S3					
120002-282	282	1	H8-H9	S5/S3		L6/L3/L4/L	C3		
120002-282	282	1	H8	S4					
120002-282	282	1	H5	S4					
120002-282	282	1							
120002-282	282	1							
120002-282	282	1	H8	S5	F4-F5	L3-L6	C2-C3	D2	T2-T3/T6
120002-282	282	1	H8						
120002-282	282	1							
120002-282	282	1	H8	S3	F3	L4	C3		
120002-282	282	1	H8	S3-S4	F3	L3/L4/L6	C3	D3	T6/T3-T5
120003-282	282	1	H8	S2-S5	F3	<b></b>	C3		T6
120003-282	282	1	H8	S2-S5	F3		C3		T6
120003-282	282	1	H8	S4	F3-F4	L6	C3		Т3
	-		-			-			-

								seasonal_	temperatu
	customer			saprobien			communit	distributio	re_spectru
tracking_id	_id	job_id	halobion	_spectrum	flow	location	У	n	m
120003-282	282	1	H8	S4					
120003-282	282	1	H8	S3					
120003-282	282	1	H8	S2	F4		C3		T2/T4
120003-282	282	1	H8	S1-S2	F3	L3-L4	C2-C3	D2-D4	T6
120003-282	282	1	H8	S4					
120003-282	282	1	H8	S7	F2	L3	C3		
120004-282	282	1	H8	S4	F3-F4	L6	C3		Т3
120004-282	282	1	H8	S3					
120004-282	282	1	H8	S2-S5	F3		C3		T6
120004-282	282	1	H8						
120004-282	282	1	H8	S2-S5	F3		C3		Т6
120004-282	282	1	H8	S4					
120004-282	282	1	H8	S2	F4		C3		T2/T4
120004-282	282	1	H8	S4	•				
120004-282	282	1	H8	S7	F3		C3		
120005-282	282	1	H8		•				
120005-282	282	1	H8	S2-S5	F3		C3		Т6
120005-282	282	1	H8	S4	F3-F4	L6	C3		Т3
120005-282	282	1	H8	S7	F3		C3		
120005-282	282	1	H8	S4-S5	F3		C1		
120005-282	282	1	•		•				
120005-282	282	1	H8	S2-S5	F3		C3		T6
120005-282	282	1	H8	S5	F1-F2	L3	C3		
120005-282	282	1	H8-H9	S5/S3	•	L6/L3/L4/L	C3		
120005-282	282	1	H8	S4					
120005-282	282	1	H9	S5	F2	L2-L3		•	
120005-282	282	1	H8	S4					
120005-282	282	1	H8	S3				•	
120005-282	282	1	H8	S4-S5	F3-F4		C3/C7	D4	

			oxygen_r	nitrogen_u		functional			macroinv
	customer		equireme	ptake_met		_feeding_	habit_beh		ertebrate
tracking_id	_id	job_id	nts	abolism	author	group	avior	life_cycle	_habitat
120001-282	282	1	01	Y2	*1				
120001-282	282	1	01	Y1	*1				
120001-282	282	1			*1				
120001-282	282	1	03	Y2					
120001 202	282	1	$\bigcirc 2$	V1	•			•	•
120001-202	202	1	02		•	•	•	•	•
120001-202	202	1		V2	•	•	•	•	•
120001-202	202	1	03	12 V2	*1	•	•	•	•
120001-202	202	1	V2	02	۱ *1	•	•	•	•
120001-202	202	1	15	03	1	•	•	•	•
120001-202	202	1			*1		•	•	
120001-202	202	1	04	ΪZ	I		•	•	
120001-262	202	1			•		•	•	
120001-282	282	1	01	YZ	•	•	•	•	•
120001-282	282	1	01	Y1	•	•	•	•	•
120001-282	282	1	04	Y3	*1		•	•	•
120001-282	282	1	02		•				•
120001-282	282	1	01	Y2	*1				•
120001-282	282	1		•	*1	•	•	•	•
120001-282	282	1		•	•	•	•	•	•
120001-282	282	1	03	Y2	•		•	•	
120001-282	282	1							
120001-282	282	1							
120002-282	282	1	O3	Y2	*1				
120002-282	282	1	01	Y2	*1				
120002-282	282	1	O4	Y3	*1				
120002-282	282	1	01	Y2	*1				
120002-282	282	1	01	Y2					
120002-282	282	1							
120002-282	282	1	02	Y2	*1				
120002-282	282	1	01						
120002-282	282	1	02	Y2					
120002-282	282	1	01	Y1	*1				
120002-282	282	1			*1				
120002-282	282	1			*1				
120002-282	282	1	03	Y2	•			•	
120002-282	282	1	00		•		•	•	•
120002-282	282	1	03	Y2	*1	•	•	•	•
120002-202	282	1	02	V3	•		•	•	•
120002-202	202	1	02	V2	•	•	•	•	•
120002-202	202	1	02	12	•	•	•	•	•
120002-202	202	1	•	•	•	•	•	•	•
120002-202	202	1	02	V2	• *1		•	•	•
120002-202	202 202	1	02	١Z	I	•	•	•	•
120002-282	202	1	•	•	•	•	•	•	•
120002-282	202	1			*4	•		•	•
120002-282	282	1	03	Y2	"] *4	•	•	•	•
120002-282	282	1	Y3	03	^] *4	•	•	•	•
120003-282	282	1	01	YZ	°1	•	•	•	•
120003-282	282	1	01	Y2	*1	•			•
120003-282	282	1	01	Y2					

			oxygen_r	nitrogen_u		functional			macroinv
	customer		equireme	ptake_met		_feeding_	habit_beh		ertebrate
tracking_id	_id	job_id	nts	abolism	author	group	avior	life_cycle	_habitat
120003-282	282	1							
120003-282	282	1	03	Y2					
120003-282	282	1	04	Y3	*1				
120003-282	282	1	O4	Y2	*1				
120003-282	282	1	02	Y2					
120003-282	282	1	01	Y1					
120004-282	282	1	01	Y2					
120004-282	282	1	O3	Y2					
120004-282	282	1	01	Y2	*1				
120004-282	282	1							
120004-282	282	1	01	Y2	*1				
120004-282	282	1							
120004-282	282	1	O4	Y3	*1				
120004-282	282	1	02	Y3					
120004-282	282	1	02	Y1	*1				
120005-282	282	1							
120005-282	282	1	01	Y2	*1				
120005-282	282	1	01	Y2					
120005-282	282	1	02	Y1	*1				
120005-282	282	1	02	Y2	*1				
120005-282	282	1							
120005-282	282	1	01	Y2	*1				
120005-282	282	1	02	Y1	*1				
120005-282	282	1	O3	Y2	*1				
120005-282	282	1	O2	Y1					
120005-282	282	1	01	Y1	*1				
120005-282	282	1							
120005-282	282	1	O3	Y2					
120005-282	282	1	O3	Y2	*1				

		customer		sample_i				sample_d			
raw_id trac	king_id	_id	job_id	d	system_name	site	station	ate	calculation_type	level_	replicate_
1 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
2 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
3 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
4 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
5 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
6 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
7 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
8 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
9 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
10 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
11 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
12 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
13 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
14 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
15 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
16 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
17 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
18 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
19 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
20 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
21 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
22 120	001-282	282	1	D2	Little River	Westfield	LITR00.1	7/2/2012	Periphyton - Diatom Only	Benthic	
23 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
24 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
25 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
26 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
27 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
28 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
29 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
30 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
31 120	002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	

	customer		sample_i				sample_d			
tracking_id	_id	job_id	d	system_name	site	station	ate	calculation_type	level_	replicate_
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120002-282	282	1	D1	Westfield River	Westfield	WSFR10.3	7/2/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120003-282	282	1	D3	Westfield River	Westfield	WSFR12.7	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
120004-282	282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
	tracking_id 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120002-282 120003-282	customertracking_id_id120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120002-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120003-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120004-282282120	customer         tracking_id       _id       job_id         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120002-282       282       1         120003-282       282       1         120003-282       282       1         120003-282       282       1         120003-282       282       1         120003-282	customersample_itracking_id_idjob_idd120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D1120002-2822821D3120003-2822821D3120003-2822821D3120003-2822821D3120003-2822821D3120003-2822821D3120003-2822821D3120003-2822821D3120003-2822821D4120004-2822821D4120004-2822821D4120004-2822821D4120004-2822821D4120004-2822821D4120004-2822821D4120004-2822821D4120004-282282 <td< td=""><td>customer         sample_i           tracking_id         _id         job_id         d         system_name           120002-282         282         1         D1         Westfield River           120002-282         282         1         D3         Westfield River</td><td>customersample_Itracking_ididjob_iddsystem_namesite120002-2822821D1Westfield RiverWestfield120002-2822821D1Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120003-2822821D3Westfield RiverWestfield120003-2822821D3Westfield RiverWestfield120003-2822821<td>tracking_id         job_id         d         system_name         site         station           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3           120002-282         282         1         D1         Westfield River         <t< td=""><td>sample_1         sample_1         sample_1         sample_1           tracking_id         id         job_id         d         system_name         site         station         ate           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3</td></t<><td>Customer         Sample_1         sample_1         sample_1           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1<td>customersample_lsample_lsample_lsample_lsample_lsample_llevel_l120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-282282</td></td></td></td></td<>	customer         sample_i           tracking_id         _id         job_id         d         system_name           120002-282         282         1         D1         Westfield River           120002-282         282         1         D3         Westfield River	customersample_Itracking_ididjob_iddsystem_namesite120002-2822821D1Westfield RiverWestfield120002-2822821D1Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120002-2822821D3Westfield RiverWestfield120003-2822821D3Westfield RiverWestfield120003-2822821D3Westfield RiverWestfield120003-2822821 <td>tracking_id         job_id         d         system_name         site         station           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3           120002-282         282         1         D1         Westfield River         <t< td=""><td>sample_1         sample_1         sample_1         sample_1           tracking_id         id         job_id         d         system_name         site         station         ate           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3</td></t<><td>Customer         Sample_1         sample_1         sample_1           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1<td>customersample_lsample_lsample_lsample_lsample_lsample_llevel_l120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-282282</td></td></td>	tracking_id         job_id         d         system_name         site         station           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3           120002-282         282         1         D1         Westfield River <t< td=""><td>sample_1         sample_1         sample_1         sample_1           tracking_id         id         job_id         d         system_name         site         station         ate           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3</td></t<> <td>Customer         Sample_1         sample_1         sample_1           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1<td>customersample_lsample_lsample_lsample_lsample_lsample_llevel_l120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-282282</td></td>	sample_1         sample_1         sample_1         sample_1           tracking_id         id         job_id         d         system_name         site         station         ate           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3	Customer         Sample_1         sample_1         sample_1           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1         Westfield River         Westfield         WSFR10.3         7/2/2012         Periphyton - Diatom Only           120002-282         282         1         D1 <td>customersample_lsample_lsample_lsample_lsample_lsample_llevel_l120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-282282</td>	customersample_lsample_lsample_lsample_lsample_lsample_llevel_l120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-2822821D1Westfield RiverWestfieldWSFR10.37/2/2012Periphyton - Diatom OnlyBenthic120002-282282

	customer		sample_i				sample_d			
raw_id tracking_id	_id	job_id	d	system_name	site	station	ate	calculation_type	level_	replicate_
63 120004-282	2 282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
64 120004-282	2 282	1	D4	Westfield River	Westfield	WSFR17.2	7/11/2012	Periphyton - Diatom Only	Benthic	
65 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
66 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
67 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
68 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
69 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
70 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
71 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
72 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
73 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
74 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
75 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
76 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
77 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	
78 120005-282	2 282	1	D5	Westfield River	Huntington	WB01	7/9/2012	Periphyton - Diatom Only	Benthic	

							pollution_	t			
		customer		report_no		custom_t	olerance_				
raw_id	tracking_id	_id	depth fraction	te	taxa_id	axa_id	class	organism	habitat	phylum	division
1	120001-282	282	0.		9349		3	Algae	Freshwater		Bacillariophyta
2	120001-282	282	0.		9013		3	S Algae	Freshwater		Bacillariophyta
3	120001-282	282	0.		9334		2	Algae	Freshwater		Bacillariophyta
4	120001-282	282	0.		1013		3	Algae	Freshwater		Bacillariophyta
5	120001-282	282	0.		1061		3	Algae	Freshwater		Bacillariophyta
6	120001-282	282	0.		9212		3	S Algae	Freshwater		Bacillariophyta
7	120001-282	282	0.		9218		3	S Algae	Freshwater		Bacillariophyta
8	120001-282	282	0.		1095		2	2 Algae	Freshwater		Bacillariophyta
9	120001-282	282	0.		1119		3	8 Algae	Freshwater		Bacillariophyta
10	120001-282	282	0.		1000646		3	8 Algae	Freshwater		Bacillariophyta
11	120001-282	282	0.		1157		3	8 Algae	Freshwater		Bacillariophyta
12	120001-282	282	0.		9397		2	2 Algae	Freshwater		Bacillariophyta
13	120001-282	282	0.		10379		2	2 Algae	Freshwater		Bacillariophyta
14	120001-282	282	0.		1160		3	Algae	Freshwater		Bacillariophyta
15	120001-282	282	0.		1161		1	Algae	Freshwater		Bacillariophyta
16	120001-282	282	0.		9055		3	S Algae	Freshwater		Bacillariophyta
17	120001-282	282	0.		9057		3	S Algae	Freshwater		Bacillariophyta
18	120001-282	282	0.		1193		(	Algae	Freshwater		Bacillariophyta
19	120001-282	282	0.		9072		2	2 Algae	Freshwater		Bacillariophyta
20	120001-282	282	0.		9093		3	S Algae	Freshwater		Bacillariophyta
21	120001-282	282	0.		1222		2	2 Algae	Freshwater		Bacillariophyta
22	120001-282	282	0.		9506		2	2 Algae	Freshwater		Bacillariophyta
23	120002-282	282	0.		1000755		(	) Algae	Freshwater		Bacillariophyta
24	120002-282	282	0.		1013		3	8 Algae	Freshwater		Bacillariophyta
25	120002-282	282	0.		1343		3	8 Algae	Freshwater		Bacillariophyta
26	120002-282	282	0.		9212		3	8 Algae	Freshwater		Bacillariophyta
27	120002-282	282	0.		1098		2	2 Algae	Freshwater		Bacillariophyta
28	120002-282	282	0.		1095		2	Algae	Freshwater		Bacillariophyta
29	120002-282	282	0.		1000646		3	Algae	Freshwater		Bacillariophyta
30	120002-282	282	0.		1140		3	Algae	Freshwater		Bacillariophyta
31	120002-282	282	0.		9397		2	Algae	Freshwater		Bacillariophyta

							pollution_	t			
		customer		report_no		custom_t	olerance_				
raw_id	tracking_id	_id	depth fraction	on te	taxa_id	axa_id	class	organism	habitat	phylum	division
32	120002-282	282	0.		1152		3	B Algae	Freshwater		Bacillariophyta
33	120002-282	282	0.		1161		1	Algae	Freshwater		Bacillariophyta
34	120002-282	282	0.		9055		3	B Algae	Freshwater		Bacillariophyta
35	120002-282	282	0.		1193		(	) Algae	Freshwater		Bacillariophyta
36	120002-282	282	0.		1201		3	8 Algae	Freshwater		Bacillariophyta
37	120002-282	282	0.		9072		2	2 Algae	Freshwater		Bacillariophyta
38	120002-282	282	0.		1000065		2	2 Algae	Freshwater		Bacillariophyta
39	120002-282	282	0.		1369		2	2 Algae	Freshwater		Bacillariophyta
40	120002-282	282	0.		9482		1	Algae	Freshwater		Bacillariophyta
41	120002-282	282	0.		9124		3	3 Algae	Freshwater		Bacillariophyta
42	120002-282	282	0.		9771		2	2 Algae	Freshwater		Bacillariophyta
43	120002-282	282	0.		1477		3	3 Algae	Freshwater		Bacillariophyta
44	120002-282	282	0.		9506		2	2 Algae	Freshwater		Bacillariophyta
45	120002-282	282	0.		1331		3	3 Algae	Freshwater		Bacillariophyta
46	120002-282	282	0.		1653		(	) Algae	Freshwater		Chrysophyta
47	120003-282	282	0.		1014		3	B Algae	Freshwater		Bacillariophyta
48	120003-282	282	0.		1000755		(	) Algae	Freshwater		Bacillariophyta
49	120003-282	282	0.		1013		3	B Algae	Freshwater		Bacillariophyta
50	120003-282	282	0.		1095		2	2 Algae	Freshwater		Bacillariophyta
51	120003-282	282	0.		9397		2	2 Algae	Freshwater		Bacillariophyta
52	120003-282	282	0.		1161		1	Algae	Freshwater		Bacillariophyta
53	120003-282	282	0.		9072		2	2 Algae	Freshwater		Bacillariophyta
54	120003-282	282	0.		9123		1	Algae	Freshwater		Bacillariophyta
55	120003-282	282	0.		9124		3	3 Algae	Freshwater		Bacillariophyta
56	120004-282	282	0.		1013		3	8 Algae	Freshwater		Bacillariophyta
57	120004-282	282	0.		1000755		(	) Algae	Freshwater		Bacillariophyta
58	120004-282	282	0.		1343		3	3 Algae	Freshwater		Bacillariophyta
59	120004-282	282	0.		108384		2	2 Algae	Freshwater		Bacillariophyta
60	120004-282	282	0.		1095		2	2 Algae	Freshwater		Bacillariophyta
61	120004-282	282	0.		9397		2	2 Algae	Freshwater		Bacillariophyta
62	120004-282	282	0.		1161		1	Algae	Freshwater		Bacillariophyta

raw_id         tracking_id         id         depth         fraction         te         taxa_id         axa_id         class         organism         habitat         phylum         division           63         120004-282         282         0         .         9055         .         3         Algae         Freshwater         .         Bacillarion           64         120004-282         282         0         .         9072         .         2         Algae         Freshwater         .         Bacillarion           65         120005-282         282         0         .         4275         .         3         Algae         Freshwater         .         Bacillarion           66         120005-282         282         0         .         1013         .         0         Algae         Freshwater         .         Bacillarion           67         120005-282         282         0         .         1000755         .         0         Algae         Freshwater         .         Bacillarion           68         120005-282         282         0         .         1099         .         3         Algae         Freshwater         .         Bacillarion <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>pollution_</th> <th>_t</th> <th></th> <th></th> <th></th>									pollution_	_t			
raw_id       tracking_id       id       depth       fraction       te       taxa_id       axa_id       class       organism       habitat       phylum       division         63       120004-282       282       0       .       9055       .       3 Algae       Freshwater       .       Bacillarion         64       120004-282       282       0       .       9072       .       2 Algae       Freshwater       .       Bacillarion         65       120005-282       282       0       .       4275       .       3 Algae       Freshwater       .       Bacillarion         66       120005-282       282       0       .       1013       .       3 Algae       Freshwater       .       Bacillarion         67       120005-282       282       0       .       1000755       .       0 Algae       Freshwater       .       Bacillarion         68       120005-282       282       0       .       108384       .       2 Algae       Freshwater       .       Bacillarion         70       120005-282       282       0       .       1099       .       3 Algae       Freshwater       .       Bacillarion			customer			report_no		custom_t	olerance	_			
63       120004-282       282       0       9055       3 Algae       Freshwater       Bacillarion         64       120004-282       282       0       9072       2 Algae       Freshwater       Bacillarion         65       120005-282       282       0       4275       3 Algae       Freshwater       Bacillarion         66       120005-282       282       0       1013       3 Algae       Freshwater       Bacillarion         67       120005-282       282       0       1013       3 Algae       Freshwater       Bacillarion         68       120005-282       282       0       1000755       0 Algae       Freshwater       Bacillarion         69       120005-282       282       0       108384       2 Algae       Freshwater       Bacillarion         70       120005-282       282       0       1099       3 Algae       Freshwater       Bacillarion         71       120005-282       282       0       1095       2 Algae       Freshwater       Bacillarion         73       120005-282       282       0       1140       3 Algae       Freshwater       Bacillarion         74       120005-282       282       <	raw_id	tracking_id	_id	depth	fraction	te	taxa_id	axa_id	class	organisn	habitat	phylum	division
64       120004-282       282       0       9072       2       Algae       Freshwater       Bacillarion         65       120005-282       282       0       4275       3       Algae       Freshwater       Bacillarion         66       120005-282       282       0       1013       3       Algae       Freshwater       Bacillarion         67       120005-282       282       0       1000755       0       Algae       Freshwater       Bacillarion         68       120005-282       282       0       1000755       0       Algae       Freshwater       Bacillarion         69       120005-282       282       0       108384       2       Algae       Freshwater       Bacillarion         70       120005-282       282       0       1099       3       Algae       Freshwater       Bacillarion         71       120005-282       282       0       1095       2       Algae       Freshwater       Bacillarion         72       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         73       120005-282       282       0       1152       3	63	120004-282	282	0			9055			3 Algae	Freshwater		Bacillariophyta
65       120005-282       282       0       .       4275       .       3 Algae       Freshwater       Bacillarion         66       120005-282       282       0       .       1013       .       3 Algae       Freshwater       Bacillarion         67       120005-282       282       0       .       1000755       0 Algae       Freshwater       Bacillarion         68       120005-282       282       0       .       108384       2 Algae       Freshwater       Bacillarion         69       120005-282       282       0       .       1099       3 Algae       Freshwater       Bacillarion         70       120005-282       282       0       .       1099       3 Algae       Freshwater       Bacillarion         71       120005-282       282       0       .       1095       2 Algae       Freshwater       Bacillarion         72       120005-282       282       0       .       1140       3 Algae       Freshwater       Bacillarion         73       120005-282       282       0       .       1152       3 Algae       Freshwater       Bacillarion         74       120005-282       282       0 <td>64</td> <td>120004-282</td> <td>282</td> <td>0</td> <td></td> <td></td> <td>9072</td> <td></td> <td></td> <td>2 Algae</td> <td>Freshwater</td> <td></td> <td>Bacillariophyta</td>	64	120004-282	282	0			9072			2 Algae	Freshwater		Bacillariophyta
66       120005-282       282       0       .       1013       .       3 Algae       Freshwater       Bacillarion         67       120005-282       282       0       .       1000755       0 Algae       Freshwater       Bacillarion         68       120005-282       282       0       .       108384       2 Algae       Freshwater       Bacillarion         69       120005-282       282       0       .       9212       .       3 Algae       Freshwater       Bacillarion         70       120005-282       282       0       .       1099       .       3 Algae       Freshwater       Bacillarion         71       120005-282       282       0       .       1095       .       2 Algae       Freshwater       Bacillarion         72       120005-282       282       0       .       1095       .       2 Algae       Freshwater       Bacillarion         73       120005-282       282       0       .       1140       .       3 Algae       Freshwater       Bacillarion         74       120005-282       282       0       .       1152       .       3 Algae       Freshwater       Bacillarion	65	120005-282	282	0			4275			3 Algae	Freshwater		Bacillariophyta
67       120005-282       282       0       1000755       0       Algae       Freshwater       Bacillarion         68       120005-282       282       0       108384       2       Algae       Freshwater       Bacillarion         69       120005-282       282       0       9212       3       Algae       Freshwater       Bacillarion         70       120005-282       282       0       1099       3       Algae       Freshwater       Bacillarion         71       120005-282       282       0       1095       2       Algae       Freshwater       Bacillarion         72       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         73       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         73       120005-282       282       0       1152       3       Algae       Freshwater       Bacillarion         74       120005-282       282       0       1152       3       Algae       Freshwater       Bacillarion         75       120005-282       282       0       9055       3       Alg	66	120005-282	282	0			1013			3 Algae	Freshwater		Bacillariophyta
68       120005-282       282       0       108384       2       Algae       Freshwater       Bacillarion         69       120005-282       282       0       9212       3       Algae       Freshwater       Bacillarion         70       120005-282       282       0       1099       3       Algae       Freshwater       Bacillarion         71       120005-282       282       0       1095       2       Algae       Freshwater       Bacillarion         72       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         73       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         73       120005-282       282       0       1140       3       Algae       Freshwater       Bacillarion         74       120005-282       282       0       1152       3       Algae       Freshwater       Bacillarion         75       120005-282       282       0       9055       3       Algae       Freshwater       Bacillarion         76       120005-282       282       0       9057       3       Algae<	67	120005-282	282	0			1000755			0 Algae	Freshwater		Bacillariophyta
69       120005-282       282       0       .       9212       .       3 Algae       Freshwater       Bacillarion         70       120005-282       282       0       .       1099       .       3 Algae       Freshwater       Bacillarion         71       120005-282       282       0       .       1095       .       2 Algae       Freshwater       Bacillarion         72       120005-282       282       0       .       1140       .       3 Algae       Freshwater       .       Bacillarion         73       120005-282       282       0       .       1140       .       3 Algae       Freshwater       .       Bacillarion         73       120005-282       282       0       .       9397       .       2 Algae       Freshwater       .       Bacillarion         74       120005-282       282       0       .       1152       .       3 Algae       Freshwater       .       Bacillarion         75       120005-282       282       0       .       .       9055       .       3 Algae       Freshwater       .       Bacillarion         76       120005-282       282       0       .	68	120005-282	282	0			108384			2 Algae	Freshwater		Bacillariophyta
70       120005-282       282       0       .       1099       .       3 Algae       Freshwater       Bacillarion         71       120005-282       282       0       .       1095       .       2 Algae       Freshwater       Bacillarion         72       120005-282       282       0       .       1140       .       3 Algae       Freshwater       .       Bacillarion         73       120005-282       282       0       .       1140       .       3 Algae       Freshwater       .       Bacillarion         73       120005-282       282       0       .       9397       .       2 Algae       Freshwater       .       Bacillarion         74       120005-282       282       0       .       1152       .       3 Algae       Freshwater       .       Bacillarion         75       120005-282       282       0       .       9055       .       3 Algae       Freshwater       .       Bacillarion         76       120005-282       282       0       .       9057       .       3 Algae       Freshwater       .       Bacillarion         76       120005-282       282       0       .	69	120005-282	282	0			9212			3 Algae	Freshwater		Bacillariophyta
71       120005-282       282       0       1095       2 Algae       Freshwater       Bacillarion         72       120005-282       282       0       1140       3 Algae       Freshwater       Bacillarion         73       120005-282       282       0       9397       2 Algae       Freshwater       Bacillarion         74       120005-282       282       0       1152       3 Algae       Freshwater       Bacillarion         75       120005-282       282       0       9055       3 Algae       Freshwater       Bacillarion         76       120005-282       282       0       9057       3 Algae       Freshwater       Bacillarion         76       120005-282       282       0       9057       3 Algae       Freshwater       Bacillarion         76       120005-282       282       0       9057       3 Algae       Freshwater       Bacillarion	70	120005-282	282	0			1099			3 Algae	Freshwater		Bacillariophyta
72 120005-282 282       0.       1140       3 Algae       Freshwater       Bacillarion         73 120005-282 282       0.       9397       2 Algae       Freshwater       Bacillarion         74 120005-282 282       0.       1152       3 Algae       Freshwater       Bacillarion         75 120005-282 282       0.       9055       3 Algae       Freshwater       Bacillarion         76 120005-282 282       0.       9057       3 Algae       Freshwater       Bacillarion         76 120005-282 282       0.       9057       3 Algae       Freshwater       Bacillarion	71	120005-282	282	0			1095			2 Algae	Freshwater		Bacillariophyta
73 120005-282 282       0 .       9397       2 Algae       Freshwater .       Bacillarion         74 120005-282 282       0 .       1152       3 Algae       Freshwater .       Bacillarion         75 120005-282 282       0 .       9055       3 Algae       Freshwater .       Bacillarion         76 120005-282 282       0 .       9057       3 Algae       Freshwater .       Bacillarion	72	120005-282	282	0			1140			3 Algae	Freshwater		Bacillariophyta
74 120005-282 282       0.       .       1152 .       3 Algae       Freshwater .       Bacillarion         75 120005-282 282       0.       .       9055 .       3 Algae       Freshwater .       Bacillarion         76 120005-282 282       0.       .       9057 .       3 Algae       Freshwater .       Bacillarion         76 120005-282 282       0.       .       9057 .       3 Algae       Freshwater .       Bacillarion	73	120005-282	282	0			9397			2 Algae	Freshwater		Bacillariophyta
75 120005-282 282       0 .       .       9055 .       3 Algae       Freshwater .       Bacillarion         76 120005-282 282       0 .       .       9057 .       3 Algae       Freshwater .       Bacillarion	74	120005-282	282	0			1152			3 Algae	Freshwater		Bacillariophyta
76 120005-282 282 0 9057 . 3 Algae Freshwater Bacillarion	75	120005-282	282	0			9055			3 Algae	Freshwater		Bacillariophyta
	76	120005-282	282	0			9057			3 Algae	Freshwater		Bacillariophyta
77 120005-282 282 0 9072 . 2 Algae Freshwater . Bacillarior	77	120005-282	282	0			9072			2 Algae	Freshwater		Bacillariophyta
78 120005-282 282 0 9776 . 2 Algae Freshwater . Bacillariop	78	120005-282	282	0			9776			2 Algae	Freshwater		Bacillariophyta

	customer		subclass						
raw_id tracking_id	_id	class_	_	order_	suborder	family	subfamily	tribe	genus
1 120001-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
2 120001-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
3 120001-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
4 120001-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
5 120001-282	282	Bacillariophyceae		Achnanthales		Cocconiedaceae			Cocconeis
6 120001-282	282	Bacillariophyceae		Achnanthales		Cocconiedaceae			Cocconeis
7 120001-282	282	Bacillariophyceae		Achnanthales		Cocconiedaceae			Cocconeis
8 120001-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
9 120001-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
10 120001-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Diatoma
11 120001-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
12 120001-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
13 120001-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
14 120001-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
15 120001-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
16 120001-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
17 120001-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
18 120001-282	282	Coscinodiscophyceae		Melosirales		Melosiraceae			Melosira
19 120001-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
20 120001-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
21 120001-282	282	Bacillariophyceae		Bacillarales		Bacillariaceae			Nitzschia
22 120001-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Synedra
23 120002-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
24 120002-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
25 120002-282	282	Bacillariophyceae		Thalassiophysales		Catenulaceae			Amphora
26 120002-282	282	Bacillariophyceae		Achnanthales		Cocconiedaceae			Cocconeis
27 120002-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
28 120002-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
29 120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Diatoma
30 120002-282	282	Bacillariophyceae		Eunotiales		Eunotiaceae			Eunotia
31 120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria

		customer		subclass						
raw_id	tracking_id	_id	class_	_	order_	suborder	family	subfamily	tribe	genus
32	120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
33	120002-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
34	120002-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
35	120002-282	282	Coscinodiscophyceae		Melosirales		Melosiraceae			Melosira
36	120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Meridion
37	120002-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
38	120002-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
39	120002-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
40	120002-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
41	120002-282	282	Bacillariophyceae		Bacillarales		Bacillariaceae			Nitzschia
42	120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Synedra
43	120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Synedra
44	120002-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Synedra
45	120002-282	282	Fragilariophyceae		Tabellariales		Tabellariaceae			Tabellaria
46	120002-282	282	Chrysophyceae							
47	120003-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
48	120003-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
49	120003-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
50	120003-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
51	120003-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
52	120003-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
53	120003-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
54	120003-282	282	Bacillariophyceae		Bacillarales		Bacillariaceae			Nitzschia
55	120003-282	282	Bacillariophyceae		Bacillarales		Bacillariaceae			Nitzschia
56	120004-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
57	120004-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
58	120004-282	282	Bacillariophyceae		Thalassiophysales		Catenulaceae			Amphora
59	120004-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Anomoeoneis
60	120004-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
61	120004-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
62	120004-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema

	customer		subclass						
raw_id tracking_id	_id	class_	_	order_	suborder	family	subfamily	tribe	genus
63 120004-282	282	Bacillariophyceae	÷	Cymbellales		Gomphonemataceae	÷		Gomphonema
64 120004-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
65 120005-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
66 120005-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
67 120005-282	282	Bacillariophyceae		Achnanthales		Achnanthaceae			Achnanthes
68 120005-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Anomoeoneis
69 120005-282	282	Bacillariophyceae		Achnanthales		Cocconiedaceae			Cocconeis
70 120005-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
71 120005-282	282	Bacillariophyceae		Cymbellales		Cymbellaceae			Cymbella
72 120005-282	282	Bacillariophyceae		Eunotiales		Eunotiaceae			Eunotia
73 120005-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
74 120005-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Fragilaria
75 120005-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
76 120005-282	282	Bacillariophyceae		Cymbellales		Gomphonemataceae			Gomphonema
77 120005-282	282	Bacillariophyceae		Naviculales		Naviculaceae			Navicula
78 120005-282	282	Fragilariophyceae		Fragilariales		Fragilariaceae			Synedra

		customer			subspeci					colonialit	
raw_id	tracking_id	_id	subgenus	species	es	variety	form_	morph	coloniality	y_state	structure_
1	120001-282	282		biasolettiana					Cell-Nonmotile	Free	Vegetative
2	120001-282	282		bioreti					Cell-Nonmotile	Free	Vegetative
3	120001-282	282		delicatula		•			Cell-Nonmotile	Free	Vegetative
4	120001-282	282		minutissima		•			Cell-Nonmotile	Free	Vegetative
5	120001-282	282		neothumensis		•			Cell-Nonmotile	Free	Vegetative
6	120001-282	282		placentula		lineata			Cell-Nonmotile	Free	Vegetative
7	120001-282	282		placentula		pseudolineata			Cell-Nonmotile	Free	Vegetative
8	120001-282	282		silesiaca					Cell-Nonmotile	Free	Vegetative
9	120001-282	282		sinuata					Cell-Nonmotile	Free	Vegetative
10	120001-282	282		moniliformis					Cell-Nonmotile	Free	Vegetative
11	120001-282	282		brevistriata					Lateral-Filament	Attached	Vegetative
12	120001-282	282		capucina		vaucheriae			Lateral-Filament	Attached	Vegetative
13	120001-282	282		capucina		mesolepta			Lateral-Filament	Attached	Vegetative
14	120001-282	282							Cell-Nonmotile	Free	Vegetative
15	120001-282	282		parvulum					Cell-Nonmotile	Free	Vegetative
16	120001-282	282		pumilum					Cell-Nonmotile	Free	Vegetative
17	120001-282	282		truncatum					Cell-Nonmotile	Free	Vegetative
18	120001-282	282		varians					Filament	Attached	Vegetative
19	120001-282	282		cryptotenella		•			Cell-Motile	Free	Vegetative
20	120001-282	282		rhynchocephala					Cell-Motile	Free	Vegetative
21	120001-282	282		gracilis					Cell-Motile	Free	Vegetative
22	120001-282	282		ulna		ulna			Cell-Nonmotile	Free	Vegetative
23	120002-282	282		minutissima		robusta			Cell-Nonmotile	Free	Vegetative
24	120002-282	282		minutissima					Cell-Nonmotile	Free	Vegetative
25	120002-282	282		pediculus					Cell-Nonmotile	Free	Vegetative
26	120002-282	282		placentula		lineata			Cell-Nonmotile	Free	Vegetative
27	120002-282	282		caespitosa					Cell-Nonmotile	Free	Vegetative
28	120002-282	282		silesiaca					Cell-Nonmotile	Free	Vegetative
29	120002-282	282		moniliformis					Cell-Nonmotile	Free	Vegetative
30	120002-282	282							Cell-Nonmotile	Free	Vegetative
31	120002-282	282		capucina		vaucheriae			Lateral-Filament	Attached	Vegetative

		customer			subspeci					colonialit	
raw_id	tracking_id	_id	subgenus	species	es	variety	form_	morph	coloniality	y_state	structure_
32	120002-282	282		crotonensis					Lateral-Filament	Attached	Vegetative
33	120002-282	282		parvulum					Cell-Nonmotile	Free	Vegetative
34	120002-282	282		pumilum					Cell-Nonmotile	Free	Vegetative
35	120002-282	282		varians					Filament	Attached	Vegetative
36	120002-282	282		circulare					Cell-Nonmotile	Free	Vegetative
37	120002-282	282		cryptotenella					Cell-Motile	Free	Vegetative
38	120002-282	282		cryptotenelloides					Cell-Motile	Free	Vegetative
39	120002-282	282		pupula					Cell-Motile	Free	Vegetative
40	120002-282	282		salinarum					Cell-Motile	Free	Vegetative
41	120002-282	282		recta					Cell-Motile	Free	Vegetative
42	120002-282	282		arcus		arcus	•		Cell-Nonmotile	Free	Vegetative
43	120002-282	282		filiformis					Cell-Nonmotile	Free	Vegetative
44	120002-282	282		ulna		ulna			Cell-Nonmotile	Free	Vegetative
45	120002-282	282		fenestrata					Complex-Filament	Free	Vegetative
46	120002-282	282							Cell-Nonmotile	Free	Cyst
47	120003-282	282		clevei					Cell-Nonmotile	Free	Vegetative
48	120003-282	282		minutissima		robusta			Cell-Nonmotile	Free	Vegetative
49	120003-282	282		minutissima					Cell-Nonmotile	Free	Vegetative
50	120003-282	282		silesiaca					Cell-Nonmotile	Free	Vegetative
51	120003-282	282		capucina		vaucheriae		•	Lateral-Filament	Attached	Vegetative
52	120003-282	282		parvulum				•	Cell-Nonmotile	Free	Vegetative
53	120003-282	282		cryptotenella				•	Cell-Motile	Free	Vegetative
54	120003-282	282		palea					Cell-Motile	Free	Vegetative
55	120003-282	282		recta					Cell-Motile	Free	Vegetative
56	120004-282	282		minutissima					Cell-Nonmotile	Free	Vegetative
57	120004-282	282		minutissima		robusta			Cell-Nonmotile	Free	Vegetative
58	120004-282	282		pediculus					Cell-Nonmotile	Free	Vegetative
59	120004-282	282		vitrea					Cell-Nonmotile	Free	Vegetative
60	120004-282	282		silesiaca					Cell-Nonmotile	Free	Vegetative
61	120004-282	282		capucina		vaucheriae			Lateral-Filament	Attached	Vegetative
62	120004-282	282	•	parvulum					Cell-Nonmotile	Free	Vegetative

		customer			subspeci					colonialit	
raw_id	tracking_id	_id	subgenus	species	es	variety	form_	morph	coloniality	y_state	structure_
63	120004-282	282		pumilum	•				Cell-Nonmotile	Free	Vegetative
64	120004-282	282		cryptotenella	•				Cell-Motile	Free	Vegetative
65	120005-282	282		flexella	•				Cell-Nonmotile	Free	Vegetative
66	120005-282	282		minutissima	•				Cell-Nonmotile	Free	Vegetative
67	120005-282	282		minutissima	•	robusta			Cell-Nonmotile	Free	Vegetative
68	120005-282	282		vitrea	•				Cell-Nonmotile	Free	Vegetative
69	120005-282	282		placentula	•	lineata			Cell-Nonmotile	Free	Vegetative
70	120005-282	282		cistula	Ē				Cell-Nonmotile	Free	Vegetative
71	120005-282	282		silesiaca					Cell-Nonmotile	Free	Vegetative
72	120005-282	282			Ē				Cell-Nonmotile	Free	Vegetative
73	120005-282	282		capucina	Ē	vaucheriae			Lateral-Filament	Attached	Vegetative
74	120005-282	282		crotonensis	Ē				Lateral-Filament	Attached	Vegetative
75	120005-282	282		pumilum	Ē				Cell-Nonmotile	Free	Vegetative
76	120005-282	282		truncatum					Cell-Nonmotile	Free	Vegetative
77	120005-282	282		cryptotenella					Cell-Motile	Free	Vegetative
78	120005-282	282		ulna		acus			Cell-Nonmotile	Free	Vegetative
	customer	physiologi		assay_cl	palmer_in						
--------------------	----------	------------	--	----------	-----------						
raw_id tracking_id	_id	cal_state	taxonomic_authority	ass	dex						
1 120001-282	282	Live	(Ktz.) Grunow	1	0						
2 120001-282	282	Live	Germain	1	0						
3 120001-282	282	Live	(Kutzing) Grunow	1	0						
4 120001-282	282	Live	Kutzing	1	0						
5 120001-282	282	Live	Krammer	1	0						
6 120001-282	282	Live	(Ehrenb.) Van Heurck	1	0						
7 120001-282	282	Live	Geitler	1	0						
8 120001-282	282	Live	Bleisch	1	0						
9 120001-282	282	Live	Gregory	1	0						
10 120001-282	282	Live	Kutzing 1833	0	0						
11 120001-282	282	Live	Grunow in Van Heurck	1	0						
12 120001-282	282	Live	(Kutzing) Lange-Bertalot	1	0						
13 120001-282	282	Live	Rabenh.	1	0						
14 120001-282	282	Live	C. Agardh	1	1						
15 120001-282	282	Live	(Ktz.) Ktz.	1	1						
16 120001-282	282	Live	(Grunow) Reichardt & Lange-Bertalot	1	0						
17 120001-282	282	Live	Ehrenb.	1	0						
18 120001-282	282	Live	C. A. Agardh (for genus)	1	1						
19 120001-282	282	Live	Lange-Bert.	1	0						
20 120001-282	282	Live	Ktz.	1	0						
21 120001-282	282	Live	Hantzsch	1	0						
22 120001-282	282	Live	(Nitzsch) Ehrenb.	1	3						
23 120002-282	282	Live	Hustedt	0	0						
24 120002-282	282	Live	Kutzing	1	0						
25 120002-282	282	Live	(Kutzing) Grunow	1	0						
26 120002-282	282	Live	(Ehrenb.) Van Heurck	1	0						
27 120002-282	282	Live	(Kutzing) Brun	1	0						
28 120002-282	282	Live	Bleisch	1	0						
29 120002-282	282	Live	Kutzing 1833	0	0						
30 120002-282	282	Live	N"rpell-Schempp & Lange-Bert. in Lange-Bert.	1	0						
31 120002-282	282	Live	(Kutzing) Lange-Bertalot	1	0						

	customer	physiologi		assay_cl	palmer_in
raw_id tracking_id	_id	cal_state	taxonomic_authority	ass	dex
32 120002-282	282	Live	Kitton	1	0
33 120002-282	282	Live	(Ktz.) Ktz.	1	1
34 120002-282	282	Live	(Grunow) Reichardt & Lange-Bertalot	1	0
35 120002-282	282	Live	C. A. Agardh (for genus)	1	1
36 120002-282	282	Live	(Greville) C. Agardh	1	0
37 120002-282	282	Live	Lange-Bert.	1	0
38 120002-282	282	Live		0	0
39 120002-282	282	Live	Kutzing	1	0
40 120002-282	282	Live	Grunow	1	0
41 120002-282	282	Live	Hantzsch	1	0
42 120002-282	282	Live	(Ehrenberg) Cleve	1	0
43 120002-282	282	Live	Grunow	1	0
44 120002-282	282	Live	(Nitzsch) Ehrenb.	1	3
45 120002-282	282	Live	(Lyngb.) Ktz.	1	0
46 120002-282	282		N/A	1	0
47 120003-282	282	Live	Grunow	1	0
48 120003-282	282	Live	Hustedt	0	0
49 120003-282	282	Live	Kutzing	1	0
50 120003-282	282	Live	Bleisch	1	0
51 120003-282	282	Live	(Kutzing) Lange-Bertalot	1	0
52 120003-282	282	Live	(Ktz.) Ktz.	1	1
53 120003-282	282	Live	Lange-Bert.	1	0
54 120003-282	282	Live	(Ktz.) W. Sm.	1	3
55 120003-282	282	Live	Hantzsch	1	0
56 120004-282	282	Live	Kutzing	1	0
57 120004-282	282	Live	Hustedt	0	0
58 120004-282	282	Live	(Kutzing) Grunow	1	0
59 120004-282	282	Live	(Grunow) Ross	1	0
60 120004-282	282	Live	Bleisch	1	0
61 120004-282	282	Live	(Kutzing) Lange-Bertalot	1	0
62 120004-282	282	Live	(Ktz.) Ktz.	1	1

	customer	physiologi		assay_cl	palmer_in
raw_id tracking_id	_id	cal_state	taxonomic_authority	ass	dex
63 120004-282	282	Live	(Grunow) Reichardt & Lange-Bertalot	1	0
64 120004-282	282	Live	Lange-Bert.	1	0
65 120005-282	282	Live	Brun	1	0
66 120005-282	282	Live	Kutzing	1	0
67 120005-282	282	Live	Hustedt	0	0
68 120005-282	282	Live	(Grunow) Ross	1	0
69 120005-282	282	Live	(Ehrenb.) Van Heurck	1	0
70 120005-282	282	Live	(Ehrenberg in Hemprich & Ehrenberg) Kirchner in Cohn	1	0
71 120005-282	282	Live	Bleisch	1	0
72 120005-282	282	Live	N"rpell-Schempp & Lange-Bert. in Lange-Bert.	1	0
73 120005-282	282	Live	(Kutzing) Lange-Bertalot	1	0
74 120005-282	282	Live	Kitton	1	0
75 120005-282	282	Live	(Grunow) Reichardt & Lange-Bertalot	1	0
76 120005-282	282	Live	Ehrenb.	1	0
77 120005-282	282	Live	Lange-Bert.	1	0
78 120005-282	282	Live	(Nitzsch) Ehrenb.	1	3

			area_per_u	volume_pe	area_to_v	biovolume
	customer		nit_square	r_unit_cubi	olume_rati	_to_volum
raw_id tracking_id	_id	environmental_tolerance_class	_um	c_um	0	e_ratio
1 120001-282	282	P4,N3,H8,.,.,,,,,,,,,,	104.5522	51.4719	2.031	1
2 120001-282	282	P3,N3,H8,S5,.,,,,,,01,Y1,.	273.4442	257.3593	1.063	1
3 120001-282	282	P5,.,H4,.,,,,,,,,,,,,,	126.669	86.8588	1.458	1
4 120001-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	72.4828	31.1445	2.327	1
5 120001-282	282	.,.,H8,.,,,,,,,,,,,,	84.446	36.1911	2.333	1
6 120001-282	282	P4,N1,H8,S4-S5,F3-F4,.,C3/C7,D4,.,O3,Y2,*1	802.3376	1098.7632	0.73	1
7 120001-282	282	P4,N1,H8,S5,F3-F4,.,C3,D4,.,O3,Y2,.	1105.8406	1608.4954	0.688	1
8 120001-282	282	P3,.,H8,S3,.,,,,,,O3,Y2,.	213.5254	165.1826	1.293	1
9 120001-282	282	P3,N3,H8,S4,F1,.,C3,.,T2-T3,O1,Y2,*1	284.8	250.88	1.135	1
10 120001-282	282	P4-P5,.,H9,S7,.,,,,,T3-T4,,,,*1	144.2619	95.1023	1.517	1
11 120001-282	282	P4,N1,H8,S5,F3,L3-L4,C3,.,,,O1,Y1,*1	145.7699	120.6372	1.208	1
12 120001-282	282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	231.1298	162.4946	1.422	1
13 120001-282	282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	765.1412	490.3498	1.56	1
14 120001-282	282	.,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	412.9739	338.9924	1.218	1
15 120001-282	282	P3,N1,H8,S2,F4,.,C3,.,T2/T4,O4,Y3,*1	442.5957	406.6276	1.088	1
16 120001-282	282	.,.,H8,.,,.,.,.,.,.,	300.9538	176.134	1.709	1
17 120001-282	282	P4,N2,H8,S4,.,.,,,02,Y1,.	787.7686	1003.0645	0.785	1
18 120001-282	282	P4, N1, H8, S3-S4, F3, L3/L4/L6, C3, D3, T6/T3-T5, Y3, O3, *1	2488.1414	8980.7662	0.277	1
19 120001-282	282	P4,N0,H8,S4,.,,,,,,,,,,	343.8159	260.5763	1.319	1
20 120001-282	282	P4,N1,H7-H8,S4,.,L4,C3,D2/D4,.,O4,Y2,*1	659.4831	669.1341	0.986	1
21 120001-282	282	P3,N3,H6,S4,.,,,,,,,O2,.,.	309.6354	160.8495	1.925	1
22 120001-282	282	P4,N1,H8,S4-S5,F3,.,C1,D2-D4,T2-T3,.,,,*1	1399.296	1648.64	0.849	1
23 120002-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	112.2931	72.0204	1.559	1
24 120002-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	98.6209	45.8019	2.153	1
25 120002-282	282	P4,N1,H8,S4,.,,,,,,O2,Y3,.	42.6598	25.0925	1.7	1
26 120002-282	282	P4,N1,H8,S4-S5,F3-F4,.,C3/C7,D4,.,O3,Y2,*1	425.4973	392.5399	1.084	1
27 120002-282	282	.,.,H8,S3,,,,,,,,,,,	1085.6233	1754.624	0.619	1
28 120002-282	282	P3,.,H8,S3,.,.,,,O3,Y2,.	95.5423	41.2672	2.315	1
29 120002-282	282	P4-P5,.,H9,S7,.,,,,,T3-T4,,,,*1	160.8495	115.8117	1.389	1
30 120002-282	282	P2-P3,N1-N5,H8-H9,S5/S3,.,L6/L3/L4/L5/L7,C3,.,.,O3,Y2,*1	865.2197	1186.2654	0.729	1
31 120002-282	282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	176.9345	128.6796	1.375	1

