BAYLOR
UNIVERSITY

27 December 2013

TO: Members of the *Oklahoma Scenic Rivers Joint Phosphorus Criteria Study* Committee:
FROM: Ryan S. King, Ph.D. Associate Professor of Biology, Baylor University
RE: Request for Statement of Qualifications

I am writing in response to the Request for Statement of Qualifications pertaining to the Oklahoma Scenic Rivers Joint Phosphorus Criteria Study. My research team is uniquely qualified to lead this study. . Here, I briefly outline our relevant qualifications.

- I have authored or coauthored 40 peer-reviewed papers or reports that focus explicitly on the nutrient criteria development or effects of nutrient enrichment on freshwater ecosystems (Appendix 1).
- I have led two previous field studies in Texas, one funded by US EPA Region 6, the other by Texas Commission on Enviromental Quality, specifically designed to identify levels of nitrogen and phosphorus in wadeable streams and rivers that were associated with undesirable changes in the natural species composition of algae and fishes and sharp, nonlinear increases in the frequency and biomass of filamentous, nuisance algae (King et al. 2009a; King et al. 2009b). Most recently, we published some of the data from these studies in *Freshwater Biology*, one of the first studies to link low level phosphorus enrichment to synchronous changes in periphyton chemistry, algal species composition, and fish assemblages (Taylor et al. 2014).
- My interdisciplinary training allows me to direct every aspect of large-scale field studies such as the one that is the subject of this RFQ. I have been responsible for study designs (utilizing Geographic Information Systems), site selections, data collections, data quality assurance protocols, database design and data entry methods, data analysis (including development of analytical techniques specifically related to criteria development, programming in R) and writing of reports and publications. I submit that few, if any, other investigators have the breadth of technical training and experience necessary to effectively lead all aspects of a nutrient criteria field study.
- I have been actively engaged since 1998 in the ongoing dialogue among states and the US EPA regarding numerical nutrient criteria development. I currently served as a member of the Regional Technical Advisory Group (RTAG) related to nutrient criteria for EPA Region 6. I am an invited member of the US EPA Expert Workgroup on Nutrient Indicators in Streams. I have a contract with Tetra Tech, a consultant for US EPA, to review every state's numeric nutrient criteria document. To date, I have reviewed only 3 because so few states have completed their criteria development for streams. Finally, I served as an invited reviewer for US EPA's *Using stressor-response relationships to derive numeric nutrient criteria (EPA 820-S-10-001),* a document that is cited as the cornerstone of the Oklahoma Scenic Rivers phosphorus study (and in which 2 of my papers are cited therein). Thus, I am viewed by both state and federal scientists engaged in criteria development as an expert and leader in the field.

- I have particular expertise in the analysis of ecological data, particularly detecting and interpreting nonlinear responses and ecological thresholds. I teach *Advanced Ecological Data Analysis* for graduate students. The course is taught entirely in the software package R, which is free. I am quite familiar with all of the statistical methods described in the EPA stressor-response document, and one of which I helped popularize (King and Richardson 2003). Recently, the most notable contribution to methods to support criteria development are a series of papers with Matthew E. Baker on a new analytical method (and software) that we developed for detecting and interpreting ecological thresholds called Threshold Indicator Taxa Analysis (TITAN; Baker and King 2010, King and Baker 2010, King and Baker 2011, King et al. 2011, Bernhardt et al. 2012, Baker and King 2013, King and Baker 2014). TITAN has been well received (e.g., the focal method in a recent PNAS paper by Payne et al. 2013 on nitrogen deposition thresholds in Europe) and highly cited (45 citations as of 12/27/13). Several several state agencies are employing TITAN to assist with environmental criteria development (e.g., Connecticut, Massachusetts). TITAN is fundamentally different than any other method available, and is perfectly suited for identifying synchronous changes in algal species abundances along phosphorus gradients (see King et al. 2009a, b, Taylor et al. 2014). TITAN would be one of several methods I would employ to develop a weight of evidence supporting a particular concentration of total phosphorus that results in undesirable aesthetic or water quality conditions in the Designated Scenic Rivers.
- I am also a strong advocate of causal analysis and linking field studies with in situ or mesocosm experiments. To this end, I led the design and construction of one of the largest outdoor experimental stream facilities in North America (Baylor Experimental Aquatic Research (BEAR)). The facility is located near Baylor at the Lake Waco Wetlands. We have completed several experiments related to nutrients (King et al. 2009a, Taylor et al. 2012a, b), with current research focused on the interaction between nutrients and emerging contaminants such as nanoparticles.
- On a broader scale, I have been active in professional and community service, serving as Associate Editor of a top journal in my field (*Ecological Applications*, an Ecological Society of America publication; prior to that, I was Associate Editor for *Freshwater Science*), refereeing manuscripts from 2 dozen other journals, reviewing proposals for funding agencies such as NSF and US EPA, and serving on several departmental and university committees. I am routinely invited to serve on expert workgroups and panels at a national level. I have been repeatedly asked to provide expert testimony in State (WV) and Federal court due to my expertise in stream ecosystem structure and function and how it is affected by Mountaintop Removal Mining in Appalachia.
- My lab group and affiliates at Baylor would provide an impressive level of expertise and experience in supporting this study.
    - Dr. Jeffrey A. Back serves as the Center for Reservoir and Aquatic Systems Research (CRASR) laboratory manager. Dr. Back is an expert on nutrient chemistry analyses. He has run tens of thousands of dissolved and total phosphorus samples during his 9 years as laboratory manager. Dr. Back is also an exceptional scientist, playing an integral role in field collections for our previous nutrient criteria work in Texas streams. He is also a world-class macroinvertebrate taxonomist.
    - Dr. Barbara Winsborough is an independent consultant who works with my lab group on a regular basis. Dr. Winsborough is arguably one of the top diatomists in the world,

and is highly adept at identifying all types of algae and cyanobacteria as well. Dr. Winsborough would be the lead algal taxonomist for this project.

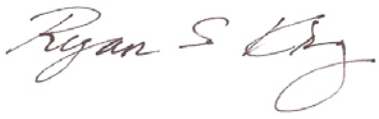
- Graduate students. I have been fortunate to have recruited many excellent graduate students. I currently have four students in the lab, any of which could play a role on this project.
- Technicians. We recently hired a technician, Byron Griffin, to facilitate a new experimental stream study. Byron will be well trained by the time this study begins, and would be a strong candidate for a technician position on the study. I envision hiring at least one other technician to support the field and laboratory work necessary to complete this project.
- Facilities. Baylor has state-of-the-science analytical lab capabilities, including a Lachat Quik-Chem Flow-Injection Autoanalyzer for nutrients, Shimadzu TOC analyzer, a Stable Isotope Mass Spectrometry lab, and most other instruments typically associated with freshwater chemical research. Our field equipment is also top-notch, including 13 brand-new YSI EXO1 data sondes equipped with optical DO sensors, portable weather stations, 12 ISCO water samplers, and conventional equipment required to complete field studies. We also have access to a fleet of field vehicles, including 3 Toyota Tundra extended cab 4x4 trucks and a Chevy Tahoe.

In closing, I encourage each committee member to give my statement of qualifications serious consideration. I am very interested in leading this project. I have much experience doing exactly what this study requires. I understand what works and what to avoid. Few, if any, other investigator could be more prepared to lead this study than I and my research team.

I also encourage you to review my CV, appendix 1, and the 5 attached PDFs which are included to add further support to my qualifications.

Thank you for considering my qualifications.

Sincerely,



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Appendix 1. List of peer-reviewed publications or reports authored or co-authored by Ryan S. King that are directly related to nutrient criteria development or effects of nutrients on freshwater ecosystems. The most important papers dealing with approaches (study design, data analysis framework, specific methods) specifically related to nutrient criteria development are highighted. **Bold**=Members of the King Aquatic Ecology Lab. * = student or postdoc.

**Taylor, J. M**.*, **R.S. King**, A. A. Pease, and K.O. Winemiller. 2014. Nonlinear responses of stream ecosystem structure to low level phosphorus enrichment. *Freshwater Biology* (in press).

**King, R.S**. and M.E. Baker. 2014. Use, misuse, and limitations of Threshold Indicator Taxa Analysis (TITAN) for natural resource management. In: G. Guntenspergen (editor), *Ecological Thresholds for Resource Management*. In press.

**Back, J.A***., and **R. S. King**. 2013. Sex and size matter: Ontogenetic patterns of nutrient content of aquatic insects. *Freshwater Science* 32:837-848

Baker, M.E., and **R. S. King**. 2013. Of TITAN and straw men: an appeal for greater understanding of community data. *Freshwater Science* 32:489-506.

**Lang, D.A.*, R.S. King**, and J.T. Scott. 2012. Divergent responses of biomass and enzyme activities suggest differential nutrient limitation in stream periphyton. *Freshwater Science* 31:1096-1104.

**Taylor, J. M.*, J. A. Back*,** T. W. Valenti*, and **R. S. King.** 2012. Fish-mediated nutrient cycling and benthic microbial processes: Can consumers influence stream nutrient cycling at multiple spatial scales? *Freshwater Science* 31:928-944.

Studds, C. E., W. V. DeLuca, M. E. Baker, **R. S. King,** and P. P. Marra. 2012. Land cover and rainfall interact to shape waterbird community composition. *PLoS One* 7.e35969, doi:10.1371/journal.pone.0035969.

Taylor, J. M.*, **J. A. Back*,** and **R. S. King.** 2012. Grazing minnows increase benthic autotrophy and enhance response of periphyton elemental composition to experimental phosphorus additions. *Freshwater Science* doi 10.1899/11-055.1.

**Shaftel, R. S.*, R. S. King, and J. A. Back*.** 2012. Alder cover drives nitrogen availability in Kenai Peninsula headwater streams, Alaska. *Biogeochemistry* 107:135-148

Valenti, T. W.*, **J.M. Taylor*, J.A. Back*, R.S. King**, and B. W. Brooks. 2011. Hydrological and nutrient influences on diel pH in wadeable streams: Implications for ecological risk assessment of ionizable contaminants. *Integrated Environmental Assessment and Management* DOI: 10.1002/ieam.202. Press coverage: *Science Daily.*

**Shaftel, R. S.*, R. S. King, and J. A. Back***. 2011. Breakdown rates, nutrient quality, and macroinvertebrate colonization of bluejoint grass litter in headwater streams of the Kenai Peninsula, Alaska. *Journal of the North American Benthological Society* 30:386-398.

**King, R. S.^** and M. E. Baker^. 2011. An alternative view of ecological community thresholds and appropriate analyses for their detection. *Ecological Applications* doi:10.1890/10-0882.1 ^equal contributors

**King, R. S**.^ and M. E. Baker.^ 2010. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. *Journal of the North American Benthological Society* 29:998-1008. ^Joint first-authors,

Dodds, W.K., W. H. Clements, K. Gido, B. Hilderbrand, and **R. S. King**. 2010. Thresholds, breakpoints, and nonlinearities in aquatic ecosystems as related to management. *Journal of the North American Benthological Society* 29:988-997.

Baker, M. E.^ and **R. S. King^.** 2010. A new method for identifying and interpreting biodiversity and ecological community thresholds. *Methods in Ecology and Evolution* 1.25-37. Press coverage: *NPR, Baltimore Sun, Yahoo News, Environmental Protection Magazine*. Method was centerpiece of 2013 *PNAS* paper.
^Joint first-authors, listed in alphabetical order.

Fulton, B. A.*, R. A. Brain, S. Usenko**, J. A. Back*, R. S. King**, and B. W. Brooks. 2009 Influence of N and P concentrations and ratios on *Lemna gibba* growth responses to triclosan in laboratory and stream mesocosm experiments. *Environmental Toxicology and Chemistry* 28:2610-2621

**King, R. S.,** B. W. Brooks, **J. A. Back*, J. M Taylor*,** and B.A Fulton*. 2009. Linking Observational and Experimental Approaches for the Development of Regional Nutrient Criteria for Wadeable Streams. Section 104(b)(3) Water Quality Cooperative Agreement #CP-966137-01 U. S. EPA Region 6, Dallas, TX.

**King, R. S.,** K. O. Winemiller, **J.M Taylor*, J. A. Back*** and A. Pease*. 2009. Development of biological indicators of nutrient enrichment for application in Texas streams. §106 Water Pollution Control Grant # 98665304, Texas Commission on Environmental Quality, Austin, TX.

**Scott, J. T.*, D. A. Lang*, R. S. King**, and R. D. Doyle. 2009. Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: Evidence for differential nutrient limitation in stream benthos. *Journal of the North American Benthological Society* 28:57-68.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2008. Response to comment on "Estimating ecological thresholds for phosphorus in the Everglades". *Environmental Science & Technology* 42: 6772-6773.

**Back, J. A.*, J. M. Taylor*, R. S. King**, E. Hintzen*, and K. Fallert*. 2008. Ontogenetic differences in mayfly stoichiometry influence growth rates in response to phosphorus. *Fundamental and Applied Limnology (Archiv fur Hydrobiologie)* 171:233-240.

**Scott, J. T.*, J. A. Back*, J. M. Taylor*,** and **R. S. King**. 2008. Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society* 27:332-334.

**King, R. S**. and C. J. Richardson. 2008. Macroinvertebrate responses along a gradient of long-term nutrient additions and altered hydroperiod. Pp 277-320 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

**King, R. S.** and C. J. Richardson. 2008. Macroinvertebrate and fish responses to experimental P additions in Everglades sloughs. Pp 477-504 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2008. An ecological basis for establishment of a phosphorus threshold for the Everglades ecosystem. Pp 595–620 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Kaštovský, J., K. Kaštovsk , M. Bastl, J. Vymazal, and **R. S. King**. 2008. Experimental assessment of phosphorus effects on algal assemblages in dosing mesocosms. Pp 461-476 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Richardson, C. J., **R. S. King**, J. Vymazal, E. A. Romanowicz, and J W. Pahl. 2008. Macrophyte community responses in the Everglades with an emphasis on cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*) interactions along a gradient of long-term nutrient additions, altered hydroperiod and fire. Pp 215-260 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

**King, R. S,** and C. J. Richardson. 2007. Subsidy-stress response of macroinvertebrate-assemblage biomass to a phosphorus gradient in an oligotrophic wetland ecosystem. *Journal of the North American Benthological Society* 26:491-508.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2007. Estimating ecological thresholds for phosphorus in the Everglades. *Environmental Science &*

*Technology* 41:8084-8091 (cover photo, featured paper). Press coverage: *American Chemical Society*

**King, R. S.**, D. F. Whigham, W. V. DeLuca, and P. P. Marra. 2007. Threshold effects of coastal urbanization on *Phragmites australis* (common reed) abundance and foliar nitrogen in Chesapeake Bay. *Estuaries and Coasts* 30:469-481 (cover photo, featured paper). Press coverage: *Coastal and Estuarine News*.

**King, R. S.**, M. E. Baker, D. F. Whigham, D. E. Weller, T. E. Jordan, P. F. Kazyak, and M. K. Hurd. 2005. Spatial considerations for linking watershed land cover to ecological indicators in streams. *Ecological Applications* 15:137-153. Press coverage: *Faculty of 1000* (recommended).

**King, R. S.**, C. J. Richardson, D. L. Urban, and E. A. Romanowicz. 2004. Spatial dependency of vegetation-environment linkages in an anthropogenically influenced wetland ecosystem. *Ecosystems* 7:75-97 (cover photo, featured paper).

Qian, S. S., Y. Pan, and **R. S. King**. 2004. Soil phosphorus threshold in the Everglades: a Bayesian changepoint analysis for multinomial response data. *Ecological Indicators* 4:29-37.

**King, R. S.** and C. J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environmental Management* 31:795-809.

Qian, S. S., **R. S. King**, and C. J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166:87-97.

**King, R. S.** and C. J. Richardson. 2002. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *Journal of the North American Benthological Society* 21:150-171.

Lemly, A. D. and **R. S. King**. 2000. An insect-bacteria bioindicator for assessing detrimental nutrient enrichment in wetlands. *Wetlands* 20:91-100.

**King, R. S.** and J. C. Brazner. 1999. Coastal wetland insect communities along a trophic gradient in Green Bay, Lake Michigan. *Wetlands* 19:426-437.

# RYAN S. KING

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Lab webpage: www.baylor.edu/aquaticlab

## RESEARCH INTERESTS

**Aquatic community ecology**: linking landscape & geologic history, speciation and community assembly; ecological community thresholds; species responses to novel environmental gradients; new techniques in multivariate community analysis.

**Biogeochemistry and food webs**: terrestrial–aquatic energy pathways; consumer–resource stoichiometry, role of microbes in stream ecosystem function.

**Landscape ecology**: linkages between catchments and stream ecosystems; use of spatial data for predicting ecological condition of aquatic habitats.

## EDUCATION

**Doctor of Philosophy**, Ecology. September 2001
Department of the Environment, Duke University, Durham, NC
*Dissertation*: Dimensions of invertebrate assemblage organization across a phosphorus-limited Everglades landscape. *Advisor*: Curtis J. Richardson.

**Master of Environmental Management**, Water Resources Management. May 1996
Nicholas School of the Environment, Duke University
*Thesis*: Spatial and diel variability in the availability of flying insects as duckling food in prairie pothole wetlands of North Dakota. *Advisor:* Robert G. Qualls.

**Bachelor of Science**, Biology, *summa cum laude*. August 1994
Harding University, Searcy, AR
*Thesis*: Relationship between rainbow trout food habits and benthic macroinvertebrates in the Greer's Ferry Tailwater, AR. *Advisor:* Ronald Doran.

## PROFESSIONAL EXPERIENCE

**Associate Professor,** Department of Biology, Baylor University. August 2009 – .

**Visiting Scholar,** Institute for the Environment, University of North Carolina. 2010.

**Assistant Professor**, Department of Biology, Baylor University. 2004 – 2009.

**Ecologist,** Smithsonian Environmental Research Center, Edgewater, MD. 2001–2004.

**Research Associate**, Duke Wetland Center, Nicholas School of the Environment, Duke University. 1996–2001

**NNEMS Fellow**, Wetland Function Group, Mid-Continent Ecology Division, United States Environmental Protection Agency, Duluth, MN. 1995 – 1996.

## COURSES TAUGHT

**Advanced Ecological Data Analysis** (BIO 5413). Lecture/lab, 4 credits
**Stream Ecology** (BIO 5405). Lecture/lab. 4 credits
**Aquatic Biology** (BIO 4406). Lecture/lab, 4 credits
**Modern Concepts of Bioscience II** (BIO 1306). Lecture. 3 credits
**Visualizing Data in R** (BIO 5100). Graduate seminar, 1 credit.
**Field Methods in Stream Ecology** (BIO 5100). Graduate seminar, 1 credit.
**Taxonomy of Aquatic Insects** (BIO 5100). Graduate seminar, 1 credit.

**PROFESSIONAL ACTIVITIES**

**Subject-Matter (Associate) Editor**, *Ecological Applications*, 2013 – .
**Ad-hoc Subject-Matter Editor,** *Ecological Applications.* 2011, 2012
**Associate Editor**, *Journal of the North American Benthological Society*. 2003–2006.
**Manuscript Referee**: *Annals of the Entomological Society of America; Aquatic Microbial Ecology; Archiv für Hydrobiologie; Biogeochemistry; Ecological Applications; Ecological Monographs; Ecology; Ecology of Freshwater Fish, Ecosystems; Environmental Entomology; Environmental Management; Environmental Science & Technology; Hydrobiologia; Journal of Applied Ecology; Journal of Environmental Quality; Journal of Environmental Statistics, Journal of the North American Benthological Society;* Kluwer Academic Monograph Series; *Landscape Ecology*; *Lakes and Reservoirs: Research and Management*; *Limnology and Oceanography*; *Methods in Ecology and Evolution*, *Oikos*, *Plant Ecology*, *Science of the Total Environment, Soil Science Society of America Journal*; *Southeastern Naturalist*; Springer-Verlag Ecological Monograph Series; *Transactions of the American Fisheries Society*, United States Environmental Protection Agency; *Wetlands*
**Board of Directors,** Society for Freshwater Science, 2011 – 2012.
**Executive Committee,** North American Benthological Society. 2009 – 2011.
**Scientific Society Member**: Society for Freshwater Science
**Proposal Referee/Grant Panelist:** National Science Foundation, US Environmental Protection Agency, Earthwatch Institute.
**Expert Witness,** Ohio Valley Environmental Coalition, West Virginia Highlands Conservancy,and Sierra Club, v. Elk Run Coal Company, Inc., and Alex Energy, Inc, US District Court, Huntington, WV. May-Dec 2013
**Expert Witness,** Sierra Club et al. v. USACOE & Highland Mining Co, US District Court, Huntington WV. April-May 2012.
**Expert Witness,** Sierra Club v. Patriot Mining; State Environmental Board, Charleston, WV, December 2010
**Workgroup Member,** Expert Workgroup on Nutrient Indicators in Streams, USEPA, Washington, DC, April 2013.
**Workgroup Member,** Healthy Watersheds, US EPA. 2010.
**Review Team Member,** Texas Instream Flow Desktop Methodology, Texas Commission on Environmental Quality. 2007 – 2008.
**Workgroup Member,** Tiered Aquatic Life Use (TALU), Subhumid Agricultural Plains (SAP), US Environmental Protection Agency Region 6. 2006 – .
**Workgroup Member,** Regional Technical Advisory Group (RTAG), Nutrient Criteria, US Environmental Protection Agency Region 6. 2004 – .
**Workgroup Member,** Maryland Biological Stream Survey, Maryland Department of Natural Resources, Annapolis, MD. 2003-2004.
**Workgroup Member,** Biological Assessment of Wetlands Workgroup (BAWWG), US Environmental Protection Agency, Washington, DC. 1997–2001.
**Peer-Review Consultant,** United States Environmental Protection Agency "State of Science" publications and State Numeric Nutrient Criteria documents. Reviews conducted through Versar, Inc., Springfield, VA and Tetra Tech, Owings Mills, MD. 2000–2001, 2003, 2007, 2009-2013.