			area_per_u	volume_pe	area_to_v	biovolume
	customer		nit_square	r_unit_cubi	olume_rati	_to_volum
raw_id tracking_id	_id	environmental_tolerance_class	_um	c_um	0	e_ratio
32 120002-282	282	P4,N1-N3,H8,S4-S5,F3,.,C1,.,,O2,Y2,*1	419.0131	300.7082	1.393	1
33 120002-282	282	P3,N1,H8,S2,F4,.,C3,.,T2/T4,O4,Y3,*1	315.7063	260.2776	1.213	1
34 120002-282	282	.,.,H8,.,,,,,,,,,,,,,	333.4611	236.3709	1.411	1
35 120002-282	282	P4, N1, H8, S3-S4, F3, L3/L4/L6, C3, D3, T6/T3-T5, Y3, O3, *1	2010.6193	6433.9818	0.313	1
36 120002-282	282	P4,N1,H8,S5,F4-F5,L3-L6,C2-C3,D2,T2-T3/T6,O2,Y2,*1	770.2057	321.6991	2.394	1
37 120002-282	282	P4,N0,H8,S4,.,.,,,,,,,,	295.561	221.9724	1.332	1
38 120002-282	282	• • • • • • • • • • • • • • • • • • • •	271.4336	202.6704	1.339	1
39 120002-282	282	P3,N1,H8,S3,F3,L4,C3,.,,O3,Y2,*1	623.292	764.0353	0.816	1
40 120002-282	282	P3,N1,H5,S4,.,,,,,,O2,Y2,.	380.007	289.5292	1.313	1
41 120002-282	282	P4,.,H8,S4,,,,,,,,O2,Y2,.	464.9557	301.5929	1.542	1
42 120002-282	282	• • • • • • • • • • • • • • • • • • • •	967.68	1105.92	0.875	1
43 120002-282	282	P2,N5,H8,S5,F4,.,,,,,O1,,,	291.84	114.688	2.545	1
44 120002-282	282	P4,N1,H8,S4-S5,F3,.,C1,D2-D4,T2-T3,.,,,*1	3991.68	6359.04	0.628	1
45 120002-282	282	P2,N0-N6,H9,S4-S5/S7,F1,L3-L4,C3/C9,D2/D4,.,O1,Y1,*1	2292.48	3939.84	0.582	1
46 120002-282	282	., ., ., ., ., ., ., ., ., ., .	48.9331	32.1833	1.52	1
47 120003-282	282	P4,N1,H8,S7,F2,L3,C3,,O1,Y1,.	237.2531	218.7554	1.085	1
48 120003-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	95.806	50.3459	1.903	1
49 120003-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	62.9232	23.3597	2.694	1
50 120003-282	282	P3,.,H8,S3,.,.,,,O3,Y2,.	240.9279	181.2252	1.329	1
51 120003-282	282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	127.3727	87.7836	1.451	1
52 120003-282	282	P3,N1,H8,S2,F4,.,C3,.,T2/T4,O4,Y3,*1	290.6813	200.1184	1.453	1
53 120003-282	282	P4,N0,H8,S4,.,,,,,,,,,	325.7203	246.0998	1.324	1
54 120003-282	282	P3,N1,H8,S1-S2,F3,L3-L4,C2-C3,D2-D4,T6,O4,Y2,*1	188.9982	96.5097	1.958	1
55 120003-282	282	P4,.,H8,S4,,,,,,,,O2,Y2,.	425.246	325.7203	1.306	1
56 120004-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	84.4209	39.2976	2.148	1
57 120004-282	282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	106.9649	58.6297	1.824	1
58 120004-282	282	P4,N1,H8,S4,.,,,,,,,O2,Y3,.	175.6307	144.4965	1.215	1
59 120004-282	282	P3-P5,N1-N5,H8,S7,F3,.,C3,.,.,O2,Y1,*1	289.5292	217.1469	1.333	1
60 120004-282	282	P3,.,H8,S3,,,,,,,,,O3,Y2,.	278.6795	237.2949	1.174	1
61 120004-282	282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	135.7796	76.4035	1.777	1
62 120004-282	282	P3,N1,H8,S2,F4,.,C3,.,T2/T4,O4,Y3,*1	304.7738	202.7678	1.503	1

		area_per_u	volume_pe	area_to_v	biovolume
custome	r	nit_square	r_unit_cubi	olume_rati	_to_volum
raw_id tracking_id _id	environmental_tolerance_class	_um	c_um	0	e_ratio
63 120004-282 282	.,.,H8,,,,,,,,,,,,,,	421.0042	333.089	1.264	1
64 120004-282 282	P4,N0,H8,S4,,,,,,,,,,,,	298.577	224.3851	1.331	1
65 120005-282 282	P2-P3,N5,H9,S5,F2,L2-L3,.,.,,O1,Y1,*1	382.0177	442.3362	0.864	1
66 120005-282 282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	56.2973	19.503	2.887	1
67 120005-282 282	P3,N6,H8,S2-S5,F3,.,C3,.,T6,O1,Y2,*1	95.806	50.0644	1.914	1
68 120005-282 282	P3-P5,N1-N5,H8,S7,F3,.,C3,.,.,O2,Y1,*1	224.5192	163.3628	1.374	1
69 120005-282 282	P4,N1,H8,S4-S5,F3-F4,.,C3/C7,D4,.,O3,Y2,*1	1570.7963	2827.4334	0.556	1
70 120005-282 282	P4,N1,H8,S5,F1-F2,L3,C3,.,.,O2,Y1,*1	363.955	241.2544	1.509	1
71 120005-282 282	P3,.,H8,S3,.,,.,,O3,Y2,.	313.5868	269.6533	1.163	1
72 120005-282 282	P2-P3,N1-N5,H8-H9,S5/S3,.,L6/L3/L4/L5/L7,C3,.,,,O3,Y2,*1	1068.0912	2508.0465	0.426	1
73 120005-282 282	P4,N1,H8,S4,F3-F4,L6,C3,.,T3,O1,Y2,.	138.9338	86.2154	1.611	1
74 120005-282 282	P4,N1-N3,H8,S4-S5,F3,.,C1,.,,O2,Y2,*1	302.3217	173.3154	1.744	1
75 120005-282 282	.,.,H8,,,,,,,,,,,,,,	378.1649	273.4492	1.383	1
76 120005-282 282	P4,N2,H8,S4,,,,,,,,O2,Y1,.	1767.2267	2597.9089	0.68	1
77 120005-282 282	P4,N0,H8,S4,,,,,,,,,,,,	325.7203	246.0998	1.324	1
78 120005-282 282	• • • • • • • • • • • • • • • • • • • •	307.2	229.376	1.339	1

	concentrat										
					average_	ion_natura	total_area_s		total_volume		total_biovolu
			biovolume_	biomass_	cells_per	l_unit_per	quare_um_p	relative_total	_cubic_um_	relative_total	me_cubic_u
		customer	per_unit_c	per_anim	_natural_	_square_c	er_square_c	_area_perce	per_square_	_volume_pe	m_per_squa
raw_id	tracking_id	_id	ubic_um	al_ug	unit	m_	m_	nt	cm_	rcent	re_cm_
1	120001-282	282	51.4719	0	1	2	209.1044	0.00220216	102.9438	0.0010429	102.9438
2	120001-282	282	257.3593	0	1	2	546.8884	0.0057595	514.7186	0.00521449	514.7186
3	120001-282	282	86.8588	0	1	1	126.669	0.001334	86.8588	0.00087995	86.8588
4	120001-282	282	31.1445	0	1	400	28993.12	0.30533832	12457.8	0.12620689	12457.8
5	120001-282	282	36.1911	0	1	1	84.446	0.00088934	36.1911	0.00036664	36.1911
6	120001-282	282	1098.7632	0	1	4	3209.3504	0.03379897	4395.0528	0.04452519	4395.0528
7	120001-282	282	1608.4954	0	1	3	3317.5218	0.03493817	4825.4862	0.04888581	4825.4862
8	120001-282	282	165.1826	0	1	17	3629.9318	0.03822829	2808.1042	0.02844821	2808.1042
9	120001-282	282	250.88	0	1	2	569.6	0.00599869	501.76	0.00508321	501.76
10	120001-282	282	95.1023	0	1	2	288.5238	0.00303856	190.2046	0.00192692	190.2046
11	120001-282	282	120.6372	0	1	2	291.5398	0.00307032	241.2744	0.00244429	241.2744
12	120001-282	282	162.4946	0	1	53	12249.8794	0.12900845	8612.2138	0.08724821	8612.2138
13	120001-282	282	490.3498	0	2	13	9946.8356	0.10475416	6374.5474	0.06457896	6374.5474
14	120001-282	282	338.9924	0	1	2	825.9478	0.00869839	677.9848	0.0068685	677.9848
15	120001-282	282	406.6276	0	1	9	3983.3613	0.0419504	3659.6484	0.03707499	3659.6484
16	120001-282	282	176.134	0	1	6	1805.7228	0.0190168	1056.804	0.01070622	1056.804
17	120001-282	282	1003.0645	0	1	3	2363.3058	0.02488893	3009.1935	0.03048539	3009.1935
18	120001-282	282	8980.7662	0	1	4	9952.5656	0.10481451	35923.0648	0.36392768	35923.0648
19	120001-282	282	260.5763	0	1	7	2406.7113	0.02534605	1824.0341	0.01847884	1824.0341
20	120001-282	282	669.1341	0	1	1	659.4831	0.00694528	669.1341	0.00677883	669.1341
21	120001-282	282	160.8495	0	1	1	309.6354	0.0032609	160.8495	0.00162953	160.8495
22	120001-282	282	1648.64	0	1	6	8395.776	0.08841933	9891.84	0.10021178	9891.84
23	120002-282	282	72.0204	0	1	236	26501.1716	0.23597605	16996.8144	0.14304366	16996.8144
24	120002-282	282	45.8019	0	1	46	4536.5614	0.04039519	2106.8874	0.01773137	2106.8874
25	120002-282	282	25.0925	0	1	1	42.6598	0.00037986	25.0925	0.00021118	25.0925
26	120002-282	282	392.5399	0	1	39	16594.3947	0.14776251	15309.0561	0.12883964	15309.0561
27	120002-282	282	1754.624	0	1	3	3256.8699	0.02900035	5263.872	0.04430027	5263.872
28	120002-282	282	41.2672	0	1	4	382.1692	0.00340297	165.0688	0.0013892	165.0688
29	120002-282	282	115.8117	0	1	2	321.699	0.00286452	231.6234	0.00194932	231.6234
30	120002-282	282	1186.2654	0	1	2	1730.4394	0.01540846	2372.5308	0.01996701	2372.5308
31	120002-282	282	128.6796	0	1	7	1238.5415	0.01102842	900.7572	0.00758069	900.7572

	concentrat										
					average_	ion_natura	total_area_s		total_volume		total_biovolu
			biovolume_	biomass_	cells_per	l_unit_per	quare_um_p	relative_total	_cubic_um_	relative_total	me_cubic_u
		customer	per_unit_c	per_anim	_natural_	_square_c	er_square_c	_area_perce	per_square_	_volume_pe	m_per_squa
raw_id	tracking_id	_id	ubic_um	al_ug	unit	m_	m_	nt	cm_	rcent	re_cm_
32	120002-282	282	300.7082	0	1	22	9218.2882	0.08208298	6615.5804	0.05567613	6615.5804
33	120002-282	282	260.2776	0	1	13	4104.1819	0.03654513	3383.6088	0.02847615	3383.6088
34	120002-282	282	236.3709	0	1	20	6669.222	0.05938517	4727.418	0.03978552	4727.418
35	120002-282	282	6433.9818	0	1	2	4021.2386	0.03580657	12867.9636	0.10829563	12867.9636
36	120002-282	282	321.6991	0	1	1	770.2057	0.00685819	321.6991	0.00270739	321.6991
37	120002-282	282	221.9724	0	1	6	1773.366	0.01579069	1331.8344	0.0112086	1331.8344
38	120002-282	282	202.6704	0	1	3	814.3008	0.00725083	608.0112	0.00511697	608.0112
39	120002-282	282	764.0353	0	1	1	623.292	0.00555002	764.0353	0.00643005	764.0353
40	120002-282	282	289.5292	0	1	2	760.014	0.00676744	579.0584	0.0048733	579.0584
41	120002-282	282	301.5929	0	1	3	1394.8671	0.0124204	904.7787	0.00761454	904.7787
42	120002-282	282	1105.92	0	1	1	967.68	0.00861657	1105.92	0.00930732	1105.92
43	120002-282	282	114.688	0	1	1	291.84	0.00259865	114.688	0.0009652	114.688
44	120002-282	282	6359.04	0	1	6	23950.08	0.21326021	38154.24	0.32110265	38154.24
45	120002-282	282	3939.84	0	1	1	2292.48	0.02041307	3939.84	0.03315734	3939.84
46	120002-282	282	32.1833	0	1	1	48.9331	0.00043572	32.1833	0.00027085	32.1833
47	120003-282	282	218.7554	0	1	1	237.2531	0.00598715	218.7554	0.01031856	218.7554
48	120003-282	282	50.3459	0	1	287	27496.322	0.69387795	14449.2733	0.68156379	14449.2733
49	120003-282	282	23.3597	0	1	88	5537.2416	0.13973396	2055.6536	0.09696398	2055.6536
50	120003-282	282	181.2252	0	1	3	722.7837	0.01823966	543.6756	0.02564486	543.6756
51	120003-282	282	87.7836	0	1	14	1783.2178	0.04500004	1228.9704	0.05796982	1228.9704
52	120003-282	282	200.1184	0	1	6	1744.0878	0.04401258	1200.7104	0.05663681	1200.7104
53	120003-282	282	246.0998	0	1	4	1302.8812	0.0328786	984.3992	0.04643354	984.3992
54	120003-282	282	96.5097	0	1	2	377.9964	0.00953885	193.0194	0.00910461	193.0194
55	120003-282	282	325.7203	0	1	1	425.246	0.01073121	325.7203	0.01536404	325.7203
56	120004-282	282	39.2976	0	1	79	6669.2511	0.1332011	3104.5104	0.10646787	3104.5104
57	120004-282	282	58.6297	0	1	302	32303.3998	0.64517716	17706.1694	0.60722559	17706.1694
58	120004-282	282	144.4965	0	1	1	175.6307	0.00350777	144.4965	0.00495545	144.4965
59	120004-282	282	217.1469	0	1	1	289.5292	0.0057826	217.1469	0.00744696	217.1469
60	120004-282	282	237.2949	0	1	2	557.359	0.01113181	474.5898	0.01627586	474.5898
61	120004-282	282	76.4035	0	1	10	1357.796	0.02711848	764.035	0.02620226	764.035
62	120004-282	282	202.7678	0	1	2	609.5476	0.01217414	405.5356	0.01390767	405.5356

concentrat											
					average_	ion_natura	total_area_s		total_volume		total_biovolu
			biovolume_	biomass_	cells_per	l_unit_per	quare_um_p	relative_total	_cubic_um_	relative_total	me_cubic_u
		customer	per_unit_c	per_anim	_natural_	_square_c	er_square_c	_area_perce	per_square_	_volume_pe	m_per_squa
raw_id	tracking_id	_id	ubic_um	al_ug	unit	m_	m_	nt	cm_	rcent	re_cm_
63	120004-282	282	333.089	0	1	15	6315.063	0.12612711	4996.335	0.1713472	4996.335
64	120004-282	282	224.3851	0	1	6	1791.462	0.03577984	1346.3106	0.04617115	1346.3106
65	120005-282	282	442.3362	0	1	2	764.0354	0.01792748	884.6724	0.02957756	884.6724
66	120005-282	282	19.503	0	1	276	15538.0548	0.36458795	5382.828	0.17996598	5382.828
67	120005-282	282	50.0644	0	1	54	5173.524	0.12139258	2703.4776	0.09038632	2703.4776
68	120005-282	282	163.3628	0	1	12	2694.2304	0.06321795	1960.3536	0.06554119	1960.3536
69	120005-282	282	2827.4334	0	1	1	1570.7963	0.03685747	2827.4334	0.09453058	2827.4334
70	120005-282	282	241.2544	0	1	1	363.955	0.00853991	241.2544	0.00806594	241.2544
71	120005-282	282	269.6533	0	1	2	627.1736	0.01471612	539.3066	0.01803083	539.3066
72	120005-282	282	2508.0465	0	1	2	2136.1824	0.0501238	5016.093	0.1677048	5016.093
73	120005-282	282	86.2154	0	1	21	2917.6098	0.06845937	1810.5234	0.06053187	1810.5234
74	120005-282	282	173.3154	0	1	15	4534.8255	0.10640603	2599.731	0.08691772	2599.731
75	120005-282	282	273.4492	0	1	6	2268.9894	0.05324001	1640.6952	0.05485394	1640.6952
76	120005-282	282	2597.9089	0	1	1	1767.2267	0.04146655	2597.9089	0.0868568	2597.9089
77	120005-282	282	246.0998	0	1	6	1954.3218	0.04585659	1476.5988	0.04936765	1476.5988
78	120005-282	282	229.376	0	1	1	307.2	0.0072082	229.376	0.00766881	229.376

				total_anim		algal_cell_	algal_bioma				
				al_biomas	relative_to	concentrat	ss_concentr	relative_alga	relative_alga		
			relative_total	s_ug_per	tal_animal	ion_cells_	ation_mg_p	I_cell_conce	I_biomass_c	relative_con	
		customer	_biovolume_	_square_c	_biomass	per_squar	er_square_c	ntration_per	oncentration	centration_p	average_
raw_id	tracking_id	_id	percent	m_	_percent	e_cm_	m_	cent	_percent	ercent	gald_um
1	120001-282	282	0.0010429	0	0	2	0.0000001	0.00359712	0.00101308	0.00368324	12.8
2	120001-282	282	0.00521449	0	0	2	0.00000051	0.00359712	0.00516668	0.00368324	16
3	120001-282	282	0.00087995	0	0	1	0.0000009	0.00179856	0.00091177	0.00184162	9.6
4	120001-282	282	0.12620689	0	0	400	0.00001246	0.71942446	0.12622918	0.73664825	10.32
5	120001-282	282	0.00036664	0	0	1	0.00000004	0.00179856	0.00040523	0.00184162	8
6	120001-282	282	0.04452519	0	0	4	0.0000044	0.00719424	0.04457531	0.00736648	25.92
7	120001-282	282	0.04888581	0	0	3	0.00000483	0.00539568	0.04893153	0.00552486	32
8	120001-282	282	0.02844821	0	0	17	0.00000281	0.03057554	0.02846741	0.03130755	15.0667
9	120001-282	282	0.00508321	0	0	2	0.0000005	0.00359712	0.00506538	0.00368324	16
10	120001-282	282	0.00192692	0	0	2	0.00000019	0.00359712	0.00192484	0.00368324	14.8
11	120001-282	282	0.00244429	0	0	2	0.00000024	0.00359712	0.00243138	0.00368324	9.6
12	120001-282	282	0.08724821	0	0	53	0.00000861	0.09532374	0.08722578	0.09760589	23.4182
13	120001-282	282	0.06457896	0	0	26	0.00000637	0.04676259	0.06453289	0.02394107	43.28
14	120001-282	282	0.0068685	0	0	2	0.00000068	0.00359712	0.00688891	0.00368324	27.2
15	120001-282	282	0.03707499	0	0	9	0.00000366	0.01618705	0.03707855	0.01657459	25.6
16	120001-282	282	0.01070622	0	0	6	0.00000106	0.01079137	0.0107386	0.01104972	28
17	120001-282	282	0.03048539	0	0	3	0.00000301	0.00539568	0.03049356	0.00552486	33.2
18	120001-282	282	0.36392768	0	0	4	0.00003592	0.00719424	0.36389663	0.00736648	36.2667
19	120001-282	282	0.01847884	0	0	7	0.00000182	0.01258993	0.01843797	0.01289134	28.8
20	120001-282	282	0.00677883	0	0	1	0.00000067	0.00179856	0.0067876	0.00184162	41.6
21	120001-282	282	0.00162953	0	0	1	0.00000016	0.00179856	0.00162092	0.00184162	40
22	120001-282	282	0.10021178	0	0	6	0.00000989	0.01079137	0.10019314	0.01104972	75.2
23	120002-282	282	0.14304366	0	0	236	0.000017	0.55791962	0.14307047	0.55791962	9.44
24	120002-282	282	0.01773137	0	0	46	0.00000211	0.10874704	0.01775757	0.10874704	13.28
25	120002-282	282	0.00021118	0	0	1	0.0000003	0.00236407	0.00025248	0.00236407	4.8
26	120002-282	282	0.12883964	0	0	39	0.00001531	0.09219858	0.12884758	0.09219858	19.3333
27	120002-282	282	0.04430027	0	0	3	0.00000526	0.0070922	0.04426769	0.0070922	36.8
28	120002-282	282	0.0013892	0	0	4	0.00000017	0.00945626	0.0014307	0.00945626	12.4
29	120002-282	282	0.00194932	0	0	2	0.0000023	0.00472813	0.00193566	0.00472813	14.4
30	120002-282	282	0.01996701	0	0	2	0.00000237	0.00472813	0.01994571	0.00472813	80
31	120002-282	282	0.00758069	0	0	7	0.0000009	0.01654846	0.00757432	0.01654846	16

			total_anim		algal_cell_	algal_bioma				
			al_biomas	relative_to	concentrat	ss_concentr	relative_alga	relative_alga		
		relative_total	s_ug_per	tal_animal	ion_cells_	ation_mg_p	I_cell_conce	I_biomass_c	relative_con	
	customer	_biovolume_	_square_c	_biomass	per_squar	er_square_c	ntration_per	oncentration	centration_p	average_
raw_id tracking_id	_id	percent	m_	_percent	e_cm_	m_	cent	_percent	ercent	gald_um
32 120002-28	2 282	0.05567613	0	0	22	0.00000662	0.05200946	0.05571332	0.05200946	44.32
33 120002-28	2 282	0.02847615	0	0	13	0.00000338	0.03073286	0.02844578	0.03073286	20.8
34 120002-28	2 282	0.03978552	0	0	20	0.00000473	0.04728132	0.03980725	0.04728132	25.6
35 120002-282	2 282	0.10829563	0	0	2	0.00001287	0.00472813	0.10831276	0.00472813	32
36 120002-28	2 282	0.00270739	0	0	1	0.0000032	0.00236407	0.00269309	0.00236407	40
37 120002-28	2 282	0.0112086	0	0	6	0.00000133	0.0141844	0.01119316	0.0141844	24.5333
38 120002-282	2 282	0.00511697	0	0	3	0.00000061	0.0070922	0.00513371	0.0070922	22.4
39 120002-28	2 282	0.00643005	0	0	1	0.00000076	0.00236407	0.00639609	0.00236407	30.4
40 120002-28	2 282	0.0048733	0	0	2	0.00000058	0.00472813	0.00488123	0.00472813	32
41 120002-28	2 282	0.00761454	0	0	3	0.0000009	0.0070922	0.00757432	0.0070922	48
42 120002-28	2 282	0.00930732	0	0	1	0.00000111	0.00236407	0.00934166	0.00236407	48
43 120002-28	2 282	0.0009652	0	0	1	0.00000011	0.00236407	0.00092575	0.00236407	44.8
44 120002-28	2 282	0.32110265	0	0	6	0.00003815	0.0141844	0.32106697	0.0141844	156
45 120002-28	2 282	0.03315734	0	0	1	0.00000394	0.00236407	0.03315869	0.00236407	76
46 120002-282	2 282	0.00027085	0	0	1	0.0000003	0.00236407	0.00025248	0.00236407	4
47 120003-28	2 282	0.01031856	0	0	1	0.00000022	0.00246305	0.01037727	0.00246305	13.6
48 120003-28	2 282	0.68156379	0	0	287	0.00001445	0.70689655	0.68159806	0.70689655	10.72
49 120003-28	2 282	0.09696398	0	0	88	0.00000206	0.21674877	0.097169	0.21674877	10.3273
50 120003-28	2 282	0.02564486	0	0	3	0.00000054	0.00738916	0.02547148	0.00738916	18.1333
51 120003-28	2 282	0.05796982	0	0	14	0.00000123	0.03448276	0.05801838	0.03448276	11.44
52 120003-28	2 282	0.05663681	0	0	6	0.0000012	0.01477833	0.0566033	0.01477833	21.2
53 120003-28	2 282	0.04643354	0	0	4	0.0000098	0.00985222	0.04622603	0.00985222	27.2
54 120003-28	2 282	0.00910461	0	0	2	0.00000019	0.00492611	0.00896219	0.00492611	24
55 120003-28	2 282	0.01536404	0	0	1	0.0000033	0.00246305	0.01556591	0.00246305	36
56 120004-28	2 282	0.10646787	0	0	79	0.0000031	0.18899522	0.10631319	0.18899522	11.04
57 120004-28	2 282	0.60722559	0	0	302	0.00001771	0.72248804	0.60735696	0.72248804	11.52
58 120004-28	2 282	0.00495545	0	0	1	0.00000014	0.00239234	0.00480124	0.00239234	17.6
59 120004-28	2 282	0.00744696	0	0	1	0.00000022	0.00239234	0.00754481	0.00239234	24
60 120004-282	2 282	0.01627586	0	0	2	0.00000047	0.00478469	0.01611845	0.00478469	17.6
61 120004-282	2 282	0.02620226	0	0	10	0.00000076	0.02392344	0.02606388	0.02392344	16.8
62 120004-282	2 282	0.01390767	0	0	2	0.00000041	0.00478469	0.01406078	0.00478469	24

				total_anim		algal_cell_	algal_bioma				
				al_biomas	relative_to	concentrat	ss_concentr	relative_alga	relative_alga		
			relative_total	s_ug_per	tal_animal	ion_cells_	ation_mg_p	I_cell_conce	I_biomass_c	relative_con	
		customer	_biovolume_	_square_c	_biomass	per_squar	er_square_c	ntration_per	oncentration	centration_p	average_
raw_id tr	racking_id	_id	percent	m_	_percent	e_cm_	m_	cent	_percent	ercent	gald_um
63 12	20004-282	282	0.1713472	0	0	15	0.000005	0.03588517	0.17147288	0.03588517	28.64
64 12	20004-282	282	0.04617115	0	0	6	0.00000135	0.01435407	0.04629768	0.01435407	24.8
65 12	20005-282	282	0.02957756	0	0	2	0.0000088	0.005	0.02942135	0.005	17.6
66 12	20005-282	282	0.17996598	0	0	276	0.00000538	0.69	0.17987143	0.69	10.4
67 12	20005-282	282	0.09038632	0	0	54	0.0000027	0.135	0.09027005	0.135	10.56
68 12	20005-282	282	0.06554119	0	0	12	0.00000196	0.03	0.06552937	0.03	18.6667
69 12	20005-282	282	0.09453058	0	0	1	0.00000283	0.0025	0.09461639	0.0025	36
70 12	20005-282	282	0.00806594	0	0	1	0.00000024	0.0025	0.008024	0.0025	32
71 12	20005-282	282	0.01803083	0	0	2	0.00000054	0.005	0.01805401	0.005	20
72 12	20005-282	282	0.1677048	0	0	2	0.00000502	0.005	0.16783543	0.005	56
73 12	20005-282	282	0.06053187	0	0	21	0.00000181	0.0525	0.06051437	0.0525	15.2
74 12	20005-282	282	0.08691772	0	0	15	0.0000026	0.0375	0.08692672	0.0375	39.68
75 12	20005-282	282	0.05485394	0	0	6	0.00000164	0.015	0.0548307	0.015	28.8
76 12	20005-282	282	0.0868568	0	0	1	0.0000026	0.0025	0.08692672	0.0025	51.2
77 12	20005-282	282	0.04936765	0	0	6	0.00000148	0.015	0.04948136	0.015	27.2
78 12	20005-282	282	0.00766881	0	0	1	0.00000023	0.0025	0.00768967	0.0025	22.4

						customer_r			sample_	measure	
		customer	taxa_	cou		equested_		sample_	weight_u	ment_not	tally_note
raw_id ti	racking_id	_id	nt		uncertain	units	alternate_taxa_name	weight	nit	es	S
1 1	20001-282	282		2	FALSE	NU/sq. cm		0			
2 1	20001-282	282		2	FALSE	NU/sq. cm	Psammothidium bioretii	0			
3 1	20001-282	282		1	FALSE	NU/sq. cm	Achnanthes hauckiana var. rostrata	0			
4 1	20001-282	282		400	FALSE	NU/sq. cm	Achnanthidium minutissimum	0			
5 1	20001-282	282		1	FALSE	NU/sq. cm		0			
6 1	20001-282	282		4	FALSE	NU/sq. cm		0			
7 1	20001-282	282		3	FALSE	NU/sq. cm		0			
8 1	20001-282	282		17	FALSE	NU/sq. cm	Encyonema silesiacum	0			
9 1	20001-282	282		2	FALSE	NU/sq. cm		0			
10 1	20001-282	282		2	FALSE	NU/sq. cm		0			
11 1	20001-282	282		2	FALSE	NU/sq. cm	Pseudostaurosira brevistriata	0			
12 1	20001-282	282		53	FALSE	NU/sq. cm		0			
13 1	20001-282	282		13	FALSE	NU/sq. cm		0			
14 1	20001-282	282		2	FALSE	NU/sq. cm		0			
15 1	20001-282	282		9	FALSE	NU/sq. cm		0			
16 1	20001-282	282		6	FALSE	NU/sq. cm		0			
17 1	20001-282	282		3	FALSE	NU/sq. cm		0			
18 1	20001-282	282		4	FALSE	NU/sq. cm		0			
19 1	20001-282	282		9	FALSE	NU/sq. cm		0			
20 1	20001-282	282		1	FALSE	NU/sq. cm		0			
21 1	20001-282	282		1	FALSE	NU/sq. cm		0			
22 1	20001-282	282		6	FALSE	NU/sq. cm		0			
23 1	20002-282	282		236	TRUE	NU/sq. cm	Achnanthidium minutissimum v. robusta	0			
24 1	20002-282	282		46	FALSE	NU/sq. cm	Achnanthidium minutissimum	0			
25 1	20002-282	282		1	FALSE	NU/sq. cm	Amphora perpusilla	0			
26 1	20002-282	282		39	FALSE	NU/sq. cm		0			
27 1	20002-282	282		3	FALSE	NU/sq. cm		0			
28 1	20002-282	282		4	FALSE	NU/sq. cm	Encyonema silesiacum	0			
29 1	20002-282	282		2	FALSE	NU/sq. cm		0			
30 1	20002-282	282		2	FALSE	NU/sq. cm		0			
31 1	20002-282	282		7	FALSE	NU/sq. cm		0			

				customer_r			sample_	measure	
	customer	taxa_cou		equested_		sample_	weight_u	ment_not	tally_note
raw_id tracking_id	_id	nt	uncertain	units	alternate_taxa_name	weight	nit	es	S
32 120002-282	282	22	FALSE	NU/sq. cm		0			
33 120002-282	282	13	FALSE	NU/sq. cm		0			
34 120002-282	282	20	FALSE	NU/sq. cm		0			
35 120002-282	282	2	FALSE	NU/sq. cm		0			
36 120002-282	282	1	FALSE	NU/sq. cm		0			
37 120002-282	282	6	FALSE	NU/sq. cm		0			
38 120002-282	282	3	FALSE	NU/sq. cm		0			
39 120002-282	282	1	FALSE	NU/sq. cm	Sellaphora pupula	0			
40 120002-282	282	2	FALSE	NU/sq. cm		0			
41 120002-282	282	3	FALSE	NU/sq. cm		0			
42 120002-282	282	1	FALSE	NU/sq. cm		0			
43 120002-282	282	1	FALSE	NU/sq. cm		0			
44 120002-282	282	6	FALSE	NU/sq. cm		0			
45 120002-282	282	1	FALSE	NU/sq. cm		0			
46 120002-282	282	1	FALSE	NU/sq. cm		0			
47 120003-282	282	1	FALSE	NU/sq. cm	Karayevia clevei	0			
48 120003-282	282	287	TRUE	NU/sq. cm	Achnanthidium minutissimum v. robusta	0			
49 120003-282	282	88	FALSE	NU/sq. cm	Achnanthidium minutissimum	0			
50 120003-282	282	3	FALSE	NU/sq. cm	Encyonema silesiacum	0			
51 120003-282	282	14	FALSE	NU/sq. cm		0			
52 120003-282	282	6	FALSE	NU/sq. cm		0			
53 120003-282	282	4	FALSE	NU/sq. cm		0			
54 120003-282	282	2	FALSE	NU/sq. cm		0			
55 120003-282	282	1	FALSE	NU/sq. cm		0			
56 120004-282	282	79	FALSE	NU/sq. cm	Achnanthidium minutissimum	0			
57 120004-282	282	302	TRUE	NU/sq. cm	Achnanthidium minutissimum v. robusta	0	•		
58 120004-282	282	1	FALSE	NU/sq. cm	Amphora perpusilla	0	•		
59 120004-282	282	1	FALSE	NU/sq. cm	Brachysira neoexilis	0			
60 120004-282	282	2	FALSE	NU/sq. cm	Encyonema silesiacum	0			
61 120004-282	282	10	FALSE	NU/sq. cm		0			
62 120004-282	282	2	FALSE	NU/sq. cm		0			

						customer_r			sample_	measure	
		customer	taxa_c	cou		equested_		sample_	weight_u	ment_not	tally_note
raw_id	tracking_id	_id	nt	l	uncertain	units	alternate_taxa_name	weight	nit	es	S
63	120004-282	282		15	FALSE	NU/sq. cm		0	•		
64	120004-282	282		6	FALSE	NU/sq. cm		0	•		
65	120005-282	282		2	FALSE	NU/sq. cm	Eucocconeis flexella	0			
66	120005-282	282		276	FALSE	NU/sq. cm	Achnanthidium minutissimum	0			
67	120005-282	282		54	TRUE	NU/sq. cm	Achnanthidium minutissimum v. robusta	0			
68	120005-282	282		12	FALSE	NU/sq. cm	Brachysira neoexilis	0			
69	120005-282	282		1	FALSE	NU/sq. cm		0			
70	120005-282	282		1	FALSE	NU/sq. cm		0			
71	120005-282	282		2	FALSE	NU/sq. cm	Encyonema silesiacum	0			
72	120005-282	282		2	FALSE	NU/sq. cm		0			
73	120005-282	282		21	FALSE	NU/sq. cm		0			
74	120005-282	282		15	FALSE	NU/sq. cm		0			
75	120005-282	282		6	FALSE	NU/sq. cm		0			
76	120005-282	282		1	FALSE	NU/sq. cm		0			
77	120005-282	282		6	FALSE	NU/sq. cm		0			
78	120005-282	282		1	FALSE	NU/sq. cm		0			

# Statistical Considerations in Assessing the Compliance of Water Quality Criteria

Song S. Qian,<sup>\*,†</sup> Craig A. Stow,<sup>‡</sup> YoonKyung Cha,<sup>¶</sup> and Lester L. Yuan<sup>§</sup>

Department of Environmental Sciences, The University of Toledo, Toledo, OH 43606,

Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric

Administration, Ann Arbor, MI 48108, School of Natural Resources and Environment,

University of Michigan, Ann Arbor, MI 48108, and U.S. Environmental Protection Agency,

Officie of Science and Technology, 1200 Pennsylvania Avenue, N.W., Washington, DC

20460

E-mail: song.qian@utoledo.edu

#### Abstract

We discuss statistical issues related to the assessment of the compliance of numerical water quality criteria. Issues arise when rules and regulations were developed for assessing the compliance of an environmental criterion defined through water quality population characteristics (e.g., mean) that cannot be directly measured, and sample statistics were used instead. A common consequence of many currently used assessment methods is the unknown and often varying levels of confidence of compliance due to, for example, varying sample sizes. We discuss these

1

2

3

<sup>\*</sup>To whom correspondence should be addressed <sup>†</sup>The University of Toledo <sup>‡</sup>NOAA-GLERL <sup>¶</sup>SNRE, Michigan <sup>§</sup>US EPA

issues from a statistical analysis point of view and propose a Bayesian hierarchi-11 cal modeling approach for developing compliance assessment methods. Using the 12 predictive distribution of the target water quality constituent, the Bayesian ap-13 proach results in a uniform standard for compliance assessment based on available 14 information. For locations with adequate monitoring data, the posterior predic-15 tive distribution is readily available. For locations with limited monitoring data, 16 a prior predictive distribution can be derived using cross-sectional water qual-17 ity monitoring data and used to develop initial assessment method. The prior 18 predictive distribution can be subsequently updated when new observations are 19 available. 20

21

Key Words and Terms: Bayesian statistics, Clean Water Act, Compliance
 assessment, Hierarchical model, Multilevel model, nutrient criteria

## 23 Introduction

Under the implementing regulations of the U.S. Clean Water Act (CWA) numeric 24 water quality criteria are established to protect the designated uses of a waterbody 25 (1). Numeric criteria typically have three components: magnitude, duration, and 26 frequency. The magnitude is the pollutant concentration associated with protecting 27 designated uses in the water body based on available data and best scientific judgment. 28 For many pollutants, this magnitude is determined based on toxicity studies as the 29 concentration level that will not impair a water body's ability to support aquatic life. 30 These magnitudes are expressed as the Criteria Maximum Concentration (CMC) and 31 Criteria Continuous Concentration (CCC), representing the highest concentrations in 32 water to which an aquatic community can be exposed briefly (acute) and indefinitely 33 (chronic), respectively, without resulting in an unacceptable effect (2). 34

The duration, or averaging period, is defined as the length of time over which one averages environmental measurements of the pollutant when determining whether a

water body is in compliance with the criterion. Duration was originally introduced to 37 bridge the difference between the natural conditions, where concentration fluctuates, 38 and the lab test conditions, where the test animals are usually exposed to a constant 39 concentration (3). A 4-day average concentration was initially recommended for as-40 sessing the compliance of CCC, because (1) 4 day is much shorter than the 20 or 30 day 41 chronic test period (hence can capture short-term fluctuations better) and (2) damages 42 to some aquatic species are likely caused during the (initial) "sensitive life stage" (3). 43 Because the mean concentration in the field can only be estimated by a sample 44

average (a random variable), when we compute averages over the stated duration, we 45 inevitably introduce uncertainty to the assessment process. In theory, a sample average 46 is an unbiased estimate of the population mean. That is, when repeatedly sampled the 47 average of a set of sample averages will approach the true population mean. But for a 48 given sample average, we are uncertain whether the average is above or below the true 49 mean. As a result, an allowed frequency of sample averages exceeding the standard 50 is specified to allow for this uncertainty. The idea is that if only a small fraction of 51 the sample averages are above the criterion, the true mean is likely to be below the 52 criterion. 53

Specifying the appropriate frequency component of a criterion can be challenging. 54 From an ecological perspective, occasional exceedances should be accompanied by a 55 period of time for the aquatic ecosystem to recover, and some research has shown 56 that aquatic ecosystems can recover from an exceedance in about three years (4, 5). 57 Consequently, one recommendation is that the acceptable exceedance frequency be 58 set at no more than once in three years (3). The EPA guidelines explained that 59 the MDF concept is similar to the concept used in describing precipitation or stream 60 flow, where the frequency is an average recurrence period (3). That is, a criterion 61 in the form of Magnitude-Duration-Frequency (MDF) is a statement of an acceptable 62 probability (frequency) of exceeding a specific concentration (magnitude) calculated 63

over a given time period (duration). Given the frequency F, the magnitude M defines 64 the 1 - F quantile of the sampling distribution. The duration defines the levels of 65 aggregation from the raw data. In other words, the 1 - F quantile of a sampling 66 distribution is compared to the magnitude. However, the stringency of one exceedance 67 in three years depends on the intensity with which monitoring data are collected in 68 at least two ways. First, ambiguity arises if the time period stated in the frequency 69 component differs from the time period stated as the duration component. For example, 70 if duration is specified as a 4-day average, then, by sampling 4 days each week one could 71 conceivably accumulate 156  $(52 \times 3)$  averages in three years. Then, one exceedance 72 in three years would represent the 155/156, or 99.4 percentile of the distribution of 73 averages. More realistically, one might expect to collect one 4-day average each year, 74 and one exceedance in three years would represent the 67 percentile. Thus, the same 75 frequency specification can be associated with very different percentiles of the sampling 76 distribution, simply by changing the intensity of the monitoring effort. 77

Even when the specified time period in the frequency and duration components are 78 consistent, the number of samples collected still affects the variability of the averages 79 that are estimated. For example, an annual average computed from intensive monitor-80 ing (e.g., 20 samples/year) would be much less variable than the same computed with 81 only 4 sample, and hence, the distribution of the averages computed using intensive 82 monitoring data would be less variable than the distribution computed from less inten-83 sive monitoring. Consequently, the actual value associated with one exceedance in 3 84 years (or the 67 percentile of the sampling distribution) is a function of sample size. 85

Because we do not know the true underlying concentration distribution of a pollutant, we must infer the distribution from monitoring data and decide whether the distribution is consistent with the water quality criterion. Inference using limited monitoring data is uncertain, but the MDF components of the criterion can be used to ensure a degree of confidence when assessing the compliance of a water.

In this paper, we discuss statistical issues related to assessing water quality standard 91 compliance with a focus on the estimation of pollutant concentration variances using 92 existing regional water quality monitoring data. We use phosphorus monitoring data 93 from streams and rivers in the Great Lakes region as an example to illustrate (1) the use 94 of a Bayesian hierarchical model to partially pool data from multiple sites to quantify 95 variances of the target pollutant concentration, (2) how to derive compliance assessment 96 rules from the resulting model, and (3) how to update compliance assessment rules for 97 sites with little or no existing monitoring data. 98

## <sup>39</sup> Statistical Considerations

A variation of the MDF requirement in assessing compliance is the raw score method 100 discussed in Smith et al. (6). The raw score method declares a water body in compliance 101 when less than 10% of the samples exceeding a numeric criterion. This method has a 102 duration of one day (assuming daily sample) and a frequency of 10%. As a result, we are 103 comparing the 90 percentile of the population distribution to the criterion. Statistical 104 problems of this approach is well documented (6). As a result, the raw score method 105 is replaced by statistical hypothesis testing procedures for testing whether the criterion 106 is exceeded less than 10% of the time. As a statistical decision problem, Smith et al 107 showed that the use of the raw score method resulted in unacceptably high error rates. 108 This result is expected according on the Neyman-Pearson Lemma (7), because the 109 raw score method rejection region (>10%) of the sample exceeding the criterion) was 110 not selected to have a fixed type I error probability. A typical water quality criterion 111 is expressed in terms of the mean concentration of a water body over a time period 112 (e.g., one year), which cannot be measured. When using the hypothesis testing method 113 discussed by Smith et al (6) (with the null hypothesis that the true 90th percentile is 114 less than or equal to the criterion), we are effectively comparing the lower confidence 115

<sup>116</sup> bound of the estimated 90th percentile to the criterion. In doing so, we assume that if
<sup>117</sup> the lower bound is less than the criterion, the mean will also be less than the criterion.
<sup>118</sup> The distance between the lower bound and the mean is, however, a function of the
<sup>119</sup> sample size. Gibbons (8) showed that the lower bound is

$$LCL_{1-\alpha,p} = \bar{y} + K_{\alpha,p}s,$$

where  $LCL_{1-\alpha,p}$  is the lower  $(1-\alpha)$  confidence bound of the p(100)th percentile of 120 the distribution,  $\bar{y}$  is the sample mean, s is the sample standard deviation, and  $K_{\alpha,p}$ 121 is a normal tolerance limit factor (a function of  $\alpha, p$ , and sample size). Because a 122 typical water quality criterion is specified in terms of mean concentrations, the use 123 of the hypothesis testing approach (i.e., LCL) is equivalent to use the sample mean 124 plus a safety factor. But the safety factor increases as the sample size increases (see 125 Table 1 in reference (8)). (Just as in the case of the 1 exceedance in three years 126 statement of the EPA guidelines (3), the more data we have, the more stringent the 127 test is.) This behavior is counterintuitive as our confidence on the sample average 128 as an estimate of the population mean increases as sample size increases. The cause 129 of this behavior is that we have moved the target from population mean to the 90th 130 percentile of the population distribution. The use of the 90th percentile is a means for 131 providing a margin of safety. Just as the selection of the frequency in EPA's guidelines 132 (3) determines one of many factors affecting the level of safety, the selection of p(100)133 percentile determines one aspect of the safety factor. In both cases, we did not target 134 the quantity of interest (the population mean) directly. As a result, the actual levels of 135 safety with regard to the population mean varies. In other words, using a hypothesis 136 testing method may not fully alleviate the problem of a varying level of confidence, 137 which often penalizes a thorough study (large sample size). 138

<sup>139</sup> The statistical problem is, however, to establish a connection between population

mean (regulated by the criterion) and a sample statistics (sample average), such that 140 we have a certain level of confidence that the criterion is met when the sample statistics 141 is within certain range. This is a statistical modeling problem. We most likely need to 142 have case-specific models to account for local and regional differences. Both classical 143 and Bayesian statistics approaches can be used. Under a classical statistics frame-144 work, we can use the confidence interval to describe the uncertainty. In a Bayesian 145 framework, we use the posterior distribution of the population mean directly. In many 146 circumstances, the confidence interval and the posterior credible intervals are similar. 147 However, using a Bayesian approach, we not only have a much simpler conceptual in-148 terpretation, but also can build a more realistic model. For example, Gronewold and 149 Borsuk (9) used a Bayesian hierarchical model for linking the observed colony forming 150 unit and multiple tube fermentation results (used for estimating the most probable 151 number, or MPN) to the underlying true fecal indicator bacteria concentration. The 152 posterior distribution of the true concentration is used for inference. 153

We apply the Bayesian hierarchical modeling approach to regional nutrient monitoring data to discuss statistical issues related to numerical nutrient criteria. Our emphasis is on the statistical concepts related to the MDF requirement and how the MDF concept is related to the variance components of nutrient monitoring data. We find that the Bayesian statistics framework is more suited for describing the issues, in that, we can directly quantify the uncertainty we have on the quantity of interest, rather than through the confidence interval of sample statistics.

## <sup>161</sup> Methods and Materials

## <sup>162</sup> Example Data and Data Analysis

The data used in this paper were first reported by Frey et al. (10), including nutrients (TP and TN) concentration data from 64 sampling sites (each with at least 6 samples per year) in wadeable streams in ecoregions surrounding the Great Lakes. The sampling sites represent three broad nutrient ecoregions: VI – combelt and northern Great
Plains, VII – mostly glaciated dairy region, and VIII – nutrient poor largely glaciated
upper Midwest and Northeast. These sampling sites are part of the USGS National
Water Quality Assessment (NAWQA) program long-term monitoring network. The
data in the USGS report (10) were sampled between 1993 and 2006.

## <sup>171</sup> Variance Components

We use the multilevel/hierarchical models (11) to model distributions of TP in the 172 dataset. We assume that nutrient concentration variables are log-normally distributed 173 (12, 13). As a result, we work in a (natural) logarithmic scale. In the U.S., long-174 term nutrient monitoring data are widely available from networks managed by local, 175 regional, and federal governments. The multilevel model partially pools data from 176 various sources (region, monitoring station, season, year, and so on) to improve the 177 overall model performance. For our purpose, we use the multilevel model to quantify 178 relative strengths of various sources of variation. The results will help us to develop 179 regional and local compliance assessment rules. 180

In a typical cross-sectional data set, an observation can be simplified as a sum of
 several additive components:

$$y_{ijkl} = \beta_0 + \beta_{1i} + \beta_{2j} + \beta_{3k} + \epsilon_{ijkl} \tag{1}$$

where  $y_{ijkl}$  is the *l*th observed log concentration value in *k*th month (or season), *j*th year, and *i*th site,  $\beta_0$  is the overall mean for all site, year, and month,  $\beta_{1i}$  is the site effect (e.g.,  $\beta_{13} = -2$  indicates the mean of site 3 is 2 units below overall average),  $\beta_{2j}$ is the year effect,  $\beta_{3k}$  is the seasonal effect, and  $\epsilon_{ijkl}$  is a normal random variable with mean 0 and a constant variance ( $\sigma_0^2$ ) representing the measurement error and other random noise. Using a Bayesian hierarchical modeling approach, we further impose common prior distributions on  $\beta_{1i}, \beta_{2j}$ , and  $\beta_{3k}$ :

$$\beta_{1i} \sim N(0, \sigma_1^2) \tag{2}$$

$$\beta_{2j} \sim N(0, \sigma_2^2) \tag{3}$$

$$\beta_{3k} \sim N(0, \sigma_3^2) \tag{4}$$

which is equivalent to the classical approach of constraining  $\sum_i \beta_{1i} = 0, \sum_j \beta_{2j} =$ 190  $0, \sum_k \beta_{3k} = 0$ . But the common prior distributions in equations (2) to (4) have been 191 shown to improve the model's overall performance (predictive accuracy and robust-192 ness against outliers) (14, 15). When using cross-sectional data covering distinct geo-193 graphic/climate subregions (e.g., ecoregions, states), the between site variance  $\sigma_1^2$  can 194 be further divided into between sub-region variance  $(\sigma_{11}^2)$  and a nested between site 195 variance  $(\sigma_{12}^2)$ . This simplified model can be used to derive the true annual mean and 196 its spatiotemporal variation. For a given site i = I, the estimated long-term mean is a 197 normal random variable with mean  $\beta_0 + \beta_{1I}$  and variance  $\sigma_2^2 + \sigma_3^2$ , or 198

$$\mu_I \sim N(\beta_0 + \beta_{1I}, \sigma_2^2 + \sigma_3^2).$$
(5)

The estimated posterior distribution of  $\mu_I$  from equation (5) is the distribution of interest for site *I*. If the  $(1-\alpha)100$  percentile of the distribution is less than or equal to the water quality criterion, the criterion is exceeded less than the acceptable frequency of  $\alpha$ .

When the cross-sectional data are from a region of similar environmental conditions, the hierarchical modeling approach can be used for developing regional nutrient criterion compliance assessment rules. If the cross-sectional data represent sub-regions with different environmental conditions (e.g., multiple ecoregions), we can add a subregion effect term and use a nested formulation to account for such differences. These models can be implemented either using MCMC algorithms [e.g., in WinBUGS (16), JAGS (17), or Stan (18, 19)] or using the MLE estimator (e.g., the function lmer in R package lme4 (20)) when the number of sites, years, and regions are large (11). See online Supporting Information for detail, where we also discuss how to use equation (5) to derive the acceptable exceedance frequency for sample averages.

We analyze the total phosphorus (TP) concentration data using the hierarchical 213 model discussed in this section. Two models were fit. Sampling sites were grouped 214 by states in one model and by ecoregion in the other. In these models, a regional 215 (state or ecoregion) mean terms is added to the model in equation (1) by dividing the 216 site mean term  $\beta_{1i}$  into a sum of two terms:  $\beta_{1i} = \beta_{1r}^R + \beta_{1i}^S$ . As a result, we have 21 a set of regional means  $(\beta_{1r}^R)$  and each with a number of (nested) site means  $(\beta_{1i}^S)$ . 218 When developing regional (state or ecoregion) assessment rules, we use the posterior 219 distribution of regional mean: 220

$$\mu_r \sim N(\beta_0 + \beta_{1r}^R, \sigma_{12}^{r^2} + \sigma_2^2 + \sigma_3^2).$$
(6)

 $_{221}$  where the between site variance  $\sigma_{12}^{r\ 2}$  can be site-specific.

In addition to site-specific and region-specific posterior distributions of mean con-222 centrations, the resulting models can also be used for developing informative prior 223 distributions for all model coefficients ( $\beta$ 's and  $\sigma$ 's). These informative priors can be 224 directly used for regions (sites) with little or no monitoring data. When new data are 225 available, new posterior distributions can be developed for these regions or sites. The 226 use of the Bayesian updating process was discussed by Qian and Reckhow (21), where 227 posterior predictive distributions of chlorophyll a concentration in the Neuse River 228 Estuary was updated each year and the 90th percentiles of these distributions were 229 compared to the North Carolina eutrophication standard (chlorophyll a concentration 230 below 40  $\mu g/L$ ). 23

For a site (I) represented in the data used for developing the model, observations (log concentrations) in a future year can be modeled as:

$$y_{Ijkl} \sim N(\mu_I, \sigma_0^2)$$

$$\mu_I \sim N(\beta_0 + \beta_{1I}, \sigma_2^2 + \sigma_3^2)$$
(7)

For a site (i) not represented in the data used for developing the model, observations (log concentrations) in a future year can be modeled as:

$$y_{ijkl} \sim N(\mu_i, \sigma_0^2)$$

$$\mu_i \sim N(\beta_0, \sigma_1^2 + \sigma_2^2 + \sigma_3^2)$$
(8)

In both cases, the hierarchical model provides necessary information for deriving in-236 formative prior distributions of  $\beta_0, \beta_{1I}$  and  $\sigma_0, \sigma_1, \sigma_2, \sigma_3$ . The posterior distribution of 237  $\mu_I$  or  $\mu_i$  can be used for standard compliance assessment by comparing the  $(1 - \alpha)100$ 238 percentile to the standard. In the analysis, we set aside data from New York as an 239 example for updating. Only one monitoring station from New York (Ecoregion 83) was 240 included in the data set. The site reported 27 observations from 1995 to 1997. For TP 241 concentrations, 15 of the 27 values are below detection limit of 0.01 mg/L. We used the 242 censored data computation method described in references (22) and (23). 243

We note that EPA recommended nutrient criteria are not derived from toxicity 244 tests, rather they are derived from existing data. For stream nutrient criteria, EPA 245 recommended to use the 75th percentiles of nutrient concentration data from reference 246 area as nutrient criteria. When data from reference area are unavailable, 25th per-247 centiles of all available data are used as the recommended nutrient criteria. In other 248 words, the recommended nutrient criteria are associated with uncertainty. We derive 249 posterior distributions of nutrient criteria using simulation. Details of the derivation 250 are summarized in the online supporting materials. 25

## 252 Results

We fit the hierarchical models using two different methods for dividing the study area into subregions: by state and by level 3 Ecoregion. The estimated variance components of the two models (Table 1) can be visualized through the estimated "random effects" (i.e., the estimated  $\beta_1^R$ ,  $\beta_2$ , and  $\beta_3$ ). The ecoregion-based random effects for monitoring sites in Ohio (Figure 1) suggest that between monitoring sites variation is the largest, followed by between season, and between year variances.

As an example of model updating, we set aside the data from the only site from New York when developing the Ecoregion-based model. We derive prior distributions of model parameters for the New York site (in Ecoregion 83) using model parameters estimated for Ecoregion 83. As there was only one other site in the same ecoregion (in Ohio with a sample size of 33), sample size from this Ohio site was used as the prior sample size (see reference (21) for discussion on prior parameter selection).

The posterior distribution of the site mean  $\mu_i$  (equation (8)) for the New York site, 265 as well as the posterior predictive distribution of individual observation y are compared 266 to the EPA recommended TP criterion for the ecoregion (0.02413 mg/L (24)) (Figure 267 2). Compared to the Ohio site in the same ecoregion (Figure 2), the New York site has 268 a much lower TP concentration. Assuming that we use  $\alpha = 0.1$ , the 90th percentile of 269 the posterior site mean distribution is -3.61 (or 0.027 mg/L), above the recommended 270 criterion of 0.02413 mg/L. Without considering the uncertainty in the TP criterion, 27 the probability of the site mean exceeding the criterion is 0.167. When considering the 272 uncertainty in the TP criterion, the estimated probability that the site mean exceeds 273 the criterion is 0.161. 274

## 275 Discussion

Assessing the compliance of an environmental standard is a problem of decision-making 276 with imperfect information because the use of sampling statistics. Quantifying uncer-277 tainty is naturally a statistical problem, and we opt to use the Bayesian posterior 278 distribution of the quantity of interest. The Bayesian paradigm is increasingly used 279 in environmental research (25-27). The use of the posterior distribution to present 280 the uncertainty of the parameter of interest is particularly appealing. In our case, the 281 Bayesian model results in a posterior distribution of TP concentration, from which we 282 estimated the probability of the mean exceeding the criterion. This probability not only 283 clearly summarizes the uncertainty we have, but also provides a tool for risk manage-284 ment decision (as long as we state the probability that we are comfortable with). The 285 Bayesian approach directly targeting the quantity of interest, thereby avoids a varying 286 level of confidence in compliance assessment using many classical statistics approaches 287 (28, 29).288

Applications of Bayesian statistics in environmental sciences often evade a diffi-289 culty of the Bayesian statistics – the selection of prior distributions – by using "non-290 informative" or flat priors. The controversy of the Bayesian approach is largely focused 29 on the use of prior distribution when there is no obvious choice for such a distribution 292 (30). A non-informative prior for a parameter can be informative when the parameter 293 is transformed. In our professional work, we learned that eliciting prior distributions is 294 a difficult task, because (1) we often need prior distributions on model parameters of 295 which we have little knowledge, and (2) summarizing expert knowledge into a proba-296 bility distribution can be a daunting task if not impossible (31). In fitting a Bayesian 297 hierarchical model using cross-sectional data, we need only apply prior distributions on 298 hyper-parameters. In our example, the hyper-parameters represent a spatial or tem-299 poral aggregation and flat or "non-informative" priors can often be interpreted as an 300 empirical Bayes approach for estimating parameters at site level (32, 33). As a result, 30

the resulting posterior distributions of the hyper-parameters can be used as proper informative priors for sites without data and site-specific posterior distributions can be used as priors for future assessment. In other words, the Bayesian hierarchical modeling approach also allows us to develop informative prior distributions using cross-sectional data.

The Bayesian hierarchical modeling approach provides a framework for pooling re-307 sources at local, regional, and national levels for better assessing water quality status. 308 For example, the federal government can support the development of models using large 309 cross-sectional data based on, perhaps, level II ecoregions (with level III ecoregions as 310 the local level). The resulting level III models can then be used by State governments 31 to develop prior distributions for appropriate level III ecoregion hierarchical models 312 (with level IV ecoregions as the local level). For level IV ecoregions with little or no 313 monitoring data, the respective level III ecoregion model can be used to provide ini-314 tial assessment and the Bayesian updating process in equations (7) and (8) provides a 315 process of gradually moving towards site-specific nutrient mean concentration distribu-316 tions. As environmental conditions change over time, this Bayesian updating approach 317 can be used as part of the periodical review and updating of water quality criteria 318 required by the CWA. 319

### 320 Acknowledgement

SSQ's work is partly supported by a grant from the Research Council of the University of
 Toledo. We thank Robert Miltner for constructive discussions and insightful comments.

## 323 References

(1) U.S. EPA, Quality Criteria for Water; U.S. Environmental Protection Agency,
 1976.

- (2) U.S. EPA, National Recommended Water Quality Criteria; United States Envi ronmental Protection Agency: Office of Water, Office of Science and Technology,
   2009.
- (3) Stephan, C.; Mount, D.; Hansen, D.; Gentile, J.; Chapman, G.; Brungs, W. Guide *lines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic* Organisms and Their Uses; US Environmental Protection Agency: Office of Re search and Development, Cincinnati, OH, 1985.
- (4) Hutchens, J.; Chung, K.; Wallace, J. Temporal variability of stream macroinver tebrate abundance and biomass following pesticide disturbance. Journal of the
   North American Benthological Society 1998, 17, 518–534.
- (5) Wallace, J.; Vogel, D.; Cuffney, T. Recovery of a headwater stream from an insec ticide induced community disturbance. Journal of North American Benthological
   Society 1986, 5, 115–126.
- (6) Smith, E.; Ye, K.; Hughes, C.; Shabman, L. Statistical assessment of violations
   of water quality standards under section 303(d) of the Clean Water Act. *Environ- mental Science and Technology* 2001, 35, 606–612.
- (7) DeGroot, M. Probability and Statistics, 2nd ed.; Addison-Wesley Publishing Com pany, Inc., 1986; p 723.
- (8) Gibbons, R. A statistical approach for performing water quality impairment assessment. Journal of the American Water Resources Association 2003, 39, 841–
  849.
- (9) Gronewold, A.; Borsuk, M. Improving water quality assessment through a hier archical Bayesian analysis of variability. *Environmental Science and Technology* 2010, 44, 7858–7864.

- (10) Frey, J.; Bell, A.; Hambrook-Berkman, J.; Lorenz, D. Assessment of nutrient enrichment by ese of algal-, invertebrate-, and fish-community attributions in wadeable streams in ecoregions surrounding the Great Lakes; Scientific Investigations
  Report 2011-5009; National Water-Quality Assessment Program, U.S. Geological
  Survey: Reston, Virginia, 2011.
- (11) Gelman, A.; Hill, J. Data Analysis Using Regression and Multilevel/Hierarchical
   Models; Cambridge University Press, New York, 2007.
- (12) Ott, W. Environmental Statistics and Data Analysis; Lewis Publishers, Boca Ra ton, 1995.
- (13) van Belle, G. Statistical Rules of Thumb, 2nd ed.; Wiley, 2008.
- (14) Box, G.; Tiao, G. Bayesian Inference in Statistical Analysis; Addison-Wesley,
   Reading, MA, 1973.
- (15) Gelman, A.; Carlin, J.; Stern, H.; Rubin, D. Bayesian Data Analysis, 2nd ed.;
  Chapman & Hall: London, 2003.
- (16) Lunn, D.; Thomas, A.; Best, N.; Spiegelhalter, D. WinBUGS a Bayesian modelling framework: concepts, structure, and extensibility. *Statistics and Computing* **2000**, 10, 325 337.
- (17) Plummer, M. JAGS: A Program for Analysis of Bayesian Graphical Models Using
   Gibbs Sampling. Proceedings of the 3rd International Workshop on Distributed
   Statistical Computing (DSC 2003). Vienna, Austria, 2003; ISSN 1609-395X.
- (18) Hoffman, M.; Gelman, A. The No-U-Turn Sampler: Adaptively Setting Path
   Lengths in Hamiltonian Monte Carlo. Journal of Machine Learning Research In
   press,

- 373 (19) Stan Development Team, Stan Modeling Language User's Guide and Reference
  374 Manual, Version 1.0. 2012.
- <sup>375</sup> (20) Bates, D. *lme4: Mixed-effects modeling with R*; Springer, 2010.
- Qian, S.; Reckhow, K. H. Combining model results and monitoring data for water
   quality assessment. *Environmental Science and Technology* 2007, *41*, 5008–5013.
- (22) Qian, S.; Schulman, A.; Koplos, J.; Kotros, A.; Kellar, P. A hierarchical modeling approach for estimating national distributions of chemicals in public drinking
  water systems. *Environmental Science and Technology* 2004, *38*, 1176–1182.
- Wu, R.; Qian, S.; Hao, F.; Cheng, H.; Zhu, D.; Zhang, J. Modeling Contaminant
   Concentration Distributions in China's Centralized Source Waters. *Environmental Science and Technology* 2011, 45, 6041–6048.
- U.S. EPA, Ambient Water Quality Criteria Recommendations Information Sup porting the Developlment of State and Tribal Nutrient Criteria Rivers and
   Streams in Nutrient Ecoregion VII; EPA 822-B-00-018; U.S. EPA Office of Water:
   Washington, CD.C., 2000.
- (25) Scavia, D.; Evans, M. A.; Obenour, D. R. A Scenario and Forecast Model for
   Gulf of Mexico Hypoxic Area and Volume. *Environmental Science and Technology* 2013, 47, 10423–10428.
- (26) Francis, R. A.; Van Briesen, J. M.; Small, M. J. Bayesian Statistical Modeling
   of Disinfection Byproduct (DBP) Bromine Incorporation in the ICR Database.
   *Environmental Science and Technology* 2010, 44, 1232–1239.
- (27) Starrfelt, J.; Borgå, K.; Ruus, A.; Fjeld, E. Estimating Trophic Levels and Trophic
   Magnification Factors Using Bayesian Inference. *Environmental Science and Tech- nology* 2013, 47, 11599–11606.

- (28) Mcbride, G. B.; Ellis, J. C. Confidence of compliance: a Bayesian approach for
   percentile standards. *Water Research* 2001, 35, 1117 1124.
- McBride, G. B. Confidence of compliance: parametric versus nonparametric approaches. Water Research 2003, 37, 3666 3671.
- (30) Efron, B. Controversies in the foundations of statistics. The American Mathemat *ical Monthly* 1978, 85, 231–246.
- (31) Kahneman, D.; Slovic, P.; Tversky, A. Judgment Under Uncertainty: Heuristics
   and Biases; Cambridge University Press: Cambridge CB2 2RU, UK, 1982; p 555.
- (32) Efron, B.; Morris, C. Stein's estimation rule and its competitors an empirical
  Bayes approach. Journal of the American Statistical Association 1973, 68, 117–
  130.
- (33) Efron, B.; Morris, C. Data analysis using Stein's estimator and its generalizations.
   Journal of the American Statistical Association 1975, 70, 311–319.

## $_{410}$ Tables

Model	$\sigma_0$	$\sigma_{11}$	$\sigma_{12}$	$\sigma_2$	$\sigma_3$	$\beta_0$
By State	0.7781	0.5124	0.7723	0.0599	0.2450	-2.4583
By Ecoregion	0.7782	0.7022	0.6625	0.0595	0.2446	-2.5364

Table 1: Estimated Variances

## 411 Figures



Figure 1: Estimated random effects from the Ecoregion-based model for sites in Ohio. The left panel shows the site effect (the two digits before the dash "—" are the level 3 ecoregion number), the middle panel is the seasonal effect, and the right panel is the year effect. Note that the x-axis scales are different. The solid dot is the estimated mean and the thin lines are mean  $\pm$  standard error.



Figure 2: Posterior distributions of annual mean TP concentration (dark-shaded histogram) and posterior distribution of TP criterion (light-shaded histogram) for the New York sampling site (Level III Ecoregion 83) are compared to the 90th percentile of the estimated posterior mean TP distribution (the black vertical line). The prior distribution of the site mean has a 64% credible interval of (0.07, 0.22)  $(e^{\beta_0 \pm \sqrt{\sigma_1^2 + \sigma_2^2 + \sigma_3^2}}, equation (8))$ . The y-axis is the (unit-less) probability density.

Supporting Information Available
The nutrient part of the USGS database
Deriving sample average exceedance rate based on acceptable risk
R code for calculating exceedance frequency
R and Stan code for Bayesian updating (New York example)
This material is available free of charge via the Internet at http://pubs.acs.
org/.


Available online at www.sciencedirect.com



Ecological Modelling 166 (2003) 87-97



www.elsevier.com/locate/ecolmodel

# Two statistical methods for the detection of environmental thresholds

Song S. Qian<sup>a,\*</sup>, Ryan S. King<sup>b,1</sup>, Curtis J. Richardson<sup>b</sup>

 <sup>a</sup> The Cadmus Group, Inc., 6330 Quadrangle Drive, Suite 180, Chapel Hill, NC 27517, USA
 <sup>b</sup> Duke University Wetland Center, Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708, USA

Received 31 July 2001; received in revised form 22 July 2002; accepted 11 March 2003

#### Abstract

A nonparametric method and a Bayesian hierarchical modeling method are proposed in this paper for the detection of environmental thresholds. The nonparametric method is based on the reduction of deviance, while the Bayesian method is based on the change in the response variable distribution parameters. Both methods are tested using macroinvertebrate composition data from a mesocosm experiment conducted in the Everglades wetlands, where phosphorus is the limiting nutrient. Using the percent of phosphorus tolerant species and a dissimilarity index as the response variables, both methods resulted in a similar and well-defined TP concentration threshold, with a distribution function that can be used to determine the probability of exceeding the threshold.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Bray–Curtis dissimilarity; Changepoint; Deviance; Everglades; Gibbs sampling; Macroinvertebrate; Nonparametric; Total phosphorus

#### 1. Introduction

Anthropogenic perturbations such as landscape fragmentation, cultural eutrophication, or introduction of toxic substances often cause changes in structure and function of aquatic and terrestrial ecosystems. For example, in the USA it is estimated that nearly 40% of all water bodies are ecologically impaired by various pollutants (USEPA, 1998a). Studies of how an ecosystem responds to such disturbances have important management implications, such as the es-

\* Corresponding author. Tel.: +1-919-403-5105;

fax: +1-919-401-5867.

<sup>1</sup> Present address: Smithsonian Environmental Research Center, 647 Contees Wharf Road, Box 28, Edgewater, MD 21037, USA. tablishment of water-quality or emission standards for particular geographic regions (Adams and Greeley, 2000). One common approach to establishing environmental criteria is to examine changes of selected population, community, or ecosystem attributes along a gradient of environmental conditions (e.g. Karr and Chu, 1997). Ecological attributes often show little change until a critical environmental value, or threshold, is reached (Fore et al., 1996; Richardson and Qian, 1999). Thus quantitative description of such exposure–response relationships can be very useful in support of the development of numerical environmental criteria (Suter, 1993; USEPA, 1998a).

Although the identification of environmental thresholds is deeply rooted in ecological risk assessment (Suter, 1993), surprisingly few statistical techniques are appropriate for their detection. Many traditional

E-mail address: sqian@cadmusgroup.com (S.S. Qian).

statistical techniques are not suitable for estimating such thresholds, nor are they adequate for estimating uncertainty in their predictions, both of which are critical components in ecological risk assessment (Bartell et al., 1992; Suter, 1993, 1996; Lemly and Richardson, 1997; USEPA, 1998b). Also, most methods require parametric assumptions, such as normality and linearity, that ecological data rarely meet (e.g. Clarke, 1993). Rather, ecological responses to environmental gradients often are nonlinear, non-normal, and heteroscadistic (Legendre and Legendre, 1998).

We propose two statistical methods for detecting a changepoint along an environmental gradient. The methods presented here can be seen as part of the larger effort of using mathematical and statistical models to study wetland ecology (e.g. Wang and Mitsch, 2000; Shukla, 1998; Buzzelli et al., 2000; Loiselle et al., 2000; Moreno-Grau et al., 1996) and to make risk assessment (e.g. Findlay and Zheng, 1999). The second method is also part of the increasing interest in the application of Bayesian statistics in the Ecol. Model. community (e.g. Omlin and Reichert, 1999; Prato, 2000; Reichert and Omlin, 1997; Steinberg et al., 1997; Borsuk et al., 2001; Aldenberg et al., 1995).

Our methods are, however, explicitly designed to detect ecological changes along an environmental gradient, and may be useful in criteria development because they (1) estimate discrete, numerical values of the predictor variable that lead to ecological changes, (2) provide an estimate of uncertainty by generating confidence limits, and (3) make very few assumptions regarding properties of the data.

The first method is a nonparametric approach that finds a changepoint that results in the largest reduction in the deviance of the response variable. The second method is based on a Bayesian hierarchical modeling approach. We present the statistical basis of these two methods and demonstrate the application using a data set collected from an experimental nutrient gradient in an Everglades wetland.

#### 2. Method

Let  $y_1, \ldots, y_n$  be the sequence of the ecological response variable observed along the ordered environmental gradient  $x_1, \ldots, x_n$ . A changepoint problem is to find r  $(1 \le r \le n)$  that separates the response variable into two groups:  $y_1, \ldots, y_r$  and  $y_{r+1}, \ldots, y_n$ , each with distinct characteristics such as the mean and the variance. The corresponding value of the environmental variable  $x_r$  is the threshold.

#### 2.1. The nonparametric deviance reduction approach

The nonparametric deviance reduction approach is based on the idea that a structural change in an ecosystem may result in the change of both the mean and the variance of the ecological response variable used to indicate the change. When the observations from multiple sites are ordered along the gradient, the threshold or changepoint separates the observations into two groups. Each is relatively homogeneous. This idea was inspired by the tree-based modeling approach (Breiman et al., 1984), where binary splits were used to construct a predictive regression or classification model. Guisan and Zimmermann (2000) and Qian and Anderson (1999) are two examples of application of tree-based model in ecological studies. The concept of deviance reduction was used to develop the method for environmental threshold estimation and a bootstrap method was used to quantify uncertainty about the threshold.

The deviance (Venables and Ripley, 1994), a measure of homogeneity, is defined for a continuous variable, as:

$$D = \sum_{k=1}^{n} (y_k - \mu)^2$$
(1)

where *D* is the deviance, *n* is the sample size, and  $\mu$  is the mean of the *n* observations *y<sub>k</sub>*. For a categorical variable, the deviance is defined as:

$$D = -2\sum_{k=1}^{g} n_k \log(p_k)$$
 (2)

where g is the number of classes,  $p_k$  is the proportion of observations and  $n_k$  is the number of observations in class k, respectively.

When the response data are divided into two groups, the sum of the deviance for the two subgroup is always less than or equal to the deviance of the entire data. Each possible changepoint is associated with a deviance reduction:

$$\Delta_i = D - (D_{\le i} + D_{>i})$$

where *D* is the deviance of the entire data set  $y_1, \ldots, y_n$ ,  $D_{\leq i}$  is the deviance of the sequence  $y_1, \ldots, y_i$ , and  $D_{>i}$  is the deviance of the sequence  $y_{i+1}, \ldots, y_n$ , where  $i = 1, \ldots, n$ . The changepoint *r* is the *i* value that maximizes  $\Delta_i : r = \max_i \Delta_i$ .

Uncertainty about the changepoint can be estimated by using a bootstrap simulation (Efron and Tibshi rani, 1993) and expressed as a 90% confidence interval. This uncertainty may be interpreted as a recognition that a changepoint may be best represented as a small range of values rather than one discrete value. A second consideration is that the deviance reduction approach will always find a changepoint no matter whether there is a real ecological change or not. Thus, we use the approximate  $\chi^2$  test to judge whether the resulting changepoint is statistically significant. The  $\chi^2$  test is based on the fact that the deviance reduction divided by the scale parameter is approximately  $\chi^2$  distributed (d.f. = 1) (Venables and Ripley, 1994). A large deviance reduction will result in a small *P*-value, thus the rejection of the null hypothesis of no changepoint.

Our method is consistent with the tree-based modeling approach. In fact, the changepoint is the first split of a tree model when x is used as the single predictor variable. As a result, the commonly available tree model software (e.g. rpart in S-Plus and R) can be used. In this paper, we wrote an S-Plus function to calculate the changepoint and used the S-Plus function bootstrap to evaluate the uncertainty.

#### 2.2. The Bayesian hierarchical modeling approach

Under a Bayesian framework, we make specific probabilistic assumptions about the ecological response variable. Specifically, we assume that the response variable values,  $y_1, \ldots, y_n$ , collected from the *n* sites along the gradient of interest, are random samples from the sequence of random variables  $Y_1, \ldots, Y_n$ . In other words, we define a random variable for each site, and assume that these random variables belong to the same family of distributions with parameter  $\theta$ .

The term "site" is used to refer a sampling location that has a distinct predictive variable value. Depending on the scale of the study, a site can be a  $1 \text{ m} \times 1 \text{ m}$ sampling grid as in the Everglades example, or reaches of streams miles apart. The random variables  $Y_1, \ldots, Y_n$  have a changepoint  $r (1 \le r \le n)$  if the parameter value changes at r:  $Y_1, \ldots, Y_n \simeq \pi(Y_n|q_n)$ 

$$(3)$$
$$\frac{Y_{r+1}, \dots, Y_n \sim \pi(Y_i | \theta_2)}{Y_{r+1}, \dots, Y_n \sim \pi(Y_i | \theta_2)}$$

Theoretical background on this type of changepoint analysis can be found in Smith (1975), Raftery and Akman (1986), Carlin et al. (1992). Extension to a multiple changepoint problem can be found in Stephens (1994). In this paper, we summarize the results of changepoint analysis presented in Smith (1975) and use the newly developed Gibbs sampling procedure for parameter estimation.

In our example, the response variables can be approximated by a normal distribution or a binomial distribution (see Section 3.2). Accordingly, we present the details of a changepoint problem for normal and binary response variables.

#### 2.3. Normal distribution model

When the random variables  $Y_1, \ldots, Y_n$  are from a normal distribution family, the changepoint problem is defined as follows:

$$Y_i \sim \begin{cases} N(\mu_1, \sigma_1^2), & i = 1, \dots, r\\ N(\mu_2, \sigma_2^2), & i = r+1, \dots, n \end{cases}$$
(4)

Let  $\lambda_1 = 1/\sigma_1^2$  and  $\lambda_2 = 1/\sigma_2^2$ . As a result, model parameters are  $\theta = (\mu_1, \lambda_1, \mu_2, \lambda_2)$ . Assume the prior is of the form

$$\pi(\theta, r) \propto \pi(\lambda_1)\pi(\lambda_2)$$

In addition, let the prior distributions of  $\lambda_1$  and  $\lambda_2$  be from a gamma distribution family, i.e.  $\lambda_1 \sim \gamma(\alpha'_1, \beta'_1)$ and  $\lambda_2 \sim \gamma(\alpha'_2, \beta'_2)$ . The proper prior distributions for  $\lambda_1$  and  $\lambda_2$  ensures a proper posterior distribution for *r*. In practice, values of the parameters  $(\alpha'_1, \beta'_1)$  and  $(\alpha'_2, \beta'_2)$  can be chosen to make the prior distributions nearly flat. We used 0.001 for all four parameters.

The joint distribution of data and parameters is proportional to the product of prior and likelihood:

$$\prod_{i=1}^{n} \pi(\theta, r) \pi(Y_{i}|r, \theta) \propto \lambda_{1}^{r/2 + \alpha_{1}' - 1} \\ \times e^{[-(1/2)r\lambda_{1}(\mu_{1} - \bar{Y}_{1})^{2}]} e^{(-\lambda_{1}\delta_{1})} \\ \times \lambda_{2}^{(n-r)/2 + \alpha_{2}' - 1} e^{[-(1/2)(n-r)\lambda_{2}(\mu_{2} - \bar{Y}_{2})^{2}]} \\ \times e^{(-\lambda_{2}\delta_{2})}$$
(5)

and the marginal distribution of r is:

$$\operatorname{pr}(r|Y) \propto \left\{ \begin{array}{l} \frac{1}{r^{1/2}} \frac{1}{(n-r)^{1/2}} \frac{\Gamma(\gamma_1)}{\delta_1^{\gamma_1}} \frac{\Gamma(\gamma_2)}{\delta_2^{\gamma_2}}, & \text{for } r < n \\ \frac{1}{n^{1/2}} \frac{\Gamma(\gamma_n)}{\delta_n^{\gamma_n}} \frac{\Gamma(\alpha'_2)}{\beta'_2^{\alpha'_2}}, & \text{for } r = n \end{array} \right.$$
(6)

where

$$\begin{split} \bar{Y}_1 &= \frac{1}{r} \sum_{i=1}^r Y_i, & \bar{Y}_2 &= \frac{1}{n-r} \sum_{i=r+1}^n Y_i, \\ \gamma_1 &= \frac{r-1}{2} + \alpha'_1, & \delta_1 &= \frac{1}{2} \left[ \sum_{i=1}^r Y_i^2 - r\bar{Y}_1^2 \right] + \beta'_1, \\ \gamma_2 &= \frac{n-r-1}{2} + \alpha'_2, & \delta_2 &= \frac{1}{2} \left[ \sum_{i=r+1}^n Y_i^2 - (n-r)\bar{Y}_2^2 \right] + \beta'_2 \\ \gamma_n &= \frac{n-1}{2} + \alpha'_1, & \delta_n &= \frac{1}{2} \left[ \sum_{i=1}^n Y_i^2 - n\bar{Y}_1^2 \right] + \beta'_1 \end{split}$$

and  $\Gamma(\cdot)$  represents the Gamma function.

This is a discrete probability distribution. Because the order of the response variable is the same as the environmental gradient variable, the probability of  $x_i$ being the threshold is also defined by Eq. (6). We may choose to use the mode of the distribution as the estimate of the changepoint, or the expected value of the corresponding environmental gradient variable.

The posterior conditional distributions of parameters  $\theta$  are:

$$\mu_1|\mu_2, \lambda_1, \lambda_2, r \sim N(Y_1, r\lambda_1),$$
  

$$\lambda_1|\mu_1, \mu_2, \lambda_2, r \sim \gamma(\gamma_1, \delta_1),$$
  

$$\mu_2|\mu_1, \lambda_1, \lambda_2, r \sim N(\bar{Y}_2, (n-r)\lambda_1), \text{ and }$$
  

$$\lambda_2|\mu_1, \mu_2, \lambda_1, r \sim \gamma(\gamma_2, \delta_2)$$

#### 2.4. Binomial distribution model

When the random variables  $Y_1, \ldots, Y_n$  are from binomial distributions, the changepoint problem becomes

$$Y_i \sim \begin{cases} \text{binomial}(\theta_1, N_i), & i = 1, \dots, r\\ \text{binomial}(\theta_2, N_i), & i = r+1, \dots, n \end{cases}$$
(7)

where  $\theta_1$  and  $\theta_2$  are probabilities of success before and after the change, respectively. Assuming a uniform prior on r,  $\theta_1$ , and  $\theta_2$ , the joint distribution of data and parameter is proportional to:

$$\pi(\theta_{1}, \theta_{2}, r)L(Y; \theta_{1}, \theta_{2}, r) \propto \theta_{1}^{\sum_{i=1}^{r} Y_{i}}$$

$$\times (1 - \theta_{1})^{\sum_{i=1}^{r} (N_{i} - Y_{i})} \theta_{2}^{\sum_{i=r+1}^{n} Y_{i}}$$

$$\times (1 - \theta_{2})^{\sum_{i=r+1}^{n} (N_{i} - Y_{i})} = \theta_{1}^{S_{11}}$$

$$\times (1 - \theta_{1})^{S_{12}} \theta_{2}^{S_{21}} (1 - \theta_{2})^{S_{22}}$$
(8)

where  $S_{11} = \sum_{i=1}^{r} Y_i$ ,  $S_{12} = \sum_{i=1}^{r} (N_i - Y_i)$ ,  $S_{21} = \sum_{i=r+1}^{n} Y_i$ , and  $S_{22} = \sum_{i=r+1}^{n} (N_i - Y_i)$ .

Integrating out  $\theta_1$  and  $\theta_2$  from the joint distribution (8), the posterior marginal distribution of *r* is

$$\pi(r|Y) \propto \int \theta_1^{S_{11}} (1-\theta_1)^{S_{12}} \theta_2^{S_{21}} (1-\theta_2)^{S_{22}} d\theta_1 d\theta_2$$
  
$$\propto \frac{\Gamma(S_{11}+1)\Gamma(S_{12}+1)}{\Gamma(S_{11}+S_{12}+2)} \frac{\Gamma(S_{21}+1)\Gamma(S_{22}+1)}{\Gamma(S_{21}+S_{22}+2)}$$
(9)

The conditional posterior distributions of  $\theta_1$  and  $\theta_2$  are available:

$$\pi(\theta_i | r, Y) = \text{beta}(S_{j1}+1, S_{j2}+1), \text{ for } j=1, 2$$
 (10)

Inference on whether a changepoint exists can be made by the probability of no changepoint pr(n|Y).

Inference on the posterior distributions of r,  $\mu_1$ ,  $\mu_2$ ,  $\lambda_1$ , and  $\lambda_2$  for the normal model, and r,  $\theta_1$ , and  $\theta_2$  for the binomial model are made by using the Gibbs sampler, a Markov chain Monte Carlo simulation (MCMC) method (Gelfand and Smith, 1990; Gelfand et al., 1990; Smith and Roberts, 1993). Casella and George (1992) provides an intuitive exposition of the Gibbs sampler. Examples of environmental ap-

plication of the Gibbs sample are found in Qian and Reckhow (1998), Qian and Richardson (1997), and Qian et al. (2000).

We note that multiple samples from each site can be (and should be) directly used in both methods' computation procedures.

For the binomial model, we wrote S-Plus functions to calculate the changepoint distribution (Eq. (9)) and to sample the posterior distributions of the parameters (Eq. (10)). The calculation can be done using any software that evaluates a  $\Gamma$ -function and generates random samples from a beta distribution. For the normal model, we used WinBUGS (Spiegelhalter et al., 2000), a freely available software for Bayesian analysis using Gibbs sampler.

#### 3. The Everglades example

#### 3.1. Introduction

Numerous studies have shown that the Everglades is a phosphorus limited ecosystem (e.g. Richardson et al., 1999; Steward and Ornes, 1975a,b; Swift and Nicholas, 1987; Flora et al., 1988). One of the most publicized sources of perturbation to the Everglades ecosystem has been excessive inputs of phosphorus. Thus, as part of the 1994 Everglades Forever Act (EFA, Florida Administrative Code, 17-302.530), the state of Florida mandated that a numerically defensible water-column total phosphorus (TP) standard be established by 2001. Moreover, as a Florida Class III waterbody, legislation has specifically mandated that the TP concentration selected as a standard must not result in an imbalance of flora or fauna.

In accordance with this legislation, a phosphorus dosing study was established in the Water Conservation Area-2A (WCA-2A) in the northern Everglades to experimentally examine changes in biological attributes in response to TP concentrations. Two dosing facilities, each with five walled mesocosms and one unwalled control, were constructed in adjacent sloughs in southern part of WCA-2A where anthropogenic impact is negligible. Mesocosms were 2-m wide and 8-m long flumes, continuously dosed with P from the north ends. Each walled mesocosm was assigned a soluble reactive phosphate (SRP) treatment in one facility and replicated in the other. These treatments ranged from background concentrations (walled control) to approximately  $125 \mu g/l$  in the highest treatment. Water-column SRP and TP were measured biweekly throughout the duration of the study at multiple stations down the length of each mesocosm. The dosing study was inaugurated on 30 November 1992 and terminated on 21 September 1998. Greater detail of the dosing study experimental design and operation can be found in Richardson et al. (2000) and Pan et al. (2000).

#### 3.2. Data

While several levels of biological organization were studied to develop a phosphorus threshold for the Everglades, here we only consider the relationship between macroinvertebrate assemblages and TP as a test for the two statistical methods. Macroinvertebrates are one of the most widely used biological indicators in aquatic systems (Rosenberg and Resh, 1993; Barbour et al., 1999), thus were expected to be useful monitors of ecological condition in the Everglades. We initiated the macroinvertebrate component of the phosphorus dosing study in 1996, 4 years after dosing had begun. Samples were collected at the 2, 4, and 6-m stations within each mesocosm (including unwalled control) on four dates, two wet season (2 September 1996, 21 September 1998) and two dry season (8 January 1997, 4 February 1998). Thus, there were 36 observations on each date. Greater detail on sampling design and methods are described elsewhere (Richardson et al., 2000; King and Richardson, in press).

To assess dose–response relationships between macroinvertebrate communities and TP, 2 metrics were calculated using the species abundance data: (1) Bray–Curtis dissimilarity (BCD) and (2) percent tolerant individuals.

BCD was selected as a metric because it has been shown to be one of the most robust and ecologically interpretable measures for species abundance data (Bray and Curtis, 1957; Faith et al., 1987; Clarke, 1993; Legendre and Legendre, 1998; Legendre and Anderson, 1999). Before calculation, a  $\log_{10}(x + 1)$ transformation was applied to taxon abundances to increase the relative contribution of the uncommon and rare taxa (e.g. Gauch et al., 1982; Efron and Tibshirani, 1989; Cao et al., 1998). Since BCD is based on pairwise comparisons between all sample pairs, samples were ordinated using nonmetric multidimensional scaling (nMDS), rotated using varimax rotation to maximize variation along nMDS Axis 1, and extracted as univariate scores along nMDS Axis 1 (McCune et al., 1997; Legendre and Legendre, 1998). Because the standardized log-abundance variables are approximately normal, nMDS Axis 1, a linear combination of these nearly normal variables, is approximately normal as well. The objective in the use of nMDS was to recover a multivariate assemblage pattern that could potentially be attributed to a gradient in TP concentration, and to reduce dimensionality to allow for univariate changepoint analysis.

Percent tolerant individuals was calculated using a list of taxa shown to be highly abundant in high phosphorus, eutrophic areas in the Everglades but uncommon in low phosphorus areas (King, 2001; King and

Richardson, in press). This metric was recorded in the form of two counts: number of tolerant species and total number of individuals. Because the nature of the data is binary (a subject is either phosphorus tolerant, success, or non-tolerant, failure) and we are interested in the proportion of tolerant species (or the probability of success), a binomial distribution is appropriate.

In accordance with the Everglades Forever Act (Florida Administrative Code 17-373.4592), longterm geometric mean values of TP were used as predictors in this analysis. We define the "long-term" as the approximate life span of most long-lived taxa present at the dosing study, which is about 6 months. Each geometric mean corresponded to the precise location of each macroinvertebrate sample collected from the mesocosms.

Table 1

Changepoint estimation results for macroinvertebrate responses in a phosphorus dosing mesocosm in the Everglades

Sample no.	TP threshold (µg/l)	Intervals		
	Nonparametric	Bayesian	Nonparametric	Bayesian
	Response variable: BCD			
1	$12.25 \ (P = 0.00123)$	$10.23 \ (P < 0.00001)$	10.05, 18.38	10.05, 10.55
2	11.60 $(P < 0.00001)$	11.81 ( $P < 0.00001$ )	11.12, 12.76	11.27, 12.76
3	$10.53 \ (P = 0.00073)$	$10.68 \ (P < 0.00001)$	10.07, 10.68	10.61, 11.59
4	10.81 $(P = 0.00073)$	13.94 ( $P < 0.00001$ )	8.31, 13.94	10.55, 13.94
	Response variable: percent ph	osphorus tolerant species		
1	$12.11 \ (P < 0.00001)$	$10.54 \ (P < 0.00001)$	10.05, 18.05	10.05, 10.55
2	$14.04 \ (P < 0.00001)$	$13.47 \ (P < 0.00001)$	11.27, 16.43	12.72, 15.21
3	$10.67 \ (P < 0.00001)$	$10.68 \ (P < 0.00001)$	9.07, 11.99	10.61, 10.68
4	$10.69 \ (P < 0.00001)$	11.80 $(P < 0.00001)$	7.12, 14.40	8.31, 12.38

#### Table 2

Mean response variable values

Sample no.	BCD (left)		BCD (right)		
	Nonparametric	Bayesian	Nonparametric	Bayesian	
	Response variable: BCD				
1	$-0.80 \pm 0.33$	$-0.82 \pm 0.36$	$0.35 \pm 0.63$	$0.34\pm0.66$	
2	$-0.95 \pm 0.41$	$-0.92\pm0.48$	$0.48 \pm 0.40$	$0.49\pm0.41$	
3	$-0.76 \pm 0.74$	$-0.74\pm0.78$	$0.55 \pm 0.44$	$0.56\pm0.45$	
4	$-0.65\pm0.71$	$-0.53\pm0.74$	$0.46\pm0.42$	$0.52\pm0.41$	
	Response variable: percen	t phosphorus tolerant species			
1	$0.044 \pm 0.032$	$0.041 \pm 0.008$	$0.231 \pm 0.109$	$0.221\pm0.012$	
2	$0.070 \pm 0.041$	$0.071 \pm 0.013$	$0.224 \pm 0.086$	$0.216\pm0.015$	
3	$0.026 \pm 0.027$	$0.033 \pm 0.007$	$0.182 \pm 0.147$	$0.178\pm0.011$	
4	$0.056\pm0.043$	$0.079\pm0.016$	$0.194\pm0.096$	$0.197\pm0.017$	



Fig. 1. Changepoint distributions estimated for BCD using the nonparametric (dashed lines) and the Bayesian (the solid lines) methods. Data are shown as shaded dots.



Fig. 2. Changepoint distributions estimated for percent phosphorus tolerant species using the nonparametric (dashed lines) and the Bayesian (the solid lines) methods. Data are shown as shaded dots.

#### 3.3. Results

For each of the four sampling events, we present the changepoint estimated using both the nonparametric and the Bayesian methods (Table 1), as well as the estimated mean and standard deviation of the BCD and percent phosphorus tolerant species on both sides of the changepoint (Table 2). Uncertainty in the estimated changepoint are presented by using (1) the range of the middle 90% of the 1000 bootstrap simulation replicates, and (2) the 90% credible intervals for the Bayesian estimates (under the column Intervals in Table 1). The values of P under the two TP threshold columns in Table 1 are the P-value for testing whether a changepoint exists estimated based on the approximate  $\chi^2$  test discussed in the method section for the nonparametric method, and is the probability of no changepoint, pr(n|Y), for the Bayesian method.

Figs. 1 and 2 present the threshold distributions along with the BCD and percent tolerant species data, respectively, for all four sampling events. In the figures, the bootstrap simulation results for the nonparametric method were shown by the dashed lines (the estimated probability density function of the 1000 bootstrap simulation replicates); the Bayesian method results are shown by the vertical lines with the height representing the probability of the corresponding TP value being the threshold; the shaded circles are the data used for the analysis.

The results from the nonparametric method are comparable to those of the Bayesian method. This is expected since the probability distribution assumptions (normal and binomial) made on the response variables under the Bayesian method are appropriate. Because the Bayesian method uses the distributional information, it resulted in narrower 90% intervals for the changepoint. When the proper response variable probability distribution cannot be ascertained, the nonparametric method should be used.

#### 4. Discussions

We presented two statistical methods for quantifying environmental thresholds using data from an experiment conducted in the Everglades as an example. Both methods are designed specifically for the detection of change in the selected ecological response along a gradient. The nonparametric method does not make probabilistic assumptions about the response data; it is therefore more robust. Computation of the nonparametric method is straightforward and can be done without special software. However, it is necessary to identify the type of response data (e.g. continuous, counts, or categorical) in order to calculate the deviance properly. Because the nonparametric method does not make use of information about the probabilistic distribution of the response variable, it is less efficient than the Bayesian method when such information exist. The Bayesian method requires specific information on the distribution of the response variable. This information is, however, readily available for most ecological data, either from past experience (e.g. log-normal is a good approximation for concentration variables), or the nature of data (e.g. binomial distribution is appropriate for binary response variables and Poisson distribution is often used for counts data). Computation of the Bayesian method is more intense and complicated than the computation of the nonparametric method. If only the changepoint is of interest, any software that is capable of evaluating the Gamma function is sufficient (Eqs. (6) and (9)). We recommend that both methods be used in order to fully explore all possible outcomes.

From the probability density (or distribution) functions (Figs. 1 and 2) one can determine the most likely threshold values and their uncertainty. From a risk assessment view point, a cumulative density or distribution function (CDF) can then be used to directly read out the probability of exceeding the threshold (Fig. 3). The CDFs for the percent tolerant species presents the risk exceeding the threshold at various TP concentrations.

It is more often the case that a threshold is not well defined. As a result, the change in the selected response variable may be gradual. If the data cover the change well, i.e. there are enough data points to described both before and after the change, our methods will work well, resulting in a flatter changepoint distribution.

The EFA requires the TP threshold to be set to prevent flora and fauna imbalance. However, there was no definition about what constitutes an imbalance. In the example, we selected the percent phosphorus tolerant macroinvertebrate species and the Bray–Curtis dissimilarity index as the response variables. A change



Fig. 3. Cumulative changepoint distributions estimated for percent phosphorus tolerant species using the nonparametric (dashed lines) and the Bayesian (the solid lines) methods, indicating the risk of exceeding the TP threshold.

in the two response variables we used only represents an "imbalance" in the macroinvertebrate composition metrics we tested, which may or may not be the same imbalance as defined in the EFA. The results presented in Table 1 indicated a TP threshold value of slightly above 11  $\mu$ g/l. Separately using data from the same mesocosm experiment, we estimated the TP threshold values using additional variables representing various ecological trophic levels. We found that, in general, the TP thresholds were different for response variables representing different trophic levels or the community level, which indicates that an environmental threshold estimated using our methods should not be isolated from the intended response variable.

Dimension reduction is a common practice in quantitative ecology (Legendre and Legendre, 1998). In our case, we used Bray–Curtis dissimilarity index and percent tolerant individuals. Reducing the dimension of the data will inevitably lead to loss of information. Therefore, using the full species composition is advisable. We will report our work on using species composition data for estimating threshold in a separate paper (Qian et al., 2003). In the mean time, we suggest that multiple metrics (e.g. abundance, number of taxa) be used for estimating the same threshold to better understand what aspect of the ecosystem changes at which TP concentration. For the same reason, the definite total phosphorus threshold for the Everglades ecosystem should be estimated using response variables from multiple trophic levels.

Three most commonly seen types of data are: (1) continuous data whose distribution can be approximated by a normal distribution, (2) counts data that can be approximated by a Poisson distribution, and (3) binomial or multinomial data (e.g. presence/absence of a particular species and counts of several species found in a site). Both the nonparametric and the Bayesian methods can be applied to all three types of data. We presented the normal and binomial response variable cases in the Everglades example. When the response variable is counts, a log transformation should be performed before applying the nonparametric method. The Bayesian changepoint method for counts data can be found in Raftery and Akman (1986). The application of Bayesian hierarchical modeling approach to

multinomial or categorical data can be found in Qian et al. (2003).