**ACADEMIC APPOINTMENTS AND SERVICE**

**Graduate Program Director,** Department of Biology, Baylor University. June 2012 –.
**Adjunct Faculty Appointment,** Duke University, Graduate Program in Ecology. 2012–
**Chair,** Microbial Ecology search committee, Department of Biology, Baylor University, 2011-2012.
**Member,** Chair of the Department of Biology search committee, Baylor University. 2011- present.
**Outside member,** Departmental Tenure Committee, Environmental Science (2

faculty). 2008-present.

**Interim Graduate Program Director**, The Institute of Ecological, Environmental, and Earth Sciences. 2008-2009

**Graduate Committee,** Department of Biology, Baylor University. 2006 – 2008.

**Library Committee,** Department of Biology, Baylor University. 2004 – 2007

**Webpage Committee,** Department of Biology, Baylor University. 2004–2008.

**Principal Investigator and Director** of the Baylor Experimental Aquatic Research (BEAR) stream facility at the Lake Waco Wetlands. 2005 –.

**Director**, Native Plant Sale, Baylor Biological Honor Society (Tri-Beta), Baylor University. 2008–2011.

**Advisor**, Delta Phi Omega national sorority, Baylor University. 2008-2013.

## PUBLICATIONS

***bold**=member of King lab*
**=students or postdocs*

**Taylor, J. M**.*, **R.S. King**, A. A. Pease, and K.O. Winemiller. 2014. Nonlinear responses of stream ecosystem structure to low level phosphorus enrichment. *Freshwater Biology* (in press).

**King, R.S**. and M.E. Baker. 2014. Use, misuse, and limitations of Threshold Indicator Taxa Analysis (TITAN) for natural resource management. In: G. Guntenspergen (editor), *Ecological Thresholds for Resource Management*. In press.

Richardson, C. J. and **R. S. King**. 2014. A primer on sampling plant communities in wetlands. In: *Methods in Biogeochemistry of Wetlands*. Soil Science Society of America. In press.

**Back, J.A***., and **R. S. King**. 2013. Sex and size matter: Ontogenetic patterns of nutrient content of aquatic insects. *Freshwater Science* 32:837-848

Baker, M.E., and **R. S. King**. 2013. Of TITAN and straw men: an appeal for greater understanding of community data. *Freshwater Science* 32:489-506.

**Lang, D.A.*, R.S. King**, and J.T. Scott. 2012. Divergent responses of biomass and enzyme activities suggest differential nutrient limitation in stream periphyton. *Freshwater Science* 31:1096-1104.

Husemann, M.*, **J. W. Ray*, R. S. King, E. Hooser*,** and P.D. Danley. 2012. Comparative biogeography reveals differences in population genetic structure of five species of stream fishes. *Biological Journal of the Linnean Society* DOI: 10.1111/j.1095-8312.2012.01973.x

Bernhardt, E. S., B. D. Lutz, **R. S. King**, A. M. Helton, C. A. Carter, J. P. Fay, D. Campagna, J. Amos. 2012. How many mountains can we mine? Assessing the regional degradation of Central Appalachian rivers by surface coal mining. *Environmental Science & Technology* 46: 8115–8122 (*Science Daily* and others)

**Ray, J.W.*,** M. Husemann*, **R. S. King**, and P. D. Danley. 2012. Genetic analysis reveals dispersal of Florida bass haplotypes from reservoirs to rivers in central Texas. *Transactions of the American Fisheries Society* 141:1269–1273

**Stanley, C. E.*, J. M. Taylor*,** and **R. S. King.** 2012. Coupling fish community structure with instream flow and habitat connectivity between two hydrologically extreme years. *Transactions of the American Fisheries Society* 141:1000-1015.

**Taylor, J. M.*, J. A. Back*,** T. W. Valenti*, and **R. S. King.** 2012. Fish-mediated nutrient cycling and benthic microbial processes: Can consumers influence stream nutrient cycling at multiple spatial scales? *Freshwater Science* 31:928-944.

Studds, C. E., W. V. DeLuca, M. E. Baker, **R. S. King,** and P. P. Marra. 2012. Land cover and rainfall interact to shape waterbird community composition. *PLoS One* 7.e35969, doi:10.1371/journal.pone.0035969.

**King, R. S**., C. M. Walker, D. F. Whigham, S. Baird, and **J. A. Back*.** 2012. Catchment topography and wetland geomorphology drive macroinvertebrate community structure and juvenile salmonid distributions in southcentral Alaska headwater streams. *Freshwater Science* 31:341-364.

Whigham, D. W., C. M. Walker, **R. S. King**, and S. Baird. 2012. Multiple scales of

influence on wetland vegetation associated with headwater streams in Alaska, USA. *Wetlands* 10.1007/s13157-012-0274-z.

Taylor, J. M.*, **J. A. Back*,** and **R. S. King.** 2012. Grazing minnows increase benthic autotrophy and enhance response of periphyton elemental composition to experimental phosphorus additions. *Freshwater Science* doi 10.1899/11-055.1.

Walker, C. M., **R. S. King**, Whigham, D. W, and S. Baird. 2012. Landscape and wetland influences on headwater stream chemistry in the Kenai Lowlands, Alaska. *Wetlands* 32:301-310.

**Dekar, M.P.*, R. S. King**, C. M. Walker, D. W. Whigham, and **J. A. Back*.** 2012. Allochthonous inputs from grass-dominated wetlands support juvenile salmonids in headwater streams: evidence from stable isotopes of carbon, hydrogen, and nitrogen. *Freshwater Science* 31:121-132

**Shaftel, R. S.*, R. S. King, and J. A. Back*.** 2012. Alder cover drives nitrogen availability in Kenai Peninsula headwater streams, Alaska. *Biogeochemistry* 107:135-148

**King, R. S.,** M. E. Baker, P. F. Kazyak, and D. E. Weller. 2011. How novel is too novel? Stream community thresholds at exceptionally low levels of watershed urbanization. *Ecological Applications*. 21:1659-1678, doi:10.1890/10-1357.1. Press coverage: *Science Daily, Faculty of 1000 (recommended)*

Pease, A. A*, **J. M. Taylor*, R. S. King,** and K. O. Winemiller. 2011. Multiscale environmental influences on fish community structure in central Texas streams. *Transactions of the American Fisheries Society* 140:1409-1427.

Valenti, T. W.*, **J.M. Taylor*, J.A. Back*, R.S. King**, and B. W. Brooks. 2011. Hydrological and nutrient influences on diel pH in wadeable streams: Implications for ecological risk assessment of ionizable contaminants. *Integrated Environmental Assessment and Management* DOI: 10.1002/ieam.202. Press coverage: *Science Daily*.

**Shaftel, R. S.*, R. S. King, and J. A. Back***. 2011. Breakdown rates, nutrient quality, and macroinvertebrate colonization of bluejoint grass litter in headwater streams of the Kenai Peninsula, Alaska. *Journal of the North American Benthological Society* 30:386-398.

**King, R. S.^** and M. E. Baker^. 2011. An alternative view of ecological community thresholds and appropriate analyses for their detection. *Ecological Applications* doi:10.1890/10-0882.1 ^equal contributors

**King, R. S**.^ and M. E. Baker.^ 2010. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. *Journal of the North American Benthological Society* 29:998-1008. ^Joint first-authors,

Dodds, W.K., W. H. Clements, K. Gido, B. Hilderbrand, and **R. S. King**. 2010. Thresholds, breakpoints, and nonlinearities in aquatic ecosystems as related to management. *Journal of the North American Benthological Society* 29:988-997.

Baker, M. E.^ and **R. S. King^.** 2010. A new method for identifying and interpreting biodiversity and ecological community thresholds. *Methods in Ecology and Evolution* 1.25-37. Press coverage: *NPR, Baltimore Sun, Yahoo News, Environmental Protection Magazine.* Method was centerpiece of 2013 *PNAS* paper. ^Joint first-authors, listed in alphabetical order.

Whigham, D.F., M. C. Whigham, I. Feller, W. Rodriguez, and **R. S. King**. 2009. Ecological characteristics of *Batis maritima* in Florida and Belize. *Smithsonian Contributions to Marine Sciences* 38: 491-499.

Fulton, B. A.*, R. A. Brain, S. Usenko**, J. A. Back*, R. S. King**, and B. W. Brooks. 2009 Influence of N and P concentrations and ratios on *Lemna gibba* growth responses to triclosan in laboratory and stream mesocosm experiments. *Environmental Toxicology and Chemistry* 28:2610-2621

**Scott, J. T.*, D. A. Lang*, R. S. King**, and R. D. Doyle. 2009. Nitrogen fixation and

phosphatase activity in periphyton growing on nutrient diffusing substrata: Evidence for differential nutrient limitation in stream benthos. *Journal of the North American Benthological Society* 28:57-68.

DeLuca, W. V., C. Studds, **R. S. King**, P. P. Marra. 2008. Coastal development and the integrity of estuarine waterbird communities: threshold responses and the importance of scale. *Biological Conservation* 141:2669-2678.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2008. Response to comment on "Estimating ecological thresholds for phosphorus in the Everglades". *Environmental Science & Technology* 42: 6772-6773.

**Back, J. A.*, J. M. Taylor*, R. S. King**, E. Hintzen*, and K. Fallert*. 2008. Ontogenetic differences in mayfly stoichiometry influence growth rates in response to phosphorus. *Fundamental and Applied Limnology (Archiv fur Hydrobiologie)* 171:233-240.

**Scott, J. T.*, J. A. Back*, J. M. Taylor*,** and **R. S. King**. 2008. Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society* 27:332-334.

**King, R. S**. and C. J. Richardson. 2008. Macroinvertebrate responses along a gradient of long-term nutrient additions and altered hydroperiod. Pp 277-320 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

**King, R. S.** and C. J. Richardson. 2008. Macroinvertebrate and fish responses to experimental P additions in Everglades sloughs. Pp 477-504 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2008. An ecological basis for establishment of a phosphorus threshold for the Everglades ecosystem. Pp 595–620 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Kaštovský, J., K. Kaštovsk , M. Bastl, J. Vymazal, and **R. S. King**. 2008. Experimental assessment of phosphorus effects on algal assemblages in dosing mesocosms. Pp 461-476 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

Richardson, C. J., **R. S. King**, J. Vymazal, E. A. Romanowicz, and J W. Pahl. 2008. Macrophyte community responses in the Everglades with an emphasis on cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*) interactions along a gradient of long-term nutrient additions, altered hydroperiod and fire. Pp 215-260 In: *The Everglades Experiments: Lessons for Ecosystem Restoration (Ecological Studies 201)*, C. J. Richardson. New York. Springer-Verlag.

**King, R. S,** and C. J. Richardson. 2007. Subsidy-stress response of macroinvertebrate-assemblage biomass to a phosphorus gradient in an oligotrophic wetland ecosystem. *Journal of the North American Benthological Society* 26:491-508.

Richardson, C. J., **R. S. King**, S. S. Qian, P. Vaithiyanathan, R. G. Qualls, and C. A. Stow. 2007. Estimating ecological thresholds for phosphorus in the Everglades. *Environmental Science & Technology* 41:8084-8091 (cover photo, featured paper). Press coverage: *American Chemical Society*

**King, R. S.**, D. F. Whigham, W. V. DeLuca, and P. P. Marra. 2007. Threshold effects of coastal urbanization on *Phragmites australis* (common reed) abundance and foliar nitrogen in Chesapeake Bay. *Estuaries and Coasts* 30:469-481 (cover photo, featured paper). Press coverage: *Coastal and Estuarine News*.

Rosenberg, D. M., V. H. Resh, and **R. S. King.** 2007. Use of aquatic insects in biomonitoring. pp. 123-137 *In*: R. W. Merritt and K. W. Cummins (editors). *An*

*Introduction to the Aquatic Insects of North America, 4th Edition.* Kendall/Hall, Dubuque, IA, USA.

**King, R. S.**, M. E. Baker, D. F. Whigham, D. E. Weller, T. E. Jordan, P. F. Kazyak, and M. K. Hurd. 2005. Spatial considerations for linking watershed land cover to ecological indicators in streams. *Ecological Applications* 15:137-153. Press coverage: *Faculty of 1000* (recommended).

**King, R. S.**, A. H. Hines, F. D. Craige, and S. Grap*. 2005. Regional, watershed, and local correlates of blue crab and bivalve abundances in subestuaries of Chesapeake Bay, USA. *Journal of Experimental Marine Biology and Ecology* 319:101-116. Press coverage: *Bay News Journal*.

**King, R. S.**, J. Beaman, D. F. Whigham, A. H. Hines, M. E. Baker, and D. E. Weller. 2004. Watershed land use is strongly linked to PCBs in white perch in Chesapeake Bay subestuaries. *Environmental Science & Technology* 38:6546-6552. Press coverage*: Smithsonian Magazine.*

**King, R. S.**, C. J. Richardson, D. L. Urban, and E. A. Romanowicz. 2004. Spatial dependency of vegetation-environment linkages in an anthropogenically influenced wetland ecosystem. *Ecosystems* 7:75-97 (cover photo, featured paper).

Qian, S. S., Y. Pan, and **R. S. King**. 2004. Soil phosphorus threshold in the Everglades: a Bayesian changepoint analysis for multinomial response data. *Ecological Indicators* 4:29-37.

**King, R. S.** and C. J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environmental Management* 31:795-809.

Qian, S. S., **R. S. King**, and C. J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166:87-97.

**King, R. S.** and C. J. Richardson. 2002. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *Journal of the North American Benthological Society* 21:150-171.

**King, R. S.**, K. T. Nunnery, and C. J. Richardson. 2000. Macroinvertebrate assemblage response to highway crossings in forested wetlands: implications for biological assessment. *Wetlands Ecology and Management* 8:243-256.

Lemly, A. D. and **R. S. King**. 2000. An insect-bacteria bioindicator for assessing detrimental nutrient enrichment in wetlands. *Wetlands* 20:91-100.

**King, R. S.** and J. C. Brazner. 1999. Coastal wetland insect communities along a trophic gradient in Green Bay, Lake Michigan. *Wetlands* 19:426-437.

**King, R. S.** and D. A. Wrubleski. 1998. Spatial and diel availability of flying insects as potential duckling food in prairie wetlands. *Wetlands* 18:100-114.

**MANUSCRIPTS UNDER INTERNAL OR PEER REVIEW**

***bold**=member of King lab*
**=students or postdocs*

**Dekar, M. P.*, C. McCauley*, J. W. Ray*, and R. S. King**. Differential survival, competition, and recruitment among cyprinids exposed to chronic heat stress in experimental streams.

Pease, A. A., **J. M. Taylor*,** K. O. Winemiller, and **R. S. King**. Functional trait diversity and trait-environment relationships in central Texas stream fish assemblages: implications for biomonitoring.

**Hiatt, D., P. Kostka, R. S. King** et al. Catchment alder cover influences periphyton enzyme activity in headwater streams.

**McCauley, C., M. P. Dekar, and R. S. King**. Nutrient enrichment accelerates leaf-litter breakdown in natural streams and in experimental streams lacking macroinvertebrates.

Labay, B., D. Hendrickson, A. Cohen, K. O. Winemiller, **R. S. King,** T. H. Bonner, G. Wilde et al. Toward bioassessment without reference sites using species distribution

modeling.

Callahan, M.K., M. C. Rains, J. C. Bellino, C. M. Walker, D. F. Whigham and **R. S. King.** Trends and Controls of Surface Water Temperatures in Headwater Streams in Two Common Geomorphic Settings, Kenai Peninsula.

**Ray, J. W.,** M. Husemann, **R. S. King,** and P. D. Danley. Genetic impoverishment of spotted bass Micropterus punctulatus in Texas and a reevaluation of the relationships of *Micropterus* species found in Texas.

**Back, J. A.,** J. M. Taylor, and R. S. King. Sex and size, subsized: Effect of stream phosphorus enrichment on P content and maximum size of two mayfly species.

**GRANTS AND CONTRACTS**

Nation Science Foundation. *It's hot and there's not a lot of water: optimizing water availability and quality for future generations.* Water Sustainability and Climate (WSC) program. D. L. Roelke, B. W. Brooks. **R. S. King** et al.. $5,000,000 (pending).

City of Austin. *Refining the Ecological Integrity Index (EII) for biological assessment of Austin streams*. $37,500. **R.S. King.** 2015-2016 (pending).

Syngenta, Inc. *Effects of Pulsed Atrazine Exposures on Aquatic Plant Communities Using Field-Based Simulated Streams.* **R. S. King** (PI), B. W. Brooks, and C. K. Chambliss. $1,000,000. 2013-2014.

National Science Foundation, *Center for Environmental Implications of Nanomaterials.* C.W. Matson and **R. S. King.** $400,000. 2013-2018.

US Fish and Wildlife Service, Alaska State Wildlife Fund. *Genetic variation of populations of juvenile coho salmon and Dolly Varden within and among river basins on the lower Kenai Peninsula.* P.D. Danley and **R. S. King,** $99,879. 2013-2014.

City of Austin. *Physiographic classification of catchments for refining biological assessment of Austin streams.* **R.S. King.** $34,000. 2012-2014.

Alaska Sustainable Salmon Fund. *Juvenile salmon use of headwater streams as rearing habitat.* C. Walker, **R. S. King,** D. F. Whigham, $315,000-. 2012-2014.

Alaska Sustainable Salmon Fund. *Tools for ecosystem-based management of wetlands on the Kenai Lowlands, Alaska*: *Understanding upland-wetland-stream connection.* C. Walker, **R. S. King,** D. F. Whigham, M. Rains. $299,824. 2010-2012.

University Research Committee (URC), Baylor University. *Using stable hydrogen isotopes to identify energy pathways in small streams of the Kenai Peninsula, Alaska.* **R.S. King (PI).** $7,500. 2009-2010

Texas Commission on Environmental Quality (TCEQ). *Development of biological indicators of nutrient enrichment for application to Texas streams, phase II.* **R.S. King (PI**). $34,000. 2008-2009.

U. S. EPA Science to Achieve Results (STAR) Fellowship. **Jason M. Taylor** (mentor, **R.S. King**). $111,000. 2008-2010.

U. S. EPA – Regional Wetlands Grant Program via Alaska Department of Fish and Game. *Headwater stream wetland settings and shallow ground water influence: relationships to juvenile salmon habitat on the Kenai Peninsula, Alaska.* C. Walker (PI), **R. S. King (co-PI)**, D. F. Whigham (co-PI), M. Rains (co-PI). $139,997. 2007-2009.

Texas Commission on Environmental Quality (TCEQ). *Development of biological indicators of nutrient enrichment for application to Texas streams.* **R.S. King (PI).** $46,769. 2007-2008.

Texas Commission on Environmental Quality (TCEQ) via Texas A&M University. *Refinement and Validation of Habitat Quality Indices (HQI) and Aquatic Life Use (ALU) Indices for Application to Assessment and Monitoring of Texas Surface Waters.* K. Winemiller (PI), **R.S. King (co-PI).** $385,500. 2006-2009.

U. S. EPA – Water Quality Cooperative Agreement Program. *Linking observational*

*and experimental approaches for the development of regional nutrient criteria for wadeable streams.* **R. S. King** (**PI**) and B. W. Brooks (co-PI). $155,000. 2006-2007.

U.S. EPA – Science to Achieve Results (STAR) Program via Smithsonian Environmental Research Center (D. E. Weller, SERC PI). *A watershed classification system for improved monitoring and restoration: landscape indicators of watershed impairment.* **R. S. King (PI**, Baylor subcontract) $20,116**.** 2006.

U. S. EPA – Regional Wetlands Grant Program via Alaska Fish and Game Department. *The functional significance of low-order streams and associated riparian wetlands in supporting fish and invertebrate populations in the Kenai Lowlands, Alaska: Attributing the Kenai Lowlands Wetland Management Tool.* C. Walker (PI), D. F. Whigham (co-PI), **R. S. King (co-PI)**. $81,630. 2006-2007.

Baylor University – University Research Committee. *Influence of riparian wetlands on sources and quality of food for juvenile salmon in headwater streams of southeast Alaska.* $6,000. **R. S. King (PI)**. 2006-2007.

Altria Group, Inc. – 2004 Environment/Water Conservation. *Baylor Experimental Aquatic Research support grant*. **R. S. King (PI)**, B. W. Brooks, R. D. Doyle. $35,000. 2005-2006.

Baylor University – Faculty Research Investment Program. *A flow-injection autoanalysis system to support multidisciplinary aquatic research at Baylor*. $15,000. **R. S. King (PI)**, B. W. Brooks, R. D. Doyle. 2004-2005.

U.S. EPA – Science to Achieve Results (STAR) Program via Smithsonian Environmental Research Center (D. F. Whighm, SERC PI). *Ecological and socioeconomic indicators for integrated assessment of aquatic ecosystems of the Atlantic Slope.* **R. S. King** (**PI**, Baylor subcontract). $19,953. 2005.

Center for Transportation and the Environment Graduate Fellowship. *A functional and biological assessment of wetland ecosystem response to highways: before, during, and after construction*. $15,000. **R. S. King**. 1999-2000.

U. S. EPA — National Network for Environmental Management Studies Fellowship. *The effects of sedimentation, turbidity, and vegetation microhabitat structure on availability of emerging insects as duckling food in prairie wetlands.* $6,000. **R. S. King.** 1995.

**PRESENTATIONS (FIRST AUTHOR ONLY**)

King, R. S. A framework for developing regional nutrient criteria for wadeable streams. American Fisheries Society National Meeting, Little Rock Arkansas, September 2013.