Because TP is the limiting nutrient in the Everglades ecosystem, it is expected that the ecosystem will respond to the elevated TP concentration. Because BCD summarizes the community pattern of the macroinvertebrate assemblage data, the variable is a ranking of some sort. A clear and well-defined changepoint along the TP gradient (Figs. 1 and 2 and Table 2) indicates the existence of a TP threshold and supports the theory of a phosphorus assimilative capacity for wetlands as proposed by Richardson and Qian (1999).

#### Acknowledgements

We thank E. Conrad Lamon, Sujit Ghosh, Craig A. Stow, and Yangdong Pan for their constructive comments and suggestions on an earlier version of the manuscript. The comments and suggestions from two reviewers and the editor are greatly appreciated. Qian's work is supported by US EPA (STAR Grant #R827898). Funding for the Everglades research was provided by a grant to the Duke University Wetland Center from the Everglades Area Environmental Protection District of Florida.

#### References

- Adams, S.M., Greeley, M.S., 2000. Ecotoxicological indicators of water quality: using multi-response indicators to assess the health of aquatic ecosystems. Water Air Soil Pollut. 123, 103– 115.
- Aldenberg, T., Janes, J.H., Kramer, P.R.G., 1995. Fitting the dynamic model PClake to a multi-lake survey through Bayesian statistics. Ecol. Model. 78, 83–99.
- Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 841-0B-99-002.
- Bartell, S.M., Gardner, R.H., O'Neill, R.V. (Eds.), 1992. Ecological Risk Estimation. Lewis Publishers, Chelsea, MI.
- Borsuk, M.E., Higdon, D., Stow, C.A., Reckhow, K.H., 2001. A Bayesian hierarchical model to predict benthic oxygen demand from organic matter loading in estuaries and coastal zones. Ecol. Model. 143, 165–181.
- Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325– 349.

- Breiman, L., Friedman, J.H., Olshen, R., Stone, C.J., 1984. Classification and Regression Trees. Wadsworth International Group, Belmont, CA.
- Buzzelli, C.P., Childers, D.L., Dong, Q., Jones, R.D., 2000. Simulation of periphyton phosphorus dynamics in Everglades National Park. Ecol. Model. 134, 103–115.
- Cao, Y., Williams, D.D., Williams, N.E., 1998. How important are rare species in aquatic community ecology and bioassessment? Limnol. Oceanogr. 43, 1403–1409.
- Carlin, B.P., Gelfand, A.E., Smith, A.F.M., 1992. Hierarchical Bayesian analysis of changepoint problems. Appl. Statist. 41, 389–405.
- Casella, G., George, E., 1992. Explaining the Gibbs sampler. The Am. Statist. 46, 167–174.
- Clarke, K.R., 1993. Nonparametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143.
- Efron, B., Tibshirani, R.J., 1993. An Introduction to the Bootstrap. Chapman and Hall, London.
- Faith, D.P., Norris, R.H., 1989. Correlation of environmental variables with patterns of distribution and abundance of common and rare freshwater macroinvertebrates. Biol. Conserv. 50, 77–98.
- Faith, D.P., Minchin, P.R., Belbin, L., 1987. Compositional dissimilarity as a robust measure of ecological distance. Vegetatio 69, 57–68.
- Findlay, C.S., Zheng, L., 1999. Estimating ecosystem risks using cross-validated multiple regression and cross-validated holographic neural networks. Ecol. Model. 119, 57–72.
- Flora, M.D., Walker, D.R., Scheidt, D.J., Rice, R.G., Landers, D.H., 1988. The response of the Everglades marsh to increased nitrogen and phosphorus loading: Part I. Nutrient dosing, water chemistry, and periphyton productivity. National Park Service, South Florida Research Center, Everglades National Park, Homestead, FL.
- Fore, L.S., Karr, J.R., Wisseman, R.W., 1996. Assessing invertebrate responses to human activities: evaluating alternative approaches. J. North Am. Benthol. Soc. 15, 212–231.
- Gauch, H.G., 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge, UK.
- Gelfand, A.E., Smith, A.F.M., 1990. Sampling-based approaches to calculating marginal densities. J. Am. Statist. Assoc. 85 (410), 398–409.
- Gelfand, A.E., Hills, S.E., Racine-Poon, A., Smith, A.F.M., 1990. Illustration of Bayesian inference in normal data models using Gibbs sampling. J. Am. Statist. Assoc. 85 (412), 972–985.
- Guisan, A., Zimmermann, N.E., 2000. Predictive habitat distribution models in ecology. Ecol. Model. 135, 147–186.
- Karr, J.R., Chu, E.W., 1997. Biological monitoring and assessment: using multimetric indexes effectively. University of Washington, Seattle, WA. EPA 235-R97-001.
- King, R.S., 2001. Dimensions of invertebrate assemblage organization in a phosphorus-limited Everglades landscape. Ph.D. disseration. Duke University, Durham, NC.
- King, R.S., Richardson, C.J., in press. Macroinvertebrate and fish responses to experimental P additions in Everglades sloughs. In: Richardson, C.J. (Ed.), The Everglades Experiments: Lessons for Ecosystem Restoration. Springer-Verlag, New York.

- Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24.
- Legendre, P., Legendre, L., 1998. Numerical Ecology, 2nd ed. Elsevier, Amsterdam, The Netherlands.
- Lemly, A.D., Richardson, C.J., 1997. Guidelines for risk assessment in wetlands. Environ. Monit. Assess. 47, 117–134.
- Loiselle, S., Carpaneto, G.M., Hull, V., Waller, T., Rossi, C., 2000. Feedback analysis in reserve management: studying local myths using qualitative models. Ecol. Model. 129, 25–37.
- McCune, B., Dey, J.P., Peck, J.E., Cassell, D., Heiman, K., Will-Wolf, S., Neitlich, P.N., 1997. Repeatability of community data: species richness versus gradient scores in large-scale lichen studies. The Bryologist 100, 40–46.
- Moreno-Grau, S., Garcia-Sanchez, A., Moreno-Clavel, J., Serrano-Aniorte, J., Moreno-Grau, M.D., 1996. A mathematical model for waste water stabilization ponds with macrophytes and microphytes. Ecol. Model. 91, 91–99.
- Omlin, M., Reichert, P., 1999. A comparison of techniques for the estimation of model prediction uncertainty. Ecol. Model. 115, 45–59.
- Pan, Y., Stevenson, R.J., Vaithiyanathan, P., Slate, J., Richardson, C.J., 2000. Changes in algal assemblages along observed and experimental phosphorus gradients in a subtropical wetland, USA. Freshwater Biol. 44, 339–353.
- Prato, T., 2000. Multiple attribute Bayesian analysis of adaptive ecosystem management. Ecol. Model. 113, 181–193.
- Qian, S.S., Anderson, C.W., 1999. Exploring factors controlling the variability of pesticide concentrations in the Willamette River Basin using tree-based models. Environ. Sci. Technol. 33, 3332–3340.
- Qian, S.S., Richardson, C.J., 1997. Estimating the long-term phosphorus accretion rate in the Everglades: a Bayesian approach with risk assessment. Water Resour. Res. 33 (7), 1681–1688.
- Qian, S.S., Lavine, M.L., Stow, C.A., 2000. Bayesian noparametric binary response regression models with application in environmental management. Environ. Ecol. Statist. 7, 75–89.
- Qian, S.S., Pan, Y., King, R.S., 2003. Soil total phosphorus threshold in the Everglades: a Bayesian changepoint analysis for multinomial response data. Ecological Indicators (to appear).
- Raftery, A.E., Akman, V.E., 1986. Bayesian analysis of a Poisson process with a change-point. Biometrika 73, 85–89.
- Reichert, P., Omlin, M., 1997. On the usefulness of overparameterized ecological models. Ecol. Model. 95, 289– 299.
- Richardson, C.J., Qian, S.S., 1999. Long-term phosphorus assimilative capacity in freshwater wetlands: a new paradigm for sustaining ecosystem structure and function. Environ. Sci. Technol. 33 (10), 1545–1551.
- Richardson, C.J., Ferrell, G.M., Vaithiyanathan, P., 1999. Nutrient effect on stand structure, resorption efficiency, and secondary

compounds in Everglades sawgrass. Ecology 80, 2182-2192.

- Richardson, C.J., Vaithiyanathan, P., Stevenson, R.J., King, R.S., Stow, C.A., Qualls, R.G., Qian, S.S., 2000. The ecological basis for a phosphorus (P) threshold in the Everglades: directions for sustaining ecosystem structure and function. Duke University, Durham, NC. Duke Wetland Center Publication 00-02.
- Rosenberg, D.M., Resh, V.H. (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.
- Shukla, V.P., 1998. Modeling the dynamics of wetland macrophytes: Keoladeo National Park Wetland, India. Ecol. Model. 109, 99–114.
- Smith, A.F.M., 1975. A Bayesian approach to inference about a change-point in a sequence of random variables. Biometrika 62, 407–416.
- Smith, A.F.M., Roberts, G.O., 1993. Bayesian computation via the Gibbs sampler and related Markov chain Monte Carlo methods. J. Roy. Stat. Soc. B 55 (1), 3–23.
- Spiegelhalter, D., Thomas, A., Best, N., 2000. WinBUGS: Version 1.3 User Manual. http://www.mrc-bsu.cam.ac.uk/bugs.
- Steinberg, L.J., Reckhow, K.H., Wolpert, R.L., 1997. Characterization of parameters in mechanistic models: a case study of a PCB fate and transport model. Ecol. Model. 97, 35–46.
- Stephens, D.A., 1994. Bayesian retrospective multiple-changepoint identification. Appl. Statist. 43, 159–178.
- Steward, K.K., Ornes, W.H., 1975a. Assessing a marsh environment for wastewater renovation. J. Water Pollut. Control Federation 47, 1880–1891.
- Steward, K.K., Ornes, W.H., 1975b. The autecology of sawgrass in the Florida Everglades. Ecology 56, 162–171.
- Suter, G.W. 1993. Ecological Risk Assessment. Lewis Publishers, Chelsea, MI.
- Suter, G.W., 1996. Abuse of hypothesis testing statistics in ecological risk assessment. Hum. Ecol. Risk Assess. 2, 331– 347.
- Swift, D.R., Nicholas, R.B., 1987. Periphyton and water quality relationships in the Everglades Water Conservation Areas, 1978–1982. South Florida Water Management, West Palm Beach, FL.
- Venables, W.N., Ripley, B.D., 1994. Modern Applied Statistics with S-Plus. Springer, New York.
- USEPA (United States Environmental Protection Agency), 1998a. National strategy for the development of regional nutrient criteria. Office of Water, Washington, DC. EPA 822-R-98-002.
- USEPA (United States Environmental Protection Agency), 1998b. Guidelines for ecological risk assessment. Risk Assessment Forum, Washington, DC.
- Wang, N., Mitsch, W.J., 2000. A detailed ecosystem model of phosphorus dynamics in created riparian wetlands. Ecol. Model. 126, 101–130.

## Importance of landscape variables and morphology on nutrients in Missouri reservoirs

J.R. Jones, M.F. Knowiton, D.V. Obrecht, and E.A. Cook

Abstract: The proportion of cropland cover in the catchments of Missouri reservoirs, a surrogate for non-point-source nutrient loss from agricultural watersheds, accounts for some 60%-70% of the cross-system variance in long-term averages of total phosphorus and total nitrogen (n = 126, ln transformation for nutrients and logit for cropland). The addition of dam height and an index of flushing rate improved  $r^2$  values to ~77% for both nutrients. Even among reservoir catchments with >80% grass and forest cover, cropland accounted for most of the variation in nutrients. Reservoir nutrients showed a strong negative relation to forest cover. Relations between grass cover and nutrients were positive but weak, and grass had no significant statistical effect once cropland was taken into account. Residual analysis suggests that urban reservoirs would have about twice the nutrient level of reservoirs in non-cropland basins (forest and grass). The increase in nutrients with the proportion of cropland and the decrease with forest cover have previously been documented in Missouri streams.

**Résumé :** La proportion des terres agricoles dans les bassins versants des réservoirs du Missouri, une variable de remplacement pour les pertes diffuses de nutriments dans les bassins versants agricoles, explique environ 60 % – 70 % de la variances des quantités moyennes à long terme de phosphore total et d'azote total dans les bassins (n = 126, transformation ln des valeurs de nutriments et transformation logit dans le cas des terres agricoles). L'addition de la hauteur des barrages et d'un indice de vidange améliore les valeurs de  $r^2$  à ~77 % pour les deux variables de nutriments. Même dans les bassins versants de réservoirs avec >80 % de couverture de prairie ou de forêts, les terres agricoles expliquent la plus grande partie de la variation des nutriments. Il y a une forte relation négative entre les nutriments dans les réservoirs et la couverture forestière. Les relations entre la prairie et les nutriments sont positives, mais faibles, et tout effet statistiquement significatif disparaît lorsqu'on tient compte des terres agricoles. Une analyse résiduelle indique que les réservoirs urbains auraient environ le double des concentrations de nutriments des réservoirs dans les bassins versants sans terres agricoles (donc de forêts et de prairies). L'augmentation des nutriments en fonction de la proportion des terres agricoles et leur diminution en fonction de la couverture forestière avaient déjà été démontrées dans les cours d'eau du Missouri.

[Traduit par la Rédaction]

#### Introduction

A central paradigm of modern limnology is that external nutrient loading, modified by morphology and hydrology, determines the trophic state of a lake (Edmondson 1961; Vollenweider 1975). Increased biological production resulting from nutrient enrichment is a major theme in freshwater ecology (Likens 1972; Smith 1998), and nutrient control to reduce excessive productivity is the principal focus of applied limnology (Sas 1989; Cooke et al. 1993). Anthropogenic activity has accelerated eutrophication, and lake management has typically addressed this problem by reducing excessive nutrient loading associated with point-source discharge from municipalities. This approach been successful in reversing eutrophication in Lake Washington (Edmondson 1994), in areas of the Laurentian Great Lakes (Nicholls et al. 2001), in several major European lakes (Sas 1989), and in other areas.

Recently, aquatic scientists have determined that nonpoint-source nutrient inputs from agricultural and urban sources are a leading cause of the nation's remaining water quality problems (Novotny and Chesters 1989; Soranno et al. 1996; Carpenter et al. 1998). Studies have quantified the interdependence of land cover and nutrient export coefficients from a variety of landscapes modified by human activity (Beaulac and Reckhow 1982; Frink 1991). It can be inferred from paleolimnological records and historic waterquality data that lake enrichment, by way of non-pointsource mechanisms at the landscape level, has resulted from conversion of land from native cover to agriculture and urban use (Stoermer et al. 1993; Schelske and Hodell 1995;

Received 20 June 2003. Accepted 6 April 2004. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 2 November 2004. J17579

J.R. Jones,<sup>1</sup> M.F. Knowlton, and D.V. Obrecht. Department of Fisheries and Wildlife Sciences, School of Natural Resources, University of Missouri, Columbia, MO 65211, USA.

E.A. Cook. US Department of Agriculture, Natural Resources Conservation Service, Parkade Center, Suite 250, 601 Business Loop 70 W, Columbia, MO 65203, USA.

<sup>1</sup>Corresponding author (e-mail: jonesj@missouri.edu).

Can. J. Fish. Aquat. Sci. 61: 1503-1512 (2004)

Reavie and Smol 2001). Specific studies have linked changes in lakes to intensified land-use practices within their catchments; in these examples the pathway for increased nutrient and organic-matter loading was from non-point sources (Mitchell and Galland 1981; Soranno et al. 1996; Carignan et al. 2000).

Limnological research in Missouri has demonstrated that stream nutrient levels are tied to land cover through nonpoint processes (Smart et al. 1985; Perkins et al. 1998; Lohman and Jones 1999). The general pattern is that nutrient levels increase in streams as a function of the proportion of cropland within the watershed and decline with forest cover. Relative to forest, row-crop agriculture represents a major disturbance to the landscape, with frequent tillage and fertilizer application (Turner and Rabalais 1991; Howarth et al. 1996). Missouri analyses suggest that these two contrasting cover types account for much of the measured variance in nutrient levels among streams within the state.

In this paper, we extend the land cover nutrient analysis to Missouri reservoirs, which vary from oligo- to hypereutrophic (Jones and Knowlton 1993). Few reservoirs in Missouri have point-source inputs, so quantifying the link between land cover (an indirect measure of external nutrient input from non-point-source anthropogenic activities) and large-scale patterns of reservoir trophic state is essential for interpreting factors regulating regional water quality (Jones and Knowlton 1993). Our objective was to determine whether land cover in the watershed, and measures of morphology and (or) hydrology, could account for among-system variation in reservoir nutrient levels within the state. Our approach is consistent with the concepts that underpin simple empirical loading models (Edmondson 1961; Vollenweider 1975). We use land cover as a surrogate for nutrient input, with the supposition that cropland will be the dominant source of external nutrients. In this large-scale comparative lake study we are identifying the influence of watershed characteristics on reservoir limnology (Jones et al. 1998), and the effects of unmeasured variables remain as residual error. Surprisingly, few studies have linked land cover to lake trophic state and there is only one study of this nature on reservoirs (Knoll et al. 2003).

#### Methods

#### Limnology data

Limnology data used in this analysis come from 135 Missouri reservoirs that represent the range of reservoir resources within the state, including those used for water supply, recreation, and multipurpose Corps of Engineers reservoirs (Fig. 1). Reservoirs were sampled seasonally on three or four occasions during May-August from the surface layer at a site near the dam. Individual reservoirs were represented in the data set by collections ranging from 4 to 21 summer seasons during the period 1978-2002; most reservoirs were represented by data from ≥10 seasons. To limit the effects of temporal variation (Knowlton et al. 1984) on this analysis we confined our study to those reservoirs with four or more summer seasons' data. Samples were processed by standard methodology (Knowlton and Jones 1995). Analyses are based on lake means for total phosphorus (TP) and total nitrogen (TN) (Table 1). Lake means were calculated as nested

averages over the period of record for each reservoir by calculating the geometric mean (ln-transformed) for each summer and then calculating the geometric mean across all summers to estimate the lake mean. Morphometric variables (volume and dam height) were provided by the Missouri Department of Natural Resources. Bathymetric maps were available for 26 reservoirs. For this subset, mean depth and dam height were strongly correlated (r = 0.96), with mean depth averaging about one-fourth of dam height. A hydrologic flushing index was estimated for each reservoir by using the regional runoff coefficient (Missouri Department of Natural Resources 1986), watershed area, and reservoir volume.

#### Watershed data

Geographic Information Systems and remote-sensing techniques were used to characterize cover types within the watersheds of Missouri reservoirs (forest, cropland, grass (which includes pastures), urban area, and water). Watersheds were digitized in a heads-up fashion using ESRI's Arc Info software (Environmental Systems Research Institute 1997) and United States Geological Survey 1:24 000 topographic maps in digital raster graphic format. Topology was built for each digitized watershed and a grid coverage  $(30 \text{ m} \times 30 \text{ m})$ was created using Arc Info software. Streams within the watersheds were digitized in the same fashion as the watersheds. The arcs depicting streams were buffered at two different distances, to create polygons around the streams that represented riparian zones of 75 and 150 m. Topology was built for the buffered streams and grid coverages were created  $(30 \text{ m} \times 30 \text{ m})$ . Watershed and buffered-stream coverages were then imported into ERDAS IMAGINE software (Leica Geosystems GIS & Mapping 1997) and masked with a land-use coverage (1993 data) created by the Missouri Resources Assessment Program. A report was created for each masked coverage, providing the area classified in each cover-type category. The total area that was classified as water was divided into reservoir and non-reservoir categories.

Relations between landscape variables and nutrients were examined by least-squares methods of single and stepwise multiple regression with p < 0.01 unless otherwise stated. Data were transformed using ln or logit (adding 0.003 to cover types measured to avoid zero values) where appropriate. All analyses were performed with SPSS<sup>®</sup> for Windows version 11 (SPSS Inc. 2001).

#### Results

#### **Reservoir and watershed characteristics**

The median Missouri reservoir in this analysis is eutrophic, with 705  $\mu$ g L<sup>-1</sup> TN and 39  $\mu$ g L<sup>-1</sup> TP. Among the study reservoirs, however, nutrient values ranged 12- to 30-fold (Table 1), TP being more variable than TN (coefficient of variation = 73% vs. 44%).

The median watershed had 31% grass, 24% forest, 13% cropland, and <1% urban area, but individual proportions ranged from zero to >74% in each cover type (Table 1). Collectively, forest and agriculture (cropland plus grass) jointly composed >85% of the land area in most (80%) study basins (Fig. 2*a*), the remaining area being classified as water (median 5%) and urban area. Some 80% of the catchments had

Fig. 1. Map of Missouri, USA, showing the location of the 135 reservoirs considered in this study.



Table 1. Summary statistics for limnological data, land cover, and watershed-morphology data for Missouri reservoirs (n = 135).

	Media	an	Mean		Minir	num	25%	75%	Maximum
Nutrient data									
Total phosphorus (µg·L <sup>-1</sup> )	39		45		6		21	58	182
Total nitrogen ( $\mu g \cdot L^{-1}$ )	705		725		200		500	920	2 330
Land cover (%)									
Forest	4	4.5	35		0		12	54	95
Grass	31		32		0		19	46	78
Cropland	13		19		0		5	32	74
Urban area	0		7		0		0	3	96
Water	5		6		0		3	8	25
Watershed-morphology data									
Reservoir area (ha)	42		750		2		17	114	21 787
Dam height (m)	14	1 A.L	16		5		10	19	77
Volume ( $m^3 \times 10^4$ )	208	. 1	7 787		6		67	509	333 319
Watershed area (ha)	1028		37 781		33		393	3857	1 875 178
Ratio of watershed to reservoir area	21		48		4		15	39	592
Flushing index (year <sup>-1</sup> )	1.	1	3.'	7	0.1		0.5	5 2.	5 87.1

Fig. 2. (a) Plot of the proportion of forest cover in the catchments of 135 Missouri reservoirs against the proportion of the catchment in agriculture (cropland plus grass). The nine data points enclosed by an ellipse have >50% urban area in the catchment. The broken line represents 85% land cover jointly in forest and agriculture. (b) Ternary diagram showing the proportions of cropland, forest, and grass in the catchments of the Missouri reservoirs.







<5% urban area, but nine reservoirs located within metropolitan Kansas City had 50–96% urban area (mostly residential; Fig. 2*a*); this unique subset is treated separately in the land cover – nutrient analysis. Forest and grass jointly composed 75–99% of the catchment area in half of the basins (Fig. 2*b*); within this subset these two cover types were negatively correlated (r = -0.96, n = 66) and across the continuum of possible combinations, forest and grass were well represented (low, high, and equal proportions of each type). Grass and cropland jointly composed 75–93% of the catchment area in ~20% of the basins (Fig. 2*b*) in combinations of 20–70% of either cover type, which were negatively correlated (r = -0.95, n = 32). Forest showed a hyperbolic relation to cropland (r = -0.86, logit-transformed, urban reservoirs excluded); with the exception of a few highly forested catchments, combinations of these two cover types did not dominate the study basins.

Among catchments with >5% cropland (n = 82) the proportion of cropland within the 75-m corridor consistently averaged about 20% less than within the overall watershed. Similar minor differences were found among catchments such that cropland within the 75- and 150-m corridors was highly correlated with cropland in the entire drainage basin  $(r \ge 0.89, n = 108)$ . Comparisons also showed that the proportion of each cover type in the riparian zone was similar to its proportion in the entire basin (grass, forest, and urban area,  $r \ge 0.85$ ). These strong correlations limit the suitability of the data set to test the role of riparian land cover on reservoir nutrients at this scale of analysis.

There are strong correlations among the physical features of reservoirs and their watersheds (ln-transformed, n = 135; Table 1). Dam height was significantly correlated with reservoir surface area (r = 0.71), volume (r = 0.83), and watershed area (r = 0.57). Reservoir surface area was correlated with both watershed area (r = 0.89) and storage volume (r = 0.96). The ratio of watershed area to reservoir surface area (both in hectares) ranged from 4 to 592, with a median of 21 and an average of 48. The flushing index, expressed as the ratio of average inflow volume to reservoir volume, ranged from 0.1 to 87, with a median of 1.1 and mean of 3.7 (year<sup>-1</sup>).

#### Limnological characteristics and land cover

As expected, both TP and TN in Missouri reservoirs were positively related to the proportion of cropland within their watersheds (r > 0.61, n = 126), which does not include the nine reservoirs with >50% urban area; Figs. 3a and 3b), with a pattern of increasing variance with increasing cover. Minimum nutrient levels were strongly influenced by cropland; reservoirs with >50% cropland had minimum TP and TN of 43 and 830  $\mu$ g L<sup>-1</sup>, respectively. Nutrient concentrations were also weakly correlated with grass (Figs. 3c and 3d), but minimum values were low over the entire range in the data set. Both nutrients were negatively correlated with forest (r > -0.59; Figs. 3e and 3f), and variation declined with increasing forest. For reservoirs with >90% forest, TP and TN were less than 15 and 335  $\mu$ g·L<sup>-1</sup>, respectively, with little among-system variation. These results parallel the pattern seen in Missouri streams, where cropland tends to be the largest source of nutrients and forest the smallest (Perkins et al. 1998). Grass is an intermediate nutrient source, but is more similar to forest than to cropland, at least in terms of minimum observed concentrations. Neither nutrient showed a significant correlation with the proportion of urban area; reservoirs with >50% urban area had nutrient levels of 16-55  $\mu$ g·L<sup>-1</sup> TP and 390–900  $\mu$ g·L<sup>-1</sup> TN, which rank low to intermediate within the overall data set.

When transformed variables (In for nutrients and logit for land cover) were used, cropland accounted for 62% and 71% of the variation in TP and TN, respectively (n = 126, not including reservoirs with >50% urban area; Fig. 4, Table 2). Multiple regression showed that the inclusion of dam height (In-transformed), a surrogate term for lake morphometry and

Fig. 2. (a) Plot of the proportion of forest cover in the catchments of 135 Missouri reservoirs against the proportion of the catchment in agriculture (cropland plus grass). The nine data points enclosed by an ellipse have >50% urban area in the catchment. The broken line represents 85% land cover jointly in forest and agriculture. (b) Ternary diagram showing the proportions of cropland, forest, and grass in the catchments of the Missouri reservoirs.





<5% urban area, but nine reservoirs located within metropolitan Kansas City had 50–96% urban area (mostly residential; Fig. 2*a*); this unique subset is treated separately in the land cover – nutrient analysis. Forest and grass jointly composed 75–99% of the catchment area in half of the basins (Fig. 2*b*); within this subset these two cover types were negatively correlated (r = -0.96, n = 66) and across the continuum of possible combinations, forest and grass were well represented (low, high, and equal proportions of each type). Grass and cropland jointly composed 75–93% of the catchment area in ~20% of the basins (Fig. 2*b*) in combinations of 20–70% of ei-

ther cover type, which were negatively correlated (r = -0.95, n = 32). Forest showed a hyperbolic relation to cropland (r = -0.86, logit-transformed, urban reservoirs excluded); with the exception of a few highly forested catchments, combinations of these two cover types did not dominate the study basins.

Among catchments with >5% cropland (n = 82) the proportion of cropland within the 75-m corridor consistently averaged about 20% less than within the overall watershed. Similar minor differences were found among catchments such that cropland within the 75- and 150-m corridors was highly correlated with cropland in the entire drainage basin  $(r \ge 0.89, n = 108)$ . Comparisons also showed that the proportion of each cover type in the riparian zone was similar to its proportion in the entire basin (grass, forest, and urban area,  $r \ge 0.85$ ). These strong correlations limit the suitability of the data set to test the role of riparian land cover on reservoir nutrients at this scale of analysis.

There are strong correlations among the physical features of reservoirs and their watersheds (ln-transformed, n = 135; Table 1). Dam height was significantly correlated with reservoir surface area (r = 0.71), volume (r = 0.83), and watershed area (r = 0.57). Reservoir surface area was correlated with both watershed area (r = 0.89) and storage volume (r = 0.96). The ratio of watershed area to reservoir surface area (both in hectares) ranged from 4 to 592, with a median of 21 and an average of 48. The flushing index, expressed as the ratio of average inflow volume to reservoir volume, ranged from 0.1 to 87, with a median of 1.1 and mean of 3.7 (year<sup>-1</sup>).

#### Limnological characteristics and land cover

As expected, both TP and TN in Missouri reservoirs were positively related to the proportion of cropland within their watersheds (r > 0.61, n = 126), which does not include the nine reservoirs with >50% urban area; Figs. 3a and 3b), with a pattern of increasing variance with increasing cover. Minimum nutrient levels were strongly influenced by cropland; reservoirs with >50% cropland had minimum TP and TN of 43 and 830  $\mu$ g·L<sup>-1</sup>, respectively. Nutrient concentrations were also weakly correlated with grass (Figs. 3c and 3d), but minimum values were low over the entire range in the data set. Both nutrients were negatively correlated with forest (r > -0.59; Figs. 3e and 3f), and variation declined with increasing forest. For reservoirs with >90% forest, TP and TN were less than 15 and 335  $\mu$ g·L<sup>-1</sup>, respectively, with little among-system variation. These results parallel the pattern seen in Missouri streams, where cropland tends to be the largest source of nutrients and forest the smallest (Perkins et al. 1998). Grass is an intermediate nutrient source, but is more similar to forest than to cropland, at least in terms of minimum observed concentrations. Neither nutrient showed a significant correlation with the proportion of urban area; reservoirs with >50% urban area had nutrient levels of 16-55  $\mu$ g·L<sup>-1</sup> TP and 390–900  $\mu$ g·L<sup>-1</sup> TN, which rank low to intermediate within the overall data set.

When transformed variables (In for nutrients and logit for land cover) were used, cropland accounted for 62% and 71% of the variation in TP and TN, respectively (n = 126, not including reservoirs with >50% urban area; Fig. 4, Table 2). Multiple regression showed that the inclusion of dam height (In-transformed), a surrogate term for lake morphometry and



Fig. 3. Linear plots of total phosphorus and total nitrogen against the proportions of cropland, grass, and forest in the catchments.

(b)

2000

1500



a correlate of watershed physiography, improved the  $r^2$  values to 73% for TP and 76% for TN (Table 2).

The addition of the flushing index (ln-transformed) improved the explanation of among-system variation in TP to 77% (Table 2) but was not significant in the TN analysis. Among these independent variables, only dam height and flushing index were significantly correlated (r = 0.38, p < 0.0001). Analysis of multicollinearity showed that parameter estimates were not adversely affected in regressions that included both dam height and flushing index. Given the intercorrelation among the physical and morphological features of these systems, watershed area (ln-transformed,  $r^2 = 0.78$ ) or the ratio of watershed to reservoir surface area (ln-transformed,  $r^2 = 0.77$ ) performed similarly to the flushing index in the TP analysis.

Collectively, morphology variables, and for TP, hydrology variables, accounted for ~20%-40% of the variance not accounted for by non-point-source features of cropland (Table 2). Adding other land-use categories to these models did not appreciably reduce the residual variance (only ~1% improvement in  $r^2$  values) and were not always significant over just cropland and morphology. Even among reservoir systems dominated by grass and forest (<20% cropland, n = 75), cropland accounted for >50% of the variation in TP

(52%) and TN (57%), and adding dam height improved the models to  $\sim$ 70% for both nutrients.

Reservoir nutrients were negatively related to proportion of forest (logit-transformed,  $r^2 = 0.49-0.62$ ; Fig. 4), and with dam height and watershed area included, multiple regression explained ~70% of the among-system variation in nutrient content of the Missouri reservoirs (models not shown). Relations of grass (logit-transformed) with nutrients were positive but much weaker ( $r^2 = <0.2$ ) than those with cropland or forest, and multiple regressions using the proportion of agricultural land in the watershed (cropland plus grass) explained less variance than did cropland alone. Relations with the proportion of urban area were not significant.

For the nine reservoirs with >50% urban area, the statewide regression models consistently underpredicted TP and TN. Residuals (observed minus predicted) were nearly all positive (Fig. 5) and were strongly correlated with the proportion of urban cover (r = ~0.9 for models 3 and 5 from Table 2). This result suggests that urban catchments export proportionately more TP and TN than other non-cropland cover types (forest and grass).

For non-urban catchments with cover-type measurements in the riparian corridor (n = 99), residuals from the statewide models were only weakly related to land use in the 75- and



Fig. 4. Total phosphorus and total nitrogen plotted against the proportions of cropland and forest. Nutrients are In-transformed and cover type was logit-transformed.

Table 2. Regression models for total phosphorus and total nitrogen (TP and TN, respectively, In-transformed) based on the proportion of cropland (%crop, logit-transformed), morphology (dam height, DH), and hydrology (flushing index, FI).

Relation	$r^2$	SE
1: TP = 4.27 + 0.36 % crop	0.62	0.47
2: $TP = 5.53 + 0.33$ % crop - 0.50 DH	0.73	0.40
3: $TP = 5.20 + 0.35$ % crop - 0.37 DH + 0.12 FI	0.77	0.37
4: $TN = 6.96 + 0.24$ % crop	0.71	0.26
5: $TN = 7.51 + 0.23 \% crop - 0.22 DH$	0.76	0.23

Note: Model number, standard error (SE), and coefficient of determination  $(r^2)$  are provided for each regression. Units are shown in Table 1.

150-m corridors. Residuals from the TP and TN models (models 3 and 5 from Table 2) were positively correlated with grass in the 150-m corridor (r = 0.27-0.31, p < 0.05), residuals from the TN model (model 5 from Table 2) were also correlated with grass in the 75-m corridor (r = 0.26, p < 0.260.05), and residuals from the TP and TN models were correlated with urban cover in the 150-m corridor (model 5 from Table 2, r = 0.2, p < 0.05). Proportions of forest and cropland in the riparian corridor had no significant influence on residuals from any of the models.

#### Discussion

Collectively, these empirical analyses specific to Missouri reservoirs support limnological theory in that nutrient concentrations are largely determined by external inputs as modified by morphology and hydrology (Edmondson 1961; Vollenweider 1975). In this statewide analysis, cropland accounts for some 60-70% of the variance in reservoir nutrient concentration. These relations with cropland were expected, but are surprisingly powerful given that they are limited to a single catchment feature, a surrogate of the degree of nonpoint-source loading. The models presented herein ignore nutrient contributions from non-cropland cover, which account for >75% of the land area considered in this study. The pattern, however, directly parallels findings that cropland was highly correlated with nutrients in Missouri streams (Perkins et al. 1998).

80 90

Undoubtedly, cropland is a greater relative source of nutrients than the other dominant cover types in our analysis, grass and forest. Even among reservoirs where cropland composed <5% of the basin (n = 32, urban reservoirs excluded), variation in cropland was a much stronger correlate of TP and TN than variation in other cover types. Row-crop agriculture represents a continuous disturbance to the landscape that is intensively managed by tilling, harvest, and application of nitrogen-rich fertilizers (Turner and Rabalais 1991; Howarth et al. 1996). Researchers have consistently found nutrient export from cropland to be several times that from grass and forest (Beaulac and Reckhow 1982; Frink 1991), and our analysis supports this pattern. Among Missouri reservoirs, TP and TN increased strongly with cropland and decreased with forest, resulting in about a 7-fold minimum difference in nutrients between a reservoir dominated by forest and one dominated by cropland. Regression equations based on forest cover and morphology were slightly more variable than those based on cropland but suggest that this land cover type was the smallest nutrient source in our catchments.

The influence of grass on reservoir nutrient levels was less apparent. Reservoirs in catchments dominated by grass had Jones et al.

Fig. 5. Effect of the proportion of urban cover on residuals (observed – predicted, ln-transformed data) from models 3 (a; r = 0.91) and 5 (b; r = 0.89) in Table 2 calculated for nine urban reservoirs not included in the statewide land cover – nutrient analysis. Solid lines were fitted by least squares.





about triple the TN and less than double the TP of those dominated by forest, suggesting that exports from grass were intermediate between those from forest and cropland. The literature suggests that nutrient flux from grass is not necessarily uniform. Losses are much greater from intensively fertilized and grazed pastures relative to grass fields set aside for conservation (Sharpley et al. 1994; Watson and Foy 2001). This entire continuum of grassland management is known to occur within Missouri, but data from specific basins are not available. In our analyses, variation in grass had no significant statistical effect once cropland was taken into account. This finding suggests that nutrient export from grass is consistently low relative to that from cropland or that loss rates from grass are strongly correlated with those from cropland among the various catchments. Cropland in Missouri is predominantly located in regions of rich soils (Jones and Knowlton 1993), and nutrient flux from agricultural areas (cropland plus grass) in a given basin may be highly influenced by ambient soil fertility; however, we cannot test this viable hypothesis with our data set.

Nutrient export from urban catchments often equals or exceeds that from agriculture (Beaulac and Reckhow 1982; Frink 1991), and impervious surfaces increase runoff relative to other cover types. Most of our catchments had <5% urban area, but nine reservoirs in metropolitan Kansas City were unique, with >50% urban cover, and were excluded from the statewide analysis. Compared with models based on the proportion of cropland in the catchment these reservoirs had higher TP and TN than predicted, the difference increasing directly with the proportion of urban cover. Residual analysis suggests that a reservoir in an urban catchment (zero cropland) would have twice the nutrient level of a reservoir in a non-cropland basin (forest and grass) represented in our statewide regressions (e.g., In residual >0.69). Data from Missouri streams support this pattern (Smart et al. 1985). This preliminary analysis suggests that there are strong effects of urbanization on reservoir nutrients, but most Missouri impoundments are in rural regions where urban cover is a minor component of land cover.

In this analysis the percentage of cropland is a surrogate for nutrient concentration in the inflowing streams (Perkins et al. 1998). In steady-state models, lake nutrient concentrations are a function of inflow values minus sedimentation (Vollenweider 1975). Sedimentation is governed by several physical processes such as settling velocity, settling distance (mean depth), time available for settling to occur (flushing rate), and several biological processes such as zooplankton feeding and resuspension by bottom-feeding fish. In this presentation dam height serves as a surrogate for mean depth and is a correlate of reservoir volume, stratification potential, and hydrology. Flushing index is inflow volume relative to reservoir volume and is correlated with watershed area and the ratio of watershed area to lake surface area. In all cases these physical metrics explained additional variance in reservoir nutrients not accounted for by cover type.

Loss of nutrients as a result of sedimentation should decrease with increasing flushing rates, making flushing a positive influence on in-reservoir nutrient levels. Consistent with this expectation, the coefficient for the flushing index is positive (Table 2, model 3). For a given flushing rate and inflow concentration, the expected effect of increasing lake depth is also positive (Cooke et al. 1993). With increasing watercolumn depth there is a decreasing probability that sedimenting particles will reach the bottom before water exits the lake. In our regression models, however, the coefficients for depth (dam height) differ by being negative. For example, based on model 3 the median Missouri reservoir (13% cropland, dam height 14 m, and flushing index 1.1) would have 36  $\mu$ g·L<sup>-1</sup> TP, but when other features are held constant, a reservoir with half that dam height would have 46  $\mu g \cdot L^{-1}$ TP and a reservoir with twice the dam height would have 28  $\mu$ g·L<sup>-1</sup> TP. Internal nutrient loading is an inverse function of mean depth (Nürnberg 1984) and may contribute to this apparent disparity between our results and convention. Also, inputs to reservoirs are often affected by plunging inflows, yielding interflow or underflow currents that deliver incoming nutrients to subsurface strata with little effect on the surface conditions reflected in our data (Knowlton and Jones

1995). The phenomenon is likely of greater consequence in deeper water bodies relative to shallower ones, and this inflow pattern likely contributes to the large coefficients used to describe sedimentation processes in reservoirs relative to natural lakes (Jones and Bachmann 1978; Canfield and Bachmann 1981). We cannot quantify the role of either process with these data, but nearly all Missouri reservoirs develop subsurface anoxia during stratification, thereby increasing the potential for internal loading, and plunging inflows are common.

Several landscape-level factors, outside the scope of this analysis, likely contributed to unaccounted-for variation in our relations. Among these, variation in rates and timing of fertilizer application and tillage practices, all major factors in determining yields (Howarth et al. 1996; Baker and Richards 2002; Richards et al. 2002), would have contributed to non-uniform losses from agricultural fields (both cropland and grass). Changes in land use during the study period would also have contributed to residual error. In addition, the spatial pattern of land cover within the catchments, referred to by Johnson et al. (1997) as patch density, would be a source of additional variation in chemical flux from catchments (Soranno et al. 1996).

Land use within the riparian zone can be a critical factor in determining nutrient export from a watershed. Land away from the stream channel is thought to have less impact on stream nutrients, and hence flux of materials to downstream reservoirs, than land close to the channel (Osborne and Wiley 1988). We had expected that our characterization of land use within the riparian zone of these basins would be a significant factor in our models. Our data, however, are not well suited for quantifying the effects of vegetated buffers because of the strong correlation between the major cover type in the entire catchment and that found in the riparian zone. Although most watersheds had a larger proportion of forest and grass relative to cropland in the riparian corridor (median values within the 75-m corridor were 42% forest, 31% grass, and 10% cropland), the range of conditions in our data set was too narrow to provide a strong test of riparian buffers. Others have quantified the attenuation of nutrient export by vegetated buffers (Osborne and Kovacic 1993), whereas in some large-scale studies there is no evidence of reduction (Omernik et al. 1981).

Volatilized nitrogen from fertilizers and animal-waste facilities within the state could be an unmeasured atmospheric source of nitrogen to the basins and directly to the reservoirs (Howarth et al. 1996). Biotic factors may also influence among-system variance though sediment-feeding fish (Michaletz 1997; Schaus et al. 1997) that would contribute to internal loading. In two reservoirs, known introductions of grass carp (Ctenopharyngodon idella) have reduced littoral vegetation, with concurrent increases in pelagic nutrients and algal biomass (J. Jones, unpublished data) during a period when the proportion of cropland in both catchments decreased. Several reservoirs were intentionally fertilized to improve the fishery, but removing these systems from the analysis resulted in only small improvements in cover type nutrient relations. Several reservoirs receive inputs from municipal effluents, but this information did not improve the models. Lastly, our limnological collections are from the down-lake zone of these impoundments, near the dam. They represent conditions after in-reservoir processes such as sedimentation, uptake, and dilution have altered the chemistry and suspended-solids content of inflow from the catchment (Jones and Knowlton 1993), thereby blunting the relation between nutrient flux from the catchment and reservoir measurements. Surrogate measures of morphology and hydrology, dam height, and flushing accounted for variance attributed to these processes in our models.

Our land cover – nutrient relations, modified by physical features, match or exceed the level of explanation provided by similar cover-type relations in Connecticut lakes (Field et al. 1996), where relations were negative with forest and positive with urban cover and agriculture, Ontario (Dillon and Molot 1997), where peatlands explained among-lake variation in TP, Quebec (Carignan et al. 2000), where forest harvest was important in determining TP, Alberta (Prepas et al. 2001) where wetlands were correlated with TP, and Ohio (Knoll et al. 2003), where agriculture explained variation in maximum TP among 12 reservoirs. Similarly, Meeuwig and Peters (1996) successfully used empirical land-use models based on catchment characteristics and morphometry to model chlorophyll in a broad suite of lakes. Predictions of coastal eutrophication from land use account for about the same amount of variation as our analogous reservoir relations (Meeuwig 1999).

About 90% of all Missouri reservoirs were constructed in the past 50 years and many are half that age. Land-use practices have not been constant over this period, but preimpoundment land cover was undoubtedly similar to current conditions, being dominated by forest or cropland with interspersed grass. These land-use patterns would have directly influenced stream water quality (Perkins et al. 1998) and, beginning with impoundment, determined reservoir water quality. In this respect, Missouri reservoirs differ from many temperate lakes that have experienced cultural eutrophication resulting from post-settlement changes in land use (Field et al. 1996; Soranno et al. 1996). Land-cover data from a subset of the catchments (n = 29) that coincide with the period of our water-quality information (ca. 1980 - present) show virtually no change in the proportion of forest (a median increase of 2%) but a small decline in cropland and a concurrent increase in the proportion of grass (median changes of -8% and +12%, respectively). This shift in cover type is consistent with changes in agricultural policy over the period. The inference is that in the near term, cropland will not expand but large-scale declines in row-crop agriculture are also unlikely. Our analysis suggests that efforts to improve reservoir water quality in the state should focus on minimizing non-point nutrient sources. Research on nutrient loss from agriculture has shown that small "hot spots" often account for most nutrient export (Gburek et al. 2000) and that measures such as riparian-zone management can decrease nutrient loss (Osborne and Kovacic 1993; Soranno et al. 1996). The full potential for what are called best management practices (e.g., Mostaghimi et al. 2001) to control non-pointsource nutrients has not been realized. When technological nutrient management is routinely practiced, the importance of cropland to nutrient levels in Missouri reservoirs will likely diminish.

Jones et al.

#### Acknowledgments

This study of cover types was funded by the Missouri Department of Natural Resources, the US Environmental Protection Agency, and the Missouri Agricultural Experiment Station. Reservoir data come from long-term projects funded by the Missouri Department of Natural Resources, Missouri Department of Conservation, and Missouri Water Resources Research Center. We thank Bruce Perkins for compiling reservoir data, Tony Thorpe for crafting Fig. 1, Stephen Allen for making helpful comments, Tracy Wiggins for assisting with watershed data, and Daniel Zerr of the University of Missouri Center for Agricultural, Resource, and Environmental Systems for helping with land-cover information.

#### References

- Baker, D.B., and Richards, R.P. 2002. Phosphorus budgets and riverine phosphorus export in northwestern Ohio watersheds. J. Environ. Qual. 31: 96-108.
- Beaulac, M.N., and Reckhow, R.H. 1982. An examination of land use – nutrient export relationships. Water Resour. Bull. 18: 1013– 1024.
- Canfield, D.E., Jr., and Bachmann, R.W. 1981. Prediction of total phosphorus concentrations, chlorophyll *a*, and Secchi depths in natural and artificial lakes. Can. J. Fish. Aquat. Sci. **38**: 414–423.
- Carignan, R., D'Arcy, P., and Lamonagne, S. 2000. Comparative impacts of fire and forest harvesting on water quality in Boreal Shield lakes. Can. J. Fish Aquat Sci. 57(Suppl. 2): 105–117.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., and Smith, V.H. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8: 559–568.
- Cooke, G.D., Welch, E.B., Peterson, S.A., and Newroth, P.R. 1993. Restoration and management of lakes and reservoirs. 2nd ed. Lewis Publishers, Boca Raton, Fla.
- Dillon, P.J., and Molot, L.A. 1997. Effect of landscape form on export of dissolved organic carbon, iron and phosphorus from forested stream catchments. Water Resour. Res. 33: 2591–2600.
- Edmondson, W.T. 1961. Changes in Lake Washington following an increase in the nutrient income. Verh. Int. Ver. Limnol. 14: 167–175.
- Environmental Systems Research Institute. 1997. ARC/INFO. Version 7.1. Environmental Systems Research Institute, Redlands, Calif.
- Edmondson, W.T. 1994. Sixty years of Lake Washington: a curriculum vitae. Lake Reserv. Manag. 10: 75–84.
- Field, C.K., Siver, P.A., and Lott, A.-M. 1996. Estimating the effects of changing land use patterns on Connecticut lakes. J. Environ. Qual. 25: 325–333.
- Frink, C.R. 1991. Estimating nutrient exports to estuaries. J. Environ. Qual. 20: 717–724.
- Gburek, W.J., Sharpley, A.N., and Golmar, G.J. 2000. Critical areas of phosphorus export from agricultural watersheds. *In* Agriculture and phosphorus management: the Chesapcake Bay. *Edited by* A.N. Sharpley. Lewis Publishers, Boca Raton, Fla.
- Howarth, R.W., Gillen, G., Swaney, D., Townsend, A., Jaworski, N., et al. 1996. Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. Biogeochemistry, 35: 75–139.
- Johnson, L.B., Richards, C., Host, G.E., and Arthur, J.W. 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshw. Biol. **37**: 193–208.