King, R. S. A framework for developing regional nutrient criteria for wadeable streams. North Carolina Forum on Nutrient Over-enrichment, Raleigh, NC. May 2012. **Invited.**

King, R. S. and M. E. Baker. Multiple lines of evidence for nonlinear community responses to novel environmental gradients. Society for Freshwater Science meeting, Louisville, KY. May 2012

King, R. S. Fishes of central Texas streams: diversity, distribution, and their responses to drought and pollution. 2012 H.O.T. H20 Lecture Series, hosted by Heart of Texas Master Naturalists, Lake Waco Wetland Center, April 2012**. Invited.**

King, R. S. Ecosystem thresholds in response to low level phosphorus enrichment in subtropical limestone streams, Texas. Jackson School of Geosciences Seminar Series, U. of Texas at Austin, April 2011. **Invited.**

King, R. S. Synchronous changes in periphyton autotrophy, carbon:nutrient ratios, and species composition reveal stream ecosystem thresholds at low levels of phosphorus enrichment. Smithsonian Environmental Research Center, Edgewater, MD. December 2010 **(Invited).**

King, R. S. An alternative view of ecological community thresholds and appropriate analyses for their detection. UNC Institute for the Environment, Chapel Hill, NC. November 2010 **(Invited).**

King, R. S. Wetlands are strongly linked to headwater stream ecosystems in the Kenai Lowlands, Alaska. Duke University Wetland Center Speaker Series, Durham, NC. November 2010. **(Invited)**

King, R. S. Effects of urbanization on stream communities. Kachemak Bay Research Reserve seminar series, Homer, AK. October 2010. **(Invited)**

King, R. S. Linking observational and experimental approaches for the development of regional nutrient criteria for wadeable streams. Texas Parks and Wildife Regional Directors meeting, Buda, TX. June 2010. **(Invited)**

King, R. S, and M. E. Baker. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. Joint meeting of ASLO/NABS, Santa Fe, NM. June 2010.

King, R. S. Linking observational and experimental approaches for the development of regional nutrient criteria for wadeable streams. Regional Technical Advisory Group (RTAG) for nutrient criteria, EPA Region 6, Dallas, TX. February 2010. **(Invited)**

King, R. S. Linking observational and experimental approaches for the development of regional nutrient criteria for wadeable streams. Webcast and conference call presentation to regional nutrient coordinators from all EPA regions and headquarters. October 2009. **(Invited)**

King, R. S. Wetland linkages to juvenile salmon habitat in headwater streams of the Kenai lowlands, Alaska. Dept of Biology Seminar Series, Baylor University. September 2009. **(Invited)**

King, R. S., and M. E. Baker. Threshold Indicator Taxa Analysis (TITAN): A simple method for identifying and interpreting ecological community thresholds. 57th Annual Meeting of the North American Benthological Society, Grand Rapids, MI. May 2009.

King, R. S. Watershed urbanization and ecological community thresholds in streams. Department of Wildlife and Fisheries Seminar, Texas A & M University, College Station, TX. November 2008 **(Invited).**

King, R. S. Attack of the clones! Urbanization thresholds and *Phragmites* invasion of North American tidal wetlands. Ecological Thresholds Workshop, USGS/NPS, Duluth, MN. April 2008. **(Invited)**

King, R. S. Additive indicator species on environmental gradients: a new technique for estimating ecological thresholds. Ecological Thresholds Workshop, USGS/NPS, Duluth, MN. April 2008. **(Invited)**

King, R. S., J. A. Back, C. M. Walker, S. Baird, and D. F. Whigham. Wetland linkages to juvenile salmonids and macroinvertebrate communities in headwater streams of the Kenai Peninsula, Alaska. Department of Biology, Southwestern University, Georgetown, TX. November 2007. **(Invited)**

King, R. S. Nutrient and habitat criteria in central Texas streams: new perspectives. Subhumid Agricultural Plains TALU workgroup meeting, USGS, Austin, TX. November 2007. **(Invited)**

King, R. S., J. A. Back, C. M. Walker, S. Baird, and D. F. Whigham. Wetland geomorphic linkages to juvenile salmonids and macroinvertebrate communities in headwater streams of the Kenai lowlands, Alaska. 55th Annual Meeting of the North American Benthological Society, Columbia, SC. June 2007.

King, R. S. Watershed urbanization, nutrient enrichment, and ecological thresholds in streams. Special symposium on urbanization and water resources. Joint meeting of the Texas River and Reservoir Management Society (TRRMS) and Society of Environmental Toxicology and Chemistry (SETAC), SF Austin University, acogdoches, TX. May 2007. **(Invited)**

King, R. S. Urbanization, nutrients, and ecological thresholds in streams. Biology

Seminar, University of North Texas, Denton, TX. April 2007. **(Invited)**

King, R. S. Nutrient criteria development in central Texas streams. U. S. EPA Region 6, Regional Technical Advisory Group (RTAG), Dallas, Texas. February 2007. (Invited)

King, R. S. Aquatic research at Baylor: nutrient criteria, landscapes, and ecological thresholds. Baylor–USGS roundtable meeting, U. S. Geological Service, Austin, TX. January 2007 .

King, R. S. Urbanization and ecological thresholds in freshwater and estuarine ecosystems. Water Initiative Symposium (campus wide), Utah State University, Logan, UT, November 2006 **(Invited).**

King, R. S. and K. O. Winemiller. Developing and refining habitat quality and fish community indices for assessment of surface waters in Texas. Subhumid Agricultural Plains TALU workgroup meeting, USGS, Austin, TX. November 2006. **(Invited)**

King, R. S. Linking observational and experimental approaches for the development of numerical nutrient criteria in wadeable streams. TPWD nutrient criteria workgroup, Baylor. October 2006.

King, R. S. Estimating ecological thresholds for developing tiered aquatic life use (TALU) designations for wadeable streams. Subhumid Agricultural Plains TALU workgroup meeting, USGS, Austin, TX. October 2005 (Invited).

King, R. S., M. E. Baker, D. F. Whigham, et al. Watershed urbanization and ecological thresholds in streams: influences of physiography, watershed size, and spatial arrangement of impervious cover. INTECOL–ESA meeting, Montreal, Canada, August 2005

King, R. S., D. F. Whigham, W. V. DeLuca, et al. Linking watershed land cover to ecological indicators in streams and subestuaries of Chesapeake Bay. 53th Annual Meeting of the North American Benthological Society, New Orleans, LA. May 2005.

King, R. S., M. E. Baker, D. F. Whigham, et al. Watershed urbanization and biological thresholds in streams: influences of physiography, watershed size and spatial arrangement of impervious cover. Texas River and Reservoir Management Society Meeting, Waco, TX. May 2005.

King, R. S. Estimating ecological thresholds for the development of numerical nutrient criteria. Special Symposium and Panel Discussion on Nutrient Criteria, South Central Annual Meeting of the Society of Environmental Toxicology and Chemistry, Balcones Springs, TX. May 2005**. (Invited)**

King, R. S., J. R. Beaman, D. F. Whigham et al. Urbanization of estuarine watersheds is strongly linked to elevated PCBs in white perch. 4$^{th}$ All EaGLes Meeting, U.S. EPA STAR Program, Duluth, MN. September 2004.

King, R. S., M. E. Baker, D. F. Whigham, et al. Land-use thresholds and stream macroinvertebrate assemblages: influences of physiography, watershed size, and spatial arrangement. 52$^{nd}$ Annual Meeting of the North American Benthological Society, Vancouver, BC, Canada, June 2004.

King, R. S., D. F. Whigham, et al. Linking watershed land use to ecological indicators in streams and subestuaries of Chesapeake Bay. Versar, Inc., Columbia, Maryland, April 2004 **(Invited).**

King, R. S., D. F. Whigham et al. Linking watershed land use to ecological indicators in streams and subestuaries of Chesapeake Bay. Maryland Stream Monitoring Roundtable Meeting, Baltimore, Maryland. February 2004 **(Invited).**

King, R. S. Integrating bioassessment and ecological risk assessment for the development of numerical nutrient criteria for surface waters. United States Environmental Protection Agency, National Risk Management Research Laboratory, Ada, OK, February 2004 **(Invited).**

King, R. S. Phosphorus enrichment in the Everglades: Linkages among vegetation pattern, resource limitation, and aquatic consumers. Department of Biology, Baylor University, Waco, TX, January 2004 **(Invited).**

King, R. S., D. F. Whigham et al. A watershed approach to linking watershed landuse to ecological indicators in streams and estuaries. 3rd All EaGLes Meeting, U.S. EPA STAR Program, Bodega Bay, California, December 2003.

King, R. S., D. F. Whigham et al. Linking watershed land use to ecological indicators in streams and subestuaries of Chesapeake Bay. 6th Annual Baltimore Ecosystem Study Meeting (Baltimore LTER), Baltimore, Maryland, October 2003.

King, R. S., M. E. Baker, D. F. Whigham et al. Spatial considerations for linking watershed landcover to ecological indicators in streams. Special session on ecological indicators, International Association for Landscape Ecology World Congress, Darwin, Australia, July 2003 **(Invited).**

King, R. S., A. H. Hines, S. Grap, and D. F. Craige. Correlates of bivalves and blue crabs in subestuaries of Chesapeake Bay, USA. Annual Meeting of The Crustacean Society, Williamsburg, VA, June 2003.

King, R. S., M. E. Baker, D. F. Whigham, et al. Influence of spatial factors on linkages among watershed landcover, environmental conditions, and macroinvertebrate assemblages in coastal-plain streams. 51st Annual Meeting of the North American Benthological Society, Athens, GA, May 2003.

King, R. S., and C. J. Richardson. Detecting changepoints in biological attributes: an approach to developing numerical water-quality criteria. Special session on diagnosing causes of impairment in aquatic ecosystems, 50th Annual Meeting of the North American Benthological Society, Pittsburgh, PA, May 2002

King, R. S. Dimensions of invertebrate assemblage organization across a phosphorus-limited Everglades landscape. Smithsonian Environmental Research Center's Winter Seminar Series, Edgewater, MD, January 2002 **(Invited).**

King, R. S., and C. J. Richardson. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. 49th Annual Meeting of the North American Benthological Society, LaCrosse, WI. June 2001.

King, R. S., C. J. Richardson, D. L. Urban, and E. A. Romanowicz. Spatial dependency of vegetation-environment relationships in an anthropogenically influenced wetland landscape. 7th International Symposium of the Biogeochemistry of Wetlands, Durham, NC. June 2001.

King, R. S., and C. J. Richardson. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. Assessing the Health of Wetland Life: Policy, Science, and Practice, Orlando, FL. May 2001.

King, R. S., and C. J. Richardson. Macroinvertebrate and fish responses to experimental P additions in Everglades sloughs. Special session on Everglades restoration, Millennium Wetland Event, INTECOL's 6th Wetland Meeting, Quebec, Quebec, Canada, August 2000.

King, R. S., and C. J. Richardson. Influence of experimental P additions on invertebrate assemblages in sloughs of the northern Everglades. Society of Wetland Scientists 20th Annual Meeting, Norfolk, VA. June 1999.

King, R. S., and C. J. Richardson. Invertebrate assemblage response to experimental phosphorus dosing in the Everglades. 47th Annual Meeting of the North American Benthological Society, Duluth, MN. May 1999.

King, R. S., K. T. Nunnery, and C. J. Richardson. Macroinvertebrate community response to highway crossings in forested wetlands: implications for biological assessment. Connections '98: Water Quality, Wetlands, and Transportation, New Bern, NC. September 1998.

King, R. S., K. T. Nunnery, and C. J. Richardson. A comparison of methods and metrics for assessment of wetland macroinvertebrate community response to highways. 45th Annual Meeting of the North American Benthological Society, San Marcos, TX.

June 1997.

King, R. S., and D. A. Wrubleski. Diel and spatial variability in the availability of flying insects as duckling food in prairie pothole wetlands. 44th Annual Meeting of the North American Benthological Society, Kalispell, MT, USA. June 1996.

King, R. S., and D. A. Wrubleski. Diel and spatial variability in the availability of flying insects as duckling food in prairie pothole wetlands. U. S. Environmental Protection Agency, Duluth, MN, USA. May 1996.

**STUDENTS/POSTDOCS MENTORED**

Current advisees:

Leanne Baker, Ph.D. Postdoctoral Research Associate (principally advised by Cole Matson).

Daniel Hiatt. Ph. D. student, Biology. Fall 2010 –

Moncie Wright, Ph.D. student, Biology. Fall 2011 –

Caleb Robbins, Ph.D. student, Biology, Fall 2012 --

Stephen Cook, Ph.D. student, Biology. Fall 2013 --

Katherine Hooker, BS Biology student (summer undergrad research fellow, 3v90, & work study)

Amber Hearn, BS, Biology student. (research, work-study)

Jemima McCluskey, BS Biology student (research, work study)

Past advisees:

*Graduate students and postdocs*

Jeffrey A. Back. 2013. Ph.D., Biology. Effects of stream nutrient enrichment on aquatic insect stoichiometery: importance of life-history traits, sex, and ontogeny. Employer: Center for Reservoir & Aquatic Systems Research, Baylor University, Laboratory Manager and Research Associate.

Jesse Ray. Ph. D. student, Ecological, Earth, and Environmental Sciences (EEES). 2010-2012. Withdrew to accept permanent position with US Army Corps of Engineers, Walla Walla, WA.

Matthew Dekar, Ph. D. (Univ. of Arkansas, 2010). Postdoctoral Research Fellow, Baylor University. June 2010 – May 2012. Employer: U.S. Fish and Wildlife Service, Lodi, CA. Senior Fisheries Biologist, permanent.

Pamela Kostka. 2012. M. S., Biology. Grass litter breakdown in Alaskan headwater streams.

Jason M. Taylor. 2011. Ph.D. Biology, Baylor. EPA-STAR FELLOW. Incorporating trophic interactions into biological measures of nutrient enrichment in support of numerical thresholds for aquatic life use attainment in wadeable streams. Penultimate employer: Cornell University, Ithaca, NY (Postdoctoral Research Associate). Current employer: USDA National Sedimentation Laboratory, Ecologist (Permanent, Federal).

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Cagney McCauley (Biology, B.S. major, Baylor). Summer undergraduate research

fellow. 2011.
Cari Domoney (Biology, B.S. major, Baylor). 2010.
Katie Zychowski (Biology B.S. major, Baylor). 2009.
Emily Hooser (Biology B. S. major, Baylor). Summer undergraduate research fellow. 2008 (co-mentored with Dr. Patrick Danley).
Lindsey Jackson (Biology B.S. major, Baylor). 2008.
Success Sumpaongoen, B. S., Biology, Baylor. Summer undergraduate research fellow. 2007
Adolfo Flores, B. S., Biology, Baylor. Summer undergraduate research fellow. 2007
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Graduate and honors thesis committees, past:
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Kraig Martin, PhD candidate, Philosophy, Baylor, 2013.
Kyrie Cameron, B.S. Biology (Honors), Baylor. 2012.
Nick Green, Ph. D. Biology, 2012 .
Mary Sides, Ph. D. student, Biology, Baylor.
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Jason Berninger, Ph.D., BMS, Baylor. 2011.
Theodore Valenti, Ph. D. EEES, Baylor. 2011.
Barry Fulton, M. S., Environmental Science. 2009.
Jon Thomas, M. S. Biology, Baylor. 2009
Michelle Nemec. Ph.D. Biology, Baylor. 2008.
Madelon McCall, Ed.D., Baylor. 2008.
Emily Hintzen, M. S. Environmental Studies, Baylor. 2007.
Jacob Stanley. Ph. D., Biology, Baylor. 2007.
Thad Scott. Ph. D., Biology, Baylor. 2006.
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Hui Huang, M. S., Biology, Baylor. 2006.
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**STUDENT AWARDS (outside Baylor only; 15+ internal awards to date)**

Clark Hubbs Award for Best Poster Presentation, Jason Taylor. Southwestern Association of Naturalists, May 2011.
U. S. EPA Science to Achieve Results (STAR) Fellowship. Jason M. Taylor. $111,000. 2008-2010.
Best Oral Presentation (honorable mention), Texas Acad Sci. Jeffrey A. Back. 2007.

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# Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment

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# Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment

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Running title: Nutrient thresholds in streams

## SUMMARY

1. Anthropogenic inputs of nitrogen (N) and phosphorus (P) create environmental conditions that alter biological organization and ecosystem functioning in freshwaters. We studied 38 wadeable streams spanning a N and P gradient to contrast responses of algal and fish assemblages to nutrient enrichment.
2. Surface-water total P (TP) and total N (TN) concentrations represented a wide range (TP: 7-2380 µg $L^{-1}$; TN: 127-15,860 µg $L^{-1}$), and were correlated across our study sites. Total P explained significantly more variance in periphyton carbon (C) to nutrient (C:P, C:N) and N:P ratios than TN. Abrupt, nonlinear declines in these ratios were observed between 20 and 50 µg $L^{-1}$ TP and 500 - 1000 µg $L^{-1}$ TN; beyond these values, ratios exhibited minimal additional decline.
3. Algae assemblage structure was strongly linked to surface water TP, TN, periphyton nutrient ratios, and catchment-scale nutrient sources (wastewater treatment plant (WWTP) discharges and % pasture cover). In particular, there were synchronous declines in frequency and cell densities of many alga species associated with TP concentrations > 21 µg $L^{-1}$ (90% CI of 18 to 48 µg $L^{-1}$) as well as simultaneous increases in tolerant species associated with increasing enrichment.
4. Fish assemblage structure was most strongly associated with % pasture, WWTP discharges, and fine sediment cover, yet also showed significant but weaker correlations with surface-water and periphyton nutrient variables. However, 2 benthic fish species, *Etheostoma spectabile* and *Campostoma anomalum*, significantly declined with TP > 28 µg $L^{-1}$ (90% CI, 24 - 56 µg $L^{-1}$) and 34 µg $L^{-1}$ (90% CI, 21 - 56 µg $L^{-1}$), respectively.

Conversely, the tolerant minnow *Cyprinella lutrensis* and invasive carp *Cyprinus carpio* increased nonlinearly with increasing surface water TP.

5. Our results provide new insights into interpretation and analysis of assemblage-level responses to nutrient enrichment. Our findings indicate that a numerical criterion for surface-water TP of approximately 20 µg $L^{-1}$ would be needed to maintain natural algae assemblages and at least two specialist fishes within our study region. Proliferation of weedy alga species and increased abundance of invasive fishes are also likely when surface-water concentrations exceed these thresholds. While many streams likely exceed these thresholds, managers should consider potential low-level enrichment effects when developing criteria for ecosystems to protect existing nutrient-limited streams.

Key words: eutrophication, running waters/rivers/streams, attached algae, fish, stoichiometry

## Introduction

Humans have altered the input of nitrogen (N) and phosphorus (P) to freshwater systems, and the resulting eutrophication is a major obstacle in protecting freshwater and coastal marine ecosystems (Carpenter *et al.*, 1998; Smith, Joye & Howarth 2006). In response, regulatory authorities, such as the U.S. Environmental Protection Agency (US EPA) and European Union Water Framework Directive, have charged water resource managers with developing numerical criteria for nutrients that protect physical, chemical, and biological integrity of aquatic ecosystems (US EPA, 1998; Hering *et al.*, 2010). Development of numerical nutrient criteria has progressed slowly, largely due to insufficient data on nutrients and biological endpoints, as well as inadequate statistical tools for quantifying levels of enrichment likely to cause biological impairments. In naturally nutrient-limited ecosystems, rapid ecological responses to relatively small changes in nutrient enrichment can occur and may be indicative of ecological thresholds. While observed nonlinear responses may not necessarily represent a system change in stable states, they can serve as useful benchmarks for protecting surface water from nutrient enrichment or other impacts, and more studies that explore techniques for detecting thresholds in freshwater ecosystems are needed (Dodds *et al.*, 2010).

Natural inputs of inorganic N and P to freshwater systems are influenced by catchment geology and vegetation. Whereas N requirements of freshwater organisms may be augmented by fixation of atmospheric $N_2$, P is largely derived from the weathering of phosphate-bearing rocks that are patchily distributed (Notholt, Sheldon & Davidson, 1989). As a result, most freshwater species assemblages have evolved in low-P environments. For example, periphyton communities dominated by species with low P requirements are common in regions where biogeochemistry is

driven by high Ca and low P availability (Noe, Childers & Jones, 2001). Relatively small increments of anthropogenic P can create novel environmental conditions for algae species that have evolved under low nutrient conditions, and often induce changes in species composition at relatively low levels of enrichment (Gaiser *et al.*, 2005; Richardson *et al.*, 2007). Shifts in primary producer species composition and biomass in response to P enrichment also can have substantial effects on ecosystem processes that influence higher trophic levels (Carpenter *et al.*, 1998; Miltner & Rankin 1998; Smith *et al.*, 2006). For example, changes in relative and absolute abundances of primary producers associated with nutrient enrichment can influence consumer assemblages through altered consumer-resource stoichiometric relationships (Cross *et al.*, 2005; Evans-White *et al.*, 2009), habitat structure (Mittelbach, 1984), and production-respiration dynamics (Miltner & Rankin, 1998). All of these factors can influence resource availability and habitat suitability for higher consumers such as fishes.

Species-specific responses to nutrient enrichment through direct (species optima for growth) or indirect (ecosystem changes associated with enrichment) mechanisms likely culminate in shifts in species assemblage composition involving both producers and consumers. Quantifying these patterns will promote understanding of how nutrient subsidies alter stream ecosystems. In regions of the world that experience seasonal low-flow periods, understanding how nutrient subsidies influence biotic communities is particularly germane. In some catchments, wastewater discharges now account for > 90% of instream flow during low-flow periods (Brooks, Riley & Taylor, 2006). Southern regions of North America are expected to experience reduced magnitude and increased frequency and duration of low stream flows due to climate change (Sun *et al.*, 2008). This emerging threat, combined with increased water

consumption and nutrient enrichment associated with rising human population densities, will have significant impacts on freshwater biodiversity (Palmer *et al.*, 2009; Dudgeon, 2010).