- Jones, J.R., and Bachmann, R.W. 1978. Phosphorus removal by sedimentation in some Iowa reservoirs. Verh. Int. Ver. Limnol. 20: 1576–1580.
- Jones, J.R., and Knowlton, M.F. 1993. Limnology of Missouri reservoirs: an analysis of regional patterns. Lake Reserv. Manag. 8: 17–30.
- Jones, J.R., Knowlton, M.F., and Kaiser, M.S. 1998. Effects of aggregation on chlorophyll-phosphorus relation in Missouri reservoirs. Lake Reserv. Manag. 14: 1-9.
- Knoll, L.B., Vanni, M.J., and Renwick, W.H. 2003. Phytoplankton primary production and photosynthetic parameters in reservoirs along a gradient of watershed land use. Limnol. Oceanogr. 48: 608–617.
- Knowlton, M.F., and Jones, J.R. 1995. Temporal and spatial dynamics of suspended sediment, nutrients, and algal biomass in Mark Twain Lake, Missouri. Arch. Hydrobiol. 135: 145–178.
- Knowlton, M.F., Hoyer, M.V., and Jones, J.R. 1984. Sources of variability in phosphorus and chlorophyll and their effects on use of lake survey data. Water Res. Bull. 20: 397–407.
- Leica Geosystems GIS & Mapping, LLC. 1997. ERDAS IMAG-INE. Version 8.3. Leica Geosystems GIS & Mapping, LLC, Atlanta, Georgia.
- Likens, G.E. (*Editor*). 1972. Nutrients and eutrophication: the limiting-nutrient controversy. Am. Soc. Limnol. Oceanogr. Spec. Symp. No. 1.
- Lohman, K., and Jones, J.R. 1999. Nutrient sestonic chlorophyll relationships in northern Ozark streams. Can. J. Fish. Aquat. Sci. 56: 124–130.
- Meeuwig, J.J. 1999. Predicting coastal eutrophication from land-use: an empirical approach to small non-stratified estuaries. Mar. Ecol. Prog. Ser. 176: 231–241.
- Meeuwig, J.J., and Peters, R.H. 1996. Circumventing phosphorus in lake management: a comparison of chlorophyll *a* predictions from land-use and phosphorus-loading models. Can. J. Fish. Aquat. Sci. 53: 1795–1806.
- Michaletz, P.H. 1997. Factors affecting abundance, growth, and survival of age-0 gizzard shad. Trans. Am. Fish. Soc. 126: 84–100.
- Mitchell, S.F., and Galland, A.N. 1981. Phytoplankton photosynthesis, eutrophication and vertical migration of dinoflagellates in a New Zealand reservoir. Verh. Int. Ver. Limnol. **21**: 1017–1020.
- Mostaghimi, S., Brannan, K.M., Dillaha, T.A., and Bruggeman, A.C. 2001. Best management practices for nonpoint source pollution control. *In* Agricultural nonpoint source pollution: watershed management and hydrology. *Edited by* W.F. Ritter and A. Shirmohammadi. Lewis Publishers, Boca Raton, Fla. pp. 257–304.
- Nicholls, K.H., Hopkins, R.J., Standke, S.J., and Nakamoto, L. 2001. Trends in total phosphorus in Canadian near-shore waters of the Laurentian Great Lakes: 1976–1999. J. Gt. Lakes Res. 27: 402–422.
- Novotny, V., and Chesters, G. 1989. Delivery of sediment and pollutants from nonpoint sources: a water quality perspective. J. Soil Water Conserv. 6: 568-576.
- Nürnberg, G.K. 1984. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. 29: 111-124.
- Missouri Department of Natural Resources. 1986. Missouri water atlas. Missouri Department of Natural Resources, Division of Geology and Land Survey, Rolla.
- Omernik, J.M., Abernathy, A.R., and Male, L.M. 1981. Stream nutrient levels and proximity of agricultural land and forest land to streams: some relationships. J. Soil Water Conserv. **36**: 227–231.
- Osborne, L.L., and Kovacic, D.A. 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. Freshw. Biol. **29**: 243–258.

- Osborne, L.L., and Wiley, M.J. 1988. Empirical relationships between land use/cover and stream water quality in an agricultural watershed. J. Environ. Manag. **26**: 9–27.
- Perkins, B.D., Lohman, K., Van Nieuwenhuyse, E., and Jones, J.R. 1998. An examination of land cover and stream water quality among physiographic provinces of Missouri, U.S.A. Verh. Int. Ver. Limnol. 26: 940–947.
- Prepas, E.E., Planas, D., Gibson, J.J., Vitt, D.H., Prowse, T.D., Dinsmore, W.P., Halsey, L.A., McEachern, P.M., Paquet, S., Scimgeour, G.J., Tonn, W.M., Paszkowski, C.A., and Wolfstein, K. 2001. Landscape variables influencing nutrients and phytoplankton communities in Boreal Plain lakes of northern Alberta: a comparison of wetland- and upland-dominated catchments. Can. J. Fish. Aquat. Sci. 58: 1286–1299.
- Reavie, E.D., and Smol, J.P. 2001. Diatom-environmental relationships in 64 alkaline southeastern Ontario (Canada) lakes: a diatom-based model for water quality reconstructions. J. Paleolimnol. 25: 25-42.
- Richards, R.P., Calhoun, F.G., and Matisoff, G. 2002. The Lake Erie agricultural systems for environmental quality project: an introduction. J. Environ. Qual. **31**: 6–16.
- Sas, H. 1989. Lake restoration by reduction of nutrient loading: expectations, experiences, extrapolations/coordination. Akademie-Verlag, Berlin.
- Schaus, M.H., Vanni, M.J., Wissing, T.E., Bremigan, M.T., Garvey, J.E., and Stein, R.A. 1997. Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. Limnol. Ocearogr. 42: 1386–1397.
- Schelske, C.L., and Hodell, D.A. 1995. Using carbon isotopes of bulk sedimentary organic matter to reconstruct the history of nu-

trient loading and eutrophication in Lake Erie. Limnol. Oceanogr. **40**: 918–929.

- Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., and Reddy, K.R. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. 23: 437–451.
- Smart, M.M., Jones, J.R., and Sebaugh, J.L. 1985. Stream-watershed relations in the Missouri Ozark Plateau province. J. Environ. Qual. 14: 77–82.
- Smith, V.H. 1998. Cultural eutrophication of inland, estuarine and coastal waters. *In* Successes, limitations, and frontiers in ecosystem science. *Edited by* M.L. Pace and P.M. Groffman. Springer-Verlag, New York. pp. 7–49.
- Soranno, P.A., Hubler, S.L., Carpenter, S.R., and Lathrop, R.C. 1996. Phosphorus to surface waters: a simple model to account for spatial pattern of land use. Ecol. Appl. 6: 865–878.
- SPSS Inc. 2001. SPSS<sup>®</sup> for Windows. Version 11 [computer program]. SPSS Inc., Chicago.
- Stoermer, E.F., Wolin, J.A., and Schelske, C.L. 1993. Paleolimnological comparison of the Laurentian Great Lakes based on diatoms. Limnol. Oceanogr. 38: 1311–1316.
- Turner, R.E., and Rabalais, N.N. 1991. Changes in Mississippi River water quality this century. BioScience, 41: 140-147.
- Vollenweider, R.A. 1975. Input-output models: with special reference to the phosphorus loading concept in limnology. Schweiz. Z. Hydrol. 37: 53–84.
- Watson, C.J., and Foy, R.H. 2001. Environmental impacts of nitrogen and phosphorus cycling in grassland systems. Outlook Agric. 30: 117–127.

## Responses in the James River Arm of Table Rock Lake, Missouri (USA) to point-source phosphorus reduction

Daniel Obrecht, Anthony P. Thorpe and John R. Jones

#### Introduction

Missouri reservoirs span a broad range of trophic states, with cross-system variation being determined largely by nonpoint source inputs from cropland (JONES & KNOWLTON 1993, JONES et al. 2004). About 10% of the state's major reservoirs receive pointsource inputs from municipalities, and eutrophication responses to these nutrient loads have been described in a few cases (KNOWLTON & JONES 1989, KNOWLTON & JONES 1990). Table Rock Lake, a U. S. Army Corps of Engineers impoundment on the White River in southern Missouri (170 km<sup>2</sup>; Fig. 1), shows spatial variation in nutrient concentrations and



Fig. 1. Map showing seven sample sites within the James River Basin of Table Rock Lake, Missouri (USA). Site I is located directly below the City of Springfield's Southwest Treatment Plant (STP). The distance from STP is listed in parenthesis for the other sites.

algal biomass that closely matches the magnitude of cross-system variation within the entire state (KNOWLTON & JONES 1989). As a consequence of point-source input from the Springfield Southwest Treatment Plant (STP), the upper James River Arm has historically been the most enriched region of Table Rock Lake. In response to increased eutrophication, especially in the James River Arm, the Missouri Clean Water Commission regulated reductions in point-source phosphorus loading to this reservoir. The STP (permitted release ~160 million L/day) began meeting the regulated discharge total phosphorus (TP) concentration of 0.5 mg L<sup>-1</sup> in March 2001 as a consequence of a treatment plant upgrade. This paper describes the response of the James River Arm to this lake management effort.

Key words: Point-source loading, phosphorus, chlorophyll, reservoir

#### Sampling sites and methods

Discharge from the STP enters Wilsons Creek (Site I), which flows into the James River (Sites II and III) and subsequently into the James River Arm of Table Rock Lake, where samples were collected from four locations (Sites IV to VII; Fig. 1). Distance between the STP and the most down-lake site is 125 km.

Lotic data were collected by the United States Geologic Survey (USGS) at Site I (station 07052152) in Wilsons Creek immediately downstream from STP during 1993–2003 and at Site III (station 07052500) during 1999–2003. Site II was monitored during 1998–2003 by the University of Missouri's Table Rock Lake Long-Term Monitoring Program (TRM). All three lotic sites were sampled year-round (Fig. 1). USGS data were compiled from the Water Resources page of the USGS web site (water.usgs.gov). Estimated monthly phosphorus releases from STP during July 1992 through September 2003 were supplied by City Utilities of Springfield.

Lake water quality data (Sites IV-VII) were generated through two University of Missouri projects: TRM and the Lakes of Missouri Volunteer Program (LMVP). Sampling methods for LMVP and the analytical methods used by both projects are detailed in OBRECHT et al. (1998). TRM data represent a composite of three distinct samples from the epilimnion (stratified conditions) or photic zone (mixed conditions), while LMVP data represent surface grab samples. Data from Sites IV and VI are from LMVP collections during 1995-2003 and 1992-2003, respectively (Fig.1). Site VII data are from TRM during 1996–2003. Both projects contributed to 1995–2003 data from Site V, with TRM providing data from 1999-2001. Data from Sites IV, V and VI represent April-October collections. Site VII data were collected year-round, with stratified (April-October) and mixed (November-March) periods being evaluated separately.

Data were divided into pre- and post-upgrade periods using March 2001 as the cut-point and were compared (SPSS software) using the nonparametric Mann-Whitney U test with significance set at < 0.05, unless otherwise stated. Water quality trends are described using median values from the various sampling sites and periods.

#### Results

Between July 1992 and February 2001, the median monthly concentration of TP in STP discharge was  $4010 \ \mu g \ L^{-1}$  (Table 1; Fig. 3). Median total monthly discharge during this period was 12,402 kg of TP and ranged from 1890-31,103 kg (Fig.2). Since the STP upgrade, the median monthly concentration of TP has decreased to 460  $\mu$ g L<sup>-1</sup> (Table 1; Fig.3), with the median total monthly discharge decreasing to 1217 kg of TP and ranging from 797-2459 kg (Fig. 2). This 89% reduction in P load was directly reflected in Wilsons Creek (Site I), where TP levels declined 87% (median values =  $3050 \ \mu g \ L^{-1}$  vs.  $390 \ \mu g \ L^{-1}$ ; Table 1; Fig. 3). In the James River, median TP values were reduced 87% at Site II (738  $\mu$ g L<sup>-1</sup> vs. 95  $\mu$ g L<sup>-1</sup>) and 69% at Site III (295  $\mu$ g L<sup>-1</sup> vs. 90  $\mu g L^{-1}$ ; Table 1; Fig. 3). When expressed as TP concentration, this change amounts to a decrease of 2660  $\mu$ g L<sup>-1</sup> in Wilsons Creek, and respective declines of 643  $\mu$ g L<sup>-1</sup> and 205  $\mu$ g L<sup>-1</sup> at the two James River sites (Table 1; Fig.3).

Table 1. Geometric mean (Geo-mean) values are presented along with minimum, median and maximum total phosphorus data ( $\mu$ g/L) from Springfield's Southwest treatment plant (STP) and seven sites in the James River Basin, pre and post-sewage treatment plant upgrade. STP data represent monthly values, with post-upgrade starting in March 2001. Sites IV–VI were sampled during stratified conditions.

Site	Sample	Years	n	Geo-	Minimum	Median	Maximum
	renou			mean			
STP	January-	1992-2001	104	4320	270	4010	21400
	December	2001-2003	24	440	270	460	770
I	January	1993-2000	38	2730	430	3050	6000
	December	2001-2003	15	400	250	390	760
II	January–	1998-2000	19	519	119	738	1688
	December	2001-2003	21	109	42	95	325
111	January-	1999-2000	16	268	50	295	860
	December	2001-2003	30	80	20	90	170
IV	April–	1995-2000	46	129	70	126	347
	October	2001-2003	24	80	59	77	122
V	April–	1995-2000	31	68	11	70	201
	October	2001-2003	16 ·	42	23	40	78
VI	April–	1992-2000	45	39	18	34	125
	October	2001-2003	19	17	7	17	108
VII	April–	1996-2000	41 <sup>`</sup>	16	4	15	62
(stratified)	October	2001-2003	18	10	6	10	46
VII	November-	1996-2000	26	25	11	24	111
(mixis)	March	2001-2003	7	12	9	13	17



Fig.2. Monthly phosphorus releases from the Springfield Southwest Treatment Plant for the period July 1992-September 2003. Dashed line represents completion of treatment plant upgrade, March 2001.

Summer TP levels declined in the James River Arm by some 40% in response to the STP upgrade (Table 1; Fig.3), with median values declining between 33-50% at the various sites. On average, summer in-lake TP levels in the James River Arm declined by 25  $\mu$ g L<sup>-1</sup>, with the decrease showing a strong longitudinal gradient ranging from  $49 \ \mu g \ L^{-1}$  at the uplake location (Site IV) to 5  $\mu$ g L<sup>-1</sup> in the lower arm (Site VII; Fig. 3). During the unstratified period (November-March) there was a 46% decline in TP levels at Site VII (equating to 11  $\mu$ g L<sup>-1</sup>; Table 1). Summer decreases in TP, resulting from the STP upgrade, were statistically significant at all seven sampling sites, as was the decrease at Site VII during mixis (p = < 0.01).

Post-upgrade changes in other limnological parameters in the James River Arm have been consistent with responses to P-load reduction. As expected, total nitrogen (TN) levels at all sites were virtually unchanged by the upgrade, resulting in a significant increase in TN:TP ratios throughout the system. Pre- and post-upgrade TN:TP ratios increased from 5–39 in the James River at Site II (the only lotic site where TN was measured). Upper James River Arm sites (IV and V) showed small, yet statistically significant increases in TN:TP ratios, increasing from 9–11 and 12–15, respectively (Fig. 4).



Fig. 3. Changes in phosphorus for Springfield Southwest Treatment Plant (STP) and seven sites in the James River Basin shown as (a) percent decrease in median values after treatment plant upgrade and (b) pre- and post upgrade median phosphorus concentrations. Site I is in Wilsons Creek (a tributary to the James River), Sites II and III are located in the James River, and Sites IV-VII are in the James River Arm of Table Rock Lake. Data from Sites IV-VII represent April-October sampling, while all other sites were monitored yearround.

Sites in the lower James River Arm (VI and VII) showed greater change, with TN:TP ratios increasing from ~20 to > 30 (Fig. 4). Increased TN:TP marks a shift to greater P-limitation, particularly in the lower James River Arm.

Pre-upgrade, the median summertime yield of chlorophyll per unit TP (Chl:TP) was 0.5 across all sites in the James River Arm, which closely matches the average in non-turbid Missouri reservoirs (JONES & KNOWLTON 1993). Post-upgrade, the median Chl:TP ratio in-





Fig.4. Comparison of (a) Total Nitrogen (TN) to Total Phosphorus (TP) ratios and (b) Chlorophyll (Chl) to TP ratios for the four lake sites. Symbols with line represent the median values for pre- and post-treatment plant upgrade during stratified periods. Individual symbols represent median values for Site VII during mixis.

creased to 0.7 (Fig.4). Ratios of Chl:TP increased significantly at the three upper James River Arm sites (IV, V, and VI, p = < 0.01) while at Site VII the increase was minor. During stratification the increase in Chl:TP in the James River Arm was generally a result of lower TP concentrations as opposed to substantial shifts in Chl (Fig.5). Median summer Chl was unchanged at Site V (~30 µg L<sup>-1</sup>; Fig.5) and showed non-significant declines of 14% at Site IV (52.2 µg L<sup>-1</sup> vs. 44.9 µg L<sup>-1</sup>) and 6% at Site VII (7.2 µg L<sup>-1</sup> vs. 6.8 µg L<sup>-1</sup>). A 17% decline in summer Chl at Site VI was significant (median values of 16.8 µg L<sup>-1</sup> vs. 14.0 µg L<sup>-1</sup>, p =0.054), as was the decline of 58% at Site VII

Fig. 5. Changes in the (a) phosphorus-chlorophyll and (b) chlorophyll-Secchi relations for the four lake sites. Open symbols represent individual values from 18 Table Rock Lake sites while solid symbols represent median values from pre- and post-treatment plant upgrade for the four sites in the James River Arm. Arrows indicate the change in the relations after upgrade.

during mixis (median values of 9.0  $\mu$ g L<sup>-1</sup> vs. 3.8  $\mu$ g L<sup>-1</sup>, p = < 0.01; Fig. 5).

Modest post-upgrade increases in median summertime Secchi transparency at Sites IV (0.13 m) and VI (0.38 m) were significant, as was an increase of 1.46 m at Site VII during mixis (Fig. 5). Non-significant increases in median Secchi transparency of 0.13 m and 0.53 m were measured at Sites V and VII (stratified), respectively (Fig. 5). Increases in Secchi transparency are consistent with reductions in Chl within the James River Arm and follow the general hyperbolic pattern between Chl and Secchi depth in lakes (Fig. 5).

#### Discussion

Prior to this upgrade, the STP accounted for an estimated 64% of the P-load to the upper James River and 27% of the P-load to Table Rock Lake (Missouri Department of Natural Resources 2001). The 89% decrease in monthly P load from the STP matches the level of P reduction that has underpinned highly successful nutrient management practices in lakes worldwide (Edmondson 1972, SAS 1989). Reductions of this magnitude are often immediately measurable in receiving waters, as was demonstrated in the James River Arm (Fig. 3). Phosphorus reductions were sufficient at Sites IV and VI to shift trophic state classifications from hyper- to eutrophic and eu- to mesotrophic, respectively. The strong longitudinal gradient in TP concentrations along the James River Arm is characteristic of many large reservoirs in Missouri (JONES & NOVAK 1981, KNOWLTON & JONES 1989, KNOWLTON & JONES 1995).

The large response to P-load reduction at Site VII during mixis relative to the stratified summer period (Table 1) likely reflects the role of inflow patterns on in-lake P. During stratification, inflows often plunge below the epilimnion, thereby abridging their direct influence on surface waters while directly influencing the TP content of the lower, stratified water column (COOKE et al. 1993, KNOWLTON & JONES 1995). Following the upgrade, plunging inflows would have delivered less TP to the hypolimnion during summer, thereby returning less TP to surface waters with destratification. During mixis, riverine inflows are typically dispersed throughout the water column and move downlake with hydrologic flow, directly influencing surface water chemistry. These processes likely account for the greater post-upgrade reduction in TP during mixis relative to the stratified period at Site VII.

The lack of a strong statistical response in Chl levels at most lake sites may partly be attributed to the variability of Chl:TP ratios in Missouri reservoirs (JONES et al. 1998) and most temperate lakes. Also, Chl:TP generally increases directly with TN:TP ratios (SMITH 1982). Reduction in TP in the James River Arm increased both ratios and helps explain the weak Chl response.

Increases in Secchi in the James River Arm match the scale of change expected based on predictions from a Chl-Secchi model for temperate lakes (JONES & BACHMANN 1978). The Chl-Secchi relation has long been recognized as hyperbolic (EDMONDSON 1972, COOKE et al. 1993; Fig. 5), such that increases in water claritv are accelerated where Chl is  $< 10 \ \mu g \ L^{-1}$ (Site VII mixis: Fig. 5) relative to sites with Chl > 10  $\mu$ g L<sup>-1</sup>, where reductions in Chl have minor influence on transparency (Site IV; Fig. 5). As such, based on water clarity, the benefits of the STP upgrade will be more obvious in the lower reaches of the James River Arm than at up-lake sites, even though changes in TP content are much smaller (Table 1).

Overall, this study supports the theory and practice of large-scale reductions in P-load to reverse eutrophication in temperate lakes. Additional data will be collected to further document in-lake benefits of this management application, both during summer and mixis. As we continue to monitor post-upgrade conditions we will improve the power of our data set for statistical comparisons. Future assessments will also evaluate the benefit of this upgrade on the lake below the James River Arm (Fig. 1).

#### Acknowledgments

Funding was provided by the Missouri Department of Natural Resources and the University of Missouri. We thank BRUCE PERKINS and many others for field collections and laboratory work. We also appreciate comments about the text by MATTHEW KNOWLTON, EUGENE WELCH and PATRICIA CHAMBERS.

#### References

- COOKE, G.D., WELCH, E.G., PETERSON, S.A. & NEWROTH, P.R., 1993: Restoration and management of lakes and reservoirs. Lewis Publishing. Boca Raton, 548 pp.
- EDMONDSON, W.T., 1972: Nutrients and phytoplankton in Lake Washington. – In: LIKENS, G.E. (Ed.): Symposium on nutrients and eutrophication, the limiting nutrient controversy: 172–188. American Soc. of Limnol. and Oceanogr.. Spec. Symp. No. 1. Allen Press, Lawrence, Kansas.
- JONES, J.R. & BACHMANN, R.W., 1978: Trophic status of Iòwa lakes in relation to origin and glacial geology. – Hydrobiologia **57**: 267–273.
- JONES, J.R. & NOVAK, J.T., 1981: Limnological characteristics of Lake of the Ozarks, Missouri. – Verh. Internat. Verein. Limnol. 21: 919–925.

- JONES, J.R. & KNOWLTON, M.F., 1993: Limnology of Missouri reservoirs: An analysis of regional patterns. – Lake Reserv. Managem. 8: 17–30.
- JONES, J.R., KNOWLTON, M.F. & KAISER, M.S., 1998: Effects of aggregation on chlorophyll-phosphorus relations in Missouri reservoirs. Lake Reserv. Managem. 14: 1–9.
- JONES, J.R., KNOWLTON, M.F., OBRECHT, D.V. & COOK, E.A., 2004: Importance of landscape variables and morphology on nutrients in Missouri reservoirs. – Can. J. Fish. Aquat. Sci. 61: 1503–1512.
- KNOWLTON, M.F. & JONES, J.R., 1989: Summer distribution of nutrients, phytoplankton and dissolved oxygen in relation to hydrology in Table Rock Lake, a large midwestern reservoir. – Archiv. Hydrobiol. Suppl. 83: 197–225.
- KNOWLTON, M.F. & JONES, J.R., 1990: Occurrence and prediction of algal blooms in Lake Taneycomo. Lake Reserv. Manage. 6: 143–152.
- KNOWLTON, M.F. & JONES, J.R., 1995: Temporal and spatial dynamics of suspended sediment, nutrients and algal biomass in Mark Twain Lake, Missouri. – Archiv. Hydrobiol. 135: 145–178.

- Missouri Department of Natural Resources, 2001: Total maximum daily load (TMDL) for James River, Webster, Greene, Christian and Stone Counties, Missouri, Jefferson City, Missouri, 31 pp.
- OBRECHT, D.V., MILANICK, M., PERKINS, B.D., READY, D. & JONES, J.R., 1998: Evaluation of data generated from lake samples collected by volunteers. – Lake Reserv. Managem. 14: 21–27.
- SAS, H., 1989: Lake restoration by reduction of nutrient loading: expectations, experiences, extrapolations. – Academia Verlag., Richarz 497 pp.
- SMITH, V., 1982: The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. – Limnol. Oceanogr. 27: 1101–1112.

Authors' address:

DANIEL OBRECHT, ANTHONY P. THORPE, and JOHN R. JONES, Department of Fisheries and Wildlife Sciences, 302 ABNR Building, University of Missouri, Columbia, Missouri 65211, USA. E-mail: jonesj@missouri.edu

## Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management

#### John R. Jones, Matthew F. Knowlton and Daniel V. Obrecht

Department of Fisheries and Wildlife Sciences, University of Missouri, 302 Anheuser-Busch Natural Resources Building, Columbia, Missouri 65211-7240, USA

#### Abstract

Jones, J.R., M.F. Knowlton and D.V. Obrecht. 2008. Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management. Lake Reserv. Manage. 24:1–9.

Effects of nutrient input, hydraulic flushing rate and depth on reservoir nutrients were examined in the mid-continent landscape of the Ozark Highlands and Plains in Missouri and Plains of southern Iowa. Regionally the clear south-to-north increase in reservoir nutrients, amounting to a 4-fold increase in median total phosphorus (TP) and 3-fold increase in median total nitrogen (TN), showed a strong cross-system pattern with cropland cover (a surrogate for nonpoint-source nutrient loss from agricultural watersheds) but not with an index of hydraulic flushing rate. Cropland accounted for variation in TP in the Ozarks (51%) and TN in all 3 regions (Ozarks 58%, Plains 41%, Iowa 27%). Flushing accounted for variation in TP in the Missouri Plains (49%) and Iowa (29%). Our models suggest large-scale nutrient reduction will require massive changes in land cover to reduce nutrient input. In the Missouri Plains, for example, reducing cropland from 60% to 30% reduces TP and TN by only about 20% when other factors are held constant. Hydrology places added limits on reducing reservoir nutrients; consistent with theory, TP values in Missouri Plains reservoirs effectively double between flushing rates of 0.25 and 2 at any given cropland value. Dramatic nutrient reduction in these reservoirs is unlikely, and the influential role of hydraulic flushing adds additional management challenges for compliance with regional nutrient criteria. The analyses suggest hydrology must be considered when setting nutrient criteria, and it would be unreasonable to establish criteria based on water bodies with long retention time and apply them to rapidly flushed lakes.

Key words: flushing rate, hydrology, nutrient criteria, reservoirs, watersheds

A central concept of applied limnology is that lake phosphorus concentrations (TP) increase as a direct function of nutrient loading which, in turn, is modified by hydraulic retention time and sedimentation (Edmondson 1961, Vollenweider 1975, Welch and Jacoby 2004). Across the continuum of possible combinations of these deterministic variables, TP will be greatest among lakes with high inflow concentrations, low sedimentation, and rapid hydraulic flushing (Welch and Jacoby 2004). To reverse water quality problems associated with eutrophication, lake management typically focuses on reducing external nutrient loading (Sas 1989, Welch and Jacoby 2004, Cooke *et al.* 2005) because little can be done to manipulate natural sedimentation rates, and altering the hydrology of individual water bodies may be impractical or impossible.

Empirical evidence suggests phosphorus sedimentation and hydraulic flushing rates are positively correlated (Larsen and Mercer 1976, Vollenweider 1976, Canfield and Bachmann

1981). In steady-state formulations hydraulic flushing has greater influence on in-lake TP than sedimentation (Welch and Jacoby 2004), thereby allowing for TP predictions based on inflow concentration and flushing. At a given inflow concentration, in-lake TP values increase by 3-fold when flushing rate increases from 0.1 to 10 per year (Fig. 1). This second-order effect of retention time on in-lake TP is asymptotic (Fig. 1). In the overall pattern, values increase sharply among lakes with modest flushing rates such that, with constant inflow concentration, in-lake TP effectively doubles when flushing rate increases from 0.1 to 1 per year (Fig. 1). Beyond this point the rate of increase declines with ever-increasing flushing rate, becoming modest at rates >2–4-times per year. This cross-system pattern suggests that, at a given input concentration, in-lake TP values are always higher in rapidly flushed lakes than those with long retention time. Depth has long been recognized as a correlate of lake fertility (Rawson 1955, Duarte and Kalff 1989) and has also contributed to the explanatory power of empirical models.

In this paper we examine how the main effects of the deterministic variables-nutrient input, hydraulic flushing rate and depth-influence reservoir nutrients across the continuum of conditions occurring in the mid-continent landscape of Missouri and southern Iowa (Fig. 2). Impetus for this analysis is the development of regional nutrient criteria for lakes and reservoirs (Gibson et al. 2000) and subsequent management efforts that will be applied to bring lakes into compliance. The nutrient criteria process centers on a procedural protocol that identifies regionally-unique conditions in baseline reference lakes so criteria can be established that will maintain existing water quality and protect designated uses such as water supply, recreation and fisheries. Consistent with the central premise of lake management, lakes not matching regional nutrient criteria will likely undergo load reductions to reverse eutrophication and associated impairments to aquatic life. The nutrient criteria document (Gibson et al. 2000) highlights that reservoirs have a broad range of hydraulic retention times and, consistent with earlier findings (Canfield and Bachmann 1981), acknowledges this unique characteristic merits consideration. Reservoirs are built for widely differing purposes, but most have large drainage areas and short detention times relative to natural lakes (Cooke et al. 2005). Given the effect of retention time on in-lake TP it is appropriate to evaluate the potential response of mid-continent reservoirs to nutrient load reductions and address how regional conditions might shape nutrient criteria and reservoir management in this agricultural region.

Previous limnological studies have shown the character of mid-continent reservoirs differ regionally (Jones and Bachmann 1978, Jones and Knowlton 1993, Hatch 2003). As a group, Plains reservoirs in north and western Missouri are more eutrophic than southern reservoirs in the Ozark Highlands; this gradient is tied to soil fertility, which directly influences land cover (Jones and Knowlton 1993, Jones *et al.* 2004). Forests dominate many watersheds in the Ozark Highlands whereas cropland agriculture is a major feature of the Plains. Reservoirs in Southern Iowa, located on the Central Irregular Plains ecoregion are typically more fertile than Plains reservoirs in Missouri.

### **Methods**

Missouri limnology data come from a summer inventory of Missouri reservoirs (Fig. 2, n = 126) dating from 1978, characterized in Jones *et al.* (2004). For this analysis the data set was updated to include results from reservoir collections in 2003, 2004 and 2005; most reservoirs are represented by data from  $\geq$  10 summer seasons (range 4–23). Mean values of TP and total nitrogen (TN, rounded to the nearest 5 µg/L value) were calculated as nested averages over the period of record for each reservoir by calculating the geometric mean (ln-transformed) for each summer (results of 3 or 4 sampling dates) and then calculating the geometric mean



**Figure 1.**-Relation of in-lake TP as a proportion of inflow TP to flushing rate as estimated by the Vollenweider equation (Welch and Jacoby 2004).



Figure 2.-Location of Missouri and Iowa reservoirs compared in this study.

	mean	S.D.	min.	median	max.
Ozarks (n=48)					
%Crop	4.9	5.2	0.0	4.6	26.2
Flushing Rate (/y)	7.8	16.5	0.2	1.5	87.1
Mean depth (m)	4.6	3.5	1.1	3.6	19.2
TP ( $\mu$ g/L)	25	17	6	20	67
TN (µg/L)	485	210	200	480	1060
Missouri Plains (n=78)					
%Crop	30.2	17.4	0.5	28.4	73.7
Flushing Rate (/y)	1.3	1.2	0.1	0.8	6.0
Mean depth (m)	3.5	1.5	1.5	3.2	9.6
TP ( $\mu$ g/L)	59	37	14	49	200
TN (µg/L)	890	290	410	900	2195
Iowa (n=41)					
%Crop	56.4	21.7	10.2	61.1	81.7
Flushing Rate (/y)	1.4	1.1	0.3	0.9	4.9
Mean depth (m)	3.4	1.0	1.5	3.9	7.2
$TP(\mu g/L)$	119	98	27	90	474
TN (µg/L)	2180	1745	900	1600	9700

Table 1.-Summary of catchment features and mean nutrient concentrations of reservoirs in this study.

across all summers. To investigate inter-annual hydrologic influences, annual April-August total precipitation in the time series was ranked for each lake. Mean nutrient data from the years with the highest and lowest rainfall total were used to characterize conditions in "wet" and "dry" years. Morphology and hydrology data (Table 1) were from the Missouri Department of Natural Resources and are described in Jones et al. (2004), as are Geographic Information Systems and remote-sensing techniques used to characterize cover types within the watersheds, expressed as a proportion of watershed area. In this analysis we estimated mean depth at one-forth of dam height (Jones et al. 2004). Hydrologic flushing rate (FR/year) was estimated for each reservoir using regional runoff coefficients (MDNR 1986), watershed area, and reservoir volume. According to limnological theory, flushing rate exerts an asymptotic influence on lake nutrient concentrations, so we evaluated effects using a Flushing Index (FI) based on a version of the Vollenweider equation (Welch and Jacoby 2004):

Flushing Index =  $(1 + FR^{-0.5})^{-1}$ 

Based on results presented in Jones *et al.* (2004), reservoirs with >50% urban cover are unique within the data set and were excluded from this analysis. We separate Missouri reservoirs regionally into the Ozark Highlands, part of the Eastern Broadleaf Forest Ecological Province (Nigh and Schroeder 2002), and the Plains, part of the Temperate Prairie Parkland Ecological Province (Table 1; Fig. 1). This approach simplifies an earlier categorization (Jones and Knowlton 1993) that divided the Plains into glaciated and unglaciated and included an ecotonal region (Ozark Border) between the Plains and Ozarks (Thom and Wilson 1980). Iowa nutrient data were taken from Hatch (2003) and include measurements from only one summer per reservoir (1990 or 1992). Iowa morphology, hydrology and land cover data were from Bachmann *et al.* (1980). In this exploratory analysis, data were limited to reservoirs located in southern Iowa (below 42° N latitude) to most closely match the lake type, landscape and land cover immediately to the north of Missouri. We excluded data from reservoirs with large upstream impoundments (n = 9) and those with inputs dominated by groundwater (n = 2).

Relations between landscape variables and nutrients were examined by least-squares methods of single and stepwise multiple regression and analysis of covariance with p < 0.05, unless otherwise stated. Data were transformed using natural logs (ln) or logit (adding 0.003 to cover types to avoid zero values) where appropriate. All analyses were performed with SPSS for Windows (version 13) or SAS (version 9.1).

## **Results**

## Regional reservoir and watershed characteristics

Regionally, nutrient levels were least among reservoirs in the Ozark Highlands (n = 48) where median TP was 20  $\mu$ g/L and ranged from 6 to 67  $\mu$ g/L; median TN was 480  $\mu$ g/L and ranged from 200 to 1060  $\mu$ g/L (Table 1). Most watersheds in the Ozarks have <5% cropland, and only one supports

**Table 2.-**Simple and multiple regressions for cross-regional data (n = 167 reservoirs) and region-specific multiple regressions of effects on TP and TN (In-transformed) of % cropland (logit-transformed), mean depth (Z - In-transformed) and Flushing Index (FI – In-transformed. Non-significant coefficients (p > 0.05) are not shown. For TP, ANCOVA showed coefficients for depth (Z) and flushing index (FI) did not significantly differ (p > 0.05) among regions. For TN, inter-regional differences were significant for all three variables.

#	group	Regression Model	r <sup>2</sup>	RMSE
1	all	$TP_{in} = 4.312 + 0.363 \times \% crop$	0.60	0.54
2	all	$TP_{ln} = 5.032 + 0.374 \times \% crop + 1.037 \times FI_{ln}$	0.69	0.48
3	all	$TP_{in} = 5.299 + 0.357 \times \% crop + 0.740 \times FI_{in} - 0.405 \times Z_{in}$	0.73	0.45
4	Ozarks	$TP_{in} = 5.233 + 0.392 \times \% crop + 0.592 \times FI_{in} - 0.400 \times Z_{in}$	0.77	0.34
5	Plains	$TP_{ln} = 5.309 + 0.160 \times \% crop + 1.191 \times FI_{ln} - 0.306 \times Z_{ln}$	0.60	0.38
6	Iowa	$TP_{ln} = 5.927 + 2.017 \times FI_{ln}$	0.29	0.54
7	all	$TN_{ln} = 7.173 + 0.305 \times \% crop$	0.69	0.37
8	all	$TN_{in} = 7.431 + 0.309 \times \% crop + 0.372 \times FI_{in}$	0.71	0.36
9	all	$TN_{in} = 7.549 + 0.301 \times \% crop + 0.241 \times FI_{in} - 0.179 \times Z_{in}$	0.73	0.35
10	Ozarks	$TN_{ln} = 7.414 + 0.264 \times \% crop - 0.297 \times Z_{ln}$	0.74	0.23
11	Plains	$TN_{in} = 7.271 + 0.148 \times \% crop + 0.511 \times FI_{in}$	0.55	0.22
12	Iowa	$TN_{ln} = 8.300 + 0.283 \times \% crop + 1.299 \times FI_{ln}$	0.43	0.43

>17%; Flushing Rate was 0.23-87/year, and mean depth was 1.1-19.2 m (Table 1). Nutrient levels in the 78 Missouri Plains reservoirs are roughly double those measured in the Ozarks (Table 1); among these, median TP was 49 µg/L and ranged from 14 to 200 µg/L, while median TN was 900 µg/L and ranged from 410 to 2195 µg/L (Table 1). Cropland was <1-74% of total watershed area (median = 30%crop); Flushing Rate was 0.1-6/year, and mean depth was 1.5-9.6 m (Table 1). Reservoirs in southern Iowa have about twice the nutrient levels of the Missouri Plains (n = 41, median TP = 90 µg/L and median TN = 1600 µg/L). Over half the Iowa watersheds had >50% crop cover, and only 5 of 41 catchments had <30% cropland. Flushing Rate in Iowa was 0.3-4.9/year (Table 1).

#### **Cross-regional patterns**

Among these 3 regions there was a strong cross-system relation ( $r^2 \ge 0.6$ , n = 167) between reservoir nutrients and the proportions of cropland cover (%crop) in their respective catchments (Fig. 3; Table 2). A cropland-nutrient relation was detailed by Jones *et al.* (2004) for Missouri reservoirs, but the Iowa data fit nicely within the pattern and double the original range of the nutrient continuum. This cross-regional pattern shows a general increase in reservoir nutrient concentration from south-to-north such that latitude is a strong correlate of both TP<sub>in</sub> (r = 0.60) and TN<sub>in</sub> (r = 0.73), reflecting the measurable increase in cropland agriculture along this geographic axis (r = 0.72 between cropland and latitude).

In contrast, there was no strong cross-regional pattern between nutrients and Flushing Index (Fig. 4;  $r^2 \le 0.03$ , n = 167). Within regions, however, the relation between nutrients and Flushing Index was strong within the Missouri Plains (r



Figure 3.-Relation of TP and TN (In-transformed) to %crop (logit-transformed).



Figure 4.-Relation of TP and TN to Flushing Index (Intransformed).

= 0.57 for TP and 0.70 for TN, n =78), weaker among Iowa reservoirs (r = 0.54 for TP and 0.45 for TN, n = 41), and among Ozark reservoirs the correlation was nonsignificant for TN and weak for TP (r = 0.33, p = 0.02). Omitting the most rapidly flushed Ozark reservoirs (>5/year) did not change this result. For both nutrients, multiple regressions showed Flushing Index accounts for residual variation not attributable to cropland (Table 2). Within the cross-regional data set, nutrients were also negatively related to depth (r = -0.405 for TP and -0.27 for TN, n = 167) which accounts for additional residual variation in TP and TN in multiple regression models (Table 2).

#### **Region-specific patterns**

Deterministic variables accounting for cross-system patterns in reservoir nutrients differed in importance among regions. Based on partial r<sup>2</sup> values from multiple regressions (Table 2), %crop accounted for the greatest amount of explained variation for TP in the Ozarks (51%), and TN in all three regions (Ozarks 58%, Plains 41%, Iowa 27%). For TP in the Missouri Plains and Iowa, however, Flushing Index was the dominant variable accounting for 49% and 29%, respectively, of TP variability. Across the range of TP values in Iowa reservoirs, effects of %crop and mean depth were not statistically significant. In the Missouri Plains, %crop and mean depth accounted for only 7% and 4%, respectively, of TP variability. These interregional differences in explanatory power of the deterministic variables occurred even though regression slopes for Flushing Index and mean depth were not statistically different among regions for TP (ANCOVA, p > 0.05). For TN, slopes for all 3 variables differed among regions. Mean depth accounted for 16% of TN variability among Ozark reservoirs but was not significant elsewhere. Flushing Index was not significant in the Ozarks, but accounted for 13% of TN variation in the Missouri Plains and 15% in Iowa.

Differences in nutrient-%crop relations in our data suggest interregional differences in soil fertility, climate and agronomic practices influence the overall pattern. Based on the analyses, TP and TN concentrations are more sensitive to changes in %crop in the Ozarks than the Missouri Plains. Reasons for these differences are beyond the scope of this assessment but likely reflect effects of thin, permeable Ozark soils and associated higher runoff. Soils have less capacity to retain nutrients when contact with infiltrating precipitation is brief. Iowa data suggest %crop has less influence on TP and a greater influence on TN than in Missouri. In fact, due to inter-lake variation, the effect of %crop on TP in Iowa is not significant (Table 2) unless Iowa and Missouri data are combined to show the overall continuum (Fig. 3). For TN, concentrations in Iowa were much greater than Missouri reservoirs with similar catchments and morphology (Fig. 3), a difference reflected in the large intercept in the Iowa TN model (Table 2). An Iowa reservoir with median %crop and Flushing Index for this data set (25% crop, FI = 0.52) is predicted to have 1255 µg/L TN compared to 874 µg/L in the Missouri Plains. These differences likely reflect regional agronomic practices, other deterministic factors not considered, and inherent variability in limnological data. Reservoir nutrients vary widely from year to year (Knowlton et al. 1984, Knowlton and Jones 2006a, 2006b), and the Iowa data set is based on a single sampling season compared to an average of 10 years for Missouri. For this reason, we have more confidence that Missouri results reflect genuine inter-regional differences with predictive value. In the following sections quantitative analysis is restricted to Missouri reservoirs. Iowa data, however, clearly demonstrate the overall continuum of land use effects (Fig. 3) and corroborate the strong influence of hydrology among prairie reservoirs.

#### "Wet" versus "dry" years

Regression results (Table 2) indicate hydrology has a greater role in controlling nutrients in the Missouri Plains than among Ozark reservoirs. Inter-annual comparisons between the "wettest" (highest April-August rainfall) and "driest" (lowest rainfall) years in the multi-year Missouri data set (Iowa reservoirs were sampled in only one year and were not included) provide an independent test of this inter-regional difference. The expectation that flushing rates are larger in wet years, resulting in higher nutrients, was partly supported by the analysis. The results demonstrate the role of hydrology is stronger than other factors influencing temporal variation in individual reservoirs and the cross-system pattern. In the Missouri Plains some 91% of reservoirs had higher TP in the wet year, averaging 43% larger than the dry year. In the Ozarks, only 60% of reservoirs had higher TP in the wet year, with a mean difference of 16%. This result is consistent with differences in the slopes and partial r<sup>2</sup> for Flushing Index between the Plains and Ozarks (Table 2). For TN, however, wet versus dry year comparisons showed an average difference of only 5% in the Missouri Plains compared to 21% in the Ozarks. This result contrasts with the stronger effects of Flushing Index in the Plains regression analysis.

## *Implications for nutrient reduction, nutrient criteria and management*

Our predictive equations (Table 2) indicate large-scale nutrient reduction in Midwestern reservoirs will require massive changes in land use to reduce inflow concentrations. In the Missouri Plains, for example, reducing %crop from 60% to 30% (from near maximum to near median; Table 1) – reduces TP and TN by only about 20% when other factors are held constant. For Ozark reservoirs decreasing %crop from 10 to 5% (from near maximum to near median; Table 1) reduces TP by 25% and TN by 17%. These results suggest that lake trophic state improvements in the form of cropland conversions to grass or forest, or implementation of nonpoint management programs, will not be proportional to efforts required to obtain them.

Another consideration is that the models show hydrology places added limits on reducing reservoir nutrients. Consistent with theory (Fig. 1), at any given %crop level (inflow concentration), regression models for Missouri Plains reservoirs predict TP values effectively double between Flushing Index values of 0.25 and 2 (Fig. 5). Values are even broader across the full range of flushing rates within the region (Table 1). By comparison, at a fixed flushing rate value, TP would effectively double in a Plains reservoir if %crop increased from ~5% to ~70%, which is effectively the entire range of cropland within the Missouri Plains data set.

Given the influential role of Flushing Index, reservoirs with sharply differing land cover can support identical TP values. For example, the median TP value of 49  $\mu$ g/L in the Missouri Plains (Table 1) would be expected in reservoirs with 50%crop and FR = 0.5, 30%crop and FR = 0.75, 20%crop and FR = 1, or 6%crop and FR = 2. Values of TP > 49  $\mu$ g/L



**Figure 5.**-Response of TP and TN to variation in %crop and flushing rate (FR) for reservoirs in the Missouri Plains as predicted by equations 5 and 11 in Table 2.

are the general rule among reservoirs with >20% crop and FR > 1, but even Plains reservoirs with modest cropland would support such TP levels if hydraulic residence time is short (Fig. 5). These illustrations show Plains reservoirs with large catchments and minimal cropland have TP values that match levels found in impoundments located in agricultural watersheds with modest flushing rates. This comparison highlights the importance of impoundment location within the landscape as a factor determining reservoir trophic state.

The nutrient content of reservoirs of similar size and depth constructed in catchments with identical land cover will be determined by hydraulic residence time (Fig. 5). All other factors equal, the reservoir with the larger watershed will have larger flushing rate and higher nutrients than reservoirs positioned in small catchments. Consequently, rapidly flushed reservoirs are unlikely to be brought into compliance with the same nutrient standards as slowly flushed reservoirs without far greater management intervention.

The nutrient criteria effort centers on conditions in regional baseline reference lakes to establish criteria to maintain existing water quality and protect designated uses (Gibson et al. 2000). Data ranking can also be used to identify criteria and the trisection method (median of the lowest third of the ranked long-term reservoir mean values in our data set) resulted in mock reference values for the Missouri Plains reservoirs of 27 µg/L TP and 620 µg/L TN. Plains reservoirs with long-term means that fit within these mock reference criteria, however, averaged less than half the %crop, double the %forest, and hydraulic residence times 3 times longer than noncompliant reservoirs. Based on the regional regression equation (equation 5, Table 2), a Missouri Plains reservoir with median crop cover (28%), depth (3.2 m) and FR (0.8/year; Table 1) would support 61 µg/L TP, or about twice the mock target value, and would match the TP criterion only if %crop were limited to <1% of its catchment (Fig 5a). Even located in a watershed without cropland, the shallowest and most rapidly flushed Plains reservoirs support ~40 µg/L TP. Thus, for some reservoirs, complete elimination of cropland would be insufficient to meet criteria based on water bodies with lower input concentrations and longer hydraulic residence time. For reservoirs with median flushing rate, % crop would need to be <5% to meet the TN criterion (Fig 5b).

## Discussion

This analysis shows distinguishable regional differences in the relative importance of the 3 key explanatory variables—nonpoint source nutrient loading (cropland), hydrology and morphology—on reservoir nutrients (Table 2). All 3 variables are known to directly influence in-lake nutrient concentrations and have been the central metrics of empirical, cross-system lake models for several decades (Edmondson 1961, Vollenweider 1975, Cooke *et al.* 2005). The relations are based on the widespread understanding that nutrient loading and residence time largely determine lake and reservoir water quality (Jones and Bachmann 1976, Jørgensen 2003, Windolf *et al.* 1996). Most analyses have concentrated on phosphorus, but these same explanatory variables have been shown to determine in-lake nitrogen levels (Bachmann 1980, Bachmann 1984, Jensen *et al.* 1990, Windolf *et al.* 1996).

Among these Midwestern reservoirs the strong south-tonorth increase in nutrient levels (Table 1, Fig. 3), which amount to a nearly 4-fold increase in median TP and 3-fold increase in median TN, is opposite the latitude-dependent global pattern (Kalff 1991). The reservoir nutrient pattern directly parallels the increase in ambient soil fertility along this axis and the general northward increase in the intensity of agricultural practices such as crop production and associated nutrient application (Jones and Knowlton 1993, Arbuckle and Downing 2001). Strong correlations between stream nutrient concentrations in Missouri and the proportion of cropland in their catchments (Perkins et al. 1998) provide the basis for using % crop as a surrogate for inflow nutrient concentrations (Jones et al. 2004). Additional support for this approach comes from the sharp increase in stream nitrate levels in Iowa with cropland (Schilling and Libra 2000). The regional stream nutrient-cropland relation (Perkins et al. 1998) suggests baseflow concentrations of phosphorus and nitrogen double or triple across the range of crop cover found within the Missouri Plains and Iowa (Table 1). Nutrient concentration of inflowing water is considered the best single indicator of in-lake concentrations (Ahlgren et al. 1988), and high external loading from agricultural landscapes largely accounts for the mostly eutrophic and hypereutrophic condition of these Midwest reservoirs (Table 1; Fig. 3). Few reservoirs in the region have point source inputs.