In this study, we examined responses of benthic algae and fish species assemblages across 38 streams spanning a steep gradient of N and P in central Texas, USA, in hopes of identifying nutrient levels protective of naturally nutrient limited streams that might provide a basis for development of numeric nutrient criteria. We hypothesized that benthic algae assemblages would show strong relationships with surface-water nutrient concentrations and exhibit sharp, nonlinear responses to low levels of enrichment. This prediction was based on the assumption that relaxation of evolutionary constraints imposed on algae species in oligotrophic systems should results in rapid shifts in species assemblage structure (Gaiser *et al.*, 2006). We further hypothesized that due to indirect rather than direct mechanisms, nutrient enrichment effects on fish species distributions would be less predictable, resulting in weaker thresholds that are more difficult to detect.

## Methods

*Study area and field sampling*

Study streams were located in the Cross Timbers ecoregion and Brazos and Trinity River basins in Texas (Fig. 1). Hydrology within this portion of the Southern Great Plains is highly variable due to seasonal precipitation patterns that include long, hot summers (Matthews *et al.*, 2005). Study sites were selected to provide broad geographic coverage and a range of landscape features that drive nutrient enrichment (pasture and wastewater treatment plant [WWTP] discharge) resulting in a wide range of stream nutrient conditions (Fig. 1, Table 1). At each sample site, we

collected data on water chemistry, instream and riparian habitat variables, periphyton nutrient content and species composition, and fish relative abundance following Texas Commission on Environmental Quality protocols (Texas Commission on Environmental Quality (TCEQ), 2005) and protocols outlined in King *et al.*, (2009).

*Environmental variables*

We selected a suite of environmental variables that were hypothesized to be associated with changes in species composition related to nutrient enrichment gradients. We considered five classes of variables: (a) catchment physiography, (b) land cover, (c) instream and riparian habitat, (d) water chemistry and (e) periphyton biomass and tissue chemistry. We initially included a wide range of environmental variables including 23 landscape and 41 local habitat variables (Pease *et al.*, 2011), but narrowed these down to a few non-redundant variables within each broad category using scatterplot matrices and associated correlation coefficients between variables significantly correlated with periphyton or fish assemblage structure (see Appendix Table S1-S3 in Supporting Information) (see *Data analysis* section for more detail).

Surface-water nutrient sampling consisted of triplicate surface-water instantaneous grab samples. Surface-water grab samples for total phosphorus (TP) and total nitrogen (TN) were analyzed using the molybdate and cadmium reduction method, respectively, following persulfate digestion (APHA, 1998). Total alkalinity, chloride (Cl), total suspended solids (TSS), volatile suspended solids, sulfate, total dissolved solids (TDS), and fluoride were sampled and analyzed in accordance with TCEQ Surface Water Quality Monitoring Procedures (TCEQ, 2003).

*Periphyton sampling*

We collected reach-scale composite samples of epilithic periphyton for analysis of nutrient content and assemblage structure by removing material from the surface of at least 25 rocks for a composite periphyton slurry following methods outlined in King *et al.* (2009). All samples were stored in Nalgene dark bottles and transported on ice (4ºC) to the lab within 24 hours. Periphyton samples were homogenized, subsampled, and filtered onto pre-weighed Whatman GF/F (pore size = 0.7 µm) filters for quantification of chlorophyll a, dry mass, and ash-free dry mass (AFDM) following Steinman, Lamberti & Leavitt (2006). We considered bulk periphyton samples for nutrient content analyses, because periphyton collectively includes autotrophic organisms and nonalgal material, including heterotrophic microbes and detritus in the form of fine particulate organic matter (Frost, Hillebrand & Kalhert, 2005; Hillebrand, Frost & Liess, 2008), all of which can serve as a basal resource for consumers and potentially provide a reliable indicator of nutrient enrichment (Gaiser *et al.*, 2004). Previous work within the study region indicates little difference between bulk periphyton nutrient content and algae separated from fine sediments following centrifugation (Hamilton, Sippel & Bunn, 2005; King *et al.*, 2009). Subsamples of homogenized periphyton samples were dried at 60°C for 48 hours and pulverized into a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc.) for analysis of nutrient content. We measured C and N content of periphyton using a ThermoQuest Flash EA™ 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) following fuming with HCl to drive off inorganic carbonates (Hill & Middleton, 2006). Periphyton P content was analyzed using the molybdate method following a 1-hour digestion in 15 mL of distilled water with 1.8 mL of a mixture of peroxodisulphate (30 g $L^{-1}$ $K_2S_2O_8$), boric acid (50 g

$L^{-1}$ $H_3BO_3$) and sodium hydroxide (15 g $L^{-1}$ NaOH) at 121°C (Faerøvig & Hessen, 2003). Soil (Thermo Finnigan 1.99% C) and peach leaf (SRM 1547, 0.137% P, 0.298% N) standards were analyzed to assure C, N, and P recoveries met QA/QC standards (± 10%) for each sample run.

*Species assemblage structure*

Additional aliquots of homogenized periphyton samples were preserved for species identifications in accordance with taxonomic methods for soft algae and diatoms (TCEQ, 2005). One soft algae and one diatom sample were sorted and identified per stream, with at least 500 diatom and 300 soft algae cells identified per respective sample (TCEQ, 2005).

We estimated fish assemblage structure by sampling all obvious habitat components (e.g., open pools or runs, undercut banks, brush piles, rocks, riffles) within each survey reach with a backpack electrofisher (Smith-Root Model LR-24) and seine net (4.6 m x 1.8 m, or 1.8 m x 1.8 m). Reach length was determined based upon 40 times the average wetted width. Crews of 3-4 people electrofished each study reach in a single upstream pass with a minimum effort of 900 seconds. The reach was then sampled with the seine net with a minimum of six 10-m hauls that covered all habitat components within the study reach. If the sixth haul yielded species not previously collected, additional seine hauls were made until no additional species were captured (TCEQ, 2005). Fish specimens were identified, counted, and either released unharmed into the habitat or euthanized by ice-bath immersion and then preserved in 10% buffered formalin for later identification according to Hubbs, Edwards & Garrett, (1991) and Thomas, Bonner & Whiteside, (2007). Numerical abundance of each algae and fish species was recorded for each study reach for analyses of species assemblage structure. Fish and instream habitat data were part

of a previous larger-scale study examining fish-habitat relationships across several ecoregions over multiple years (Pease *et al.*, 2011).

*Data analysis*

We used a combination of generalized additive models (GAM), non-metric multidimensional scaling (nMDS) ordinations, and threshold indicator taxa analysis (TITAN), to analyze associations between ecosystem structure and water nutrients and other catchment and reach-scale variables hypothesized to be important drivers of stream communities in this region.

*Generalized Additive Modeling (GAM)*: We used GAM to model relationships between bulk periphyton nutrient content and surface water nutrients (TP and TN) because graphical evaluation of scatterplots revealed nonlinear patterns. GAMs are well suited for fitting nonlinear response relationships where the precise form between the independent and dependent variables is not known *a priori* (Zuur *et al.*, 2009). We fit responses of periphyton nutrient ratios to surface water nutrients using GAMs with the mgcv package in R 2.11.2 (Wood, 2006). We fit GAMs using the gamma distribution because periphyton nutrient content data were positively skewed. Cross-validation was used to determine the optimal amount of smoothing. We limited the number of knots (k) to 3 to avoid model over-fitting due to small sample size (Wood, 2006). P values obtained from GAM for smoothing splines are approximate, therefore we interpreted ecological significance for GAM responses at $P \leq 0.001$ (Zuur *et al.*, 2009).

*Ordination and environmental vector fitting*: We ordinated stream locations according to periphyton and fish species structure, separately, using nMDS (Minchin, 1987; Clarke, 1993). Prior to nMDS analysis, we $\log_{10}(x)$-transformed abundance of periphyton and fish species to

down-weight the contribution of numerically dominant species to the ordinations (McCune & Grace, 2002). We used Bray-Curtis dissimilarity (BCD) as the distance measure. We performed nMDS and related analyses in the vegan package in R 2.11.2 (Oksanen *et al.*, 2010; R core development team 2010). Ordination plots were rotated to have the strongest correlation with the TP along axis 1 because TP was the strongest correlate of periphyton nutrient ratios and algal species composition (see Results). We used the function 'envfit' to examine linear correlations between assemblages (nMDS axes) and environmental factors associated with nutrient enrichment, and assessed significance of the fitted environmental vectors using 1000 random permutations (Oksanen *et al.*, 2010). All significant environmental vectors were assessed for collinearity within the broad categories of catchment physiography, land cover, stream reach, water chemistry and periphyton stoichiometry using scatterplot matrices and associated correlation coefficients. A subset of significant environmental variables were retained for plotting in nMDS that did not have strong correlations ($r > 0.7$) with each other within broad environmental categories (landscape, instream habitat, water quality) (see Tables S1-S3) (Zuur *et al.*, 2009). We included TN and Cl despite strong relationships with surface water TP because we wanted to contrast assemblage responses to both nutrients, whereas Cl provides a conservative tracer of the relative contribution of wastewater discharges to stream flow (see Table S3). Additionally, we overlaid TP concentrations within site ordination plots to confirm any observed relationships with nutrient enrichment. We also explored nonlinearity in nutrient-species assemblage relationships by fitting GAM responses curves of nMDS axis 1 to surface water nutrients using methods presented above but with the Gaussian distribution.

Finally, to assess the potential confounding effect of river basin (Trinity vs. Brazos, the two major basins samples in this study) on patterns of assemblage structure, we estimated the central tendency and dispersion of assemblage composition within ordination space by river basin using permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP) with the functions 'adonis' and 'betadisper' (Oksanen *et al.*, 2010).

*Threshold Indicator Taxa Analysis (TITAN)*: We analyzed the magnitude, direction, and uncertainty of responses of individual taxa to nutrient enrichment gradients (TP, TN) using TITAN (Baker & King, 2010). TITAN identifies the value of a predictor variable that maximizes association of individual taxa with either the negative or positive side of the partition. Association is measured by IndVal, computed as the product of the percentage of sample units in which a taxon occurred and the percentage of the total number of individuals captured by each partition (Dufrêne & Legendre, 1997). Bootstrapping is used to identify reliable threshold indicator taxa. A taxon is determined to respond positively or negatively to the gradient of interest if 1) the change in frequency and abundance of the taxon is in the same direction for at least 95% of the 1000 bootstrapped runs = "high purity", and 2) at least 95% of 1000 bootstrapped runs are significantly different from a random distribution (at $p < 0.05$) = "high reliability". The sum of IndVal z scores can also be used as an indicator of assemblage-level thresholds by identifying peaks in sums of all taxa z scores along the gradient associated with the maximum decline in all negative responders (z-) or increase in frequency and abundance of all positive responders (z+). We performed TITAN on $\log_{10}(x)$-transformed abundances of

periphyton and fish taxa occurring in at least 3 sites to down-weight very large values. TITAN was run with the TITAN 2.0 package (Baker & King, 2010, 2013) in R.2.11.2.

## Results

*Surface-water nutrient concentrations*

Surface-water nutrient concentrations ranged between 7 and 2380 µg $L^{-1}$ for TP, and 127-15,860 µg $L^{-1}$ for TN (Table 1). Simple correlation analysis suggests that the observed nutrient gradient was driven primarily by differences in wastewater effluent contributions to stream flow. Both TP and TN were positively correlated with Cl concentrations (see Table S3). In general, sites with high TP also had high Cl, indicating a wastewater effluent influence on TP concentrations (see Appendix Fig. S1a in Supporting Information). Similar trends were observed for TN but were more variable (see Fig. S1d). Many sites with high nutrient concentrations also had high amounts of pasture within the watershed (see Fig. S1b,e), but those sites generally also had high Cl concentrations, and several sites with high % pasture had relatively low nutrient concentrations. Additionally, surface water TP and TN concentrations were positively correlated (Pearson's product-moment correlation, r = 0.86, $P < 0.001$) (see Table S3, Fig. S1c).

*Periphyton nutrient content*

Periphyton C:P, C:N and N:P ratios declined sharply with low levels of nutrient enrichment (Fig. 2). We observed the strongest relationship between periphyton C:P and surface water TP. Periphyton C:P ranged from 300-700 below 20 µg $L^{-1}$ TP, but rapidly approached 100-200 above 20 µg $L^{-1}$ TP and showed little additional decline with increasing TP up to 2000 µg $L^{-1}$ (Fig. 2A). Carbon:P ratios were always below 200 once surface water TP exceeded 50 µg $L^{-1}$. While we

observed similar patterns between periphyton nutrient ratios and surface water TN, TP always explained more variation (Fig. 2). This was due primarily to sites that appeared to be outliers for TN relationships. For example, periphyton nutrient ratios for one site (plotted as a triangle in Fig. 2) characterized by high surface water TN (1783 µg $L^{-1}$) but low TP (16.6 µg $L^{-1}$) followed observed patterns expected for a low TP site but was an outlier for the TN relationship, indicating that P was the most likely driver of the observed patterns. Overall, GAM fitted relationships identified large reductions (34-61 %) in nutrient content across a small range of TP (7-50 µg $L^{-1}$) and TN (127-1000 µg $L^{-1}$) concentrations (Fig. 2).

*Environmental variables and assemblage structure*

Ordination of periphyton assemblage structure sorted streams along two major axes that explained 79% of the variance among original distances in *n*-dimensional space (2-D stress = 17.7). The first axis represented a gradient primarily defined by nutrient enrichment variables, whereas the second represented a shorter gradient associated with stream discharge (Fig 3a). Variation in periphyton assemblage structure was most strongly linked to measures of nutrient enrichment defined by TP, TN, and Cl. A graphical overlay of TP on the nMDS plot helped validate the statistical relationship between TP and periphyton assemblage structure, illustrating that assemblages in sites with high TP are more similar to each other than sites with lower TP (Fig. 3a). Catchment pasture and accompanying environmental stressors (mud/silt, TSS) were closely associated with the nutrient enrichment gradient along axis 1, and WWTP density was associated with both nutrient enrichment as well as discharge on axis 2 (Fig. 3a).

Fish assemblage composition arranged sites along two major axes that explained 73% of the original distances in n-dimensional space (2-D stress = 20.9). In contrast to patterns in periphyton assemblages, composition of fish species assemblages was most strongly associated with latitudinal gradients on axis 1 and a stream size gradient on axis 2 (Fig. 3b). Percent pasture in the catchment and bank slope had significant associations with fish assemblage structure along the same trajectory as latitudinal gradients (Fig. 3b). Fish assemblage structure also was related to measures of nutrient enrichment on axis 1, with TP increasing in conjunction with increased mud/silt substrates, TSS, Cl, and WWTP density. However, the relationship between fish assemblage structure and nutrient enrichment was weaker than that observed for periphyton assemblage structure (Fig. 3b). While most sites with high TP plotted on the right side of the ordination plot (consistent with the TP vector), two sites with high TP and WWTP influence had fish species structure more similar to sites on the left side that had low values for pasture, mud/silt substrates and nutrients (Fig. 3b). Latitudinal gradients in fish species structure among river basins were indicated by separation of fish assemblages in ordination space based on river basin (PERMANOVA, $F_{1,36} = 9.51$, $P = 0.009$) (Fig. 3b) and PERMDIST results ($F_{1,36} = 0.91$, $P = 0.36$), which indicates no significant heterogeneity of variance for assemblage composition in the two basins. However, latitude was correlated with catchment % pasture (Pearson's product-moment correlation, $r = 0.76$, $P < 0.001$) (Table S1), and this pattern was driven by higher median % pasture values for sites within the Trinity Basin (Wilcoxon rank-sum test, $W = 251$, $n_1 = 9$, $n_2 = 26$, $P < 0.001$).

*Taxon and assemblage responses to nutrients*

*Periphyton*. Generalized additive model fitted relationships identified a large change in assemblage structure characterized by changes in nMDS axis 1, over a small range of TP values (10-50 µg $L^{-1}$) that was consistent with shifts in assemblage structure identified by TITAN (Fig. 4a). Trends in assemblage structure summarized by nMDS axis 1 associated with TN were less clear than those observed for TP. A potential nonlinear relationship between TN and periphyton assemblage structure was confounded by multiple sites that had high TN concentrations, but also had TP concentrations below the observed TITAN threshold and periphyton assemblages consistent with low P sites (Fig. 4b).

TITAN identified 16 of the 148 alga taxa as pure and reliable indicator taxa that declined sharply with increasing surface water TP between 16 and 52 µg $L^{-1}$ (Fig. 4c, See Appendix S4 in Supporting Information). Synchronous declines detected by TITAN resulted in an assemblage-level threshold estimate for sensitive taxa of 21µg $L^{-1}$ TP (90% CI, 18 - 48 µg $L^{-1}$) (Table 2, Fig. 4e). There was also an assemblage-level threshold expected identified at 28 µg $L^{-1}$ (90% CI, 19 and 48 µg $L^{-1}$) for the taxa that sharply increased in response to TP (Table 2, Fig. 4e). This assemblage-level threshold aligned closely with the synchronous increases in the frequency and cell density of the majority of 21 positive responding alga taxa (Fig. 4c, see Table S4).

Only seven alga taxa declined in response to increasing surface-water TN (Fig. 4d, See Appendix S5 in Supporting Information). Of these, six also had significant negative responses to TP. *Oscillatoria*, a genus capable of N-fixation in low-N environments, was the only taxon not previously associated with increasing P enrichment. Likewise, 7 taxa that increased with N enrichment also exhibited significant positive associations with increasing P enrichment (Fig. 4, see Table S5). Strong synchronous changes consistent with assemblage-level thresholds were not

observed for taxa either negatively or positively associated with surface water TN (Table 2, Fig. 4f).

*Fish*. Trends in fish assemblage structure (represented by nMDS axis 1, Fig. 3b) associated with surface water TP were less clear than trends observed for periphyton assemblage structure. There was a weaker non-linear trend in assemblage structure in response to P enrichment, but this trend was not significant (GAM, $P > 0.001$). Assemblage change appeared to be associated with low to moderate P enrichment, thereafter assemblages became highly variable regardless of TP concentrations (Fig. 5a). GAM response curves were not significant and there was also no clear trend between overall fish assemblage structure (nMDS axis 1) and surface water TN (Fig. 5b).

TITAN analysis only identified significant declines in 2 out of 31 fish taxa associated with TP. Central stonerollers (*Campostoma anomalum* Rafinesque) and orangethroat darters (*Etheostoma spectabile* Agassiz) had markedly lower occurrence and abundance at TP concentrations > 28 µg $L^{-1}$ (90% CI, 24 - 56 µg $L^{-1}$) and 34 µg $L^{-1}$ (90% CI, 21 - 56 µg $L^{-1}$), respectively (Fig. 5c, See Appendix Table S6 in Supporting Information). In contrast, TITAN revealed a significant increase in occurrence and abundance of red shiners (*Cyprinella lutrensis* Baird and Girard) that was associated with relatively low TP value of 21 µg $L^{-1}$ (90% CI,13 - 24 µg $L^{-1}$). TITAN also identified higher threshold concentrations for increasing common carp (*Cyprinus carpio* Linnaeus) at higher TP concentrations of 187 µg $L^{-1}$ (90% CI of 33 to 1069 µg $L^{-1}$) (Fig. 5c, see Table S6). TITAN estimated an assemblage-level threshold of 34 µg $L^{-1}$ for both negative and positive responding fish, but there was more uncertainty around the declining

species component (90% CI of 27 to 598 µg $L^{-1}$) than the increasing species component (90% CI of 13 to 52 µg $L^{-1}$) (Table 2, Fig. 5e).

None of the fish species revealed significant declines in association with N enrichment. Red shiners and bullhead minnows (*Pimephales vigilax* Baird and Girard) were significantly higher in occurrence and abundance when TN concentrations were greater than 280 µg $L^{-1}$, and carp increased significantly at sites where TN concentrations were greater than 546 µg $L^{-1}$ (446 - 1892 µg $L^{-1}$) (Fig. 5b, See Appendix Table S7 in Supporting Information). TITAN identified a TN threshold beyond which tolerant species such as red shiner and carp are expected to increase within the assemblage (Table 2), but this value should be treated with caution as there was no clear peak in the Sum(z) score (Fig. 5 d).

## Discussion

Low levels of nutrient enrichment, particularly P, corresponded with significant nonlinear responses of periphyton nutrient content and markedly lower abundances of many alga taxa and two benthic fish species that represent two distinct functional groups (central stoneroller = periphyton grazer; orangethroat darters = riffle-dwelling, benthic invertivore). Previous studies have reported changes in periphyton assemblages based on biotic indices at relatively low levels of P enrichment (Gaiser *et al.*, 2005; Richardson *et al.*, 2007; Stevenson *et al.*, 2008a). Our study provides new insights into periphyton assemblage responses to nutrient enrichment by demonstrating rapid nonlinear change in bulk periphyton nutrient content and abrupt, negative changes in abundance for many taxa with small increases in P. This, combined with positive changes in the abundance of several tolerant taxa associated with increasing P enrichment,

contributed to overall changes in periphyton assemblages. Two fish species revealed significant, nonlinear declines in association with low levels of P enrichment. In contrast, a tolerant native minnow (red shiners) that is invasive in many regions of North America, and an invasive exotic species (common carp) occurred more often and in greater abundance at sites with higher P. Our results suggest that large changes in assemblage structure of primary producers, as well as distributions of native and non-native fish species involved in strong interactions within stream food webs, are associated with small changes in nutrient status of streams (Fig. 6). These results can be used to help water resource managers identify ecologically relevant criteria that are protective of naturally P-limited streams.

*Phosphorus as a driver of change in stream ecosystem structure*

Nitrogen covaried with P across our study sites, and in every case of elevated P, N was also elevated above background. However, almost all of the species that had significant negative responses associated with the N-enrichment gradient were a subset of species that also had negative responses associated with P enrichment. Uncertainty around assemblage-level thresholds was much higher based on TN verses TP, and trends in overall assemblage structure (nMDS axis 1) in response to nutrient enrichment indicated that sites with high TN but low TP were associated with structures typically associated with low-P ecosystems.