These results are preliminary, and better estimates of reservoir nutrient loading and flushing rate would aid in modeling reservoir response to management and account for inconsistencies in the analysis. For example, given the stronger effect of flushing on TP and TN in the Missouri Plains than the Ozarks, we expected Plains reservoirs to show larger differences between "wet" and "dry" years. This was the case for TP but not for TN. Also our quantification of the effect of hydrology on reservoir nutrients is imprecise given that region-specific slopes for the effect of Flushing Index on TP (Table 2) range from 0.59 (Ozarks) to 2.02 (Iowa) but are not statistically significant. Regardless, these findings represent the best estimate of these deterministic variables exert controlling influence on reservoir nutrients. The analysis highlights the powerful influence of hydrology in both Missouri and Iowa. Implementation of nutrient criteria without specific consideration of hydrology runs the risk of creating unrealistic and unrealizable standards.

A key aspect of incorporating hydrology into nutrient criteria assessment is to explicitly recognize the additional potential of managing nutrients by manipulating lake morphology and hydrology. Reservoir depth, volume, and watershed size are determined by design specifications and location of the constructions site within the valley. Collectively, these features determine hydraulic retention time. For existing reservoirs, increasing the dam height to increase retention time may be a cost-effective means of improving water quality. In a well-documented example, McDaniel Lake in the Missouri Ozarks exhibited a ~40% decline in summer (July-September) mean TP after the spillway crest was raised 1.2 m (Youngsteadt 2005). During the 20-year study, multiple lake and catchment improvement measures were ongoing, but retrospective analysis ascribed 61% of the TP decline to direct effects of raising the water level and another 10% to cooler hypolimnetic temperatures resulting from the deeper basin. In contrast, substantial efforts to reduce tributary TP accounted for only 20% of the improvement. Reservoirs built with inadequate depth and residence time, or those that have lost volume through in-filling, could benefit substantially from similar modification. Compliance with nutrient criteria should recognize this potential means of water quality control.

This analysis highlights that basin depth, catchment size and resulting hydrologic features of constructed reservoirs are design decisions that have direct effects on water quality. Likewise, most reservoirs were built long after presettlement vegetation was altered for agriculture, so nutrient loads were in place prior to creating artificial lakes on the landscape. These are fundamental differences between natural and artificial lakes and should be considered when setting nutrient criteria.

## Conclusions

The major conclusion of this assessment is that dramatic nutrient reduction in Midwestern reservoirs is unlikely, particularly in the most highly flushed reservoirs. The influential role of hydraulic flushing adds additional management challenges for compliance with regional nutrient criteria. Our illustration using the trisection method to set mock nutrient criteria shows target values can be unattainable in highly flushed reservoirs. Further, the analyses suggest that hydraulic flushing rate must be considered when setting nutrient criteria. It is unreasonable to set nutrient criteria based on water bodies with long hydraulic retention time and apply them to rapidly flushed lakes. This consideration must be a factor when identifying regionally unique conditions in baseline reference lakes or by ranking regional lakes after separating them along a continuum of flushing rate values.

Another important consideration for implementation of nutrient criteria is the quantitative significance of temporal variation in measuring reservoir characteristics. As illustrated by differences between wet and dry years for TP in the Plains and TN in the Ozarks, reservoirs are not constant over time. Variation among individual (unaveraged) TP measurements can exceed 10-fold within a single reservoir (Jones and Knowlton 2005). For the typical Missouri reservoir, 5 summers of averaged data (3-6 samples per summer) are required to estimate mean TP with 95% confidence limits spanning less than a factor of 2 (Knowlton and Jones 2006b). Determining the current nutrient status of a reservoir with sufficient precision to evaluate its compliance with a given nutrient standard thus requires relatively long-term monitoring. Also, improvements resulting from efforts to comply with nutrient standards are likely to be obscured by ordinary background variation.

### References

- Ahlgren, I., T. Frisk and L. Kamp-Nielsen. 1988. Empirical and theoretical models of phosphorus loading, retention and concentration vs. lake trophic state. Hydrobiologia 170:285–303.
- Arbuckle, K.E. and J.A. Downing. 2001. The influence of watershed land use on lake N:P in a predominantly agricultural landscape. Limnol. Oceanogr. 46:970–975.
- Bachmann, R.W. 1980. The role of agricultural sediments and chemicals in eutrophication. J. Water. Pollut. Control Fed. 52:2425–2431.
- Bachmann, R.W., M.R. Johnson, M.V. More and T.A. Noonan. 1980. Clean lakes classification study of Iowa's lakes for restoration. Final Report. Iowa Cooperative Fisheries Research Unit and Department of Animal Ecology. Iowa State University. Ames, Ia.
- Bachmann, R.W. 1984. Calculation of phosphorus and nitrogen loadings to natural and artificial lakes. Verh. Internat. Verein. Limnol. 22:239–243.
- Canfield, D.E., Jr. and R.W. Bachmann. 1981. Prediction of total phosphorus concentrations, chlorophyll *a* and Secchi depths in natural and artificial lakes. Can. J. Fish. Aquat. Sci. 38:414–423.
- Cooke, G.D., E.B. Welch, S.A. Peterson and S.A. Nichols. 2005. Restoration and management of lakes and reservoirs. 3rd Ed. Taylor & Francis, Boca Raton.
- Duarte, C.M. and J. Kalff. 1989. The influence of catchment geology and lake depth on phytoplankton biomass. Arch. Hydrobiol. 115:27–40.
- Edmondson, W.T. 1961. Changes in Lake Washington following an increase in the nutrient income. Verh. Internat. Verein. Limnol. 14:167–175.
- Gibson, G., R. Carlson, J. Simpson, E. Smeltzer, J. Gerritson, S. Chapra, S. Heiskary, J. Jones and R. Kennedy. 2000. Nutrient Criteria Technical Guidance Manual Lakes and Reservoirs. U.S. Environmental Protection Agency. EPA-822-B00-001.
- Hatch, L.K. 2003. Factors affecting Iowa lake and reservoir water quality. Lake Reserv. Manage. 19:150–159.
- Jensen, J.P., P. Kristensen and E. Jeppesen. 1990. Relationships between nitrogen loading and in-lake nitrogen concentrations in shallow Danish Lakes. Verh. Internat. Verein. Limnol. 24:201–204.
- Jones, J.R. and R.W. Bachmann. 1976. Prediction of phosphorus and chlorophyll levels in lakes. J. Water Pollut. Control Fed. 48:2176–2182.
- Jones, J.R. and R.W. Bachmann. 1978. Trophic status of Iowa lakes in relation to origin and glacial geology. Hydrobiologia 57:267–273.
- Jones, J.R. and M.F. Knowlton. 1993. Limnology of Missouri reservoirs: an analysis of regional patterns. Lake Reserv. Manage. 8:17–30.
- Jones, J.R., M.F. Knowlton, D.V. Obrecht and E.A. Cook. 2004. Importance of landscape variables and morphology on nutrients in Missouri reservoirs. Can. J. Fish. Aquat. Sci. 61:1503–1512.
- Jones, J.R. and M.F. Knowlton. 2005. Chlorophyll response to nutrients and non-algal seston in Missouri reservoirs and oxbow lakes. Lake Reserv. Manage. 21:361–370.
- Jørgensen, S.E. 2003. The application of models to find the relevance of residence time in lake and reservoir management. J. Limnol. 62(Suppl. 1):16–20.
- Kalff, J. 1991. The utility of latitude and other environmental factors as predictors of nutrients, biomass and production in lakes worldwide: Problems and alternatives. Verh. Internat. Verein. Limnol. 24:1235–1239.
- Knowlton, M.F., M.V. Hoyer and J.R. Jones. 1984. Sources of variability in phosphorus and chlorophyll and their effects on use of lake survey data. Water Res. Bull. 20:397–407.
- Knowlton, M.F. and J.R. Jones. 2006a. Natural variability in lakes and reservoirs should be recognized in setting nutrient criteria. Lake Reserv. Manage. 22:161–166.
- Knowlton, M.F. and J.R. Jones. 2006b. Temporal variation and assessment of trophic state indicators in Missouri reservoirs: implication for lake monitoring and management. Lake Reserv. Manage. 22:261–271.
- Larsen, D.P. and H.T. Mercier. 1976. Phosphorus retention capacity of lakes. J. Fish. Res. Board Can. 33:1742–1750.
- [MDNR] Missouri Department of Natural Resources. 1986. Missouri Water Atlas. Divsion of Geology and Land Survey. Jefferson City.
- Nigh, T.A. and W.A. Schroeder. 2002. Atlas of Missouri Ecoregions. Missouri Department of Conservation, Jefferson City.
- Perkins, B.D., K. Lohman, E.E. Van Nieuwenhuyse and J.R. Jones. 1998. An examination of land cover and stream water quality among physiographic provinces of Missouri, U.S.A. Verh. Internat. Verein. Limnol. 26:940–947.

- Rawson, D.S. 1955. Morphometry as a dominant factor in the productivity of large lakes. Verh. Internat. Verein. Limnol. 12:164–175.
- Sas, H. 1989. Lake restoration by reduction of nutrient loading: Expectations, experiences, extrapolations. Academia Verlag Richarz, Sankt Augustin.
- Schilling, K.E. and R.D. Libra. 2000. The relationship of nitrate concentrations in streams to row crop land use in Iowa. J. Environ. Qual. 29:1846–1851.
- Thom, R.H. and J.F. Wilson. 1980. The natural divisions of Missouri. Trans. Mo. Acad. Sci. 14:9–23.
- Vollenweider, R.A. 1975. Input-output models: with special reference to the phosphorus loading concept in limnology. Schweiz. Z. Hydrol. 37:53–84.
- Vollenweider, R.A. 1976. Advances in defining critical loading levels for phosphorus in lake management. Mem. Ist. Ital. Idrobiol. 33:53–83.
- Welch, E.B. and J.M. Jacoby. 2004. Pollutant effects in freshwater: applied limnology. Spon Press, London.
- Windolf, J., E. Jeppesen, J.P. Jensen and P. Kristensen. 1996. Modelling of seasonal variation in nitrogen retention and in-lake concentration: A four-year mass balance study in 16 shallow Danish lakes. Biogeochemistry 33:25–44.
- Youngsteadt, N. 2005. Factors that influence phosphorus, filamentous cyanobacteria and odor in McDaniel Lake, a southwest Missouri water supply reservoir, 1983-2002. Lake Reserv. Manage. 21:453–464.

#### Role of contemporary and historic vegetation on nutrients in Missouri reservoirs: implications for developing nutrient criteria

#### John R. Jones<sup>1,\*</sup>, Matthew F. Knowlton<sup>1</sup>, Daniel V. Obrecht<sup>1</sup>, Anthony P. Thorpe<sup>1</sup> and James D. Harlan<sup>2</sup>

<sup>1</sup>Department of Fisheries and Wildlife Sciences, University of Missouri, 302 Anheuser-Busch Natural Resources Building, Columbia, MO 65211 <sup>2</sup>Department of Geography, University of Missouri, Stewart Hall, Columbia, MO 65211

#### Abstract

Jones, J.R., M.F. Knowlton, D.V. Obrecht, A.P. Thorpe and J.D. Harlan. 2009. Role of contemporary and historic vegetation on nutrients in Missouri reservoirs: implications for developing nutrient criteria. Lake Reserv. Manage. 25:111–118.

Using vegetative survey records from the time of Euro-American settlement (circa 1815–1850) we found the proportion of historic prairie accounted for 42% of cross-system variation in total phosphorus (TP) and 48% of total nitrogen in 156 Missouri reservoirs. When combined with dam height (surrogate for lake morphometry) and hydraulic flushing rate (TP only), 56% of variation in nutrients was explained. Consistent with previous analyses, some two-thirds of variation in nutrients was accounted for by contemporary cropland, morphometry, and hydrology (TP only). Adding prairie or historic forest cover to models based on current cropland did little to increase explained variation. The relationship between reservoir nutrients and land cover is partly an artifact of past land conversion; most arable soils with inherent fertility sufficient to generate economically viable produce and suitable topography were former prairies. The cross-system analysis of Missouri reservoirs showed that nutrients in these anthropogenic ecosystems are largely determined by nonpoint input from current land use as modified by morphology and hydrology. Historic vegetation cover, however, was our best measure of baseline conditions in the reservoir catchments and contributes to the framework for developing nutrient criteria for these artificial lakes. No natural reference conditions exist for Missouri reservoirs, and we recommend setting site-specific nutrient criteria for these constructed systems.

Key words: historic land cover, Missouri reservoirs, nutrient criteria, prairies, vegetation

Reservoirs are created in valleys with suitable hydrology and morphology for a variety of beneficial uses that range from hydroelectric power and water supply to recreation. Most impoundments in the U.S. mid-continent have been constructed in the past 60 years, so they are relatively new landscape features. With Euro-American settlement during the previous century came rapid, region-wide conversion of prairies and forests to cropland and pastures that eventually would become catchments for future impoundments. Paleoreconstruction data from natural lakes in the agricultural Midwest (e.g., Stoermer *et al.* 1993) and sediment cores from the Mississippi Delta (Turner and Rabalais 1994), the terminus of drainage from this region, show increased nutrient loading from land use changes during that time.

Reservoirs were constructed long after historic vegetation was altered and have received nutrient input from intensified agricultural practices from the time of dam closure. Studies of Midwest reservoirs show nutrient concentrations are directly correlated with contemporaneous land cover, exhibiting a positive relationship with cropland (a surrogate for nonpoint-source nutrient loss from agriculture) and a negative relationship with forest (Knoll et al. 2003, Jones et al. 2004). Including physical metrics representing morphology and hydraulic flushing rates into cross-system models accounts for additional variance in the reservoir nutrient data (Jones et al. 2004, Jones et al. 2008a). This outcome is consistent with the understanding that nutrient loading, depth and hydraulic residence time determine lake and reservoir nutrient levels (Welch and Jacoby 2004).

<sup>\*</sup>Corresponding author: jonesj@missouri.edu

Our research objective was to determine if any characteristic of historic vegetative cover from survey records at the time of Euro-American settlement (circa 1815–1850) explains variation in the nutrient levels of present day Missouri impoundments or accounts for residual variation in our contemporaneous land cover-nutrient models (Jones *et al.* 2004, Jones *et al.* 2008a). The analysis was based on an expanded data set (n = 156, Jones *et al.* 2008b). Historic vegetation cover summarized from early survey records provided our best metric of baseline conditions in the catchments of these artificial lakes and contributes to a larger framework for developing nutrient criteria in Missouri reservoirs. We outline how landscape data might be used for this purpose and recommend setting site-specific nutrient criteria for these constructed systems.

#### Methods

Summer monitoring data (1978-2007) were used to calculate the mean concentration of total phosphorus (TP) and total nitrogen (TN) in the 156 reservoirs included in this analysis (30 reservoirs have been added to the data base used in Jones et al. 2008a). Individual reservoirs are represented in the data set by collections from 4 to 27 summer seasons (described in Jones et al. 2008b). The median age of these reservoirs is 45 years (range 13-97 yr). Reservoir catchments were spatially determined in ArcInfo GIS based on 1-m resolution aerial photography and 10-m resolution digital elevation data. Dam structures were located and digitized into hydrologic cross-sections that were used to capture and delineate the areas of hydrologic flow into the dam locations; basin slope was estimated from this information. Current land use data for reservoir catchments were based on 30-m imagery from the LANDSAT thematic mapper developed by the Missouri Resource Assessment Partnership. Pre-settlement land cover was derived from original US Government Land Office survey notes and other historic sources in the Missouri Historic Landscape Project (James D. Harlan, Geographic Resources Center, University of Missouri). Current and historic cover summary statistics were calculated for each catchment along with statistics describing changes in land cover during the  $\sim$ 150-yr time period. The few largest reservoir catchments that extend beyond Missouri (i.e., Truman, Table Rock) were clipped at the current state boundary for lack of comparable historic land cover data in adjoining states. Prior to statistical analysis, land cover percentages were logit-transformed. To accommodate values of 0 and 1, 0.003 was added to values <0.5 and subtracted from values >0.5 before transformation. Flushing rate was transformed using a version of the Vollenweider equation (Jones et al. 2008a) to reflect the expected curvilinear response of nutrients to hydrology. Other variables were transformed to natural logs before analysis. Data were analyzed by simple and multiple regression. The

regional limnology of Missouri reservoirs has been recently described using recognized ecological sections (Jones *et al.* 2008b). In this analysis we grouped reservoirs into the Plains (Osage and Glacial Plains) and the Ozarks (Ozark Highlands and Ozark Border) to simplify the presentation.

#### Results

#### Land cover – historic and contemporary

Within reservoir catchments, historic prairie, forest, and scrub cover each ranged from 0 to 100%. The median historic condition was 42% prairie, 28% forest, and 8% scrub (Table 1), with prairie negatively correlated with both forest and scrub (logit transformed, n = 156, r = -0.83 and -0.26, respectively). Vegetation cover showed a strong regional pattern, with prairie dominant in the rolling topography of the Plains and forests in the more rugged Ozarks (Table 1). Basin slope was positively correlated with historic prairie (r = -0.63) and negatively correlated with historic prairie (r = -0.64).

Contemporary cropland cover in reservoir catchments ranged from 0 to 74% while forest cover ranged from 2 to 97% (Table 1). Across reservoir catchments cropland was strongly correlated with historic prairie (Fig. 1a; n = 156, r = 0.80, logit transformation). On average, 76% of current cropland in the Plains was historically prairie and 15% was forest. In the Ozarks the pattern is reversed; 72% of cropland was historically forest and 11% was prairie. Contemporary cropland was less extensive than historic prairie in most Plains catchments (89 of 100), but in most Ozarks catchments, cropland is larger than historic prairie cover (35 of 56). In reservoir catchments located statewide, cropland currently occupies about one-third of original prairie cover (limited to catchments with  $\geq 1\%$  historic prairie, median value 36%, n = 108).

Historic and current forest cover were also closely correlated in reservoir catchments (Fig. 1b; r = 0.73, logit transformation). Forest cover has changed over time, with some historically treeless catchments currently showing >50% coverage, as well as the opposite pattern. Among catchments with  $\geq 1\%$  historic forest (n = 115) the median present-day forest cover is 80% of its survey value. In the Plains, a median of 57% of current forest in the reservoir catchments is on former prairies. In the Ozarks, a median of only 9% of current forest is derived from other historic cover-types.

# Cross-system patterns of reservoir nutrients with land cover, morphology, and hydrology

Reservoir nutrients in Missouri reservoirs span a range of >30-fold for TP (6–189  $\mu$ g/L; Table 1) and >10-fold for TN

		$\begin{array}{l} \text{Statewide} \\ \text{(n}=\text{156)} \end{array}$	Plains (n = 100)	Ozarks (n = 56)
Historic Land Cov	er			
% prairie	mean	43.8	65.7	4.8
	median	41.9	73.5	0
	range	0-100	0-100	0-74.9
% forest	mean	40.9	21.5	75.5
	median	28.0	6.6	90.2
	range	0-100	0-100	0-100
% scrub	mean	15.3	12.8	19.7
	median	7.7	7.9	7.7
	range	0-100	0-92.6	0-100
Contemporary Lar	nd Cover			
% crop	mean	17.5	25.3	3.5
	median	13.3	25.4	1.0
	range	0-74.0	0.4–74.0	0-40.9
% grass	mean	34.4	38.2	27.7
	median	33.7	36.0	25.7
	range	0.6-76.7	5.3-76.7	0.6-57.8
% forest	mean	32.7	19.7	57.2
	median	23.1	15.1	55.0
	range	1.7–97.4	1.7-84.2	12.9–97.4
% urban	mean	7.3	8.5	5.2
	median	3.3	3.4	2.8
	range	0-70.5	0.3-70.5	0-34.0
Nutrients				
TP ( $\mu$ g/L)	mean	46.5	57.8	26.2
	median	38.2	47.9	20.9
	range	6.0–188.9	13.8-188.9	6.0-90.4
TN ( $\mu$ g/L)	mean	750	880	510
	median	740	860	490
	range	200-2200	380-2200	200-1060
Physical Features	C C			
flushing rate	mean	4.3	1.4	9.5
(1/year)	median	1.1	0.9	1.5
	range	0.1-142.2	0.1-6.0	0.2-142.2
dam height (m)	mean	15.5	14.0	18.2
2 /	median	13.4	12.7	14.3
	range	4.6–76.8	6.1–38.4	4.6–76.8

Table 1.-Summary of land cover and limnology data sets.

(200–2200  $\mu$ g/L, Table 1). In regression analysis the proportion of historic prairie accounted for 42% of cross-system variation in reservoir mean TP and 48% of TN variation (Table 2; Fig. 2). When combined with dam height (surrogate for lake morphometry) and hydraulic flushing rate (TP only), some 56% of the variance in nutrients was explained (Table 2). No other category of historic vegetation explained more than 2% of residual variation unless prairie was excluded. Without prairie, historic woody cover explained 31% of TP variation (partial r<sup>2</sup>, model not shown) and historic scrub an additional 5% (negative coefficients for both) in a model that included dam height (11%) and flushing rate (2%). For TN,



**Figure 1.**-Relations of current and historic cover types in catchments of 156 Missouri reservoirs. (a). Contemporary cropland versus historic prairie. (b). Contemporary forest versus historic forest. Reference lines show 1:1 ratios.

forest cover explained 39% and scrub 4% (both negative coefficients) of variation in a model including dam height (7%).

Consistent with previous analyses (Jones *et al.* 2004, Jones *et al.* 2008a), some two-thirds of cross-system variation in reservoir nutrients was accounted for by contemporary cropland in the catchments, dam height, and hydraulic flushing rate (TP only; Table 2). Adding reservoir age to the models did not significantly increase explained variation, which suggests construction date does not appreciably influence the cross-system pattern. Given the strong correlation between historic prairie and cropland (Fig. 1), predictions of TP and TN based on the two cover types were strongly correlated (Fig. 3; r > 0.85), but current cropland was the stronger predictive variable. Adding historic prairie to the cropland regressions increased explained variation by only 1% for TP and none for TN. Including historic forest cover increased

**Table 2.-**Simple and multiple regressions for TP and TN using current and historic cover metrics (n = 156 reservoirs) where % crop is percentage of current crop land (logit-transformed), % prairie is percentage of historic prairie (logit-transformed), Z<sub>In</sub> the natural log of dam height (m) and FI<sub>In</sub> is the flushing index (Jones *et al.* 2008a).

		r <sup>2</sup>	RMSE
1	$TP_{ln} = 4.224 + 0.276 \times \% crop$	0.46	0.545
2	$TP_{ln} = 5.654 + 0.254 \times \% crop - 0.569 \times Z_{ln}$	0.60	0.469
3	$TP_{ln} = 5.801 + 0.270 \times \% crop - 0.447 \times Z_{ln} + 0.633 \times Fl_{ln}$	0.65	0.444
4	$TP_{ln} = 3.692 + 0.124$ × %prairie	0.42	0.566
5	$TP_{ln} = 5.078 + 0.111$ × %prairie - 0.537 × Z <sub>ln</sub>	0.54	0.503
6	$TP_{ln} = 5.172 + 0.115$ × %prairie - 0.440 × Z_{ln} + 0.503 × FL	0.57	0.489
7	$TN_{ln} = 6.964 + 0.194 \times \% crop$	0.57	0.307
8	$TN_{ln} = 7.698 + 0.183 \times \% crop - 0.292 \times Z_{ln}$	0.67	0.272
9	$TN_{ln} = 6.588 + 0.084$ × %prairie	0.48	0.337
10	$TN_{ln} = 7.292 + 0.078$ × %prairie - 0.272 × Z <sub>ln</sub>	0.56	0.311



the  $r^2$  for the TN model by 2%. None of the other historic land cover variables added significantly to the models, and residual analysis showed no obvious regional differences in the influence of historic cover type.

These results indicate reservoirs with watersheds previously dominated by prairie had no tendency toward higher or lower nutrients relative to current cropland than those previously in forest, and vice versa. Residuals from the nutrient regressions indicated that the proportions of cropland created by plowing prairies versus that created by clearing forest or scrub did not influence reservoir TP. Among Plains reservoirs, residuals from the TN–cropland–dam height regression (equation 8, Table 2) showed a weak negative correlation (r = -0.23, p = 0.023) with the proportion of cropland derived from former forests. This trend was not evident among Ozark reservoirs. Overall, these results imply current land use is much more important than historic cover in determining reservoir nutrients and that any current influence of historic conditions is subtle.

# Characteristics of low phosphorus Missouri reservoirs

As expected from cross-system regression models (Table 2), Plains reservoirs with the lowest TP (median =  $16 \mu g/L$  for

Figure 2.-Relations of reservoir mean TP (a) and mean TN (b) to historic prairie cover.

the bottom fifth percentile, n = 5 of 101; Table 3) are deep water bodies with low flushing rates, located in catchments with less than a quarter of the median cropland found in the region (6% vs. 25%). Not surprisingly, historical survey data show the catchments of reservoirs in this group had modest prairie cover (with one exception) and an order of magnitude more forest than most Plains catchments. These reservoirs supported about one-third the TP found in the median Plains reservoir and about one-tenth of the value of shallow, nutrient-rich reservoirs situated on historic prairies (Table 3). These five reservoirs with low TP values are similar to impoundments in the Ozarks (Jones *et al.* 2008b).

The same cross-system pattern held among Ozark reservoirs; TP increased with cropland and flushing rate and was inversely tied to historic forest cover and depth. Low TP reservoirs were deep with low flushing and located in wooded valleys. Ozark reservoirs with high TP were mostly rapidly flushed, riverine impoundments or had more cropland than typical for the region.



**Figure 3.**-Comparison of predictions of TP and TN by regression models based on current cropland and historic prairie cover. Values in (a) are from equations 3 and 6 in Table 2; values in (b) are from equations 8 and 10.

#### Discussion

# Reservoir nutrients and human influences, past and present

The Missouri landscape has a long history of anthropogenic influence, with evidence of human populations for many thousands of years (O'Brien and Wood 1998), and by the early 16th century, large populations in the region modified landscapes with roads, fields, and settlements (Denevan 1992). Prairie vegetation in the Plains was likely maintained by fire at an interval of about 5–15 years (Schroeder 1982), and anthropogenic fire constituted the major influence on Ozark vegetation (Guyette *et al.* 2002).

By any measure, alteration of the Missouri landscape resulting from Euro-American settlement was drastic compared to modifications by indigenous humans. Available evidence suggests broad-scale plowing of native prairies and clearing of forests for high-intensity agriculture greatly increased nutrient loss from watersheds (Smith et al. 2003, Turner and Rabalais 2003). Consequently, the streams impounded by Missouri reservoirs currently export far greater loads of nitrogen and phosphorus than previously. Conversion of prairie to cropland in the Plains probably resulted in a several-fold increase in nutrient export. An early experimental plot study in Missouri showed conversion of prairie vegetation to continuous wheat increased N and P loss about 50-fold and continuous corn increased values about 100-fold (Miller and Krusekopf 1932). Runoff information from agricultural watersheds in the Midwest suggests soluble P loss from corn is about 10-times that from prairie (Miller and Daniel 1981). Stream nutrient data from a prairie reference site in the Kansas Flint Hills conformity showed averages of about 7  $\mu$ g/L TP and 223  $\mu$ g/L TN (Dodds and Oakes 2004); these values are considerably lower than currently measured in Missouri's agricultural streams (Perkins et al. 1998). The

**Table 3.**-Features of the five least enriched and most enriched reservoirs in the Plains region and regional medians. Units follow Table 1.

	ТР	Dam Height	Flushing Rate	% Historic Prairie	% Historic Forest	% Crop Land
Nehai Tonkeia	14	19.8	0.11	24.3	39.5	1.2
Marie	14	15.2	0.18	0.0	87.9	6.4
Lincoln	16	21.0	0.46	0.0	99.7	2.1
Weatherby	16	25.9	0.14	0.9	95.8	13.6
Fox Valley	18	15.9	0.33	0.0	84.9	12.0
Regional median	49	12.8	1.0	74.8	6.9	25.5
Ray County	152	6.1	2.1	100	0	39.6
Montrose	152	10.1	6.0	84	8.6	30.7
Maysville	162	6.1	4.2	99	0	9.4
Cameron #1	178	10.1	3.6	94	0.6	39.2
King	189	12.1	4.1	91	1.1	28.4

difference between current and pre-settlement nutrient export is probably less for the Ozarks than in the Plains. While only fragments of unbroken prairie remain in the Plains and do not constitute an entire reservoir basin, altered forests still cover much of the Ozarks (Table 1). Agricultural grasslands are a major secondary cover type in the Ozarks (Table 1) and are associated with nonpoint nutrient loss (Smart *et al.* 1985). Export coefficients consistently show plant nutrient loss from cropland is many times that from either forests or pasture (Reckhow *et al.* 1980, Alexander *et al.* 2004).

Artificial lakes in Missouri were constructed in physically favorable locations more than a century after vegetation was removed for agricultural production. Our cross-system analysis suggests the nutrient status of these mostly eutrophic (60%; Jones et al. 2008b) and mesotrophic (20%) impoundments is determined mainly by human influences. Cropland serves as a metric of nonpoint source nutrient loading from human-altered landscapes and stands out as the foremost explanatory factor in our reservoir models (Table 2; Jones et al. 2004, Jones et al. 2008a). Reservoir depth and flushing rate are a function of design and site location in the catchment and strongly influence nutrients (Jones et al. 2008a). Note that the amount of variation explained and the model parameters for this expanded data set (24% larger) are quite similar to previous analyses (Jones et al. 2004, Jones et al. 2008a), suggesting the analysis is a robust generalization of nutrient patterns in Missouri reservoirs. Nutrient levels in some reservoirs presumably have varied over time in response to changes in land use (Jones et al. 2004) and with the intensity of farming practices. Even so, impoundments in predominantly agricultural catchments have likely been fertile from the time of dam-closure. Reservoir age was not a factor in the cross-system analysis; recently constructed reservoirs fit the statewide nutrient pattern (Table 2) equally as well as those decades older. The trophic status of an individual Missouri reservoir is, in effect, determined by the decisions of choosing a location within a valley catchment and designing a dam.

Historic vegetation explained little residual variation in the nutrient–cropland regressions, suggesting that contemporary land use is the primary determinant of nutrient loss from these watersheds. Past land use can influence nutrient saturation and current loss from landscapes (Aber *et al.* 1998) and may account for the weak signal in our data, suggesting that historic forests in the Plains yield less nitrogen when converted to cropland than other cover types. But at the resolution of our analysis, historic conditions did not broadly account for variation in reservoir nutrients. This result was not surprising given that ~150 years have passed since plowing of prairies and forest clearing remade these watersheds.

Nutrients in Missouri reservoirs were related to historic prairie cover in the same general pattern, though somewhat less strongly, as they were to present-day cropland (Table 2; Fig. 3). A likely explanation is that lands most suitable for cultivation, with favorable basin slope and arable soils having inherent fertility to generate economically viable produce, were largely former prairies (Fig. 1). Within the catchments of our study reservoirs, some 76% of cropland was prairie at the time of Euro-American settlement. The relationship between reservoir nutrients and historic prairie is partly an artifact of past land conversion. Soil quality and basin topography are integral features that initially influenced cropland conversion; more recently, these same characteristics influenced which lands remained in cultivation or were converted to grasslands (including conservation reserves) and forests.

Natural reference conditions, as described by Gibson et al. (2000) for natural lakes, represent the least impacted conditions and typify ambient background or baseline nutrients. Analogous reference conditions do not exist for Missouri reservoirs. Reservoirs were built long after vegetation was altered for agriculture so that nutrient loads were in place prior to creating these artificial lakes. Human design and intentional positioning of impoundments in valleys with established land cover suggests site-specific nutrient criteria are appropriate. Site-specific assessment avoids making untenable comparisons between impoundments with different hydro- and morphology features. With other factors held equal, deep impoundments with long hydraulic retention will consistently have lower nutrients than shallow, rapidly flushed water bodies (Welch and Jacoby 2004, Jones et al. 2008a). Data from Plains reservoirs (Table 3) illustrate this fact; reservoirs with the lowest TP have physical features atypical of the region and are not examples of the nutrient condition readily achievable in most impoundments.

In estimating site-specific reservoir nutrient levels, the crosssystem, nutrient-cropland regression (Table 2) provided a quantitative framework within the context of the statewide continuum (Jones et al. 2008b) and the broader regional context (Jones et al. 2008a). The proportion of historic prairie cover could be used as a surrogate term for nutrient loading, with about the same outcome (Table 2). Prairie cover closely matched the intent of establishing a baseline conditions by representing indigenous vegetation in reservoir catchments (Gibson et al. 2000); it also provided a quantitative basis for estimating nutrient loss from the landscape at the time of Euro-American settlement. Regardless, it would be straightforward to compare nutrient levels in a given reservoir by predicting expected values based on unique design specifications (depth and volume) and edaphic features (land cover and watershed size; Table 2). Impoundments with low long-term nutrient levels or levels below the cross-system pattern might be identified for protection, consistent with the EPA antidegredation policy (Gibson *et al.* 2000). Reservoirs with nutrients in excess of the regional expectation, where nutrient-related water quality problems clearly impair designated use, might be candidates for nutrient reduction.

Several approaches have been taken to establish nutrient criteria to protect designated uses for lake water, such as water supply, recreation and aquatic life (Reckhow *et al.* 2005; Dodds *et al.* 2006; Soranno *et al.* 2008). Ideally, nutrient criteria should be tied to designated-use statements for specific impoundments. An early example was the work of Dillon and Rigler (1975) linking nutrients in boreal lakes to algal chlorophyll and recreation potential (swimming, fishing, and aesthetics). An extensive analysis of Minnesota lakes has resulted in threshold values of phosphorus, and the response variables chlorophyll and transparency, to protect use classes in the diverse ecoregions of that state (Heiskary and Wilson 2005). A similar analysis of nutrient-caused impairment is not available for Missouri reservoirs.

Designated use should reflect societal values; implementation of criteria should be technically attainable and provide a favorable ratio of water quality benefit to cost. Major nutrient reduction would not necessarily benefit reservoirs designated for warmwater recreational fisheries where production and harvest are closely tied to nutrients (Yurk and Ney 1989). About one-fourth of the impoundments in our data set were built with conservation funds and are managed with stocking and harvest regulation for recreational fishing (recreational swimming is not permitted in these impoundments). In contrast, groundwater in some areas of the Plains is naturally saline, and communities rely on surface water supplies. Several water supply reservoirs are located in valleys historically in prairie vegetation (70-100%), and cropland currently dominates their catchments (Knowlton and Jones 2007); they are eutrophic, and some samples have measurable algal toxins (Graham et al. 2004). In these nutrient impaired systems, land retirement from cropland to prairie vegetation or forest may be an appropriate tool for drastically reducing nutrient loads from agricultural catchments (Ribaudo et al. 1994).

Interestingly, implementation of stream criteria can directly improve nutrient related water quality problems in reservoirs (Dodds and Oakes 2004), which are considered major anthropogenic alterations of the landscape (Nilsson *et al.* 2005). Nonpoint nutrient loads from agriculture can be reduced using best management practices, which broadly include fertilizer, manure, and tillage management along with vegetating riparian zones and critical source areas (Pionke *et al.* 2000, Sharpley *et al.* 2001, Vidon and Smith 2007). Nutrient reductions can protect designated use in streams and, by extension, reduce eutrophication in reservoirs; therefore, a broad-scale nutrient reduction effort aimed at streams would likely reduce the slope and/or intercept of the empirical fit between reservoir nutrients and cropland (Table 2). Broad implementation of best management may represent the most readily attainable conditions in these artificial lakes without major changes in agricultural production.

Our analysis describes why nutrients differ in reservoirs statewide and provides an historical context for the crosssystem pattern. Vegetation structure from the survey at the time Euro-American settlement is our best measure of early landscape characteristics in the region and is a metric of baseline historic conditions in valleys recently impounded for the benefits that reservoirs provide society. Not surprisingly, low-nutrient reservoirs are located in deep valleys that were historically forested and remain so, while high-nutrient reservoirs are in shallow valleys that were once prairie, now converted to cropland. Once adopted, nutrient criteria enforcement will rely on the principles of applied limnology to manage nutrients or, where appropriate, improve water quality (Welch and Jacoby 2004, Cooke et al. 2005). Empirical relationships for these purposes have been developed specifically for Missouri reservoirs, including expectations for bloom frequency and summer maximum algal biomass (Jones et al. 2008b).

#### Acknowledgments

We thank the Missouri Department of Natural Resources and the Missouri Agricultural Experiment Station for support for this work.

#### References

- Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems. BioScience 48:921-934.
- Alexander, R.B., R.A. Smith and G.E. Schwarz. 2004. Estimates of diffuse phosphorus sources in surface waters of the United States using a spatially referenced watershed model. Water Sci. Tech. 49:1-10.
- Cooke, G.D., E.B. Welch, S.A. Peterson and S.A. Nichols. 2005. Restoration and management of lakes and reservoirs. 3rd ed. Taylor & Francis, Boca Raton, FL.
- Denevan, W.M. 1992. The pristine myth: the landscape of the American in 1492. Annals Assoc. Amer. Geog. 82:369-385.
- Dillon, P.J. and F.H. Rigler. 1975. A simple method for predicting the capacity of a lake for development based upon lake trophic status. J. Fish. Res. Bd. Can. 32:1519-1531.
- Dodds, W.K. and R.M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnol. Oceanogr. Methods 2:333-341.
- Dodds, W.K., E. Carney and R.T. Angelo. 2006. Determining ecoregional reference conditions for nutrients, Secchi depth

117

and chlorophyll *a* in Kansas lakes and reservoirs. Lake Reserv. Manage. 22:151-159.

- Gibson, G., R. Carlson, J. Simpson, E. Smeltzer, J. Gerritson, S. Chapra, S. Heiskary, J. Jones and R. Kennedy. 2000. Nutrient Criteria Technical Guidance Manual Lakes and Reservoirs. U.S. Environmental Protection Agency. EPA-822-B00-001.
- Graham, J.L., J.R. Jones, S.B. Jones, J.A. Downing and T.E. Clevenger. 2004. Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Res. 38:4395-4404.
- Guyette, R.P., R.M. Muzika and D.C. Dey. 2002. Dynamics of an anthropogenic fire regime. Ecosystems 5:472-486.
- Heiskary, S.A. and C.B. Wilson. 2005. Minnesota lake water quality assessment report: developing nutrient criteria. Minnesota Pollution Control Agency, St. Paul, MN. Accessed 15 Sep 2008. http://www.pca.state.mn.us/water/ lakequality.html#reports
- Jones, J.R., M.F. Knowlton, D.V. Obrecht and E.A. Cook. 2004. Importance of landscape variables and morphology on nutrients in Missouri reservoirs. Can. J. Fish. Aquat. Sci. 61:1503-1512.
- Jones, J.R., M.F. Knowlton and D.V. Obrecht. 2008a. Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management. Lake Reserv. Manage. 24:1-9.
- Jones, J.R., D.V. Obrecht, B.D. Perkins, M.F. Knowlton, A.P. Thorpe, S. Watanabe and R.R. Bacon. 2008b. Nutrients, seston, and transparency of Missouri reservoirs and oxbow lakes: An analysis of regional limnology. Lake Reserv. Manage. 24:155-180.
- Knowlton, M.F. and J.R. Jones. 2007. Temporal coherence of water quality variables in a suite of Missouri reservoirs. Lake Reserv. Manage. 23:49-58.
- Knoll, L.B., M.J. Vanni and W.H. Renwick. 2003. Phytoplankton primary production and photosynthetic parameters in reservoirs along a gradient of watershed land use. Limnol. Oceanogr. 48:608-617.
- Miller, B.A. and T.C. Daniel. 1981. An empirical equation for predicting soluble phosphorus loads from agricultural watersheds. Trans. ASAE. 375-381.
- Miller, M.F. and H.H. Krusekopf. 1932. The influence of systems of cropping and methods of culture on surface runoff and soil erosion. Columbia (MO): Missouri Agricultural Experiment Station. Res. Bull. 177.
- Nilsson, C., C.A. Reidy, M. Dynesius and C. Revenga. 2005. Fragmentation and flow regulation of the world's large river systems. Science 308:405-408.
- O'Brien, M.J. and W.R. Wood. 1998. The prehistory of Missouri. University of Missouri Press. Columbia, MO.
- Perkins, B.D., K. Lohman, E. Van Nieuwenhuyse and J.R. Jones. 1998. An examination of land cover and stream water quality

among physiographic provinces of Missouri, U.S.A. Verh. Internat. Verein. Limnol. 26:940-947.

- Pionke, H.B., W. J. Gburek and A.N. Sharpley. 2000. Critical source area controls on water quality in an agricultural watershed located in the Chesapeake Basin. Ecol. Eng. 14:325-335.
- Reckhow, K.H., M.N. Beaulac and J.T. Simpson. 1980. Modeling phosphorus loading and lake response under uncertainty: a manual and compilation of export coefficients. USEPA. 440/5-80-011. Washington, DC.
- Reckhow, K.H., G.B. Arhonditsis, M.A. Kenney. L. Hauser, J. Tribo, C. Wu, K.J. Elcock, L.J. Steinberg, C.A. Stow and S. J. McBride. 2005. A predictive approach to nutrient criteria. Environ. Sci. Tech. 39:2913-2919.
- Ribaudo, M.O., C.T. Osborn and K. Konyar. 1994. Land retirement as a tool for reducing agricultural nonpoint source pollution. Land Economics 70:77-87.
- Schroeder W.A. 1982. Presettlement Prairie of Missouri. Natural History Series, No. 2, Missouri Department of Conservation, Jefferson City, MO.
- Sharpley, A.N., R.W. McDowell and P.J.A. Kleinman. 2001. Phosphorus loss from land to water: integrating agricultural and environmental management. Plant Soil 237:287-307.
- Smart, M.M., J.R. Jones and J.L. Sebaugh. 1985. Stream-watershed relations in the Missouri Ozark Plateau Province. J. Environ. Qual. 14:77-82.
- Smith, R.A., R.B. Alexander and G.E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environ. Sci. Tech. 37:3039-3047.
- Soranno, P.A., K.S. Cheruvelil, R.J. Stevenson, S.L. Rollins, S.W. Holden, S. Heaton and E. Torng. 2008. A framework for developing ecosystem-specific nutrient criteria: Integrating biological thresholds with predictive modeling. Limnol. Oceanogr. 53:773-787.
- Stoermer, E.F., J.A. Wolin and C.L. Schelske. 1993. Paleolimnoligical comparison of the Laurentian Great Lakes based on diatoms. Limnol. Oceanogr. 38:1311-1316.
- Turner, R.E. and N.N. Rabalais. 1994. Suspended sediment, C, N, P, and Si yields from the Mississippi River basin. Hydrobiologia 511:79-89.
- Turner, R.E. and N.N. Rabalais. 2003. Linking landscape and water quality in the Mississippi River basin for 200 years. Bio-Science 53:563-572.
- Vidon, P. and A.P. Smith. 2007. Upland controls on the hydrological functioning of riparian zones in glacial till valleys of the Midwest. J. Amer. Water Resour. Assoc. 43:1524-1539.
- Welch, E.B. and J.M. Jacoby. 2004. Pollutant effects in freshwater: applied limnology. Spon Press, London.
- Yurk, J.J. and J.J. Ney. 1989. Phosphorus-fish community biomass relationships in southern Appalachian reservoirs: Can lakes be too clean for fish? Lake Reserv. Manage. 5:83-90.

#### NOTE

#### Chlorophyll maxima and chlorophyll: Total phosphorus ratios in Missouri reservoirs

#### Abstract

Reducing the magnitude and frequency of peak algal biomass is a common goal of lake management. To better quantify such conditions in Missouri reservoirs, an upper boundary delineating maximum algal chlorophyll (Chlmax) across the range of total phosphorus (TP) was developed using summer monitoring data (n = (n = 8188) and compared with 2 other Missouri datasets (n = 8188) and 5151). Typically, other factors constrain Chl below the maximum, and most samples contained a fraction of Chlmax. Near maximum conditions (Chlnm) were provisionally defined as 70% of Chlmax; individual reservoirs differ in their history of supporting Chlnm measurements (from 0 to 43% of samples) irrespective of nutrient status or the duration of summer monitoring. There was a rapid increase in the yield of Chlmax per unit TP across the oligo-mesotrophic range, while within the eutrophic range Chlmax varied with changes in TP in a near-unity response. This general pattern was similar for Chl<sub>nm</sub> and provides a basis for predicting how high Chl levels would change with nutrient management. Values of Chlmax in Missouri reservoirs are lower than lakes in Florida and larger than values in an international dataset, but the rate of change in Chl across the TP range is quite similar among these datasets, suggesting this pattern applies to different lake types.

Key words: algal biomass, chlorophyll, maximum chlorophyll, Missouri reservoirs

Lake management efforts often focus on reducing the magnitude and frequency of peak algal biomass to prevent extreme conditions considered most objectionable (Walker 1985, Bachmann et al. 2003). In this analysis we determined both maximum chlorophyll (Chlmax) values and Chl to total phosphorus ratios (Chl:TP) in association with the upper boundary of the Chl-TP distribution in large datasets from Missouri reservoirs and compared our findings with Florida lakes (Brown et al. 2000) and an international data set (Pridmore and McBride 1984). This approach treats the cross-system pattern as the potential Chl maximum at a given TP value rather than the standard approach of accounting for variation around the center of the response, as described by best-fit regression (Jones and Knowlton 2005, Jones et al. 2008). Others have considered the Chl-TP relation from the viewpoint of the upper boundary, and our analysis contributes to this line of inquiry (Hosper 1980, Smith and Shapiro 1981, Pridmore and McBride 1984, White 1989, Kaiser et al. 1994, Brown et al. 2000, Lewis 2011).

Thomson et al. (1996) promoted estimating the upper edge of data distributions where a variable, such as TP, acts as a limiting factor for a response variable, such as Chl, to better understand and quantify spatial structure in crosssystem comparisons in ecology. Evaluating response variables relative to a potential maximum is consistent with the ecological concept of limiting factors described by the phosphorus limitation paradigm and implicit in the Chl-TP relationship (Kaiser et al. 1994, Smith 2003, Sterner 2008). Variation in Chl-TP is attributed to the bioavailability of nutrient pools, nitrogen supplies relative to TP, composition of the phytoplankton community, climate, hydrology, stratification patterns, grazing pressure, and light availability, as determined by color and/or mineral particulates. Regardless of other influences, most variation in Chl is related to TP in lakes.

Maximum expression of algal biomass has been addressed based on Chl–TP ratios by White (1989) who considers potential phytoplankton biomass relative to the nutrient content of the sample. This approach differs from viewing algal blooms as a response to nutrient pulses from internal or external sources and does not imply that Chl<sub>max</sub> is necessarily associated with harmful or nuisance conditions (Smayda 1997, Carstensen et al. 2007). Alternatively, high Chl events have been characterized by quantifying the frequency that observed Chl exceeds specific threshold values in individual lakes (Walmsley 1984, Walker 1985, Walker and Havens 1995, Bachmann et al. 2003).

Using individual Chl–TP pairs from Missouri reservoirs during summer, we fitted a reference line to delineate the upper boundary of Chl (uncorrected for pheophytin, n = 8839, 0.2–447 µg/L, median 13.5 µg/L) across the range of TP (2–831 µg/L, median 36 µg/L; Fig 1a). The data were binned based on the distribution of log<sub>10</sub>TP values (n = 38 bins, each with <6% of the total observations). Within each bin, Chl–TP pairs were ranked to identify Chl<sub>max</sub> within the given nutrient range; obvious outliers were excluded. A line was fitted to the Chl<sub>max</sub> values using stepwise regression with log<sub>10</sub>TP and log<sub>10</sub>TP<sup>3</sup> (R<sup>2</sup> = 0.98, p < 0.01) to describe the upper edge of the distribution of the data; log<sub>10</sub>TP<sup>2</sup> and higher-order terms for log<sub>10</sub>TP were not significant:

$$log_{10} Chl_{max} = -0.61 + 1.62(log_{10} TP) -0.059(log_{10} TP^{3}).$$
(1)

The boundary is not distinct (Fig. 1a), and variation around the upper edge is inherent in distributions of this type (Kaiser et al. 1994). For this dataset, 1.4% (n = 129) of observed chlorophyll values (Chl<sub>obs</sub>) were greater than Chl<sub>max</sub> (Chl<sub>obs</sub>:Chl<sub>max</sub> >1); in more than half of this group Chl<sub>obs</sub> was larger than Chl<sub>max</sub> by <20%. In 8 samples Chl<sub>obs</sub> was more than double Chl<sub>max</sub>, and one was more than 4 times the



empirical limit. Review of the analytical records provided no basis to remove these samples; they likely represent rare, extreme conditions in the monitoring record. A similar presentation by Brown et al. (2000) shows a small number of observations quite distant from the edge of the data envelope, and our largest values fit within their distribution.

Most samples contained a fraction of  $\text{Chl}_{max}$ ; the median ratio of  $\text{Chl}_{obs}$ : $\text{Chl}_{max}$  was 0.31 (Fig. 1a, interquartile range 0.22–0.44, mean 0.35). This ratio was >0.8–1 in only 2.4% of the observations, and an additional 2.7% of the values had ratios >0.7<0.8. This distribution suggests values near  $\text{Chl}_{max}$  are infrequent in routine summer monitoring data. Noteworthy,  $\text{Chl}_{max}$  values and  $\text{Chl}_{max}$ :TP ratios are at least 3 times larger than the conventional limits used to categorize reservoir trophic state in Missouri reservoirs (Jones et al. 2008; Table 1). This comparison further illustrates that  $\text{Chl}_{max}$  values represent extreme conditions associated with a given nutrient value.

The empirical Chl<sub>max</sub> described by equation 1 also applies to other datasets from Missouri reservoirs. It envelops the upper boundary of data collected by citizen volunteers (n =8188, TP 3–539  $\mu$ g/L, median 31  $\mu$ g/L; Fig 1b); some 1.6% of Chlobs were larger than Chlmax and most come from Table Rock Lake, an impoundment with low mineral turbidity with large Chl:TP ratios (Thorpe and Obrecht 2008). The Chlmax boundary (equation 1) also envelops an aggregated dataset that includes daily collections from several reservoirs during summer and numerous nonsummer samples (n = 5151, TP 2–543  $\mu$ g/L, median 25  $\mu$ g/L; Fig. 1c). Some 2.6% of Chlobs was larger than Chlmax; most were from Table Rock Lake or collected during fall destratification, a period of high Chl:TP ratios (Jones and Knowlton 2005). The median ratio Chlobs:Chlmax of 0.27 was, however, somewhat lower than the other datasets (Fig. 1), in part because this dataset includes midwinter collections when low Chl:TP ratios are common (Jones and Knowlton 2005). Together, these comparisons suggest the equation for Chl<sub>max</sub> broadly applies to Missouri reservoirs.

The  $Chl_{max}$  response for Florida lakes by Brown et al. (2000) is about twice the value for the Missouri Chl–TP pattern

<sup>←</sup> **Figure 1.**-Chlorophyll (Chl) and total phosphorus (TP) from Missouri reservoirs and oxbow lakes during summer (panel a, n = 8839; Jones et al. 2008). The upper boundary on Chl in all 3 panels was described by equation 1 from the text:  $log_{10}Chl_{max} =$  $-0.61 + 1.62(log_{10}TP) - 0.059(log_{10}TP^3)$ . This upper boundary was also plotted with data from Missouri reservoirs collected by citizen volunteers (panel b, n = 8188) after TP data were increased by 2 µg/L to account for loss during storage (Obrecht et al. 1998) and data from Missouri reservoirs collected from reservoirs sampled daily during summer and seasons other than summer (panel c, n = 5151).

**Table 1.-**Trophic state criteria for Missouri reservoirs (Jones et al. 2008) with the corresponding ChI:TP ratios for conditions at the upper boundary. Maximum and near-maximum chlorophyll (see text) and corresponding ChI:TP ratios are shown for the upper TP value in each trophic state category.

	Upper Limit of Trophic State			CHL (µg/L) at		CHL:TP ratio at	
Trophic State	TP (μg/L)	CHL (µg/L)	CHL:TP	Max	Near Max	Мах	Near Max
Oligotrophic	10	3	0.30	9	6	0.90	0.62
Mesotrophic	25	9	0.36	31	22	1.25	0.86
Eutrophic	100	40	0.40	145	100	1.45	1.00

(Fig. 2a). This discrepancy likely reflects differences in climate and lake type between the 2 regions; Brown et al. (2000) previously concluded some Florida lakes have larger Chl:TP ratios than northern lakes. Suppression of Chl yields



**Figure 2.**-Plot of maximum chlorophyll (Chl<sub>max</sub>) against total phosphorus (TP) calculated using a nonlinear (upper line, panel a) equation for Florida lakes by Brown et al. (2000) and equation 1 from the text for Missouri reservoirs and for international lakes (Pridmore and McBride 1984). Data from panel a were replotted in panel b to show the rate of change in Chl<sub>max</sub> [ = (Chl<sub>max</sub>)<sub>TP</sub> - (Chl<sub>max</sub>)<sub>TP-1</sub>] / (Chl<sub>max</sub>)<sub>TP</sub>] across much of the observed TP range in the dataset.

by mineral turbidity could also reduce  $Chl_{max}$  in some Missouri reservoirs (Jones and Knowlton 2005). In contrast, values of  $Chl_{max}$  estimated for an international suite of lakes (Pridmore and McBride 1984) averaged about 60% of  $Chl_{max}$  in Missouri (Fig. 2a). Longer collection records for the Florida and Missouri datasets would increase the likelihood of sampling high Chl events, thereby contributing to larger  $Chl_{max}$  values (Brown et al. 2000, Jones et al. 2008). Regardless, these comparisons suggest regional differences in  $Chl_{max}$ .

As a preliminary approach to identify near-maximum algal biomass in Missouri reservoirs and to broaden the scope of the comparative analysis beyond  $Chl_{max}$ , we calculated the upper 95% confidence limit on mean Chl within each of the  $log_{10}TP$  bins used to generate equation 1 (mean + 1.64\*Standard Deviation). The cross-system pattern matched 70% of  $Chl_{max}$  and serves as a provisional limit for identifying near-maximum Chl ( $Chl_{nm}$ ) in these reservoirs. These data include samples within approximately 5% of  $Chl_{max}$  and those located above the upper boundary (Fig. 1a). Values of  $Chl_{nm}$  and  $Chl_{obs}$ :TP ratios are more than double the conventional limits used to categorize reservoir trophic state boundaries (Table 1).

Phytoplankton taxonomic composition varies with lake trophic state, and the cellular Chl content differs within and among species (Watson et al. 1992, 1997); both factors may influence  $Chl_{nm}$  in Missouri reservoirs. Taxonomic data from July 2003 (63 reservoirs; Jones et al. 2008) showed that 6% of the samples exceeded Chl<sub>nm</sub> criteria, as did 1 of 15 reservoirs in August 2004; these samples were from eutrophic reservoirs dominated by either *Anabaena* or *Aphanizomenon* (87–98% of total biovolume). These limited data indicate Chl<sub>nm</sub> can be exceeded when the phytoplankton community is dominated by large cyanobacteria. Additional taxonomic information is needed to determine the algal community dominating other Chl<sub>nm</sub> events, particularly in oligotrophic and mesotrophic reservoirs.

Conditions that favor  $Chl_{nm}$  in individual reservoirs may be short-lived. Daily collections from Little Dixie Lake during summer 2004 (n = 108; Fig. 3) show 9% of samples exceeded the  $Chl_{nm}$  threshold during a single event in late



**Figure 3.**-Ratio of observed chlorphyll to near-maximum chlorophyll (Chl<sub>obs</sub>:Chl<sub>nm</sub>) in daily collections from Little Dixie Lake during summer 2004. Values above the horizontal line indicate samples where Chl<sub>obs</sub> exceeded Chl<sub>nm</sub>.

July. This ephemeral peak was consistent with a bloom event wherein Chl deviates from the normal seasonal cycle for a short period of time (Hutchinson 1967, Carstensen et al. 2007). These events would not always be captured in routine summer monitoring (Knowlton and Jones 2000, Jones et al. 2008) and suggest that Chl<sub>max</sub> and Chl<sub>nm</sub> are best assessed using large datasets.

Among the most intensively sampled reservoirs in our dataset (n = 113, 33–151 summer samples, median 53), two-thirds of samples exceeding Chl<sub>nm</sub> were collected during July and August, consistent with an earlier finding that Chl increases in late summer (Jones and Knowlton 2005). Individual reservoirs in this group differ in their history to support Chl<sub>nm</sub>; 23% never expressed Chl<sub>nm</sub>, 37% supported Chl<sub>nm</sub> in 0.1–5% of samples, and 40% supported Chl<sub>nm</sub> in  $\geq$ 5–43% of samples. Interestingly, neither mean TP (6–180  $\mu$ g/L, median = 39  $\mu$ g/L) nor the number of samples collected from an individual reservoir showed a significant cor-

relation with  $\text{Chl}_{nm}$  (p > 0.05). These outcomes suggest frequency of  $\text{Chl}_{nm}$  is not a simple function of nutrient status or the duration of monitoring as represented in our summer inventory.

Lake managers have addressed undesirable algal abundance as the frequency that  $Chl_{obs}$  exceeds nuisance levels (Walmsley 1984, Walker 1985, Walker and Havens 1995, Bachmann et al. 2003). The frequency of high Chl levels is known to increase with trophic state, with large values common in enriched lakes. We followed this convention and calculated the frequency of Chl values of  $\geq 10$ ,  $\geq 20$ ,  $\geq 30$ ,  $\geq 40$ , and  $\geq 50 \,\mu g/L$  from intensively sampled reservoirs in the dataset (n = 113; Fig. 4) and found similarities with lakes in other regions and previous findings for Missouri reservoirs (Jones et al. 2008). In general, the frequency of Chl  $\geq 10 \,\mu g/L$ increased sharply among reservoirs with mean TP  $\geq 20$  $\mu g/L$  but was uncommon in reservoirs with lower mean TP (Fig. 4).

Another feature of this analysis is that within each trophic state category an increase in Chlobs represents a progressively larger ratio of Chlobs:Chlnm and therefore is less frequently observed in the data distribution (Fig. 4; Table 2). For example, among mesotrophic reservoirs, a Chl value of  $\geq 10 \ \mu g/L$  equates to nearly two-thirds of Chl<sub>nm</sub>, while 40  $\mu$ g/L Chl closely matches Chl<sub>nm</sub>, and 50  $\mu$ g/L Chl exceeds the Chl<sub>nm</sub> criteria (Table 2). This general pattern holds for Chl values across all trophic states (Table 2). Conversely, for any given Chl value, the ratio of Chlobs:Chlnm declines with trophic state (Table 2). To illustrate,  $\geq 10 \,\mu g/L$ Chl closely matches Chlnm in oligotrophic reservoirs, and equates to nearly two-thirds, half, and one-third of Chl<sub>nm</sub> in mesotrophic, eutrophic, and hypereutrophic systems, respectively (Table 2). The magnitude of these high Chl events in individual reservoirs (Fig. 4) is masked by aggregation in the presentation of the Chl-TP relationship as seasonal or long-term mean values (Jones et al. 1998, Jones and Knowlton 2005). These extreme values are the basis for estimating Chl<sub>max</sub> in summer monitoring data (Fig. 1).

**Table 2.-**Trophic state criteria for Missouri reservoirs based on TP (Jones et al. 2008) with the mean ratio of observed average chlorophyll:near-maximum chlorophyll (Chl<sub>obs</sub>:Chl<sub>nm</sub>) in Chl<sub>obs</sub> samples of  $\geq$ 10,  $\geq$ 20,  $\geq$ 30,  $\geq$ 40, and  $\geq$ 50 µg/L from intensively sampled reservoirs in the dataset (n = 113). The eutrophic category was divided at 50 µg TP/L to better illustrate the cross-system pattern in Chl<sub>obs</sub>:Chl<sub>nm</sub>.

			Chl <sub>obs :</sub> Chl <sub>nm</sub> values when Chl <sub>obs</sub>					
Trophic State	# Lakes	TP ( $\mu$ g/L) Criteria	≥10 µg/L	≥20 µg/L	≥30 µg/L	≥40 μg/L	≥50 μg/L	
Oligotrophic	9	<10	0.97	_	_	_		
Mesotrophic	22	≥10-<25	0.63	0.79	0.96	1.04	1.60	
Lower Eutrophic	45	≥25->50	0.53	0.67	0.77	0.83	0.92	
Upper Eutrophic	31	≥50-<100	0.46	0.55	0.63	0.70	0.75	
Hypereutrophic	6	>100	0.34	0.38	0.42	0.48	0.53	



**Figure 4.-**The proportion of observed chlorophyll (Chl<sub>obs</sub>) values that exceed 10, 20, 30, 40 and 50  $\mu$ g/L (panels a–e, respectively) plotted against the mean log<sub>10</sub>TP value from intensively sampled reservoirs in the dataset (n = 113). The mean ratio of Chl<sub>obs</sub> to near-maximum chlorophyll (Chl<sub>nm</sub>) was calculated for each reservoir using Chl values that exceeded the cutpoint for the respective panels. Mean Chl<sub>obs</sub>:Chl<sub>nm</sub> ratios were divided into 4 categories and are represented in the panels by unique symbols to show the cross-system pattern.

The ratio of Chlmax:TP forms a dome-shaped distribution across the range of Chlobs:TP values in the dataset when both are plotted against trophic state (as  $log_{10}TP$ ; Fig. 5a). This pattern clearly shows a rapid increase in the yield of Chl<sub>max</sub> per unit TP across the oligo- and mesotrophic ranges, followed by high ratios throughout the eutrophic range and subsequent decline among the most fertile samples. Overall, ratios of Chl<sub>max</sub>:TP increase sharply from  $\sim 0.6$  at 5  $\mu$ g TP/L to unity at 13  $\mu$ g TP/L, and the ratio increases to 1.25 at the upper boundary of the mesotrophic conditions (25  $\mu$ g TP/L). Across this range, the increase in Chl<sub>max</sub>, from 3.2 to 31.2  $\mu$ g/L, was double the 5-fold increase in TP. Between 25 and 30  $\mu$ g TP/L, the increase in Chl<sub>max</sub> was just slightly larger than the proportional increase in TP. Near the center of the data distribution, Chl<sub>max</sub>:TP forms a broad dome. Ratios were 1.4 at 44 and 125  $\mu$ g TP/L with a peak ratio of 1.46 at 74  $\mu$ g TP/L. As a consequence, Chlmax closely tracks changes in TP in a near-unity response within the eutrophic range. For example, halving TP from 100 to 50  $\mu$ g TP/L corresponds with halving Chl<sub>max</sub> (from 144 to 71  $\mu$ g/L). The decline in Chl<sub>max</sub>:TP among the most fertile samples (>125  $\mu$ g TP/L, 8% of the total; Fig. 5a) is largely a function of light limitation and available supplies of dissolved P in turbid samples (Knowlton and Jones 2000, Jones and Knowlton 2005, Jones et al. 2008).

The pattern seen in Chl<sub>max</sub>:TP ratios also holds for the 90th, 70th, and 50th percentile values in the TP bins used to generate equation 1 (Fig. 5b, values >125  $\mu$ g TP/L not shown). Within each category, Chl:TP shows a statistically significant increase with TP across oligotrophic to near-eutrophic range (r  $\geq$  0.93, n = 17, TP = 5–38  $\mu$ g/L), with a non-significant, near-flat response between 40 and 125  $\mu$ g TP/L. Collectively, these patterns illustrate how high Chl levels in Missouri reservoirs would respond to changes in TP (Fig. 5a and b).

Among samples in the 30th, 25th, and 20th percentiles in the various TP bins (Fig. 5c), the initial significant increase in Chl<sub>obs</sub>:TP with TP ( $r \ge 0.93$ , n = 17, TP = 5–38  $\mu$ g/L) was followed by a significant decline in Chl<sub>obs</sub>:TP ( $r \ge -0.94$ , n = 10). This pattern indicates the yield of Chl per unit of TP is not asymptotic across all samples within the eutrophic range. For example, halving TP from 80 to 40  $\mu$ g TP/L only results in a 20–35% reduction in Chl within the 20th to 30th percentiles within the cross-system pattern (Fig. 5c). This analysis suggests the response to phosphorus reduction would differ between the upper and lower half of the Chl–TP data distribution (Fig. 5).

Overall, this analysis proposes  $Chl_{max}$  and  $Chl_{nm}$  metrics for Missouri reservoirs that characterize peak algal biomass and serves as a basis to quantify controlling factors, assess seasonal patterns, and compare with lakes in other regions. The upper boundary on the cross-system Chl–TP pattern (Fig.



**Figure 5.-**Chlorophyll (Chl<sub>obs</sub>) and total phosphorus (TP) data from Figure 1a were replotted in panel a as the Chl<sub>obs</sub>:TP ratio against log<sub>10</sub>TP with trophic state boundaries for TP (Jones et al. 2008) shown on the x-axis. The ratio of maximum chlorophyll (Chl<sub>max</sub>) to TP (as calculated from equation 1 divided by the observed TP) forms a dome across the data distribution. Samples with the most extreme Chl<sub>obs</sub>:TP ratios were not included. In panel b, Chl<sub>obs</sub>:TP ratios for the 90th, 70th, and 50th percentile values in the TP bins used to generate equation 1 (see text) were plotted against the corresponding median TP value for each bin. In panel c, Chl<sub>obs</sub>:TP ratios for the 30th, 25th, and 20th percentile values in the TP bins were plotted against the corresponding median TP value.

1) represents the general distribution in which other factors constrain responses below the maximum. Conditions of  $Chl_{max}$  and  $Chl_{nm}$  represent near-potential algal biomass for a given TP concentration (Fig. 4; Table 2) and were generally rare in summer monitoring data (Fig. 1). An important outcome is that history and frequency of high Chl events differ among individual reservoirs and suggest system-specific constraint of Chl by biotic and abiotic factors. A detailed analysis of factors that determine the degree to which  $Chl_{obs}$ is less than  $Chl_{max}$  or  $Chl_{nm}$  in individual reservoirs is beyond the scope of this note but remains a research question.

The distribution of Chlmax:TP ratios across the TP range has management implications. The sharp increase across the least fertile samples and the near-asymptote across the eutrophic range (Fig. 5) provide a framework for interpreting how phosphorus control will reduce Chl. Interestingly, when expressing this relationship as the rate of change in Chl per unit TP, the pattern is quite similar for Missouri reservoirs, Florida lakes, and a selection of international lakes (Fig. 2b), despite large differences in actual Chlmax values among the datasets (Fig. 2a). This pattern also holds for average Chl values predicted using the least squares regression based on long-term reservoir means (Jones et al. 2008, data not shown). The clear inference is that the rate of change in Chl across observed TP values applies to a broad range of lake types and is consistent with the early finding that the slope coefficient of the Chl-TP relationship differs with TP and is nonlinear (Straskraba 1980, Watson et al. 1992, Brown et al. 2000). The transition between rapid change in Chlmax:TP and gradually declining rate of change is near the conventional boundary between meostrophic and eutrophic conditions (30 µg TP/L; Nürnberg 1996; Fig. 2b).

Control of algal biomass and associated nuisance conditions is an objective of most lake management efforts (Bachmann et al. 2003), and this analysis furthers our understanding of these issues in Missouri reservoirs.

John R. Jones, Daniel V. Obrecht, Anthony P. Thorpe

Department of Fisheries and Wildlife Sciences Anheuser-Busch Natural Resources Building University of Missouri, Columbia MO 65211-7240 Corresponding author: jonesj@missouri.edu

#### Acknowledgments

Funding was provided by the Missouri Department of Natural Resources and Missouri Agricultural Experiment Station. We thank Jennifer Graham, Joshua Millspaugh, and Robert Gitzen for advice. Roger Bachmann, Gertrud Nürnberg, and 2 anonymous reviewers provided most helpful suggestions.

#### References

- Bachmann RW, Hoyer MV, Canfield DE Jr. 2003. Predicting the frequencies of high chlorophyll levels in Florida lakes from average chlorophyll or nutrient data. Lake Reserv Manage. 19:229–241.
- Brown CD, Hoyer MV, Bachmann RW, Canfield DE Jr. 2000. Nutrient-chlorophyll relationships: an evaluation of empirical nutrient-chlorophyll models using Florida and northtemperate lake data. Can J Fish Aquat Sci. 57:1574–1583.
- Carstensen J, Henriksen P, Heiskanen A-S. 2007. Summer algal blooms in shallow estuaries: definition, mechanisms, and link to eutrophication. Limnol Oceanogr. 52:370–384.
- Hosper SH. 1980. Development and partial application of limiting values for the phosphate concentration in surface waters in the Netherlands. Hydrobiol Bull. 14:64–72.
- Hutchinson GE. 1967. A treatise on limnology. II Introduction to lake biology and the limnoplankton. New York (NY): John Wiley & Sons.
- Jones JR, Knowlton MF, Kaiser MS. 1998. Effects of aggregation on chlorophyll-phosphorus relations in Missouri reservoirs. Lake Reserv Manage. 14:1–9.
- Jones JR, Knowlton MF. 2005. Chlorophyll response to nutrients and non-algal seston in Missouri reservoirs and oxbow lakes. Lake Reserv Manage. 21:361–370.
- Jones JR, Obrecht DV, Perkins BD, Knowlton MF, Thorpe AP, Watanabe S, Bacon RR. 2008. Nutrients, seston and transparency of Missouri reservoirs and oxbow lakes: An analysis of regional limnology. Lake Reserv Manage. 24: 155–180.
- Kaiser MS, Speckman PL, Jones JR. 1994. Statistical models for limiting nutrient relations in inland waters. J Am Statist Assoc. 89:410–423.
- Knowlton MF, Jones JR. 2000. Non-algal seston, light, nutrients and chlorophyll in Missouri reservoirs. Lake Reserv Manage. 16:322–332.
- Lewis WM Jr. 2011. Global primary production of lakes: 19th Baldi Memorial Lecture. Inland Waters. 1:1–28.
- Nürnberg GK. 1996. Trophic state of clear and colored, softand hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. Lake Reserv Manage. 12:432–447.
- Obrecht DV, Milanick M, Perkins BD, Ready D, Jones JR. 1998. Evaluation of data generated from lake samples collected by volunteers. Lake Reserv Manage. 14:21–27.
- Pridmore RD, McBride GB. 1984. Prediction of chlorophyll-a concentrations in impoundments of short hydraulic retention time. J Environ Manage. 19:343–350.
- Smith VH. 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ Sci Pollut Res. 10:126–139.
- Smith VH, Shapiro J. 1981. Chlorophyll-phosphorus relations in individual lakes. Their importance to lake restoration strategies. Environ Sci Tech. 15:444–451.
- Smayda TJ. 1997. What is a bloom? A commentary. Limnol Oceanogr. 42:1132–1136.
- Sterner RW. 2008. On the phosphorus limitation paradigm for lakes. Internat Rev Hydrobiol. 93:433–445.

- Straskraba M. 1980. Effects of physical variables on production. In: LeCren ED, Lowe-McConnel RH, editors. The functioning of freshwater ecosystems. IBP 22. Cambridge (UK): Cambridge University Press. p. 13–84.
- Thomson JD, Weiblen G, Thomson BA, Alfaro S, Legender P. 1996. Untangling multiple factors in spatial distributions: lilies, gophers and rocks. Ecology 77:1698–1715.
- Thorpe AP, Obrecht DV. 2008. The Lakes of Missouri Volunteer Program 2008 data report. University of Missouri, Columbia.
- Walker WW Jr. 1985. Statistical bases for mean chlorophyll *a* criteria. In: Lake and Reservoir Management – Practical Applications. Proc. 4th Annual Conference, North American Lake Management Society, McAffee (NJ): p. 57–62.
- Walker WW Jr., Havens KE. 1995. Relating algal bloom frequencies to phosphorus concentrations in Lake Okeechobee. Lake Reserv Manage. 11:77–83.

- Walmsley RD. 1984. A chlorophyll *a* trophic status classification system for South African reservoirs. J Environ Qual. 13:97–104.
- Watson S, McCauley E, Downing JA. 1992. Sigmoid relationships between phosphorus, algal biomass, and algal community structure. Can J Fish Aquat Sci. 49:2605– 2610.
- Watson S, McCauley E, Downing JA. 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnol Oceanogr 42:487– 495.
- White E. 1989. Utility of relationships between lake phosphorus and chlorophyll *a* as predictive tools in eutrophication control studies. N Z J Mar Freshw Res. 23: 35–41.

### Appendix II – Additional Project Experience

- 1. City of Bentonville, Arkansas Regulatory Support and Biomonitoring Services
- 2. Connecticut Municipalities Narrative Nutrient Criteria and Phosphorus Limits
- 3. City of Lima, Ohio Ohio Trophic Index Review and Implementation
- 4. City of Blue Springs, Missouri –*Site-Specific Dissolved Oxygen Criteria and Wasteload Allocation Modeling Study*
- 5. City of Poplar Bluff, Missouri Main Ditch Aquatic Life Use Attainability Analysis
- 6. City of Ashland, Missouri Two Mile Prairie Stream Evaluation



Geosyntec provides regulatory support and monitoring services for the City of Bentonville wastewater and stormwater system.

#### Client: City of Bentonville, Arkansas

#### Services Provided:

- TMDL Compliance Support Services
- ✓ ADEQ and EPA Region 6 Negotiations
- Stream Biomonitoring
- Nutrient Criteria Evaluation
- ✓ MS4 Stormwater Services
- Water Quality Data Review

#### **Project Objectives**

Geosyntec<sup>▶</sup>

consultants

The City of Bentonville, Arkansas owns and operates the Bentonville Wastewater Treatment Plant. The treatment plant discharges 4.0 MGD to Town Branch, a tributary to the Elk River. In 2007, the City completed a major plant upgrade to construct a phosphorus removal system to meet new effluent limit requirements in the treatment plant's National Pollutant Discharge Elimination System (NPDES) permit. The nutrient removal system was functioning as designed and NPDES permit limits were achieved. However, in July 2010 EPA Region 6 issued a phosphorus total maximum daily load (TMDL) for Town Branch, based in part on EPA's conclusion that the biological community was impaired relative to area streams. The new phosphorus limits would require that the City meet what EPA considers to be the "limits of technology". The TMDL also placed phosphorus wasteload allocation targets on the City's stormwater runoff. These targets would require significant capital investments from the City. Because the TMDL was written in the absence of recent water quality or biological data, the City and the Arkansas Department of Environmental Quality (ADEQ) have formally commented that the stream impairment is questionable and that additional phosphorus removal will likely not result in benefit to water quality in Town Branch.

#### **Geosyntec's Scope of Services**

The City retained Geosyntec to apply our technical and regulatory expertise to satisfactorily resolve the Town Branch TMDL. Working alongside the City of Bentonville management team, Geosyntec has reviewed the historic technical basis of the TMDL and has engaged with the ADEQ water quality assessment staff to better characterize aquatic life conditions in Town Branch. In addition to providing biological and water quality monitoring services, Geosyntec is assessing the potential challenges of meeting stringent phosphorus wasteload allocation targets in municipal stormwater runoff. The project has consisted of working closely with ADEQ staff to re-evaluate the need for additional phosphorus removal to assure that the City's resources are not expended on expensive treatment plant upgrades that may have little if any water quality benefit.

#### **Notable Accomplishments**

Following an in-depth review of both the TMDL and available historical data, Geosyntec identified several significant data gaps that should be addressed to more accurately determine the water quality and biological status of Town Branch. To begin filling these data gaps, Geosyntec developed a quality assurance project plan and conducted an assessment of water quality and the aquatic community in Town Branch and previously identified reference streams. The assessment included collections of periphyton, continuous water levels and flows, and macroinvertebrates using multiple methods. Geosyntec evaluated the data collected during the preliminary assessment. Geosyntec continues to work closely with the City and ADEQ to resolve the issues regarding the TMDL.

# Geosyntec<sup>></sup>

consultants



Example of diatom data collection in Connecticut streams (CT DEEP).

Quinnipiac River looking upstream from Meriden, CT (Wikipedia). Client: Barnes & Thornburg Services Provided:

- ✓ Data evaluation
- ✓ Review of methodologies
- ✓ Technical and regulatory support

#### Project Objective

The Connecticut Department of Energy and the Environment (DEEP), in response to pressure from US Environmental Protection Agency (USEPA) Region 1, developed and revised a "Phosphorus Reduction Strategy for Inland Non-Tidal Waters". The purpose of this strategy was to develop a methodology for calculating total phosphorus limits for 45 publicly owned treatment works (POTWs). The methodology, which correlates phosphorus loads with changes in individual algal diatom taxon, results in calculated limits of 0.1 to 0.3 milligrams per liter, which require expensive capital upgrades of tens of millions of dollars and annual operation and maintenance costs. The municipalities wanted to know if the state's methodology was sound or whether there was justification for less stringent phosphorus limits (0.7 milligrams per liter). The municipalities also wanted assistance in working with the state to develop an alternative strategy to meet the intent of state legislation.

#### **Geosyntec's Scope of Services**

- Review the state's methodology and scientific literature regarding calculating thresholds in algal species
- Prepare a summary of findings and recommendations
- Review and comment on proposed National Pollutant Discharge Elimination System (NPDES) permits
- · Assist in meetings with the state to discuss findings and alternative strategies

#### **Notable Accomplishments**

Geosyntec was retained to review and comment on the state's methodology on behalf of the Cities of Danbury and Meriden and the Towns of Wallingford and Southington. The state agreed to issue permits with interim limits that the municipalities could meet and final effluent limits based on the state's existing methodology, with language that these limits could be revised based on new information. The state legislature directed that DEEP work with stakeholders. A coordinating committee has been established to update the state's strategy. The coordinating committee is being supported by a nonpoint source workgroup, a scientific methods workgroup, a municipal point source implementation workgroup, and the Connecticut Academy of Science and Engineering.

# Geosyntec<sup>▷</sup> consultants



"Natural" biolfilter established downstream of a low head dam in Ottawa River within Lima, OH

Client: City of Lima, OH Services Provided:

- ✓ Review of methodologies
- ✓ Technical and regulatory support
- ✓ Workgroup participation

#### **Project Objective**

The Ohio Environmental Protection Agency (OEPA) has been developing a trophic index condition (TIC) to aid the state in assessing attainment of aquatic life uses and address concerns about excessive instream nutrient concentrations. OEPA conducted early stakeholder outreach to obtain comments on the proposed TIC and draft implementation procedures. The City of Lima was concerned that the TIC might be adopted into the state's water quality standards regulations without a demonstration that the proposed approach was founded in sound science. This would require that cities and industries meet very low levels of phosphorus and dissolved inorganic nitrogen in wastewater treatment plant effluent and stormwater discharges, without being assured that attainment of aquatic life would significantly improve. As a result of the comments received, the state formed a Technical Advisory Group (TAG) of diverse stakeholders to assist the state in refining the TIC approach and develop implementation procedures.

#### **Geosyntec's Scope of Services**

- Review the state's methodology in developing the TIC and proposed implementation procedures
- Assist legal counsel in developing comments on behalf of the City of Lima
- Represent small publicly owned treatment works (POTWs) on the TAG
- As a member of the TAG, assist the state in developing a proposed rulemaking

#### **Notable Accomplishments**

Geosyntec was retained to review and comment on the state's methodology on behalf of the City of Lima. The state agreed to form a technical advisory group and Geosyntec is representing the state's small POTWs on the TAG.

## Geosyntec<sup>▷</sup> consultants



Geosyntec used rhodamine dye as part of a time-of-travel study on Sni-A-Bar Creek.

#### **Client: City of Blue Springs**

#### **Services Provided:**

- ✓ Site-specific criteria
- ✓ QUAL2K dissolved oxygen modeling
- Permit limit derivation
- ✓ Total Maximum Daily Load monitoring
- ✓ Quality Assurance Project Planning
- ✓ Antidegradation review
- Regulatory negotiation

#### **Project Objective**

To accommodate anticipated growth around the Kansas City metropolitan area, the City of Blue Springs (City) planned to expand and upgrade their wastewater treatment facility (WWTF), which discharges into Sni-A-Bar Creek. Perennial reaches of Sni-A-Bar Creek were included in Missouri's impaired waters list due to low dissolved oxygen (DO) concentrations. Geosyntec and agency scientists believed that Sni-A-Bar Creek did not naturally attain statewide DO criteria during summer conditions as a result of shallow depths, high residual sediment oxygen demand, low reaeration, and transient stagnant features such as beaver dams and backwater areas. To permit expansion of a WWTF that discharges to impaired reaches, state regulatory policies require intensive water quality study and evaluation of attainable conditions. Geosyntec evaluated water quality conditions within the basin, developed site-specific dissolved oxygen targets, and performed water quality modeling to assist in wastewater planning and permitting efforts.

#### **Geosyntec's Scope of Services**

Geosyntec evaluated water quality conditions within the basin, developed site-specific dissolved oxygen targets, and performed water quality modeling to assist in wastewater planning and permitting efforts. This work was one of the most intensive wasteload allocation (WLA) monitoring studies conducted in Missouri to verify a QUAL2K water quality model. WLA study efforts included multiple time-of-travel dye studies, diel DO and water chemistry measurements, flow and hydrogeometry characterization, and reaeration calculations. Geosyntec used these results to update its model of the creek and quantify attainable DO concentrations. Work was used to demonstrate that no proven or practical treatment technologies would likely achieve Missouri's statewide DO criterion.

Geosyntec recognized that a site-specific DO criterion was one of the few regulatory options available to the city that would allow expansion of the Sni-A-Bar WWTF. Therefore, Geosyntec prepared and implemented a site-specific DO criteria study in conjunction with the WLA studies. Using GIS tools, Geosyntec worked to select suitable reference streams that best exhibited natural DO conditions in the region and confirmed the choices with physical and biological habitat assessments. After sites were selected, Geosyntec conducted extensive, long-term, diel DO measurements in support of alternative DO criteria. Results from the study confirmed that conditions within this region precluded attainment of Missouri's DO criteria on a consistent basis and ultimately formed the basis for developing alternative site-specific criteria and one of Missouri's first antidegradation reviews.

#### **Notable Accomplishments**

Geosyntec's precedent-setting study and regulatory negotiations represent one of the only site-specific criteria approved by the Missouri Clean Water Commission (MCWC). This allowed Blue Springs to expand wastewater treatment capacity and prevent a large capital outlay associated with expensive and marginally beneficial treatment upgrades.

# Geosyntec Consultants



#### Client: City of Poplar Bluff, Missouri

#### Services Provided:

- ✓ Total Maximum Daily Load development
- ✓ Use Attainability Analysis evaluation
- ✓ Reference stream identification and modeling
- Comprehensive ambient water quality monitoring
- ✓ Biological assessments

Geosyntec identified reference streams using a rigorous, scientific selection process

#### **Project Objective**

Main Ditch is a channelized irrigation canal that was dredged and straightened during the early 1900's to reduce flooding and improve agricultural production in the southeast region of Missouri. Intermittent reaches of Main Ditch receive treated effluent from the City of Poplar Bluff, Missouri. Historically, periodic and intensive water quality surveys indicated that dissolved oxygen (DO) concentrations in Main Ditch were frequently below the 5.0 milligram per liter (mg/L) water quality criterion necessary to support aquatic life use designations. As a result, the Missouri Department of Natural Resources (MDNR) initiated total maximum daily load (TMDL) activities in 2002. The draft TMDL, issued in 2005, concluded that the city needed to implement significant, and potentially unaffordable, upgrades to their wastewater treatment facility to attain water quality standards in Main Ditch. The city retained Geosyntec to review the technical validity of these conclusions and evaluate the applicability of implementing use attainability analysis (UAA) and site-specific DO criteria flexibilities in the context of the TMDL conclusions.

#### **Geosyntec's Scope of Services**

Geosyntec employed a two-phased approach to address the regulatory and scientific complexities surrounding this project. In the first phase, Geosyntec conducted a technical review of the draft TMDL and developed a strategic regulatory compliance approach to address non-attainment issues. In developing the regulatory approach, Geosyntec performed a data gap analysis and reviewed relevant technical literature to outline the benefits, cost, feasibility, and scientific defensibility of performing a UAA and pursuing site-specific DO criteria development. With this information, Geosyntec coordinated meetings with the city, MDNR, USEPA Region 7, and other interested stakeholders to develop a work plan that outlined consensus-based project tasks and objectives.

The second phase of the project was divided into 1) monitoring to support UAA development, and 2) collecting data to support additional TMDL modeling activities. Using peer-reviewed selection criteria, Geosyntec identified best-available reference streams with desktop GIS analyses and verified initial conclusions with on-site biological assessments. Geosyntec continuously monitored select reference streams for more than 700 days and generated well over 200,000 individual data points. To support TMDL modeling activities, Geosyntec conducted travel time and hydrogeometry investigations in Main Ditch downstream of the city's outfall. Geosyntec also collected additional ambient water quality data in Main Ditch to supplement previous MDNR data collection efforts and increase predictive power of the Qual2K water quality model. Model calibration and verification activities are ongoing.

#### **Notable Accomplishments**

Geosyntec successfully coordinated and lead technical workgroup meetings between state, federal, and local regulatory agencies which resulted in a consensus-based approach to UAA development activities. The Missouri Clean Water Commission approved Geosyntec's site-specific dissolved oxygen criteria for Main Ditch in 2012. When these criteria are confirmed by the US EPA, the city will save over \$50 million in unnecessary treatment upgrades.



Geosyntec collaborated with Dr. Steve Chapra to modify Qual2K for shallow systems

#### Client: US Environmental Protection Agency & University of Missouri

#### **Services Provided:**

- Qual2K Water Quality Modeling
- Reference Stream Identification and Verification
- Site-Specific Dissolved Oxygen Criteria Development
- ✓ Long-Term Water Quality Monitoring
- Quality Assurance Project Plan

#### **Project Objective**

Geosyntec<sup>▶</sup>

consultants

The Two-Mile Prairie region between Columbia and Ashland, Missouri is a growing suburban area located within ecologically sensitive watersheds on the northern edge of the Ozark Plateau ecoregion. The project area is characterized by expansive forested land and relatively unimpacted watersheds. Because the area is experiencing rapid growth, local, state and federal decision-makers concluded additional water quality assessments and refinement of existing waste load allocation modeling efforts were needed to address planning and development of the Ashland wastewater treatment facility. With funding from US EPA, the University of Missouri (MU) retained Geosyntec to monitor and model water quality within stream reaches that receive treated wastewater from the Ashland facility. Data were collected to support verification of the QUAL2K surface water quality model and development of site-specific dissolved oxygen criteria.

#### **Geosyntec's Scope of Services**

Geosyntec conducted a First-Order Error Analysis (FOEA) of previous modeling efforts to evaluate the relative contribution of individual model parameters to the overall variance of predicted dissolved oxygen and ammonia concentrations.

To address uncertainties identified in the FOEA, Geosyntec extensively evaluated stream travel time and hydrogeometry under varying flow conditions to reliably and accurately describe model hydraulic parameters. Geosyntec also conducted several intensive water quality surveys during which continuous and discrete data



Geosyntec documented significant impacts of periphyton on shallow-system DO balances

(including bottom algae, or periphyton) were collected to aid in model calibration and verification. To create a more robust dataset and increase model accuracy, these studies were conducted during both summer and winter seasons. Geosyntec identified, verified, and continuously monitored best-available reference streams to determine highest attainable dissolved oxygen in the study region.

#### **Notable Accomplishments**

Geosyntec worked closely with Dr. Steve Chapra, co-creator of the Qual2K water quality model, on the Two-Mile Prairie project. Geosyntec also documented the significant impact that low periphyton densities have on shallow-stream dissolved oxygen balances and proposed site-specific DO criteria which, when implemented, will decrease wastewater treatment costs for the City.

## **Appendix III – Resumes**

- 1. Chris Zell, MS, PH
- 2. Adrienne Nemura, MS, PE
- 3. Julia Klens Caprio, MA, MBA
- 4. Mark Leisenring, MS, PE
- 5. Song Qian, PhD
- 6. Rob Annear, PhD, PE
- 7. Randy Crawford, MA
- 8. Steve Layman, PhD
- 9. Daniel Obrecht, MS
- 10. Ann St. Amand, PhD, CLP
- 11. Yangdong Pan, PhD



#### CHRISTOPHER C. ZELL, M.S., P.H.

Water Quality Standards Assessment Surface Water Quality Modeling Hydrologic Data Analysis

#### **EDUCATION**

- M.S., Natural Resources (Water Resources emphasis), University of Missouri, Columbia, Missouri, 2012
- B.S., Biology, Environmental Management, Fisheries & Wildlife Science (Minor: Chemistry), South Dakota State University, Brookings, South Dakota, 1998

#### **REGISTRATIONS AND CERTIFICATIONS**

Professional Hydrologist, American Institute of Hydrology, #10-HWQ-2016

#### CAREER SUMMARY

Mr. Zell has more than 13 years of experience in hydrology and water quality and maintains a diverse background in environmental and regulatory analysis that includes coordination of Missouri's water quality standards program, lead technical developer of water quality-based permit limits and wasteload allocations (125+), and principal investigator of nutrient, bacteria, and dissolved oxygen total maximum daily load (TMDL) studies.

#### **RELEVANT EXPERIENCE**

*Aquatic Life.* Mr. Zell continues to participate in the State of Missouri's aquatic life workgroup. Issues being discussed include designation of previously un-designated waters, development of Use Attainability Analyses protocols for aquatic life, and tiered aquatic life uses. Mr. Zell has also worked on several projects, such as the Phillips Farms Water Quality Impact Assessment, where he conducted field investigations and reviewed existing data to evaluate the biological condition of two ephemeral Ozark border streams. Work included characterizing key macroinvertebrate population metrics in the context of potential biocriteria regulations. Mr. Zell has also designed studies using EPA's stressor identification guidance to determine whether nutrients or other stressors, or a combination, are preventing attainment of aquatic life uses.

*Nutrient Criteria Policy Analysis and Negotiation.* As an invited member and speaker to Missouri's nutrient criteria committee, Mr. Zell is thoroughly familiar with criteria derivation approaches and associated challenges. He successfully challenged proposed lake nutrient criteria in Missouri following a detailed peer review of technical support documentation and numerous negotiation meetings. Such negotiations were sponsored by stakeholders including the Metropolitan St. Louis Sewer District and City of Springfield, Missouri. In addition, Mr. Zell successfully developed technical comments to the Oklahoma Water Resources Board on behalf

# CHRISTOPHER C. ZELL, M.S., P.H. Page 2



of the City of Bentonville, Arkansas that influenced the statistical expression of the scenic river phosphorus criterion.

*Nutrient Total Maximum Daily Load Development and Implementation.* Serving as Geosyntec Project Manager, Mr. Zell has directly developed or implemented over 17 nutrient and dissolved oxygen TMDLs. These projects include development of the first consensus-based nutrient TMDL in Missouri (James River), and application of numerical models to evaluate nutrient-algal relationships for locations in Missouri, Illinois, Ohio, and Oklahoma. In addition, Chris is serving as the technical advisor in a third-party review of models developed by US EPA to support the Illinois River phosphorus TMDL. The model peer-review is sponsored by the Northwest Arkansas Regional Planning Commission and under sub-contract to Wright Water Engineers.

*Watershed Protection and Restoration.* Mr. Zell has led or managed several extensive watershed monitoring and modeling studies, including multiyear investigation of urban best management practices (BMPs) focusing on nutrients (for Boone County, Section 319), evaluation of agricultural BMPs focusing on nutrients (for NRCS), evaluation of ecoregional water quality (for US EPA Region 7), and regional wastewater planning and modeling to evaluate nutrient impacts (for Missouri DNR, HDR Engineering).

#### **RECENT PEER REVIEWED PUBLICATIONS**

- Zell, C. and J. Hubbart. 2013. Considering Streamflow Trend Analyses Uncertainty in Urbanizing Watersheds: A Case Study in the Central U.S., Earth Interaction (*in Press*).
- Zell, C. and J. Hubbart. 2012. The Role of Light Intensity Functions in Determining Hypoxic Reference Stream Metabolism. Journal of Environmental Management 97:69-77
- Zell C., and J. Hubbart. 2012. Interdisciplinary Linkages of Biophysical Processes and Resilience Theory: Pursuing Predictability. Ecol. Model. 248:1-12



#### ADRIENNE NEMURA, M.S., P.E.

Water Quality Science and Engineering Water Quality Monitoring and Modeling Litigation Support and Strategic Advice

#### **EDUCATION**

M.S., Civil Engineering, Hydrosystems, Virginia Tech University, 1986 B.S., Civil Engineering, Hydrosystems, Virginia Tech University, 1984

#### **REGISTRATIONS AND CERTIFICATIONS**

Civil Engineer (P.E.), MI: 6201046150

#### **CAREER SUMMARY**

Ms. Nemura has helped municipalities, industries, agriculture, and attorneys on a wide variety of engineering, science, and policy-related projects related to implementation of the Clean Water Act. This work has included discussions on behalf of clients with numerous state and EPA personnel in regions 1, 3, 4, 5, 7 and 10, as well as EPA headquarters. She has 28 years of experience evaluating the impacts of pollutant sources on watersheds and waterways, is active in several national organizations, and is a routine speaker at national conferences on water quality issues. Ms. Nemura is known for her ability to explain complex, technical issues and to effectively advocate on clients' behalf.

#### **RELEVANT EXPERIENCE**

*National Clean Water Act Policy Change*. Ms. Nemura provided critical technical and policy support to the US Conference of Mayors Water Council in discussions with US EPA and the Department of Justice for the last four years on the need for an Integrated Planning Framework for Wastewater and Stormwater that considers affordability rather than strict compliance with water quality standards. This effort helped lead to discussions between the Mayors Water Council, National League of Cities, National Association of Counties, American Public Works Association and the Water Environment Federation (WEF), and others about redefining how EPA assesses affordability for communities in making decisions about Clean Water Act implementation. Ms. Nemura recently co-presented at a Water Environment Federation's national webinar on integrated planning and affordability, which was WEF's largest attended webinar for 2013. Ms. Nemura was also a key author of WEF's Technical Submission to EPA on *Removal of Nutrients with Currently Available Secondary Treatment Technologies*. At the time, EPA had been sued by environmental groups to add nutrient removal requirements to the Clean Water Act's secondary treatment technology (EPA later declined to require this change). Ms. Nemura has given numerous presentations at the national and state levels on Clean Water Act policy issues.

*Nutrient Criteria*. Ms. Nemura has been active at the local, state and national levels for 29 years advocating for sound science to be used in addressing issues associated with the impact of excessive nutrients on water quality. For example, she is representing the City of Lima, OH and other small publicly owned treatment works (POTWs) on the State of Ohio's Nutrient Water Quality Standards Technical Advisory Group. This work is the result of the critique of Ohio's draft trophic index criterion that she led for the City of Lima and Allen County, OH. As science advisor to the Agricultural Nutrients

#### ADRIENNE NEMURA, M.S., P.E.



Policy Council (ANPC), she co-presented a national webinar on numeric nutrient criteria development strategies (draft paper under development). Ms. Nemura led a critical review of Connecticut's phosphorus strategy on behalf of several municipal wastewater plants. This led the state to establish a workplan with the Connecticut Academy of Science and Engineers (CASE) to assist three workgroups in evaluating development of a revised strategy. Ms. Nemura assisted Clay County Utilities in reviewing and commenting on the development of numeric nutrient criteria for Florida. She also led a study of lake nutrient benchmarks for the state of Indiana to replace US EPA's ecoregional criteria with criteria based on the state's data. Ms. Nemura was actively involved in the development and review of the watershed and water quality models and the development of site-specific aquatic life uses and criteria for the Chesapeake Bay, on behalf of the metropolitan Washington D.C.'s local governments. Ms. Nemura conducted studies to evaluate management strategies to address low dissolved oxygen, bacteria, eutrophication, and pH impacts on the Anacostia and Potomac estuaries.

*Nutrient Issues and Modeling Studies.* Ms. Nemura was the primary author of a study to assist the ANPC in highlighting discrepancies in US EPA's and USDA's characterization of agricultural loads in the Chesapeake Bay watershed. For nine years, Ms. Nemura was the metropolitan Washington region's modeling and monitoring director. She helped develop and apply models for the Anacostia and Potomac estuaries, including simulation of the bluegreen algae, *Microcystis,* pH-sediment phosphorus flux, and modeling of sediment oxygen demand. She also served as the liaison between 18 local governments and the state and federal regulatory agencies on water quality issues, which included representing the governments on the Chesapeake Bay Modeling Subcommittee. Ms. Nemura managed, on behalf of affected stakeholders, the review of numerous modeling studies conducted by other consultants including nutrient modeling of High Rock Lake Reservoir in NC; phosphorus modeling of Horseshoe Lake (old Mississippi River oxbow) that would have required a \$20M upgrade for a US Steel facility; wet weather dissolved oxygen and bacteria modeling of the Chicago Area Waterways; dissolved oxygen modeling of the Cooper River in South Carolina; nutrient and dissolved oxygen modeling of the Reedy River in South Carolina;

*Total Maximum Daily Loads (TMDLs) and Watershed Implementation Plans.* Ms. Nemura led a critical review and comments on the Chesapeake Bay TMDL for five municipalities in Virginia and the District of Columbia. She also supported the American Farm Bureau Federation in their challenge of the Bay TMDL. She helped the Ohio River Valley Water Sanitation Commission (ORSANCO) POTW Advisory Committee convince EPA Region 5 to delay their development of a bacteria TMDL for 980 miles of the Ohio River to collect more data and approach the project more scientifically. Ms. Nemura was a key consultant helping Sanitation District No. 1 of Northern Kentucky develop and negotiate Watershed Plans for the country's first sewer overflow consent decree based on the principles of adaptive watershed management.