Additionally, patterns of association between periphyton nutrient content and N enrichment were always weaker than those between periphyton nutrient content and P enrichment. Uncertainty in periphyton nutrient content models based on surface-water TN was driven primarily by the fact that certain streams had high N but relatively low P, and always had

nutrient ratios consistent with low P environments rather than high N. These sites were in watersheds draining row-crop agriculture which can be a substantial non-point source of both N and P (Carpenter *et al.*, 1998). However, croplands in our study area appeared to contribute mostly N and little P to streams, and under those conditions stream biota appeared to be relatively unresponsive. Negative associations between periphyton C:N and N:P and P enrichment demonstrates that N is important in central Texas streams, but it is unlikely that there would have been a strong response to N alone under very low P. Virtually all sources of P in our study area as well as most parts of the world are also sources of N, whereas not all sources of N, particularly row-crop agriculture, are sources of P. P enrichment appears to be the primary driver of ecological responses to nutrient gradients in our study area.

*Stream assemblage patterns*

Our results contribute to growing evidence for nonlinear responses of aquatic assemblages to nutrient enrichment (King & Richardson, 2003; Richardson *et al.*, 2007; Stevenson *et al.*, 2008b; Evans-White *et al.*, 2009). Many studies have reported linear responses (Miltner & Rankin, 1998; Pan *et al.*, 2000; Justus *et al.*, 2010; Johnson & Hering, 2009) or subsidy-stress relationships (King & Richardson, 2007) between aggregate measures of stream assemblage structure (metrics, ordination axes) and increasing nutrient enrichment. Whereas linear responses provide evidence of linkages between nutrient enrichment and changes in biotic assemblages in streams, these are limited in application to nutrient criteria development, and can be an artifact of univariate measures and associated analyses techniques that simplify complex responses to novel environmental gradients (Baker & King, 2010; King & Baker 2010). We observed a nonlinear

trend in periphyton assemblage structure associated with increasing P based on an aggregate measure (nMDS axis 1) (Fig. 4a). This trend may be interpreted differently in terms of management thresholds, depending on different threshold analyses (Dodds *et al.*, 2010). For example, univariate threshold detection techniques that identify assemblage-level change based on a change in slope (piecewise regression) would likely identify a threshold at higher concentrations than assemblage-level thresholds identified by TITAN. The cumulative distribution of assemblage-level changepoints estimated by bootstrapping in TITAN represents a zone of rapid change that corresponded well with the zone of greatest change in nMDS axis 1 in the GAM model (Fig. 4a). Observed TITAN assemblage-level thresholds corresponded with the lower end of that distribution, where assemblage-level change starts to happen. Combining more traditional data analysis approaches with TITAN or other approaches that separate negative and positive responses within assemblages, will help resource managers to identify dominant negative and positive responders within the assemblage, and estimate (with confidence intervals) where major shifts in assemblage structure occur. Current studies are starting to utilize this approach in assessing assemblage-level responses to novel environmental gradients in both freshwater (King *et al.*, 2011, Kail, Arle & Jähnig, 2012; Smucker, Detenbeck & Morrison, 2013) and terrestrial (Cardoso *et al.*, 2013; Payne *et al.*, 2013; Suarez-Rubio *et al.*, 2013) ecosystems. Such an approach may lead to more proactive nutrient criteria for high quality streams by identifying TP concentrations where change starts to happen, verses concentrations where changes in assemblage structure has already occurred.

Threshold responses in periphyton assemblage structure can be used to help managers identify ecological relevant criteria that are protective of naturally P-limited streams and prevent

shifts from periphyton assemblages adapted to low-P conditions to those that flourish in eutrophic conditions. Additionally, our results may have applicability outside of our study region, as many indicator species for low and high-P conditions identified in the current study have been identified in other regional studies and at the national scale. Several of the alga taxa that had negative associations with P enrichment in our study have been shown to have optima at relatively low levels of TP (Stevenson *et al.*, 2008a; Wang *et al.*, 2009; Justus *et al.*, 2010), and all corresponded particularly well with the low-P indicator taxa identified by Potapova & Charles (2007) for streams in the United States. In every case, species with high abundance associated with low TP concentrations in our study had previously been identified as significant indicator species of low-P conditions. Species that had higher abundances in association with greater concentrations of P also agreed well with high-P indicator taxa identified by Potapova & Charles (2007) and those used by others in diatom indices that measure responses of tolerant species to nutrient enrichment (Stevenson *et al.* 2008a; Stevenson *et al.* 2008b).

Fish assemblages showed stronger relationships with pasture coverage than P enrichment or related variables, suggesting that other pasture-related stressors, such as increased bank erosion and mud/silt substrates, may have contributed to fish patterns (Fig. 3b). However, widespread application of manure on pasturelands in our study region can elevate P in shallow, sub-surface groundwater that flows into stream bank sediments during low-flow periods, and this can result in substantial inputs of sediment-bound P to streams via bank erosion (Thompson & McFarland, 2010). Observed latitudinal gradients and basin differences in fish assemblage structure may be caused, at least in part, by inter-basin differences in pasture coverage.

Despite a relatively weak correlation between P enrichment and fish assemblage structure (Figs. 3b, 5a), TITAN identified species that had negative and positive associations with increasing P. In particular, two benthic species, orangethroat darters and central stonerollers, showed markedly lower abundance as surface water TP increased beyond relatively low concentrations. In contrast, red shiners and carp were more abundant in sites with greater nutrient concentrations. Negative fish responses to nutrient enrichment may be due to intolerance to environmental conditions associated with increased primary production, particularly in streams with natural summer low flows. Valenti *et al.* (2011) observed that low-flow periods and P enrichment interact to increase the magnitude of diel fluctuations in dissolved oxygen and pH across streams within our region. Dissolved oxygen fluctuations in enriched streams could have exceeded the physiological limits of orangethroat darters and central stonerollers within isolated pools during low-flow conditions (Miltner & Rankin, 1998; Miltner, 2010). Dissolved oxygen fluctuations may have been ameliorated by flow augmentation from effluent discharge in some high-P sites, contributing to increased variation in fish assemblage structure at high P sites (Fig. 5a). Source-sink dynamics associated with variation in toxic $NH_4$ releases coupled with recolonization dynamics from adjacent low-nutrient habitats (Waits *et al*., 2008) could contribute to higher variation in fish assemblage structure at effluent-dominated sites with high P.

*What are the implications for nutrient management in streams?*

This study presents multiple lines of evidence that support the development of nutrient criteria at low levels of P (~ 20 µg $L^{-1}$) to maintain natural periphyton and fish assemblages in our study region. First, we observed non-linear changes in bulk periphyton elemental ratios at low levels of

P enrichment, particularly for C:P ratios which never fell below 300 until TP approached 20 µg $L^{-1}$. Second, there was a cumulative decline of sensitive species within periphyton assemblages around 21 µg $L^{-1}$ TP (90% CI of 18 to 48 µg $L^{-1}$) that corresponded with an overall non-linear shift in assemblage structure (depicted by nMDS Axis 1). Third, we observed significant declines in two functionally important benthic fish species (central stonerollers, orangethroat darters) associated with similar levels of P enrichment, 28 µg $L^{-1}$ (90% CI: 24-56) and 34 µg $L^{-1}$ (90% CI: 21 -56), respectively. These findings, combined with positive changes in abundance of colonizing algae species as well as tolerant minnows and invasive carp associated with increasing concentrations of TP (~21-187 µg $L^{-1}$), support our assertion that major shifts in stream ecosystem structure occur at low levels of P enrichment in streams (Fig. 6). However, it is important to recognize that observed responses are limited to our dataset and confidence intervals represent the expected range where shifts are likely to occur 90% of the time. Managers should apply a precautionary approach and consider the lower portion of these distributions when developing nutrient criteria to ensure adequate protection for nutrient-limited stream ecosystems. While meeting such criteria may be infeasible for many streams, particularly those that receive significant effluent discharge, such criteria will provide benchmarks for preventing impacts to existing low-nutrient stream ecosystems.

This approach moves beyond setting management standards for eutrophic systems by recognizing that significant changes in stream assemblages that have evolved under low-nutrient conditions can occur with slight enrichment (Fig. 6). However, managing sources of P should also include best management practices for N reduction. N enrichment covaried with P across our study sites, and Lang, King & Scott (2012) demonstrated that stream periphyton in this

region can be co-limited by both N and P. While assemblage and species-level thresholds identified in the current study may serve as starting points for establishing criteria, managers also should consider the influence of N enrichment on downstream water bodies with different nutrient limitation status when developing nutrient criteria, because N generally is the nutrient that most directly impacts estuaries and associated coastal ecosystems (Smith et al., 2006).

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Table 1. Selected physical characteristics, water chemistry and benthic nutrient content for all 38 sampling sites used in the final analyses. Surface water TN is listed for reference despite being highly correlated with phosphorus variables.

| Variable | Min | Max | Mean | Median |
|---|---|---|---|---|
| Latitude DS | 30.9 | 33.6 | 32.00 | 31.88 |
| Watershed area ($km^2$) | 68 | 6112 | 845 | 404 |
| WWTP ($1 \times 10^6$ L $d^{-1}$ $km^{-2}$) | 0 | 0.45 | 0.04 | 0 |
| % Shrub | 0 | 56.6 | 12.6 | 4.9 |
| % Pasture | 0.1 | 18.6 | 5.6 | 3.3 |
| Discharge ($m^3$ $s^{-1}$) | 0 | 24.6 | 3.5 | 1.3 |
| % Mud/silt cover | 0 | 48.3 | 8.4 | 2.2 |
| Bank Slope (%) | 17.9 | 64.3 | 34.3 | 34.1 |
| Specific Conductivity (µS $cm^{-1}$) | 184 | 1225 | 646 | 604 |
| Cl (mg $L^{-1}$) | 7 | 140 | 41 | 24 |
| TSS (mg $L^{-1}$) | 1 | 105 | 14 | 7 |
| Surface Water TP (µg $L^{-1}$) | 7 | 2380 | 296 | 29 |
| Surface Water TN (µg $L^{-1}$) | 127 | 15860 | 1635 | 463 |

Table 2. Nutrient thresholds estimated by TITAN sum ($z$-) (negative indicator taxa) and sum($z$+) (positive indicator taxa). The estimated community threshold (Obs.) is shown for each nutrient with lower (*5%*), mid (*50%*) and upper (*95%*) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates.

| Nutrient | sum ($z$-) | sum ($z$+) |
|---|---|---|
| *Periphyton* | | |
| TP | 21.43 (17.73, 25.20, 48.26) | 27.77 (19.05, 30.18, 48.13) |
| TN | 1891.67 (266.0, 434.0, 2285.0) | 440.83 (328.17, 440.83, 2393.33) |
| *Fish* | | |
| TP | 34.18 (26.78, 52.08, 598.33) | 34.18 (13.42, 27.28, 52.08) |
| TN | -- | 238.83 (238.83, 370.58, 491.16) |

*Notes:* Observed thresholds are the maximum sum ($z$). Values in parentheses are the 5$^{th}$, 50$^{th}$, and 95$^{th}$ percentiles corresponding to the frequency distribution of thresholds from 1000 bootstrap replicates. TP = surface-water total phosphorus (µg L$^{-1}$) and TN = surface-water total nitrogen (µg L$^{-1}$).

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Figure 1. Locations of the 38 study sites across the Brazos and Trinity River basins within the Cross Timbers ecoregion (Texas, U.S.A.).

Figure 2. GAM smoothers for periphyton C:P (a-b), C:N (c-d), and N:P (e-f) in response to surface-water TP and TN. Solid lines represent predicted ratios with 95% CI (dashed lines). $R^2$ = null deviance – residual deviance / null deviance and $P < 0.001$ is indicated by ***. Black triangle represents sample site with low surface water TP and high TN. Note that *x* axis is presented on log scale for ease of interpretation.

Figure 3. Nonmetric multidimensional scaling (NMS) ordination of sites based on periphyton (a) and fish (b) assemblage data. Symbol shading indicate membership among the Brazos (open) and Trinity (filled) river basins. Environmental vectors show the direction and magnitude of significant correlations between environmental factors and assemblage structure within the ordination space and associated correlation coefficients (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Ordinations are rotated on axis 1 to surface water TP (µg $L^{-1}$) which is represented by size of circles. Lat. DS = latitude in decimal degrees, WWTP = total upstream wastewater effluent per $km^2$ ($1 \times 10^6$ L $d^{-1}$), Bank Slp = bank slope (%), TSS = total suspended solids (mg $L^{-1}$), Cl = chloride (mg $L^{-1}$), TP = total phosphorus (µg $L^{-1}$), TN = total nitrogen (µg $L^{-1}$).

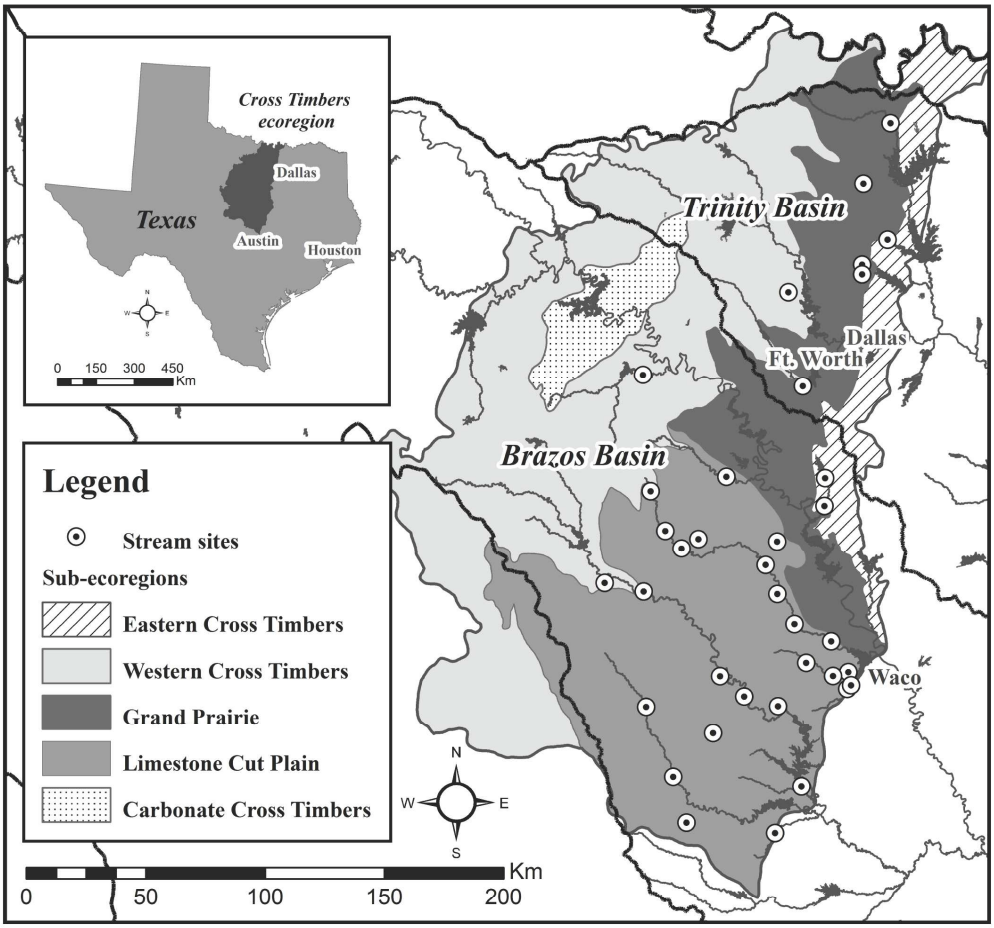
Figure 4. Change in periphyton assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN (µg $L^{-1}$) gradients spanning 38 sample sites (a-b). Solid lines represent

predicted abundance with 95% CI (dashed lines). Solid symbols represent sites below the surface-water TP (µg $L^{-1}$), assemblage-level threshold identified by TITAN. Dot sizes represent the distribution of surface water TP (µg $L^{-1}$) concentrations among sites. $R^2$ = null deviance – residual deviance / null deviance and $P < 0.001$ is indicated by ***. Note that *x* axis is presented on log scale for ease of interpretation. Significant periphyton indicator taxa identified in Threshold Indicator Taxa Analysis (TITAN) across surface water TP and TN (µg $L^{-1}$) gradients spanning 38 sample sites (c-d). Significant (purity ≥ 0.95, reliability ≥ 0.95, $P \leq 0.05$) indicator taxa are plotted in increasing order with respect to 90 % confidence in their observed change point. Solid symbols correspond to negative (z-) indicator taxa, whereas open symbols correspond to positive (z+) indicator taxa. Symbols are sized in proportion to magnitude of the response (z scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1000 bootstrap replicates. TITAN sum (*z*-) (aggregate response of negative indicator taxa, black symbols) and sum (*z*+) (positive indicator taxa, open symbols) are shown in response to TP and TN enrichment (e-f). Peak sum (*z*) scores correspond to the nutrient value resulting in the largest synchronous change among negative and positive indicator taxa, respectively. Solid (*z*-, negative indicator taxa) and dashed (*z*+, positive indicator taxa) lines represent the cumulative threshold frequency distribution of the peak sum(*z*) value obtained among 1000 bootstrap replicates for negative and positive indicator taxa, respectively (right *y* axis).

Figure 5. Change in fish assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN (µg $L^{-1}$) gradients spanning 38 sample sites (a-b). Significant fish indicator taxa identified in Threshold Indicator Taxa Analysis (TITAN) across surface water TP and TN (µg $L^{-}$

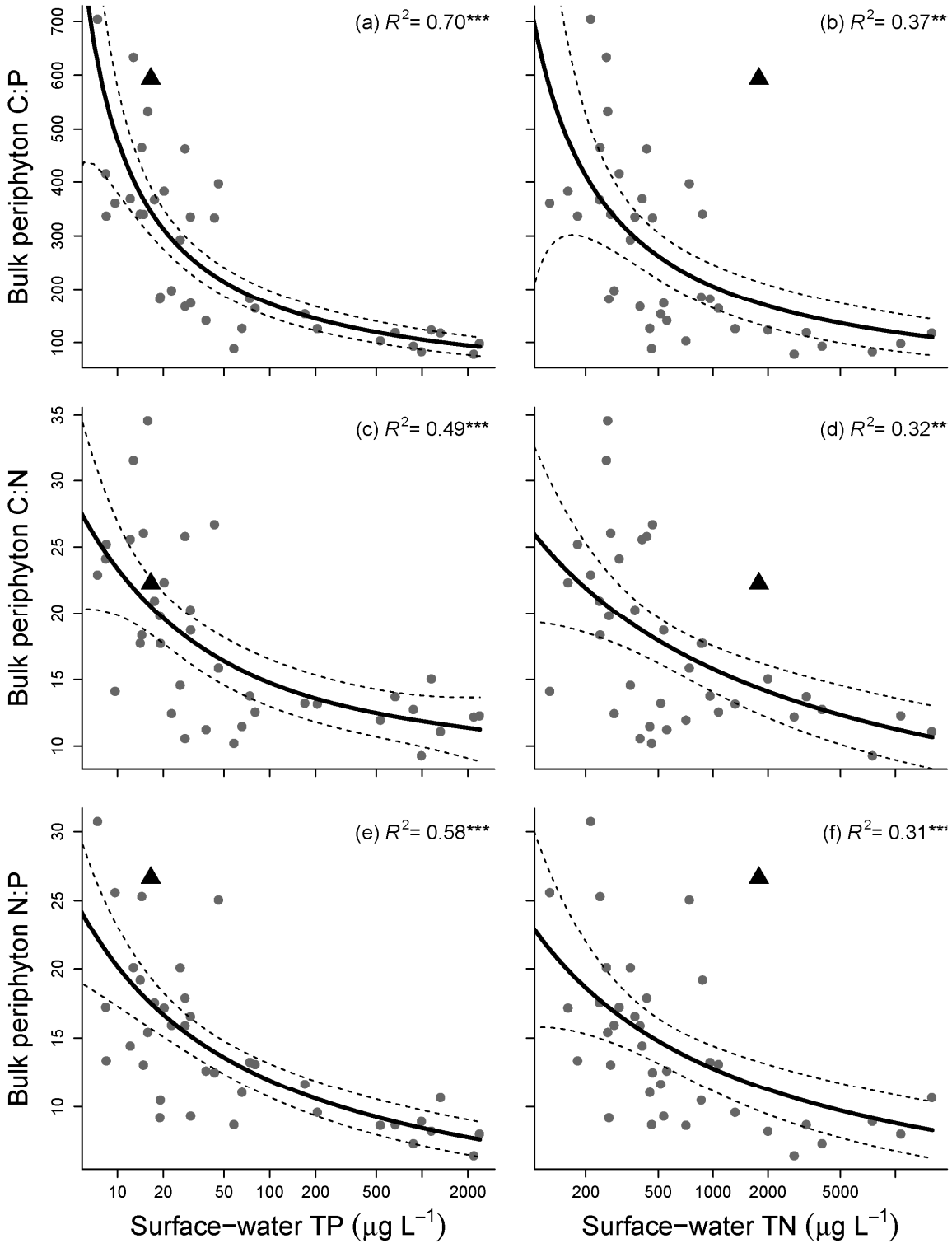
$^{1}$) gradients spanning 38 sample sites (c-d). TITAN sum (*z*-) (aggregate response of negative indicator taxa, black symbols) and sum (*z*+) (positive indicator taxa, open symbols) are shown in response to TP and TN enrichment (e-f). See Figure 4 legend for explanation of figure symbols.

Figure 6. Representative differences in stream habitat (a, b), fish assemblages (c, d), and fish indicator species (e, f) between low nutrient (TP = 7 µg $L^{-1}$; TN = 213 µg $L^{-1}$) (a, c, e) and slightly enriched (TP = 66 µg $L^{-1}$; TN = 450 µg $L^{-1}$) (b, d, f) streams from central Texas. Fish indicator species are (e) orangethroat darter (*Etheostoma spectabile*) and (f) red shiner (*Cyprinella lutrensis*).


Cross Timbers ecoregion
Dallas
Texas
Austin
Houston
0 150 300 450 Km
Trinity Basin
Dallas
Ft. Worth
Brazos Basin
Waco
Legend
Stream sites
Sub-ecoregions
Eastern Cross Timbers
Western Cross Timbers
Grand Prairie
Limestone Cut Plain
Carbonate Cross Timbers
N
W
E
S
0 50 100 150 200 Km

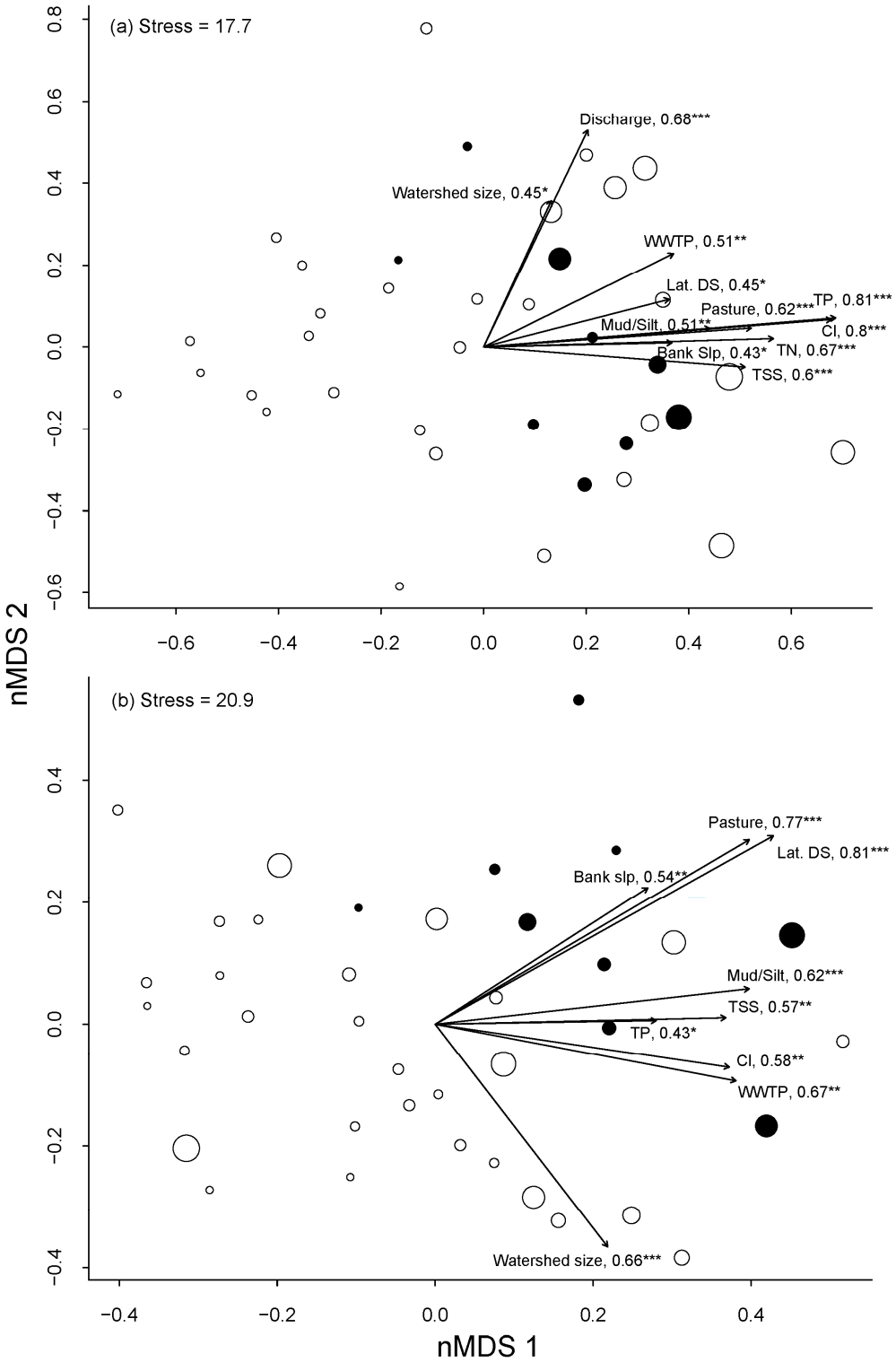
793

794 Fig. 1


(a) $R^2$= 0.70***
(b) $R^2$= 0.37**
(c) $R^2$= 0.49***
(d) $R^2$= 0.32**
(e) $R^2$= 0.58***
(f) $R^2$= 0.31***
Bulk periphyton C:P
Bulk periphyton C:N
Bulk periphyton N:P
Surface−water TP (µg L$^{-1}$)
Surface−water TN (µg L$^{-1}$)

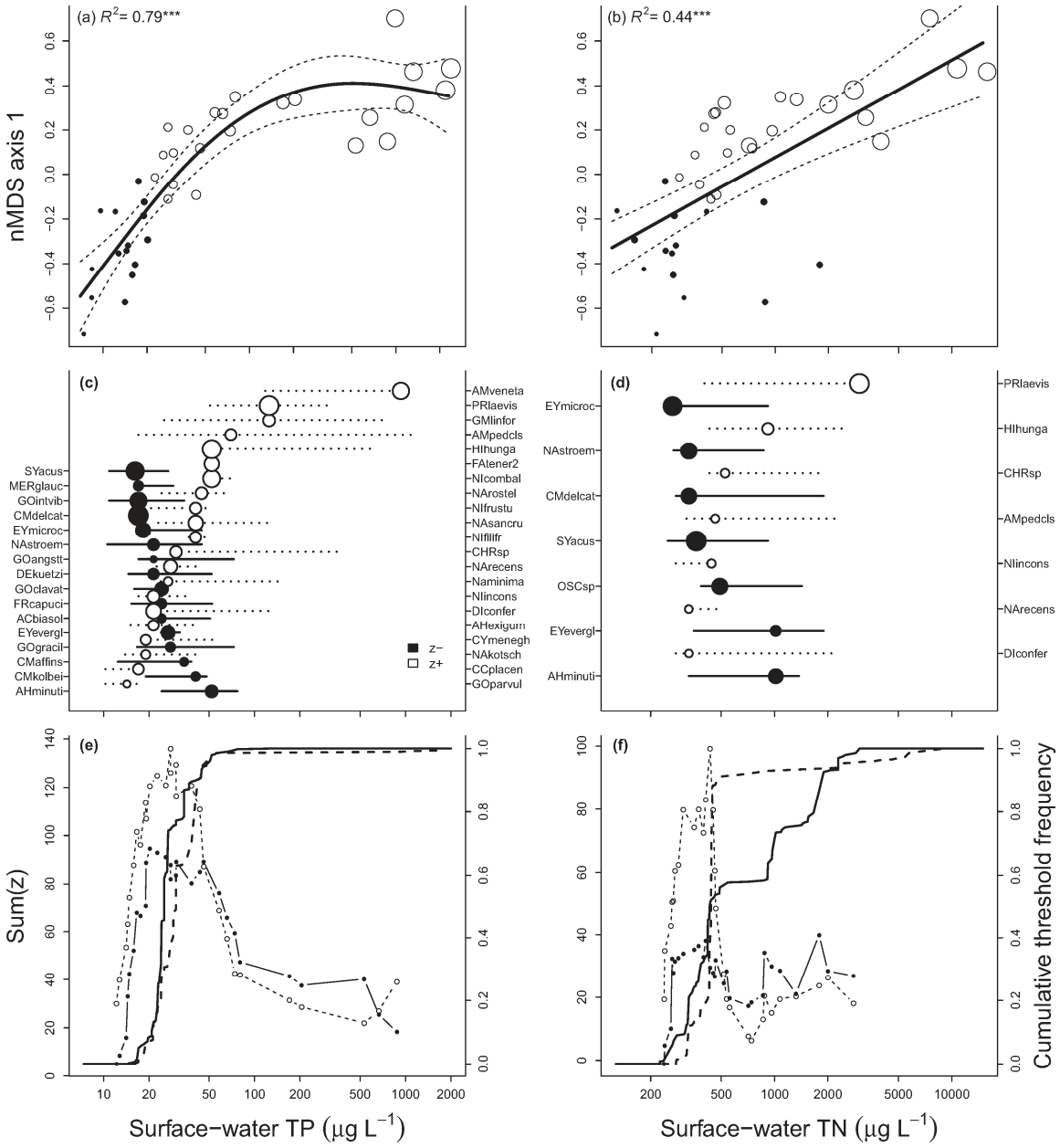
795

796 Fig. 2


(a) Stress = 17.7
Discharge, 0.68***
Watershed size, 0.45*
WWTP, 0.51**
Lat. DS, 0.45*
Pasture, 0.62***
TP, 0.81***
Mud/Silt, 0.51**
Cl, 0.8***
Bank Slp, 0.43*
TN, 0.67***
TSS, 0.6***
(b) Stress = 20.9
Pasture, 0.77***
Lat. DS, 0.81***
Bank slp, 0.54**
Mud/Silt, 0.62***
TSS, 0.57**
TP, 0.43*
Cl, 0.58**
WWTP, 0.67**
Watershed size, 0.66***
nMDS 1
nMDS 2

797

798 Fig. 3




799

800 Fig. 4

801

802

803

804

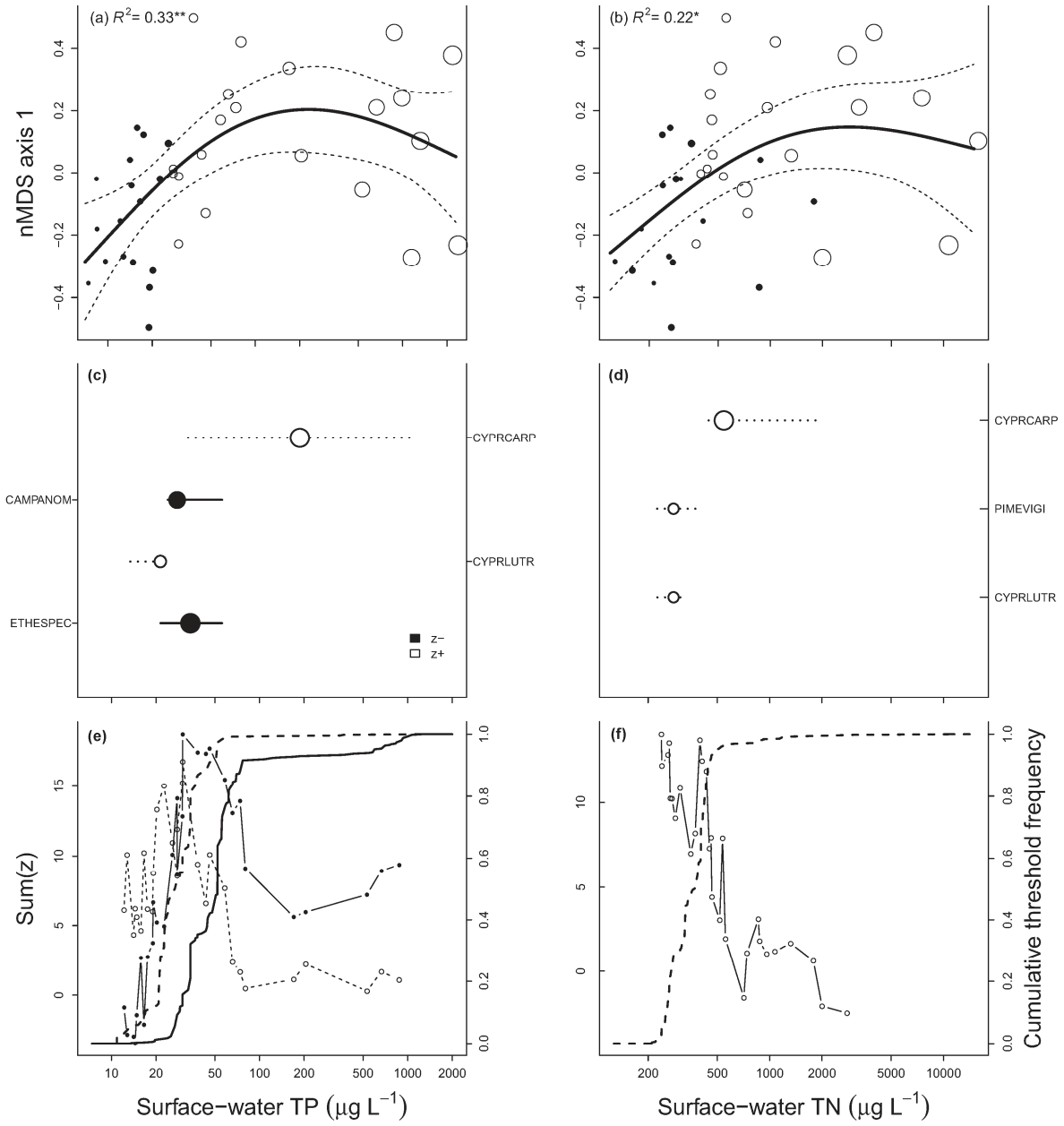

(a) $R^2$= 0.33**
(b) $R^2$= 0.22*
nMDS axis 1
(c)
(d)
CAMPANOM
ETHESPEC
CYPRCARP
CYPRLUTR
PIMEVIGI
z−
z+
(e)
(f)
Sum(z)
Cumulative threshold frequency
Surface−water TP (µg L$^{-1}$)
Surface−water TN (µg L$^{-1}$)

Fig. 5

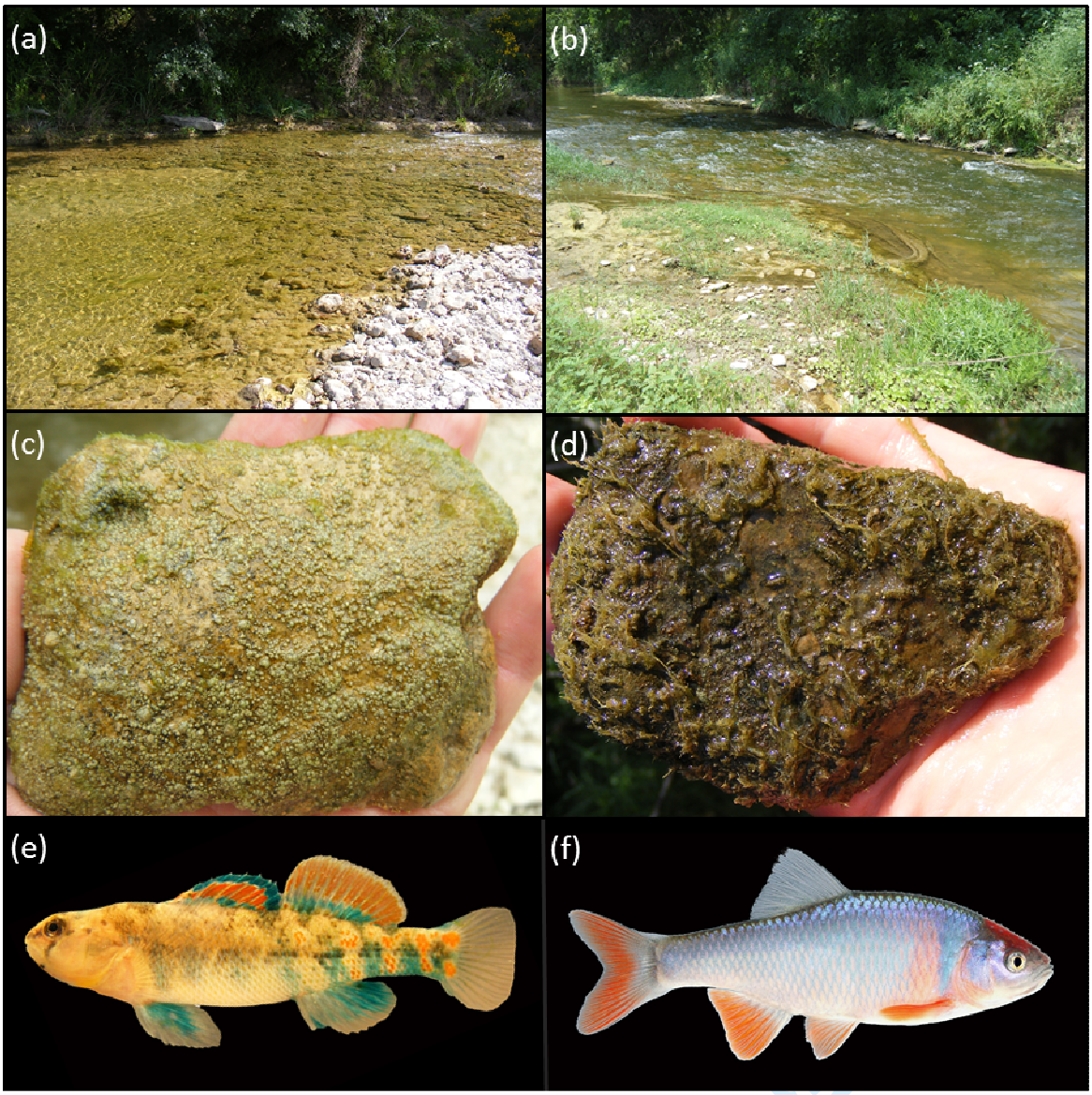



Fig. 6

Table S1. Correlation coefficients between landscape variables.

| | Latitude DS | Wshed ($km^2$) | WWTP | % Pasture |
|---|---|---|---|---|
| Latitude DS* | 1.00 | | | |
| Wshed ($km^2$) | -0.01 | 1.00 | | |
| WWTP($1 \times 10^6$ L $d^{-1}$ $km^{-2}$) | 0.41 | 0.52 | 1.00 | |
| % Pasture | 0.76 | 0.04 | 0.37 | 1.00 |

*Latitude was kept in nMDS vector fitting despite strong correlation with % Pasture to examine it as a potential confounding factor.

Table S2. Correlation coefficients between instream variables.

| | Discharge ($m^3$ $s^{-1}$) | % Mud/Silt cover | Bank slope (%) |
|---|---|---|---|
| Discharge ($m^3$ $s^{-1}$) | 1.00 | | |
| % Mud/Silt cover | -0.07 | 1.00 | |
| Bank slope (%) | 0.12 | 0.25 | 1.00 |

Table S3. Correlation coefficients between water quality variables.

| | Cl (mg $L^{-1}$) | TSS (mg $L^{-1}$) | TP (µg $L^{-1}$) | TN (µg $L^{-1}$) |
|---|---|---|---|---|
| Cl (mg $L^{-1}$) | 1.00 | | | |
| TSS (mg $L^{-1}$) | 0.36 | 1.00 | | |
| TP (µg $L^{-1}$) | 0.76 | 0.50 | 1.00 | |
| TN (µg $L^{-1}$) | 0.62 | 0.43 | 0.86 | 1.00 |

Table S4. Declining (*z*-) and increasing (*z*+) taxa results from Threshold Indicator Taxa Analysis (TITAN) of periphyton species composition in response to surface-water total phosphorus (TP, ug $L^{-1}$) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*), mid (*50%*) and upper (*95%*) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates. $R^2$ (null deviance – residual deviance / null deviance) and *P* values for corresponding Generalized Additive Models (GAM) are also presented.

| | | TITAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sp. code | Obs. | 5% | 50% | 95% | IndVa | *z* | Freq. | Purity | Rel. |
| Declining species (*z*-) | | | | | | | | | | |
| *Achnanthes biassolettiana* | ACbiasol | 24.22 | 22.38 | 24.22 | 50.73 | 71.28 | 4.13 | 18 | 0.97 | 0.96 |
| *Achnanthidium minutissimum* | AHminuti | 52.08 | 24.22 | 52.08 | 77.03 | 86.33 | 5.95 | 24 | 1.00 | 1.00 |
| *Cymbella affinis* | CMaffins | 34.18 | 12.44 | 34.18 | 38.13 | 56.43 | 4.03 | 13 | 1.00 | 0.99 |
| *C. delicatula* | CMdelcat | 17.03 | 16.15 | 17.03 | 19.60 | 98.28 | 9.39 | 15 | 1.00 | 1.00 |
| *C. kolbei* | CMkolbei | 40.73 | 19.05 | 38.13 | 48.13 | 59.09 | 4.28 | 13 | 1.00 | 1.00 |
| *Denticula kuetzingii* | DEkuetzi | 21.43 | 14.62 | 21.43 | 52.08 | 78.04 | 5.24 | 20 | 1.00 | 1.00 |
| *Encyonopsis evergladianum* | EYevergl | 26.78 | 24.00 | 26.78 | 31.96 | 95.43 | 6.38 | 23 | 1.00 | 1.00 |
| *E. microcephala* | EYmicroc | 18.23 | 17.38 | 18.23 | 44.68 | 91.37 | 7.03 | 18 | 1.00 | 1.00 |
| *Fragilaria capucina* | FRcapuci | 24.22 | 15.30 | 24.22 | 52.27 | 65.81 | 4.70 | 18 | 0.98 | 0.96 |
| *Gomphonema angustatum* | GOangstt | 21.43 | 17.03 | 23.02 | 72.92 | 55.64 | 3.07 | 13 | 1.00 | 0.99 |
| *G. clavatum* | GOclavat | 24.22 | 15.97 | 24.22 | 26.38 | 72.54 | 6.35 | 16 | 1.00 | 1.00 |
| *G. gracile* | GOgracil | 27.77 | 16.65 | 27.77 | 72.92 | 62.37 | 4.58 | 15 | 0.99 | 0.99 |
| *G. intricatum* var. *vibrio* | GOintvib | 17.03 | 10.89 | 17.03 | 34.18 | 57.27 | 8.00 | 8 | 1.00 | 0.99 |
| *Merismopedia glauca* | MERglau | 17.03 | 16.15 | 20.88 | 28.95 | 86.21 | 4.60 | 20 | 1.00 | 0.99 |
| *Sellaphora stroemii* | NAstroem | 21.43 | 10.59 | 21.43 | 44.68 | 53.24 | 5.46 | 10 | 1.00 | 0.99 |
| *Synedra acus* | SYacus | 16.18 | 10.89 | 16.18 | 26.78 | 68.39 | 8.56 | 10 | 1.00 | 1.00 |
| Increasing species (*z*+) | | | | | | | | | | |
| *Achnanthidium exiguum* | AHexigu | 21.43 | 15.12 | 21.43 | 42.08 | 63.89 | 4.01 | 18 | 1.00 | 1.00 |
| *Amphora pediculus* | AMpedcis | 69.78 | 17.03 | 69.78 | 1150.00 | 72.32 | 4.21 | 16 | 1.00 | 1.00 |
| *A. veneta* | AMveneta | 932.2 | 117.83 | 368.33 | 1012.83 | 54.67 | 6.24 | 5 | 0.99 | 0.96 |
| *Cocconeis placentula* | CCplacen | 17.03 | 10.29 | 17.03 | 17.85 | 88.29 | 4.13 | 29 | 0.99 | 0.99 |
| *Characium sp.* | CHRsp | 30.18 | 29.00 | 30.18 | 368.33 | 44.44 | 4.43 | 8 | 1.00 | 0.97 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Cyclotella meneghiniana* | CYmeme | 19.05 | 18.01 | 19.05 | 54.58 | 74.98 | 3.88 | 21 | 0.99 | 0.99 |
| *Diadesmis confervacea* | DIconfer | 21.43 | 20.80 | 23.02 | 125.08 | 82.15 | 5.68 | 21 | 1.00 | 1.00 |
| *Fallacia tenera* | FAtener2 | 52.08 | 48.13 | 52.08 | 59.97 | 42.86 | 5.57 | 6 | 1.00 | 0.96 |
| *Gomphomena parvulum* | GOparvul | 14.27 | 10.29 | 14.43 | 18.34 | 92.08 | 2.73 | 31 | 0.99 | 0.98 |
| *Gomphosphenia lingulatiformis* | GMlinfor | 125.0 | 25.2 | 52.08 | 770.33 | 47.59 | 4.59 | 9 | 1.00 | 0.98 |
| *Hippodonta hungarica* | HIhunga | 52.08 | 48.13 | 52.08 | 598.33 | 70.45 | 6.93 | 11 | 1.00 | 1.00 |
| *Navicula kotschyi* | NAkotsch | 19.05 | 13.91 | 19.05 | 40.80 | 61.02 | 3.46 | 18 | 0.99 | 0.98 |
| *Eolimna minima* | NAminim | 26.78 | 24.00 | 26.78 | 142.58 | 47.62 | 3.34 | 10 | 0.99 | 0.97 |
| *N. recens* | NArecens | 27.77 | 22.47 | 24.22 | 44.68 | 72.18 | 5.11 | 17 | 1.00 | 1.00 |
| *N. rostellata* | NArostel | 44.68 | 24.22 | 44.68 | 66.02 | 50.73 | 4.49 | 10 | 0.99 | 0.96 |
| *N. sanctaecrucis* | NAsancru | 40.73 | 23.02 | 48.13 | 125.08 | 53.12 | 5.54 | 10 | 1.00 | 0.99 |
| *Nitzschia compressa* var. *balatonis* | NIcombal | 52.08 | 48.13 | 52.08 | 72.92 | 50.00 | 6.53 | 7 | 1.00 | 0.98 |
| *N. filiformis* | NIfilifr | 40.73 | 36.73 | 40.73 | 48.13 | 43.75 | 4.25 | 7 | 1.00 | 0.97 |
| *N. frustulum* | NIfrustu | 40.73 | 18.32 | 38.13 | 48.13 | 72.69 | 4.32 | 18 | 1.00 | 1.00 |
| *N. inconspicua* | NIincons | 21.43 | 17.03 | 21.43 | 35.55 | 77.22 | 4.55 | 20 | 1.00 | 1.00 |
| *Pleurosira laevis* | PRlaevis | 125.0 | 50.73 | 125.08 | 305.92 | 84.19 | 7.21 | 12 | 1.00 | 1.00 |

Table S5. Declining (*z*-) and increasing (*z*+) taxa results from Threshold Indicator Taxa Analysis (TITAN) of periphyton species composition in response to surface-water total nitrogen (TN, ug $L^{-1}$) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*), mid (*50%*) and upper (*95%*) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates. $R^2$ (null deviance – residual deviance / null deviance) and *P* values for corresponding Generalized Additive Models (GAM) are also presented.

| | | TITAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sp. code | Obs. | 5% | 50% | 95% | IndVa | *z* | Freq. | Purity | Rel. |
| Declining species (*z*-) | | | | | | | | | | |
| *Achnanthidium minutissimum* | AHminuti | 1016.00 | 328.17 | 1016.00 | 1372.17 | 81.33 | 3.99 | 24 | 1.00 | 1.00 |
| *C. delicatula* | CMdelcat | 328.17 | 277.17 | 328.17 | 1891.67 | 62.45 | 4.23 | 15 | 0.99 | 0.97 |
| *Encyonopsis evergladianum* | EYevergl | 1016.00 | 350.83 | 973.17 | 1891.67 | 73.46 | 2.83 | 23 | 0.99 | 0.98 |
| *E. microcephala* | EYmicroc | 266.00 | 263.83 | 328.17 | 918.17 | 78.68 | 5.11 | 18 | 1.00 | 0.99 |
| *Sellaphora stroemii* | NAstroem | 328.17 | 268.89 | 328.17 | 867.83 | 46.83 | 4.44 | 10 | 1.00 | 0.97 |
| *Oscillatoria sp.* | OSCsp | 490.67 | 384.67 | 490.67 | 1427.17 | 70.19 | 4.31 | 21 | 0.99 | 0.97 |
| *Synedra acus* | SYacus | 362.00 | 249.67 | 362.00 | 918.17 | 51.61 | 5.38 | 10 | 1.00 | 0.97 |
| Increasing species (*z*+) | | | | | | | | | | |
| *Amphora pediculus* | AMpedcls | 462.50 | 318.83 | 474.00 | 2393.33 | 65.41 | 3.69 | 16 | 0.99 | 0.99 |
| *Characium sp.* | CHRsp | 525.83 | 429.00 | 500.50 | 1891.67 | 39.01 | 3.98 | 8 | 1.00 | 0.95 |
| *Diadesmis confervacea* | DIconfer | 328.17 | 277.17 | 368.50 | 2285.00 | 67.81 | 3.50 | 21 | 0.98 | 0.98 |
| *Hippodonta hungarica* | HIhunga | 918.17 | 429.00 | 918.17 | 2399.42 | 57.53 | 5.22 | 11 | 1.00 | 0.98 |
| *Navicula recens* | NArecens | 328.17 | 312.67 | 328.17 | 511.00 | 60.88 | 3.61 | 17 | 1.00 | 0.99 |
| *N. inconspicua* | NIincons | 440.83 | 277.17 | 429.00 | 448.67 | 70.30 | 3.87 | 20 | 0.99 | 0.99 |
| *Pleurosira laevis* | PRlaevis | 3016.67 | 402.50 | 1891.67 | 3373.33 | 87.90 | 8.69 | 12 | 0.99 | 0.98 |

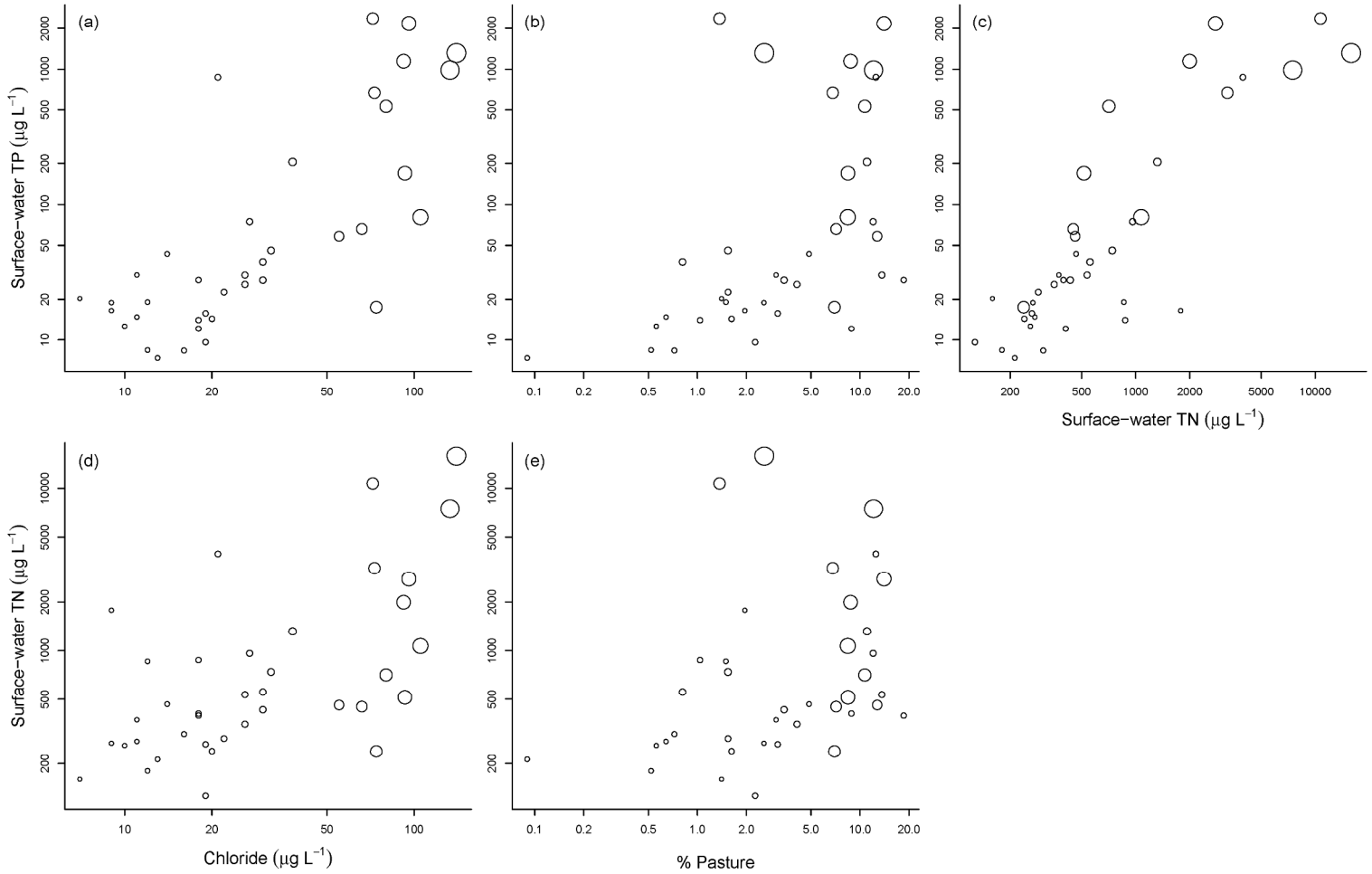
Table S6. Declining (*z*-) and increasing (*z*+) taxa results from Threshold Indicator Taxa Analysis (TITAN) of fish species composition in response to surface-water total phosphorus (TP, ug $L^{-1}$) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*), mid (*50%*) and upper (*95%*) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates. $R^2$ (null deviance – residual deviance / null deviance) and *P* values for corresponding Generalized Additive Models (GAM) are also presented.

| | | TITAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sp. code | Obs. | 5% | 50% | 95% | IndVa | *z* | Freq. | Purity | Rel. |
| Declining species (*z*-) | | | | | | | | | | |
| *Campostoma anomalum* | CAMPANO | 27.77 | 24.00 | 32.95 | 55.85 | 88.01 | 4.72 | 29 | 0.99 | 0.97 |
| *Etheostoma spectabile* | ETHESPEC | 34.18 | 21.43 | 34.18 | 55.85 | 81.63 | 5.53 | 28 | 0.99 | 0.98 |
| Increasing species (*z*+) | | | | | | | | | | |
| *Cyprinus carpio* | CYPRCARP | 187.50 | 32.95 | 187.50 | 1069.33 | 50.98 | 6.26 | 9 | 0.99 | 0.97 |
| *Cyprinella lutrensis* | CYPRLUTR | 21.43 | 13.42 | 21.43 | 24.00 | 92.22 | 3.88 | 31 | 1.00 | 0.98 |

Table S7. Declining (*z*-) and increasing (*z*+) taxa results from Threshold Indicator Taxa Analysis (TITAN) of fish species composition in response to surface-water total nitrogen (TN, ug $L^{-1}$) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*), mid (*50%*) and upper (*95%*) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates. $R^2$ (null deviance – residual deviance / null deviance) and *P* values for corresponding Generalized Additive Models (GAM) are also presented.

| | | TITAN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sp. code | Obs. | 5% | 50% | 95% | IndVa | *z* | Freq. | Purity | Rel. |
| Increasing species (*z*+) | | | | | | | | | | |
| *Cyprinus carpio* | CYPRCAR | 546.17 | 445.83 | 546.17 | 1891.67 | 45.59 | 4.87 | 9 | 1.00 | 0.99 |
| *Cyprinella lutrensis* | CYPRLUT | 280.17 | 226.13 | 280.17 | 312.67 | 92.44 | 2.85 | 31 | 0.99 | 0.97 |
| *Pimiphales vigilax* | PIMEVIGI | 280.17 | 225.33 | 280.17 | 390.50 | 84.08 | 2.87 | 29 | 0.99 | 0.95 |

Figure S1. Scatterplots of nutrient measures and dominant sources. Surface-water total phosphorus (TP, ug $L^{-1}$) with (a) chloride (Cl, ug $L^{-1}$), (b) % pasture, and (c) surface-water total nitrogen (TN, ug $L^{-1}$). Surface-water total nitrogen (TN, ug $L^{-1}$) with (d) chloride (Cl, ug $L^{-1}$) and (e) % pasture.



**Final Report**

**For the Project**

# Linking Observational and Experimental Approaches for the Development of Regional Nutrient Criteria for Wadeable Streams

**15 August 2009**

**Section 104(b)(3) Water Quality Cooperative Agreement**

**#CP-966137-01**

**U. S. EPA Region 6**

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The field component of this study was designed, implemented, and managed by RSK and members of his laboratory. Several individuals contributed significantly to the collection of the data for the field component of the project. Jeffrey A. Back and Jason M. Taylor unequivocally contributed more time in the lab and field on the field study than any other investigator. Charles E. Stanley, David A. Lang, Justin Grimm, J. Thad Scott, Emily Hooser, Adolfo Flores, Success Sumpaoengoen, Brianna Kirchner, Rebecca Shaftel, and Julie Baldizar (in approximate order of hours served) also contributed significant time to the field portion of this study.

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The experimental stream component of this study required numerous individuals and many hours of labor to accomplish. The experimental stream study was designed, implemented, and managed by RSK and BWB. We thank Barry Fulton, Theodore Valenti, Jason Berninger, and Richard Brain from Bryan Brooks' lab for leading the design and implementation of the peristaltic pump dosing system, and for contributing significantly to the routine restocking of the P stock solutions. Jason Taylor, Rebecca Shaftel, Barry Fulton, Jeff Back, Justin Grimm, Charles Stanley, Julie Baldizar, Theodore Valenti, Jason Berninger, and Richard Brain each put in significant time in the set-up, sampling, lab processing, and data entry phases of the BEAR study. Jeffrey Back performed all of the nutrient chemistry analyses during this experiment.

We especially thank Dr. Barbara Winsborough, of Winsborough Conulting, Leander, TX, for her hard work and rapid delivery of algae species identifications from both the field and

experimental studies. Dr. Winsborough conducted all algal species identifications reported in this document.

Study designs, choice of data analyses, presentation and interpretation of results, and conclusions and recommendations are those of the Principal Investigator and not those of the U.S. EPA, and no official endorsement from the U.S. EPA should be inferred.

## EXECUTIVE SUMMARY

We employed a novel approach to developing a defensible, effects-based numerical target concentration of surface-water nutrients for wadeable streams in the Cross Timbers portion of Aggregate Nutrient Ecoregion IX of U. S. EPA Region 6. Our approach relied on an integration of collected field data from wadeable streams in Texas with experimental data generated from stream mesocosms at the Baylor Experimental Aquatic Research (BEAR) facility.

### *Observational Field Study*

The observational field component of this study was conducted from August 2006 through June 2008. Data were collected quarterly (8 sampling events) from 26 wadeable streams in the Brazos River basin within the Cross Timbers Level III Ecoregion. The selected streams spanned a steep gradient of phosphorus enrichment (< 10 ug/L to >2000 ug/L TP). Sites with low, moderate, and high levels of P enrichment were widely distributed throughout the study area. The study design captured the full range of natural variability in geology, drainage networks, stream size, and other physiographic factors, while still achieving a wide spatial distribution of P enrichment and its likely sources (wastewater effluent discharges, pasture).

Field streams had similar hydrographs during the 2-year study. Differences in discharge were much greater among seasons and years rather than differences among the individual streams. August 2006-February 2007 was a drought; discharge was very low in all streams except those near effluent discharges. Heavy rains led to flooding in spring 2007, and streams maintained relatively high base-flow discharges through the remainder of the study.

Surface-water nutrients, particularly $PO_4$-P and TP, were more strongly influenced by differences among individual streams than by seasonal patterns of discharge. Streams with apparently few point or non-point sources of phosphorus exhibited consistently low TP. Streams with predominantly non-point sources of phosphorus (watersheds with pasture or other sources) showed sharp increases in TP during high discharge, reflecting transport of P from the land from surface-water runoff. Streams with point sources (effluent discharges) either had consistently high TP or had moderate declines in TP due to dilution by high surface-water runoff.

#### *Periphyton, Filamentous Algae, and Macrophyte Responses to TP*

We hypothesized excessive levels of enrichment of surface-water nutrients would be reflected in sharp changes in enrichment of the tissues of the periphyton. Indeed, sites with different levels of surface-water $PO_4$-P or TP differed markedly in their periphyton C:P (carbon-to-phosphorus) and N:P (nitrogen-to-phosphorus) molar ratios. TP consistently predicted nonlinear, threshold declines in periphyton C:P ratios. Sites with elevated surface-water TP had virtually constant periphyton C:P or N:P ratios over time (typically less than 300 and 20, respectively). Threshold declines in periphyton C:P, N:P, and C:N ratios were highly likely between 15 and 25 ug/L TP, with 19-20 ug/L representing the most consistent concentration yielding a threshold response. Moreover, despite the high variability of DIN (dissolved inorganic nitrogen) in surface waters

among sites over time, the periphyton N:P ratio was essentially identical to the C:P ratio, illustrating that P, not N, was driving differences in these ratios.

Significant threshold changes were detected in several of the periphyton, algal, and macrophyte biological response variables in response to TP. The thickness of microbial films (microalgae and other microbes; non-filamentous algae) growing on rocks showed consistently nonlinear declines in response to TP levels above 20 ug/L. This metric was computed as the percentage of observations in a reach (out of a maximum of 100 points along the reach-scale transect) in which biofilms exceeded 1 mm in thickness on rocks. Streams with TP < 20 typically had moderate to heavy growth of calcareous periphyton.

Streams above the threshold value (20 ug/L) usually had a thinner, darker layer of biofilms on the rocks but also tended to have more filamentous green algae. There were 2 distinct changepoints in filamentous algal cover: a consistent increase in cover among sites >20 ug/L TP, and a less consistent but more dramatic increase in cover at very high levels of TP (>200-1000 ug/L). Because filamentous algal cover depends highly on seasonal changes in light and water temperature, as well as sloughing from overgrowth and scouring from high flows, it is not surprising that many of the highly enriched sites had little filamentous algal cover on some dates. However, when high filamentous algal cover occurred, it was almost always associated with levels of P enrichment > 20 ug/L, and particularly at >200 ug/L.

The ratio of periphyton chlorphyll to AFDM revealed a nonlinear shift toward a greater fraction of chlorophyll-bearing organisms in the periphyton in response to TP > 20-30 ug/L. A secondary threshold appeared around 200 ug/L, likely related to the 2-tiered response of filamentous algae and chlorophyll-a at 20 and 200 ug/L TP.

One of the most important patterns that emerged from the field study was the sharp threshold declines in submersed macrophyte cover in response to TP. The two dominant submersed macrophyte taxa were *Najas* (a vascular plant) and *Chara* (a nonvascular charophyte); both virtually disappeared in streams with TP exceeding 25-54 ug/L TP.

*Diel Dissolved Oxygen Response to TP*

Interannual differences in stream discharge strongly influenced the daily (diel) variation in dissolved oxygen, water temperature, and pH. In 2006, a drought year, most of the field study streams had very low-to-no measureable discharge. This lack of flow limited the turbulent mixing of the water column. During the day, streams were supersaturated with dissolved oxygen (up 20 mg/L DO; > 250% saturation) which concomitantly caused pH to increase to relatively high levels (8.5-9.5). At night, DO was consumed and collapsed below 2 mg/L in some of the study streams. In September 2007, discharge was relatively high in all 26 study streams. Most of the streams were in flood stage for several of the preceding months and had recently subsided to wadeable flows. With higher flows and turblent mixing, minimum DO levels were rarely below 5 mg/L.

The harmful (0-2 mg/L) minimum DO values at several of the sites in 2006 were related to surface-water TP. All 6 streams with low flow and TP > 27.2 had minimum DO values < 2 mg/L, and two others with TP near 20 ug/L dropped below 3.5 mg/L DO. The four streams located immediately downstream of effluent discharges maintained relatively high minimum DO concentrations despite very high TP. This was related to reaeration associated with turbulent mixing and artificially high flows during a drought when most other streams were not flowing. However, these artificially high flows quickly declined within a few kilometers from the upstream effluent discharges due to evaporation and infiltration below the stream channel. Here the lack of reaeration by turbulent mixing coupled with high nutrient levels from the effluent caused DO to be almost completely consumed at night. Thus, effluent discharges were having dramatic effects on DO, but farther downstream than typically considered in monitoring studies.