#### JULIA KLENS CAPRIO, M.A., M.B.A.

Quality Assurance Manager Analytical Data Specialist Site Analytical Data Evaluation and Validation QA Auditing

#### **EDUCATION**

M.B.A. Quality Management, Upper Iowa University; 2009 M.A., Organizational Management, Tusculum College, Knoxville, Tennessee; 2002 B.A., Biology, Lock Haven University, Lock Haven, Pennsylvania; 1980

#### **REGISTRATIONS AND CERTIFICATIONS**

ASQ Certified Quality Manager #13876 Certified Environmental Field Sampler #0414 Certified Radiochemistry Data Validator (Radiochemistry Society) NQA-1 Lead Auditor Certified Environmental Sampler

#### **CAREER SUMMARY**

Ms. Caprio is an Associate specializing in Quality Assurance and has over 25 years of experience in the environmental field. Currently she specializes in project quality management, preparation and review of quality assurance project plans (QAPPs), quality assurance management plans (QMPs), field sampling plans, data verification, data evaluation, data validation, QA audits including laboratory audits and project on-site field audits. Her data validation experience includes chemical, radiological and geotechnical parameters for media including but not limited to sediment, soil, groundwater, surface water, biota, soil vapor and air monitoring. Ms. Caprio also provides both in-house quality assurance training through the various office locations within Geosyntec and outside quality assurance training for clients. She has over 20 years of experience as an analytical chemist in environmental and biotechnology laboratories including laboratory management, data management, quality control/quality assurance, and supervision of wet chemistry, gas chromatography, and high performance liquid chromatography departments.

#### **PROJECT EXPERIENCE**

Quality Assurance Manager for the Investigation and Characterization Former Adak Naval Complex Project, Adak, Alaska. Developed the Sampling and Analysis Plan (SAP) for the United States Department of the Navy Naval Facilities Engineering Command Northwest (NAVFAC NW) under the Environmental Multiple Award Contracts (EMAC) and under the regulatory oversight of the Alaska Department of Environmental Conservation. The SAP was specific to the investigation and characterization of the East Canal/Building T-1341. Also responsible for coordinating the data validation of the samples sent to the fixed base laboratory for analysis.

Developed the UFP-QAPP for the Ocean Cape Radio Relay System, Uakutat, Alaska. The UFP-QAPP was developed to detail the QA/QC processes and procedures implemented during the Removal Action of multiple areas of interest at the Ocean Cape Radio Relay Station Formerly Used Defense Site near Yukutat, Alaska. The project is under the supervision of the Army Corps of Engineers and under the regulatory oversight of the Alaska Department of Environmental Conservation.

Responsible for coordinating the Stage 2A data validation of the sample data from the Katzebue Federal Aviation Administration (FAA) Station, Katzebue, Alaska. The data are from a contaminated soil

removal action at the Power Plant and Carpenter Shop Areas of Concern. The validation was performed for Athna Engineering Services, LLC.

Quality Assurance Manager for the Savannah River Site Project (NQA-1). Responsible for the overall implementation of all quality assurance practices for the project including 1) on-site activities: drilling practices, sample collection, on-site project documentation, and on-site geotechnical testing/measurements 2) off site laboratory geotechnical testing, 3) engineering practices and procedures utilized for the project. Responsible for on-going audits for sub-tier contractors throughout the project, as well as audits of the project offices, both on-site and off-site.

Quality Assurance Manager responsible for preparation and implementation of the SAP (under Navy UFP-QAPP specifications) for the Naval Auxiliary Landing Field Site on San Clemente Island, CA. Also responsible for field and laboratory audits; as well as coordination of laboratory analyses and data validation.

Quality Assurance Manager for Berry's Creek Study Area – mega-sediment site, responsible for the preparation, final review and implementation of the UFP QAPP, New Jersey, EPA Region 2. Conducted field and laboratory audits against project requirements. Also responsible for the Tier III validation of the project data.

Quality Assurance Manager responsible for the preparation of the Progress Energy Site QAPP, Tampa, Florida, EPA Region 4.

Quality Assurance Manager responsible for the preparation of the West Shore Landings Site QAPP, Tampa, Florida, EPA Region 4.

Quality Assurance Manager for Geosyntec EPA Region 5 Superfund Projects

#### PUBLICATIONS

J.K. Caprio, S. Compston, S. Hill, D. Dunlap. 2012 "Selective, Low Sample Mass Invertebrate Sampling in Support of a Remedial Investigation with Potential Bioaccumulation of COPCs" Proceedings of the 2012 NEMC Conference, August.

J.K. Caprio, D. Adilman, M. Lodato. 2011. "Being Positive Your False Positives are False" Proceedings of the 2011 8<sup>th</sup> Annual DOD Environmental Data Quality Workshop, April.

J.K. Caprio. 2010 "Common Laboratory Contaminants" Proceedings of the 2010 Industrial Expo, Georgia Association of Water Professionals, March.

J.K. Caprio. 2009 "A Brief Discussion of Three Important Quality Management Concepts" Proceedings of the 2009 EPA Quality Management Conference, May.

J. Caprio. 2006, "Asking Appropriate Questions in Order to Assure Data Quality," in Proceedings of the 25<sup>th</sup> Annual Conference on Managing Environmental Quality Systems, April.



#### MARC LEISENRING, M.S., P.E.

Water Resources Engineering Environmental Data Analysis Water Quality Data Analysis and Modeling

#### **EDUCATION**

M.S., Civil and Environmental Engineering, Portland State University, 2011 B.S., Environmental Resources Engineering, Humboldt State University, 2001

#### **REGISTRATIONS AND CERTIFICATIONS**

Registered Civil Engineer, Oregon, 77895PE 8-Hour HAZWOPER Refresher, September 2011

#### CAREER SUMMARY

Mr. Leisenring has 12 years of water resources and urban stormwater quality project experience that includes a focus on urban hydrology, water quality, and best management practices (BMP) research and performance. Mr. Leisenring has a strong background in the analysis and summary of spatial and temporal environmental data with the ability to manage and query large databases and perform advanced statistical analysis techniques. He is an effective oral and written communicator, provides support and direction to junior staff, and strives to exceed the expectations of his clients and colleagues.

#### **PROJECT EXPERIENCE**

**International Stormwater BMP Database.** Water Environment Research Federation (WERF), American Society of Civil Engineers (ASCE), and U.S. EPA. In support of this ongoing project, Mr. Leisenring has been assisting in the analysis and summary of BMP performance information contained in the database. Both parametric and non-parametric data analysis techniques have been applied including robust regression on order statistics for estimating non-detects and bootstrap computations for estimating parameter variability. Recently, Mr. Leisenring has been investigating the effects of inflow concentrations on effluent quality for various BMP types and pollutant concentrations through the use of moving median smoothing techniques for reducing local variability while preserving overall data trends.

Agricultural BMP Database. Water Environment Research Federation (WERF) and the National Corn Growers Association (NCGA). Mr. Leisenring has been leading the design and development of an Agricultural BMP Database that will store water quality performance data for conservation practices. The database will be hosted on the International BMP Database website (www.bmpdatabase.org) and is anticipated to be ready for public use by January 2014.

Lake Tahoe Pollutant Load Reduction Model (PLRM). U.S. Army Corps of Engineers. Lake Tahoe is listed under Section 303(d) of the Clean Water Act for fine particulates (<20 um), nitrogen, and phosphorus, and the California Regional Water Quality Control Board and Nevada Department of

#### MARC LEISENRING, M.S., P.E.



Environmental Protection are currently collaborating on the multi-phase Total Maximum Daily Load (TMDL) program. As part of this effort, Geosyntec partnered with Northwest Hydraulic Consultants to develop the Pollutant Load Reduction Model (PLRM), a modeling tool for estimating pollutant load generation and reduction associated with source control activities and structural stormwater retrofit projects. Mr. Leisenring led the design and development of the PLRM, which is a custom interface and a Tahoe-specific backend database that interfaces directly with the U.S. EPA's SWMM5 model. The tool allows users to investigate the water quality and hydraulic effects of implementing a wide range of BMPs, including pollutant source controls, hydrologic source controls, and centralized treatment facilities.

*Critical Assessment of Stormwater Treatment and Control Selection Issues. Water Environment Research Federation (WERF).* Mr. Leisenring took the lead in the preparation of this guidance manual that examines critical factors that influence selection, sizing, and design of stormwater controls, or best management practices (BMPs), for specific locations and conditions. The manual is intended to assist stormwater managers in selection and prioritization of controls to meet the goals of protecting local receiving waters and other objectives in the most cost-effective manner possible.

Structural BMP Prioritization and Analysis Tool (SBPAT). Heal the Bay in partnership with the City and County of Los Angeles. The implementation of structural BMPs in developed areas of the Los Angeles region has been largely opportunistic and site-specific. Stormwater quality improvement projects have not strongly emphasized the selection of strategic treatment locations and BMP types based on specific water quality and watershed management goals. A technical project team led by Geosyntec and with representatives from Heal the Bay, the County of Los Angeles Department of Public Works, and the City of Los Angeles Bureau of Sanitation developed a GIS-based stormwater quality decision support tool that can be used to prioritize structural BMP retrofit projects and estimate the costs and load reductions associated with implementation. The SBPAT tool is intended to help watershed planners, stormwater managers, and stakeholders throughout Los Angeles County in conceptual planning of structural BMP retrofit projects and NPDES compliance assessments.

**PDX Phosphorus Study.** Port of Portland. Stormwater monitoring data collected at the Portland International Airport (PDX) and other surrounding properties owned and operated by the Port of Portland indicate that some of the discharge points to the Columbia Slough occasionally exceed the total phosphorus 1200-COLS NPDES permit benchmark of 0.16 mg/l. Mr. Leisenring led an assessment of the current and past land use activities, hydrology, and water quality at PDX to help identify potential sources of the high phosphorus concentrations. Water quality and flow data collected at numerous monitoring locations were statistically analyzed and discussed. The study found that the primary source of phosphorus in surface water discharges is likely regional groundwater. Additional monitoring has been recommended to further support this finding.

Stormwater Challenge - Linking BMP Performance to Receiving Water Protection. The Water Environment Research Foundation (WERF) has undertaken a project that includes the development of a modeling tool that will link existing watershed models to receiving water models such that stormwater management can be more directly linked to water quality impacts. For this ongoing project, Mr. Leisenring has been the technical lead in the development of BMP performance algorithms that account for the unit processes in various distributed and centralized treatment controls.



#### SONG S. QIAN, PH.D. Assistant Professor Department of Environmental Sciences The University of Toledo

Water Quality Modeling TMDL Development Nutrient Criterion Compliance Assessment Guidance Development Setting Environmental Standards Ecological Thresholds

#### **EDUCATION**

Ph.D., Environmental Sciences, Duke University, 1995
M.S., Statistics, Duke University, 1995
M.S., Environmental Systems Engineering, Nanjing University, 1988
B.S., Environmental Engineering, Tsinghua University, 1985

#### **CAREER SUMMARY**

Dr. Qian has been engaged in the research and practices of environmental and ecological statistics, water quality modeling and assessment, and ecological risk assessment for over 15 years. His work, including teaching, research, and consulting, is focused on environmental and ecological data analysis and modeling both for research and for environmental management. He has a long teaching career covering environmental science, water quality modeling and management, environmental and ecological data analysis and modeling, and risk assessment. Dr. Qian is known for his statistical skill reflected in his published textbook on environmental and ecological statistics and his upcoming book on the applications of bayesian hierarchical models in environmental and ecological studies. His work covers a wide range of environmental and ecological topics, including statistical issues in setting and evaluating the compliance of environmental standards, modeling phosphorus retention in the everglades wetlands, detecting and quantifying ecological thresholds, watershed modeling for Total Maximum Daily Load (TMDL) development, drinking water standard compliance study, effects of urbanization on stream ecosystem, environmental engineering, and various ecological topics. His research is focused on the development and adaptation of statistical modeling methods that are suitable for applied problems. He developed the bayesian hierarchical model for the U.S. EPA for assessing drinking water standard compliance; introduced the "hockey stick" model as a tool for developing numerical phosphorus criterion for the everglades; applied the seasonal trend analysis using loess for assessing long term trends in nutrient concentrations in the Neuse River Basin; developed the Bayesian SPARROW model, introduced the multilevel models to study the effects of urbanization on stream ecosystem; introduced the use of the change point model as a tool for nutrient criterion development; and introduced the use of several advanced statistical tools (such

Song S. Qian, Ph.D. Page 2



as multinomial regression, zero-inflated regression) for analyzing species compositional data. Dr. Qian has published over 60 peer-reviewed journal articles, and numerous book chapters and conference presentations.

#### **PROJECT EXPERIENCE**

*Environmental and Ecological Modeling* Dr. Qian is a leading expert and practitioner in environmental and ecological data analysis and modeling. Dr. Qian has supported several U.S. EPA-Office of Ground Water and Drinking Water (OGWDW) work assignments in developing statistical models supporting SDWA assessment of conventional and microbial pollutants. Dr. Qian served as Principal Investigator (PI) on several EPA-STAR (developing methods for quantifying ecological thresholds, a Bayesian SPARROW model, and performance assessment of TMDL) and U.S. Geological Survey (USGS) (developing methods for assessing the effects of urbanization on stream ecosystems) grants. These projects were focused on risk assessment.

*Environmental and Ecological Statistics* Dr. Qian is a respected environmental statistician with over 17 years of experience in teaching and research. His textbook on environmental and ecological statistics is widely used and highly praised.

*Ecological Threshold* Dr. Qian has published several papers on the use of statistical change point and "hockey stick" models for quantifying ecological thresholds for setting environmental standards. His papers were widely used by states in setting their nutrient criteria.

*Environmental Education* Dr. Qian has 10 years teaching experience at Portland State University, Duke University, and the University of Toledo, including graduate level courses in water quality management and modeling, uncertainty analysis of environmental models, environmental and ecological statistics, and advanced statistical modeling, and undergraduate courses in biodiversity, environmental sciences and environmental processes. Dr. Qian has advised over 15 master students, (co-) advised 4 Ph.D. students, and served on over 10 Ph.D. dissertation committees.

*Bayesian Hierarchical Modeling* Dr. Qian has recently evolved as a leading authority on the application of Bayesian hierarchical models in environmental and ecological studies. He is the editor of the Wiley book series on environmental and ecological data analysis and modeling and the author of the inaugural volume of the series on hierarchical/multilevel models.

*International Experience* Dr. Qian has collaborated with researchers from Finland, China, and India on various research topics. These collaborations resulted in four journal papers, including a paper on assessing China's drinking water source water quality published in ES&T. Dr. Qian served as external reviewers on two Ph.D. committees in an Indian university.

*Outreach and Technology Transfer* Dr. Qian is an active publisher, has authored/co-authored more than 50 scientific papers and more than 20 proceedings and book chapters. He has made more than 20 presentations and led technical workshops on ecological threshold for the Department of the Interior (DOI), the National Park Service (NPS) and the USGS.

Song S. Qian, Ph.D. Page 3



*Synergetic Activities* Dr. Qian serves as subject (statistics) editor of the Cambridge University Press journal *Tree Physiology*, and associate editor of the *Journal of American Water Resources Association*.

#### PUBLICATIONS

Qian, S.S., 2014. Ecological Threshold and Environmental Management; A Note on Statistical Methods for Detecting Thresholds, Ecological Indicators, 38:192-197.

Qian, S.S. and T. Cuffney, 2012. To threshold or not to threshold? That is the question. Ecological Indicators, 15:1-9.

Wu, R., S.S. Qian, F. Hao, H. Chen, D. Zhu, and J. Zhang, 2011. Modeling contaminant concentration distribution in China's centralized source waters, Environmental Science and Technology, 45:6041-6048, 2011.

Qian, S.S., Cuffney, T., Alameddine, I., McMahon, G., and Reckhow, K. 2010. On the Application of Multilevel Modeling in Environmental and Ecological Studies. Ecology, 91, 355-361

Qian, S.S., 2010. Environmental and Ecological Statistics with R. Chapman and Hall/CRC Press.

Qian, S.S. and Shen, Z. 2007. Ecological applications of multilevel analysis of variance, Ecology 8: 2489–2495

Qian, S.S., K.H. Reckhow, J. Zhai, G. McMahon, 2005. Nonlinear regression modeling of nutrient loads in streams - a Bayesian approach Water Resources Research, 41(7):W07012.

Qian, S.S., A. Schulman, J. Koplos, A. Kotros, and P. Kellar, (2004). A Hierarchical Modelling Approach for Estimating National Distributions of Chemicals in Public Drinking Water Systems. Environmental Science and Technology, 38:1176-1182.

Qian, S.S., C. Stow, and M. Borsuk, 2003. On Bayesian Inference using Monte Carlo Simulation, Ecological Modelling, 159:269-277.

Qian, S.S., R. King, and C.J. Richardson. "Two Statistical Methods for the Detection of Environmental Thresholds" Ecological Modelling, 166:87-97, 2003.


### **ROBERT L. ANNEAR, PH.D., P.E.**

Water Quality and Hydrodynamic Modeling Sediment Transport Modeling Fate and Transport Modeling Expert Consultation

### **EDUCATION**

Ph.D., Portland State University, Civil and Environmental Engineering, 2007M.S., Portland State University, Civil Engineering, 1997B.S., Boston University, Aerospace Engineering, 1993

## **REGISTRATIONS AND CERTIFICATIONS**

Environmental Engineer (P.E.), OR: 53757; ID: 14190; WA: 46812; FL: 71806 Graduate Certificate in Hydrology

# CAREER SUMMARY

As a water resources engineer, Dr. Annear is principally involved in hydrodynamic and water quality modeling with a focus on regulatory permits and requirements, stormwater management, surface water system assessments, Total Maximum Daily Loads (TMDL) development and implementation, Endangered Species Act (ESA), nutrient criteria studies, and water quality management for multiple uses (supply, salmon, recreation etc.). He has over 16 years of experience in the development and calibration of hydrodynamic and water quality models (1-D, 2-D, and 3-D) throughout the U.S. His experience includes reviewing 2-D hydrodynamic and sediment transport and fate & transport models of riverine and estuarine systems. He has also served as an Expert Witness in cases involving hydrology; water rights; and hydrodynamic, sediment transport and chemical fate and transport modeling. He is Affiliated Faculty at Portland State University, teaching undergraduate and graduate courses and has more than 10 years of experience teaching water quality modeling training workshops. Dr. Annear has considerable experience in leading multidisciplinary teams of professionals, managing projects, budgets, work flow processes, quality control and assurance, on call contracts and complex project implementation and developing monitoring plans and conducting field work. He has conducted numerous peer reviews of surface water models for agencies such as the U.S. EPA, Oregon DEO, WA Dept. of Ecology, and the U.S. Bureau of Reclamation and has also served as a reviewer for various water resource and hydrologic journals and has served on EPA national water quality grant review panels.

## **PROJECT EXPERIENCE**

*Ecosystem Threshold Technical Review*, *Barnes & Thornburg LLP*, *Senior Engineer*. Conducted a technical review of the State of Connecticut's methodology to establish phosphorus limits for publicly owned treatment works. The technical review focus on an analytical technique for identifying ecosystem thresholds along environmental gradients such as increased phosphorus concentrations on algal populations and diversity. Provided the client with list of issues, cited from literature, regarding the technical validity and regulatory applicability of the methodology.

Barney Reservoir Water Quality Data Analysis and Management, Barney Reservoir Joint Ownership

Robert L. Annear, Ph.D., P.E. Page 2



*Commission, Project Manager.* Currently conducting a detailed data analysis of water quality data collected in the reservoir over the last few years to better understand the lake limnology, seasonal trends and possible triggers for algal blooms. Conducting a critical review of the water quality database and made recommendations for database structure improvements to better meet the Commission's needs.

*Lake Chaplain*, *City of Everett, Washington. Project Manager.* Developed and calibrated a 2-D hydrodynamic and water quality model (CE-QUAL-W2) for Lake Chaplain in Northwestern Washington for a 3 year time window. Developed long term (10 year) simulations and explored impacts of withdrawal operational changes on reservoir water quality.

*Water Quality Modeling Workshop Training and Technical Support, US Army Corps of Engineers (Portland and Walla Walla District). Project Manager.* Developed curriculum for and lead a 3-day workshop on use of the 2-D hydrodynamic and water quality model, CE-QUAL-W2. The workshop included hydrodynamic and water quality modeling theory; the mechanics of using the model; recently added algorithms; and modeling studies best practices including: developing scopes and budgets, documentation, model selection, conducting model peer reviews and technical considerations for modeling projects.

*Watershed Model Update and Plan Development, Collier County, Florida. Senior Water Quality Modeler.* Provided guidance and expertise on the data collection and sampling needed to support water quality model development to meet regulatory requirements for improving storm water runoff and nutrient loading to surface and coastal waters.

**Barney Reservoir Monitoring Plan**, Joint Water Commission, City of Hillsboro, Water Department, Oregon, Project Lead. Developed a Water Quality Monitoring Plan to address multiple management objectives in a drinking water supply reservoir in Washington County, OR. Water Department staff is currently using the plan to conduct regular monitoring in the reservoir.

**DeSabla-Centerville System Temperature Model,** Pacific Gas & Electric, Consultant, Senior Modeler. Developed and calibrated a hydrodynamic and temperature model of the West Branch Feather River system including Pacific Gas & Electric hydropower facilities in CA. The model was then used to run management scenarios to improve stream temperatures in the system as part of their FERC relicensing environmental assessment and impact.

*James River, Virginia*, *MapTech, Inc., Expert Modeler*. Provided on call services to assist with the development of a 2-D hydrodynamic and water quality model (CE-QUAL-W2) of the James River in VA, including riverine and estuarine reaches.

*Kinnickinnic River, St. Paul, Minnesota, Bonestroo, Rosene, Anderlik, and Associates, Modeler.* Conducted a review of CE-QUAL-W2 application to sections of the Kinnickinnic River near the City of River Falls, WI. The model review consisted of reviewing the model files and conducting analyses to assist with debugging the model results and verifying the analyses already conducted. Recommendations on model improvements and future work for long-term development of the model.

*Willamette River main stem Temperature TMDL*, U.S. Army Corps of Engineers, Portland, Oregon and Oregon Department of Environmental Quality. Developed a 2-D hydrodynamic and water quality model (CE-QUAL-W2) of the Willamette River main stem system and part of the Lower Columbia River. The model was used by the Oregon Department of Environmental Quality to develop temperature load allocations for the Willamette River main stem system as part of the Temperature TMDL plan.



consultants

### **RANDY CRAWFORD, M.A.**

Water Quality Assessment Aquatic Biology

# **EDUCATION**

M.A., Biology, Aquatic Biology, Truman State University (formerly Northeast Missouri State University), 1976B.S.E., Biology, Truman State University (formerly Northeast Missouri State University), 1972

# **CAREER SUMMARY**

Mr. Crawford has more than 30 years of experience conducting and managing water quality monitoring assignments throughout Missouri, including Big River Experience. Prior to joining Geosyntec Consultants, Mr. Crawford managed a group of 15 environmental professionals and technicians responsible for all the Missouri Department of Natural Resources (MDNR) water quality monitoring. Since joining the firm, he has successfully managed some of Geosyntec's most intense data collection efforts.

# **PROJECT EXPERIENCE**

*Use Attainability Analyses for Missouri Department of Natural Resources, Multiple Sites.* Mr. Crawford successfully managed one of Geosyntec's most intense data collection efforts. During the recreational seasons (April –October) of 2007 and 2008, Mr. Crawford managed up to six two person crews conducting aquatic life and habitat assessments and recreational use attainability analyses at over 200 stream segments and more than 1800 miles of Missouri streams including extreme southwest Missouri, southeast Missouri, the Bootheel region, and extreme north central Missouri. All data was compiled, validated, and entered into a database for presentation to the state within the time constraints allotted.

*Kansas City Stormwater Utility Division Macroinvertebdrate Monitoring* As part of the Kansas City (KC) Stormwater Utility Division (SUD) compliance with the KCMO Municipal Separate Storm Sewer System (MS4) Permit, biological and water quality data must be monitored on headwater streams receiving MS4 discharges. Mr. Crawford led a team of Geosyntec biologists that collected aquatic macroinvertebrate samples and conducted habitat quality assessments at eight small urban streams within the MS4 jurisdiction of the City of Kansas City, Missouri. Data from these sites were compared to data collected during the same time period from three control streams, picked due to their similar size and proximity to the urban (MS4) sites. The assessment followed Missouri Department of Natural Resources (MDNR) protocols and included physical habitat characterization and aquatic macroinvertebrate evaluations. Biological data collected from each survey component formulated the basis of relative comparisons of biological community health and habitat quality in the study area. Assessment results were combined with surface water quality data and provided in a technical report for the KC SUD.

Randy Crawford, M.A. Page 2



*Metropolitan St. Louis Sewer District Water Monitoring Project.* As part of a major water quality monitoring effort to assist one of the largest metropolitan sewer districts in the nation with their program of infrastructure and capital improvements, Mr. Crawford has coordinated an intensive monitoring program that encompasses baseflow and stormflow monitoring of urban streams and two of the nations largest rivers. The baseline information collected will assist the district in developing strategies related to stormwater issues such as E. coli in streams within the district and help in the determination the effectiveness of Sanitary Sewer Overflow (SSO) and Combined Sewer Overflow (CSO) projects in controlling contaminants entering waters in the district. Mr. Crawford has successfully coordinated monitoring efforts on the six urban streams, and the Mississippi and Missouri Rivers that require specialized equipment and sampling techniques to insure that samples are collected. Sampling for trace metals requiring clean samping techniques is performed on samples at all locations. Following laboratory analysis, data are validated and entered into a dabase developed by MEC Water to accommodate the nearly 10,000 data results that are collected each year.

*Missouri Department of Natural Resources, Water Quality Monitoring Section.* As a Supervisor of Water Quality Monitoring Section (WQMS) Mr. Crawford was responsible for overseeing the activities and personnel of a Section that provides sampling and analytical support for various programs within the department as well as technical assistance to other agencies and organizations outside of the department. Under his leadership, the WQMS developed and implemented numerical biological criteria for wadeable Missouri streams using aquatic macroinvertebrates. This ongoing program will eventually be expanded to include other stream orders and will ultimately be incorporated into the Missouri Water Quality Standards. Other activities of the Section included compliance/enforcement monitoring of NPDES permitted facilities, pretreatment monitoring of industries, groundwater monitoring, water quality investigations of lakes and streams, fish tissue contaminants, technical assistance for whole-effluent toxicity testing and toxicity identification evaluations, bioassessments, volunteer monitoring, spill response, and a variety of environmental education programs.

*Hinkson Creek Phased Water Quality Investigation.* Mr. Crawford led members of his section in performing a multi-year investigation of an urban stream in Columbia, Missouri that had been placed on the Federal (303d) Impaired Waters List for unknown toxicity. Using a water quality triad approach, his team investigated the aquatic macroinvertebrate community, stormwater and instream toxicity testing and chemical analyses to investigate and pinpoint problem areas within the Hinkson Creek watershed. This high profile investigation resulted in over ten public and targeted meetings with highly diverse groups, and numerous media contacts. The results of this investigation outlined a successful approach for evaluating waters impaired by unknown pollutants.



Steven R. Layman, Ph.D.

Aquatic Ecology and Fisheries Biology Environmental Permitting and Compliance National Environmental Policy Act (NEPA) Assessment Rare, Threatened, and Endangered Species

# **EDUCATION**

Ph.D., Biological Sciences, University of Alabama, Tuscaloosa, 1994M.S., Ecology, University of Tennessee, Knoxville, 1984B.S., Biology, Bucknell University, Lewisburg, PA, 1981

# **CAREER SUMMARY**

Dr. Layman is a technology leader in applying fish biology, aquatic ecology, and ecosystem management principles to water resource projects in eastern North America. He has 22 years' experience leading ecological assessments and managing project delivery for Clean Water Act (CWA) permitting, National Environmental Policy Act (NEPA) review, facility siting and permitting, Federal Energy Regulatory Commission (FERC) hydropower licensing, and Endangered Species Act (ESA) compliance. He works collaboratively with integrated teams of client personnel, engineers, biologists, planners, and attorneys to achieve compliance objectives in a cost-effective manner while minimizing risks and meeting the expectations of regulatory agencies. Dr. Layman also assists companies in assessing water use and risks relative to facility/site operations and in documenting management strategies that promote water resource sustainability.

# **PROJECT EXPERIENCE**

*Comments on U.S. EPA Proposed Rule, Confidential Manufacturer.* Assisted a manufacturing client in preparing written comments in response to U.S. EPA's proposed CWA Section 316(b) rulemaking issued April 2011 for cooling water intake structures at existing power generating facilities and existing manufacturing and industrial facilities. Coordinated closely with the client's corporate regulatory specialist and individual facility compliance leads to address proposed regulations having the greatest impact on facility operations located in several states.

*Source Waterbody Classification White Paper, South Carolina Electric & Gas Company.* Prepared a white paper for South Carolina Electric & Gas Company (SCE&G) presenting technical arguments for reclassifying the cooling source waterbody of a 650-MW coal-fired power plant. The state permitting agency and U.S. EPA reviewed the paper and approved of reclassifying the source waterbody, thereby eliminating fish entrainment reduction as a concern. Prepared the Proposal for Information Collection and evaluated alternative best technology available (BTA), including Ristroph screens, barrier net, operational measures, and restoration, to support strategic compliance decisions. Steven R. Layman, Ph.D. Page 2



*Watershed Assessment and Modeling Project, Gwinnett County, Georgia.* Served as Assistant Project Manager and Biological Task Lead for Gwinnett County, Georgia's Watershed Assessment and Modeling Project. Led studies assessing the health of streams in the Chattahoochee, Ocmulgee, and Oconee River basins with respect to water quality, biotic integrity, and primary causes of stream impairment. This information supported modeling efforts examining options for future watershed protection strategies and development of the County's watershed management plan.

*Wastewater Discharge Evaluation, Paperboard Company, Connecticut.* For a paperboard company in Connecticut, evaluated whether certain constituents in the treated wastewater discharge from a recycled paperboard mill could interfere with the migratory behavior of anadromous American shad and river herring when diverted to the Shetucket River in the Thames River basin. Review of the scientific and technical literature determined that metals concentrations reported to disrupt olfaction and migratory behavior in anadromous fishes exceeded those anticipated in the Zone of Influence.

*EA's and EIS's, FERC, Multiple States.* Assisted FERC staff in preparing EAs and multipleproject EISs for 16 hydroelectric projects in Wisconsin, Michigan, New York, South Carolina, and Georgia. Assessed complex fisheries' issues related to turbine-induced mortality, downstream fish protection, upstream passage, and instream flow needs. Participated in NEPA scoping and resource agency 10(j) negotiations.

**Biology Sampling Programs,** Multiple Utilities, Multiple States. Designed and led biology sampling programs to support Exhibit E for several hydroelectric projects. Assessed the effects of fluctuating water levels on age and growth of game fish in four Wisconsin River impoundments; directed fisheries sampling on streams and lakes in Michigan's Upper Peninsula; and managed seasonal studies of fish, wildlife, and botanical resources for a proposed pumped storage facility in Georgia.

**Tri-State Water Allocation Environmental Impact Statements,** U.S. Army Corps of Engineers (USACE), Mobile District, Georgia, Alabama, Florida. Served as biological task lead for NEPA review of interstate water allocation agreements being negotiated for the Apalachicola-Chattahoochee-Flint (ACF) and Alabama-Coosa-Tallapoosa (ACT) river basins. Coordinated with the USACE and U.S. Fish and Wildlife Service (USFWS) in preparing the fisheries and aquatic resource sections of the Draft Environmental Impact Statements (EISs) for both basins.

**Tri-State Water Allocation Biological Assessment,** U.S. Army Corps of Engineers, Mobile District, Georgia, Alabama, Florida. Led preparation of the supporting information for the Biological Assessments (BAs) being prepared by the USACE for ACF and ACT basin water allocation under the ESA. Evaluated current and historical distribution for Federally-listed species to identify the species most likely to be affected by changes in water management, and assessed the potential for impacts to these species to assist the USFWS in making final determinations of potential effect.



DANIEL V. OBRECHT, M.S. Senior Research Associate, Limnology Laboratory Department of Fisheries and Wildlife University of Missouri

Aquatic Ecologist Field Sampling Expert Algae Sampling Design Algae Data Analysis

## **EDUCATION**

M.S., University of Missouri, Fisheries and Wildlife, 2010 B.S., University of Missouri, Fisheries and Wildlife, 1993

# **CAREER SUMMARY**

Daniel Obrecht has worked for the University of Missouri limnology laboratory for over 23 years. During this time he has garnered experience in many aspects of water quality monitoring, including the design of monitoring programs, implementation of quality control and assurance measures, data analyses, and report writing. Mr. Obrecht focuses his research on limnology and aquatic ecology processes. He has conducted research to quantify factors regulating abundance and distribution of algal biomass in freshwater systems and to determine the relationship between nutrients and algal biomass in lakes and reservoirs. His research has also included a long-term study showing the trophic state of Missouri reservoirs reflects the physiography and human alteration of their drainage basins. He also conducted research examining the proportion of cropland cover in the catchment of artificial lakes along with metrics of morphology and hydrology account for much of the among-system variation in both phosphorus and nitrogen. From October 2005 through March 2008 Mr. Obrecht took part in the development of Missouri's nutrient criteria for reservoirs. He served initially as a stakeholder and then as a member of the scientific committee that fashioned Missouri's proposed approach. Daniel ended up being the lead author on the rationale for Missouri's approach which was presented to Missouri's Clean Water Commission and submitted to EPA

# PROFESSIONAL EXPERIENCE

Senior Research Assistant/Associate - University of Missouri. Mr. Obrecht oversees the day to day operations of the Limnology Laboratory including field and laboratory techniques training, research project development and implementation, data management and analyses, and public outreach, May 1997- present.

*Coordinator of Lakes of Missouri Volunteer Program*. Mr. Obrecht identifies and trains volunteers in lake sampling and water processing, sample analysis, data management, statistical and graphical analysis, report writing, and data presentation as well as maintaining

Daniel V. Obrecht, M.S. Page 2



correspondence with volunteers. Program Coordinator Jan. 1992- May 1997; Co-coordinator May 1997- present.

*Volunteer for International Union for Conservation of Nature (IUCN)* in Nepal. Mr. Obrecht trained IUCN personnel and assisted in water sampling, laboratory analysis and in situ experimentation. He also surveyed water bodies and worked on a crocodile restoration project. Fall 1993

*Laboratory Technician at Limnology Laboratory, University of Missouri, Columbia (UMC).* Mr. Obrecht processed water samples and conducted analysis for the following parameters: total nitrogen, total dissolved nitrogen, nitrate/nitrite nitrogen, ammonia nitrogen, total phosphorus, total dissolved phosphorus, soluble reactive phosphorus, alkalinity, chloride, calcium, magnesium, potassium, sodium, carbonate/bicarbonate, chlorophyll, total suspended solids, alkaline phosphatase activity, conductivity and turbidity. 1991-1992

# PUBLICATIONS

Jones, J.R., Daniel V. Obrecht and Anthony P. Thorpe. 2011. Chlorophyll maxima and chlorophyll: total phosphorus ratios in Missouri reservoirs. Lake and Reservoir Management. 27:321-328.

Jones, J.R., Matthew F. Knowlton, Daniel V. Obrecht and Jennifer L. Graham. 2011. Temperature and oxygen in Missouri reservoirs. Lake and Reservoir Management. 27:173-182.

Jones, J.R., M.K. Knowlton, D.V. Obrecht, A.P. Thorpe and J.D. Harlan. 2009. Role of contemporary and historic vegetation on nutrients in Missouri reservoirs: implications for developing nutrient criteria. Lake and Reservoir Management. 25:111-118.

Jones, J.R., B.D. Perkins, D.V. Obrecht, M.F. Knowlton, A.P. Thorpe, S. Watanabe and R.R. Bacon. 2008. Nutrients, seston and transparency of Missouri reservoirs and oxbow lakes: an analysis of regional limnology. Lake and Reservoir Management. 24:155-180.

Jones, J.R., M.K. Knowlton and D.V. Obrecht. 2008. Role of land cover and hydrology in determining nutrients in mid-continent reservoirs: implications for nutrient criteria and management. Lake and Reservoir Management. Vol. 24:1-9.

Obrecht, D.V., A.P. Thorpe and J.R. Jones. 2005. Response in the James River Arm of Table Rock Lake, Missouri (USA) to point source phosphorus reduction. Verh. Internat. Verein. Limnol. 29:1043-1048.

Jones, J.R., M.F. Knowlton, D.V. Obrecht and E.A. Cook. 2004. Importance of landscape variables and morphology on nutrients in Missouri reservoirs. Can. J. Fish. Aquat. Sci. 61:1503-1512.



### ANN L ST. AMAND, PH.D., CLP President & Chief Scientist PhycoTech, Inc.

Algal Species Analysis for Freshwater Systems Zooplankton, Macroinvertebrates and Bacteria Analyses Head of Research

### **EDUCATION**

- Ph.D. University of Notre Dame, Notre Dame, Indiana. Aquatic Biology Program. Defense: April 12, 1990. Dissertation: Mechanisms Controlling Metalimnetic Communities and the Importance of Metalimnetic Phytoplankton to Whole Lake Primary Productivity.
- B.S. Purdue University, West Lafayette, Indiana. Ecology, Evolutionary, and Population Biology. 1984.

#### **REGISTRATIONS AND CERTIFICATIONS**

Phycologist & Certified Lake Professional (2003 to present) North American Lake Management Society

#### CAREER SUMMARY

Ann St. Amand, President of PhycoTech Inc in St. Joseph, Michigan coordinates Part 10000 Biological Examination of Standard Methods. Ann has been involved in managing lakes across the United States since 1990, specializing in aquatic sample analysis with an emphasis on freshwater phytoplankton, periphyton and zooplankton. She has processed over 33,000 freshwater and marine aquatic samples in her career and has co-chaired a workshop on freshwater algal identification at the annual NALMS symposium since 1991. She also serves on several technical and educational committees at the local and national level, including the Indiana Blue-Green Algal Task Force, and the NALMS Blue-Green Initiative. In addition, she completed two postdoctoral positions, one in surface water/groundwater interactions and the other in PCB interactions in stream systems.

### **PROJECT EXPERIENCE**

**President. PhycoTech, Inc.** 1990-Present. St. Joseph, Michigan. Provide identification and enumeration of suspended and attached algal, zooplankton and bacterial samples utilizing a unique, permanent mounting technique. Also provide photographic, statistical and interpretive services involving algal samples and ecological data.

**Research Associate. University of Notre Dame.** 1991-1995. Department of Civil Engineering/ Geological Sciences. Involved in project relating composition and biomass of periphytic biolayer in artificial stream ecosystems to PCB transfer within stream sediments.

**Research Associate. University of Notre Dame.** 1989-1991. Department of Civil Engineering. Involved in project relating groundwater quality to surface water quality including preliminary data acquisition and grant submission. Also involved in data analysis for a collaborative project on the environmental effects of oil-field brine application for road maintenance.

Ann L. St. Armand, Ph.D., CLP Page 2



**Faculty. Practicum in Aquatic Ecology, University of Notre Dame Environmental Research Center**. June 1990. Taught limnology section of summer field course.

**Research Assistant. University of Notre Dame**. 1988-1989. Identified and enumerated phytoplankton samples from three northern Wisconsin lakes.

Teaching Assistant. University of Notre Dame. 1985-1988.

**Field Intern. The Nature Conservancy (Indiana Chapter)**. November 1984-January 1985. Habitat management and landowner responsibilities within wetland and prairie habitats.

#### PUBLICATIONS

St. Amand AL, Roefer P, LaBounty JF, Tietjen T, Bolt D. 2012. Response of the algal community in Boulder Basin, Lake Mead to the introduction of Quagga Mussels and reduced water levels. Journal of Lake Reservoir. Management. In preparation.

Roy, A., Rhea, L., Mayer, A., Shuster, W., Beaulieu, J., Hopton, M., Morrison, M., and St. Amand, A. 2012. Responses of water quality and stream biota to retrofit stormwater management in a suburban neighborhood. Freshwater Biology. Submitted for review.

Bunting, L., P.R. Leavitt, B. Wissel, M.D. Graham, K.R. Laird, A. St. Amand, B.J. Hann, and D.R. Engstrom. 2012. Eutrophication of the north basin of Lake Winnipeg, Canada. Final report to Manitoba Conservation and Water Stewardship, and Environment Canada Lake Winnipeg Basin Stewardship Program, April 2012. 46 pp.

St Amand, A.L. 2011. How Algae Fit Into Food Webs. LakeLine. 31(2): 12-18.

St Amand, A.L. 2010. Chapter 7: Chlorophyta. AWWA Algae: Source to Treatment, Algal Manual M57. pp. 147-166.

### PRESENTATIONS

St. Amand, A: 2011, 2012, and 2013 Collection, Identification and Ecology of Freshwater Algae, PhycoTech, Inc. (2-day workshop)

St. Amand, A: 2012, and 2013 Collection, Identification and Ecology of Freshwater Algae Advanced Course including Blue-Greens and toxin producers, PhycoTech, Inc. (3-day workshop)

St. Amand, A, Wagner, K, Rosen, B, Chapman, A: 2012, Collection, Identification and Ecology of Freshwater Algae Advanced Course including Blue-Greens and toxin producers. Annual Meeting of the North American Lake Management Society

St. Amand, A 2012 Identification Ecology and Control of Nuisance Freshwater Algae in Illinois lakes and streams. Illinois Lake Management Association

St. Amand, A, Peter Leavitt, Joseph Eilers, and Linda Bunting (co-authors) 2011, An Introduction to Microscopy in Paleolimnology: Opportunities and Challenges for Using Soft Algae in Addition to Diatom and Chrysophyte Biomarkers. Annual Meeting of the North American Lake Management Society.



YANGDONG PAN, PH.D. Professor/Chair Department of Environmental Science and Management Portland State University, Oregon

> Algae Composition Dynamics Ecosystem Response to Nutrients Field Data Collection and Interpretation

## **CAREER SUMMARY**

Dr. Pan chairs the Department of Environmental Science and Management at Portland State University. His research centers on water resource science and conservation. Specifically he uses algal assemblages to monitor and assess ecological risk in freshwater ecosystems including both lotic and lentic systems. He and his associates have participated in several national surface water quality programs such as the US EPA's Environmental Monitoring and Assessment Programs (EMAP) in the Mid-Atlantic Region and in the western USA with a leading role on algal indicators development. Recently, he has been collaborating with Chinese environmental professionals on several water-quality projects in the Yangtze Delta region including drinking water protection for the city of Shanghai. He teaches two graduate-level courses on univariate and multivariate environmental and biological data analysis at PSU.

## **EDUCATION**

Ph.D., Biology, Bowling Green State University, Ohio, USA, 1993

Dissertation Title: The Effects of Nutrients on Periphyton (1993) Committee Chair:

Dr. Rex L. Lowe

M. S., Biology, Southern Illinois University, USA, 1988

B. S., Biology, Hangzhou Teachers College, Hangzhou, China, 1983

## **PROJECT EXPERIENCE**

**Algae Biomonitoring and Assessment of Central California Coast Watersheds**, California State University Monterey Bay, 2007-2012.

Potential Effects of Pulp and Paper Mill Effluent on Periphyton, NCASI, 2002-2012.

Pacific Northwest (PNW) Algal Sample Analysis, US EPA, 2009.

Reservoir Sediment Diatom Analysis, USGS, 2007.

**Phase I Biological Characterization of Stormwater Detention Facilities**, Clackamas County, Oregon, 2007-2008.

Algal Community Analysis, USGS, 2004-7.

YANGDONG PAN, PH.D. Page 2



**Processing, Identification, and Enumeration of Benthic Diatom Samples from the Pajaro River Watershed, California, USA**, University of California at Santa Cruz, 2006-2008.

**A Biologically Driven National Classification Scheme for US Streams and Rivers**, US EPA, 2002-2006.

**Evaluation of Periphyton-Environmental Gradients in Western Streams**, US EPA, 2001-2007.

Periphyton in Oregon Headwater Streams, USFS, 2003.

Yakima Basin NAWQA Data Analysis, USGS, 2002.

Periphyton Analysis, USGS, 2002.

**A Nonparametric Bayesian Approach for Quantifying Herbicide Exposure in Streams**, US EPA, 1999-2001.

**Determining the Chronic Effects of Individual and Mixture Herbicides on Fish and Periphyton**, Oregon Department of Transportation, 2000-2001.

**Toxicity Tests of Bromacil, Diuron, Glyphosate, and Sulfometuron in Mixtures Using Rainbow Trout and Algal Assemblages**, Oregon Department of Transportation, 1999-2000.

Enumeration and Identification of EMAP-SW Periphyton Samples, US EPA, 1998-1999.

## PUBLICATIONS

Chang, H., I. W. Jung, A. Strecker, D. Wise, M. Lafrenz, V. Shandas, A. Yeakley, Y. Pan, R. Bean, G. Johnson, and M. Psaris. 2013. Water supply, demand, and quality indicators for assessing the spatial distribution of water resource vulnerability in the Columbia River basin. Atmosphere-Ocean 51(4): 339-356.

Zhu, W., Y. Pan, J. Tao, X. Li, X. Xu, Y. Wang, and Q. Wang. 2013. Phytoplankton assemblages and their succession in a newly man-made shallow lake, Shanghai, China. Aquatic Ecology 47: 137-147.

Zhang, J., W. Ni, Y. Zhu, and Y. Pan. 2013. Effects of different nitrogen species on sensitivity and photosynthetic stress of three common freshwater diatoms. Aquatic Ecology 47: 25-35.

Gillett, N. Y. Pan, K. M. Manoylov, R. Stancheva, and C. L. Weilhoefer. 2011. The potential indicator value of rare taxa richness in diatom-based stream bioassessment. Journal of Phycology 47: 471-482.

Gillett, N. Y. Pan, K. M. Manoylov, and R. J. Stevenson. 2011. The role of live diatoms in bioassessment: a large scale study of Western US streams. Hydrobiologia 665:79–92

Stevenson, R. J., Y. Pan, and H. van Dam. 2010. Assessing environmental conditions in rivers and streams with diatoms. Pages 57-85, in Smol, J. P. & E. F. Stoermer (eds), The Diatoms: Applications for the Environmental and Earth Sciences. 2nd edition. Cambridge University Press, Cambridge.

YANGDONG PAN, PH.D. Page 3



Gillett, N. Y. Pan, C. Parker. 2009. Should only live diatoms be used in the bioassessment of small mountain streams? Hydrobiologia 620:135-147.

Stevenson, R. J., Y. Pan, K. Manoylov, C. Parker, D. Larsen, and A. Herlihy. 2008. Development of diatom indicators of ecological condition for streams of the Western United States. Journal of North American Benthological Society 27:1000-1016.

Weilhoefer, C. L. and Y. Pan. 2008. Using change-point analysis and weighted averaging approaches to explore the relationships between common benthic diatoms and in-stream environmental variables in Mid-Atlantic Highlands streams, USA. Hydrobiologia 614:259-274. Weilhoefer, C.L. and Y. Pan. 2007. A comparison of periphyton assemblages generated by two sampling protocols. Journal of North American Benthological Society 26:308-318.

Qian, S.S, and Pan, Y. 2006. Historical soil total phosphorus concentration in the Everglades. In Burk, A.R. (Ed), Focus on Ecological Research. Nova Science Publishers, New York., pp131-150.

Pan, Y., B. H. Hill, P. Husby, R. K. Hall, and P. R. Kaufmann. 2006. Relationships between environmental variables and benthic diatom assemblages in California Central Valley streams, USA. Hydrobiologia 561: 119-130.

Walker, C. and Y. Pan. 2006. Using diatom assemblages to assess urban stream conditions. Hydrobiologia 561: 179-189.

Weilhoefer, C.L. and Y. Pan. 2006. Diatom assemblages and their associations with environmental variables in Oregon coastal streams, USA. Hydrobiologia 561: 207-219.

Pan, Y., A. T. Herlihy, P. R. Kaufmann, J. Wigington, J. Van Sickle, and T. Moser. 2004. Linkages among land-use, water quality, physical habitat conditions, and lotic diatom assemblages: A multi-spatial scale assessment. Hydrobiologia 515: 59-73.

Qian, S. S., Y. Pan, and R. S. King. 2004. Soil total phosphorus threshold in the Everglades: a Bayesian changepoint analysis for multinomial response data. Ecological Indicators 4:29-37.

Pan, Y., R. J. Stevenson, B. Hill, A. Herlihy, and G. Collins. 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. Journal of North American Benthological Society 15:481-495.