Minimum DO in 2006 was also predicted significantly by mean biofilm thickness, filamentous algal cover, and the ratio of periphyton chlorophyll to AFDM. Thicker layers of calcareous periphyton were associated with low-P streams, and as TP increased, these biofilms succeeded to filamentous and colonial greens and other types of algae. Thus, these metrics appear to be sensitive to TP enrichment and may be linked quantitatively to aquatic life use standards that rely on dissolved oxygen as a measure of biological integrity.

*Algal Species Composition Response to TP*

Multivariate analyses of algal species composition during August 2006 and September 2007 continued to support the earlier conclusions that TP and related metrics had a strong influence on stream biota. In both years, most of the variance in algal species composition was related to TP amd related measures. Sites with the lowest TP and highest periphyton C:P ratios were clearly grouped away from sites with the highest TP and lowest C:P ratios, which were also distinctly grouped. Although algal species changed between years, the relative grouping of sites from low P to high P remained intact regardless of year. This was compelling evidence that algal species composition was altered by nutrient enrichment, and the magnitude of its effect was similar regardless of interannual variation in stream discharge.

Threshold Indicator Taxa Analysis (TITAN) continued to reveal stream biological thresholds at low levels of TP. Dozens of algal species essentially disappeared from streams between 12 and 30 ug/L TP, with the threshold of greatest overall decline at 19.2 (2006) and 21.6 (2007) ug/L TP, respectively. Simultaneously, numerous algal taxa showed sharp increases, either replacing taxa as they declined, or driving the declines via competitive exclusion (e.g., shading by *Cladophora*, a significant increasing taxon in both years).

Although most taxa declined or increased at relatively low levels of TP, a few taxa responded only to much higher levels of TP. These taxa were mostly associated with sites heavily influenced by effluent, and suggested a second tier of biological degradation at very high levels of TP (200-500 ug/L).

*Macroinvertebrate Taxa Responses to TP*

Results of ordinations and Threshold Indicator Taxa Analysis (TITAN) on macroinvertebrate taxonomic composition during November 2006 showed that nutrients, specifically phosphorus, corresponded to shifts in community structure. Because composition differed substantially between sites with and without flow, and because of established relationship between flow and other water chemistry variables (e.g., DO), we separated sites into groups by flow status prior to analysis.

Sites with no flow yielded more reliable relationships to TP than sites with flow, but this result should be interpreted with caution because there were only 9 sites in the group with flow. Ordinations clearly showed that in both sets of analyses, the sites with low TP and high periphyton C:P sorted together, whereas high TP and low C:P sites sorted away from the low TP sites. In both sets of TITAN analyses, more taxa were classified as negative threshold indicators (decline in response to TP) than as positive ones (increase).

## *Experimental Stream Study*

The experimental stream study was conducted from 31 January to 7 April 2008 at the Baylor Experimental Aquartic Research (BEAR) facility. Twelve streams were used in the study. The experimental streams are approximately 0.6 m wide and 20 m in length. The streams are stratified into riffle, glide, and pool sections and are designed mimic natural habitat in central Texas streams. Water is pumped from the adjacent Lake Waco Wetland and delivered to the streams via a series of 12 valves, each adjusted to regulate flow equivalently among all 12 streams (180 L/min). Before entering the streams, water iss pumped into a mixing tank where chemicals representing experimental treatments can be dosed at a fixed rate using peristaltic pumps connected to a second, adjacent tank of chemical stock solution. Once dosed with the treatment levels of non-toxic chemicals, water is released into the streams and is discharged back into the Lake Waco Wetlands.

Clean river cobble and gravel were placed in streams in January 2008 to simulate erosional habitat in natural stream in our field study. Stream flow was initiated on 31 January 2008 and calibrated to 182 L/min (+/- 5 L/min) for each stream. Streams were seeded with organic matter, periphyton, and macroinvertebrates collected from two of the intensive field sites. These sites were chosen because their P concentrations were representative of the background (control) and high P treatments to be employed in the experiment. Streams were seeded twice: 1 February and 15 February 2008. Seeding was accomplished by transplanting organisms and organic matter from five 1-$m^2$ kick screen samples collected from into each of the 12 streams.

The streams were allowed to run without any nutrient additions from 31 January to 10 March 2008 in an effort to allow growth of periphyton at low nutrient concentrations. Established periphyton communities from riffle habitat from two intensive field sites, ROCK-01 (very low ambient phosphorus levels; 1-5 ug/L PO4-P, 4-12 ug/L TP) and NBOS-03 (moderately high P levels: 50-200 ug/L PO4-P, 75-250 ug/L TP) were also transplanted into the streams. Cobbles from ROCK-01 (300) and NBOS-03 (300) were placed in random cross sections throughout the

reach of each experimental stream on 7 and 8 March, respectively, a few days prior to initiation of the experiment.

Experimental dosing of phosphorus began on 11 March 2008. Background concentration of PO4-P during the colonization phase of the study was 6 and increased to 8 ug/L during the experiment. Four streams were randomly assigned no additional phosphorus, and thus a target concentration of **8 ug/L** (**Control**). Because our field study had identified consistent nonlinear changes in numerous biological response variables between 10 and 20 ug/L PO4-P (15-25 ug/L TP), four streams were assigned a treatment of +15 ug/L PO4-P to achieve a target PO4-P concentration of **20 ug/L (Low P treatment**). The remaining four streams received +95 ug/L PO4-P to achieve a target concentration of **100 ug/L (High P treatment).**

*Periphyton and Filamentous Algae Response to P Dosing*

Periphyton growing on the ceramic tiles responded rapidly to experimental P dosing. High P treatments had significantly more chlorophyll than Low-P and Controls on Day 14. By Day 28, Low P and High P treatments were not different, but had significantly more chlorophyll-a than Controls. The lack of difference between low and high P treatments is consistent with the threshold response observed in the field study.

Periphyton (biofilm) AFDM and chlorophyll-a from the bare rocks and transplanted rocks was highly variable among P treatments and did not differ significantly. However, this was largely due to the very strong response of *Cladophora* (filamentous green algae) in the low and high P treatments. *Cladophora* biomass exploded near the end of the study, and was significantly higher in the Low and High P streams than the Controls on the bare rocks and transplant rocks (ROCK-01, NBOS-03) on Day 28.

Periphyton C:P ratios from non-transplant bare rock samples differed significantly by treatment on Day 28. High P treated streams had the lowest C:P ratio (~150), Controls had the highest (~320) whereas Low-P streams were intermediately enriched (~230). Control C:P ratios were approaching levels deemed to be near or below a C:P threshold in the field study, suggesting that even the Control PO4-P (8 ug/L) and TP (19-20 ug/L) concentrations were high enough to cause sharp changes in periphyton nutrient content.

Periphyton C:P ratios from the ROCK-01 transplants (a field site with PO4-P < 5 ug/L and TP<10 ug/L) responded very strongly to all 3 experimental treatments. On Day 0, ROCK-01 periphyton had C:P ratios above 2,000. After transplanting these rocks into the BEAR streams for just 7 days, mean C:P ratios had dropped to 689, 346, and 215 among the Control, Low P and High P treatments, respectively. By Day 28, ROCK-01 Control C:P ratios had dropped to 250, whereas Low and High P values were near 150. This illustrated the remarkable affinity of stream periphyton for phosphorus, and provided additional evidence in support of for nonlinear uptake of P as the explanation for sharp declines in periphyton C:P with small increases in surface-water TP.

In contrast, the NBOS-03 transplants (a field site with PO4-P 50-100 ug/L and TP 75-200 ug/L) did not respond to any of the experimental P treatments on Day 7 or 28, supporting the hypothesis that recycling of P within the periphyton is an important mechanism for maintaining low C:P ratios over time in streams with highly variable surface-water TP. Moreover, it also showed that periphyton from NBOS-03 was already saturated with P and thus did not sequester more P per unit carbon in the low or high P treatments.

*Algae and Macroinvertebrate Taxonomic Response to P Dosing*

Transplanted algae from ROCK-01 were significantly different among P treatments on Day 28. ROCK-01 samples that were transplanted into Low and High P streams shifted significantly away from Control streams by day 28, and increasingly resembled algal communities from P-enriched field sites. However, consistent with a nonlinear threshold response, algal species composition in Low and High P treatments did not differ. In contrast, transplanted algae species composition from NBOS-03 did not differ among treatments after 28 days of exposure.

Indicator Species Analysis revealed that at least 5 diatom species were significantly less abundant (or absent) from ROCK-01 transplants in Low P and High P streams than Controls, whearas 2 species were significantly more abundant in the Low and High P treatments. Five of these 7 taxa also were identified as significant threshold indicators in response to TP gradients in the field study, validating the responses as caused by P. All five responded in the same direction (decline, increase) in the BEAR study as in the field study.

Macroinvertebrate taxonomic composition did not differ among treatments on Day 28. The relatively short dosing period, in contrast to the relatively long life cycle of these taxa, necessarily limited the potential response of this diverse group of organisms to P treatments. Future experiments focused on the effect of low flow and P interactions, or longer dosing periods, may produce more meaningful results in the context of animal responses to experimental P enrichment.

## ***Conclusions and Recommendations***

Shifts from periphyton communities comprised of sensitive diatoms, calcareous cyanobacteria, and other non-chlorophyll bearing microbes to communities with higher chlorophyll content and more filamentous green algae was repeatably demonstrated at concentrations of surface-water TP above 20 ug/L. Streams with TP > 200-1000 ug/L likely represent a second tier of degradation, and appear at greater risk for nuisance algal growth based on our threshold analyses. However, results from the P dosing experiment suggest that concentrations as low as 20 ug/L PO4-P can lead to high levels of *Cladophora* biomass in as little as 28 days. Adding more PO4-P (100 ug/L) did not result in more *Cladophora* in a 4-week period, but did result in more *Cladophora* after only 2 weeks. Thus, because faster growth rates were observed in the 100 ug/L treatment, field streams with very high levels of TP will have a greater probability of heavy coverage of filamentous algae because biomass accumulation between scouring or sloughing events will be more rapid.

Aquatic macrophyte cover consistently declined in streams with TP > 25-50 ug/L. These submersed plants serve as important refugia for juvenile fishes and macroinvertebrates, and provide a source of dissolved oxygen during low flows. Their decline likely represents key structural and functional degradation to these stream ecosystems.

Minimum dissolved oxygen levels are highly dependent upon an interaction between flow and nutrient enrichment. Our study suggests that TP levels > 20-30 ug/L, coupled with low flows, will cause detrimental declines in minimum dissolved oxygen levels. This is particularly important in the context of minimum flows, a contentious issue in the southwestern USA. It is unlikely that studies geared toward detecting effects of nutrients on DO will adequately characterize this relationship without sampling during periods of low flow when gas exchange with the atmosphere is very slow. Distance downstream and flow status are two very important considerations when evaluating the influence of WWTP discharges on wadeable streams in semi-arid regions. Future research is needed to better quantify the interaction between minimum flows and biological integrity in streams.

The weight of evidence from both the field stream study and experimental stream study demonstrates that streams of the study area are very sensitive to phosphorus enrichment. There is a very high probability that streams exposed to surface-water TP levels exceeding 20 ug/L, and possibly 15 ug/L, will experience a sharp decline in biological integrity, including loss of characteristic structure (periphyton and macrophytes), loss of numerous species (algae and macroinvertebrates), minimum dissolved oxygen levels unsuitable for supporting native fauna during low flows, and increase likelihood of nuisance algal growth that limits recreational use of strreams. Streams exceeding 200 ug/L may represent a second tier of degradation, with more consistent nuisance algal growth and additional losses of algal and macroinvertebrate species.

Table 1. Key to variable short names used throughout this document.

| Variable | Description |
|---|---|
| SITE_ID | First 4 letters of site name followed by number (>1 for streams with more than one site) |
| NH3-N | Ammonia-nitrogen, surface water, ug/L |
| NO2NO3-N | Nitrite + nitrate-nitrogen, surface water, ug/L |
| PO4-P | Orthophosphate, surface water, ug/L |
| TN | Total nitrogen, surface water, ug/L |
| TP | Total phosphorus, surface water, ug/L |
| TURB_NTU | Turbidity, surface water, NTU |
| CHLA_UGL | Chlorophyll-a, surface water, ug/L |
| C_ALG | Total carbon, organic fraction of periphyton, % |
| C_BULK | Total carbon, bulk periphyton, % |
| C_SED | Total carbon, sediment fraction of periphyton, % |
| N_ALG | Total nitrogen, organic fraction of periphyton, % |
| N_BULK | Total nitrogen, bulk periphyton, % |
| N_SED | Total nitrogen, sediment fraction of periphyton, % |
| P_ALG | Total phosphorus, organic fraction of periphyton, % |
| P_BULK | Total phosphorus, bulk periphyton, % |
| P_SED | Total phosphorus, sediment fraction of periphyton, % |
| CN_ALG | Carbon:nitrogen ratio, OM fraction of periphyton |
| CN_BULK | Carbon:nitrogen ratio, bulk periphyton |
| CN_SED | Carbon:nitrogen ratio, sed fraction of periphyton |
| CP_ALG | Carbon:phosphorus ratio, OM fraction of periphyton |
| CP_BULK | Carbon:phosphorus ratio, bulk periphyton |
| CP_SED | Carbon:phosphorus ratio, sed fraction of periphyton |
| NP_ALG | Nitrogen:phosphorus ratio, OM fraction of periphyton |
| NP_BULK | Nitrogen:phosphorus ratio, bulk periphyton |
| NP_SED | Nitrogen:phosphorus ratio, sed fraction of periphyton |
| CHLA_M2 | Chlorophyll a, periphyton, mg/m2 (rock surface area) |
| AFDM_M2 | Ash-free dry mass, periphyton, g/m2 |
| AFDM_PCT | Percent of dry mass as organic matter |
| CHL_AFDM | Chlorophyll-a:AFDM ratio, periphyton, mg/g |
| DISCHARG | Stream discharge, cubic meters/second |
| DO_MGL | Dissolved oxygen, mg/L |
| DO_PCT | Dissolved oxygen, % |
| PH | pH |
| SALINITY | Salinity, ppt |
| SPCOND | Specific conductance, uS/cm |
| TEMP | Water temperature |
| CANOPY | Tree canopy cover, mean % |
| VELOCITY | Stream velocity, mean m/s |
| THAL_DEP | Thalweg depth, mean cm |
| WETWIDTH | Stream wetted width, mean m |
| DEPTH | Water depth, mean (100 points) |
| FLOW_N | No flow velocity detected, % (100pts) |
| FLOW_M+ | Flow velocity>0.2 m/s, % (100pts) |
| SED_CV | Sediment index, mean (100pts) |
| SED2+ | Sediment index > 3, % (100pts) |

| | |
|---|---|
| SED3+ | Sediment index > 2, % (100pts) |
| WET_PCT | % of reach with surface water (100pts) |
| BED_PCT | Bedrock, % (100pts) |
| BO_PCT | Boulder, % (100pts) |
| CO_PCT | Cobble, % (100pts) |
| GR_PCT | Gravel, % (100pts) |
| GRCO_PCT | Gravel+cobble, % (100pts) |
| SA_PCT | Sand, % (100pts) |
| SL_PCT | Silt-clay, % (100pts) |
| FINE_PCT | Silt-clay + sand, % (100pts) |
| MACALG_C | Filamentous algae cover score, mean (100pts) |
| MACALG3+ | Filamentous algae cover > 25%, % (100pts) |
| MACALG4+ | Filamentous algae cover > 50%, % (100pts) |
| MACALG5+ | Filamentous algae cover > 75%, % (100pts) |
| MICALG_R | Biofilm thickness, mean (100pts) |
| MICALG2+ | Biofilm thickness index > 0.5 mm, % (100pts) |
| MICALG3+ | Biofilm thickness index > 1 mm, % (100pts) |
| PLNT_CV | Submersed macrophyte cover index, mean (100pts) |
| PLNT1+ | Submersed macrophyte cover>0, % (100pts) |
| PLNT3+ | Submersed macrophyte cover>25%, % (100pts) |

## PROBLEM DEFINITION AND BACKGROUND

Nutrient pollution is the most common cause of degraded water quality in lakes, streams, wetlands, and estuaries in the USA. Excessive inputs of nutrients, specifically phosphorus (P) and nitrogen (N), have many negative effects on aquatic ecosystems. One of the most noticeable consequences of nutrient pollution is the accelerated growth of aquatic vegetation. Termed "eutrophication," this increase in productivity caused by nutrient enrichment produces an undesirable disturbance to the balance of organisms present in the water. For instance, eutrophication is commonly associated with explosive growths of nuisance algae that can taint drinking water supplies, cause foul odors, and even harm humans. Eutrophication can also result in dissolved oxygen shortages that kill fish and other aquatic organisms and reduce aquatic biodiversity. Thus, nutrient pollution can significantly limit the ability of a water body to support its designated uses, such as fishing, swimming, or other recreational activities.

Nutrient criteria and total maximum daily load (TMDL) implementation plans are developed by States to improve water quality in streams and reservoirs. A TMDL determines how much of a particular pollutant can be assimilated by an aquatic ecosystem and still maintain its beneficial uses and ecological integrity and is integrally related to a numerical nutrient criterion. In Texas, phosphorus has been identified by the Texas Commission on Environmental Quality as the nutrient that would have the most effect in limiting algal and plant growth in many central Texas streams. TMDL development requires that numerical criteria for the parameters of interest be established in order to calculate necessary load reductions. However, to date, numerical nutrient criteria have largely been developed subjectively and without knowledge of the biological consequences, despite the directive issued by the U.S. EPA in its National Strategy for the Development of Regional Nutrient Criteria (1998). In this document, the U.S. EPA detailed a comprehensive plan for the development of scientifically defensible, numerical water-quality criteria. The plan emphasized the need for the inclusion of endpoints in criteria development that reflect physical, chemical, and *biological* integrity of aquatic ecosystems. However, quantitative linkages between critical levels of nutrients and stream biota have not been well developed in Region 6, largely because historical sampling protocols do not include relevant ecological indicators for streams.

Another major factor limiting the development of numerical nutrient criteria is the lack of experimental evidence to support field monitoring. Experimental studies allow for controlled nutrient treatments and, when coupled with field observations, substantially improve our ability to establish causation between water quality and biological integrity. Thus, coupling experimental evidence with observations from natural streams should allow States to more effectively develop defensible nutrient criteria for streams.

In this study, we employed a novel approach to developing a defensible, effects-based numerical target concentration of surface-water phosphorus for wadeable streams in the Cross Timbers portion of Aggregate Nutrient Ecoregion IX of Region 6. Our approach relied on an integration of collected field data from wadeable streams in Texas with experimental data generated from model streams, or stream mesocosms. We used attributes of species assemblages (macrophytes, filamentous macroalgae, periphyton and macroinvertebrates) as indicators of biological condition in response to observed and experimental phosphorus gradients that spanned multiple aquatic life

use designations. We evaluated a wide range of biological attributes (e.g., periphyton nutrient content and biomass, cover or abundance of nuisance algae, community diversity, ecosystem process metrics) that are predicted by the US EPA Tiered Aquatic Life Use (TALU) conceptual model and Texas' narrative aquatic life use categories to be degraded at different stressor concentrations along a human disturbance gradient (i.e., exhibit deflections from reference at different stressor levels corresponding to aquatic life use categories). Our approach explicitly associated numerical levels of phosphorus to threshold responses in ecological indicators of aquatic life uses as defined by narrative biological criteria in the TALU framework.

The approach we employed is consistent with the mandates of the Clean Water Act and the U.S. EPA's National Strategy for the Development of Regional Nutrient Criteria because we (a) explicitly address biological integrity and associated aquatic life uses, (b) developed cause-effect linkages between nutrient pollution and stream-ecosystem responses by reproducing field responses in an experimental setting, (c) identified critical levels of nutrient pollution that caused harm to biological integrity and aquatic life uses using a weight-of-evidence approach, and (d) estimated the probability of harmful effects associated with levels of nutrient pollution so that managers and stakeholders understand the likely ecological implications of selecting target nutrient concentrations for water quality standards and future TMDLs for streams.

The purpose of this document is to report the results of this two-year study. This report is structured into two main sections:

I. Field (observational) study. This section details the study area, methods, results and interpretation of 2 years of field data collected from 26 wadeable streams in the Cross Timbers Ecoregion of the Brazos River watershed in Texas. These data represent one of the most comprehensive evaluations of the effects of nutrient enrichment on wadeable streams in the USA.
II. Experimental stream study. Part 2 of the report details the results of a short-term phosphorus dosing study conducted in the Baylor Experimental Aquatic Research (BEAR) stream facility.

The Executive Summary and Conclusions and Recommendations provide a synthesis of findings, interpretation, conclusions, and recommendations.

## SECTION I. FIELD STUDY

## STUDY AREA AND STREAM SAMPLING

Data were collected from 26 wadeable streams in the Brazos River basin within the Cross Timbers Level III Ecoregion (Figure 1; Griffin et al. 2004). The Cross Timbers ecoregion (Ecoregion 29) is a mosaic of forest, woodland, savanna, and prairie and is currently used mostly for rangeland and pastureland (Figure 2, Table 2).

Watershed variables describing physical characteristics and topography, land use, and distribution of disturbance points (outfalls and dams) were calculated for each site (Table 2). Watershed boundaries for each sample site were automatically digitized in ArcGIS 9.2 with the ArcHYDRO 9 extension using a 1:24,000 scale digital elevation model (DEM) expressed as a 30 m raster, available from the U. S. Geological Survey. Mean slope and elevation were calculated for each watershed using the digital elevation model. Mean annual precipitation was calculated for each watershed from a polygon coverage of average monthly and annual precipitation for the climatological period 1961-90. This dataset was obtained from USDA-NRCS. Number of wastewater outfalls and cumulative outfall (MGD) were calculated for each watershed based on the TCEQ municipal and industrial wastewater outfall shapefile available from http://www.tceq.state.tx.us/gis/sites.html. Landcover class percentages were calculated for each watershed using National Land Cover Database (NLCD 2001) available from http://www.mrlc.gov/nlcd_multizone_map.php. All watershed analyses were performed with ArcGIS 9.2 (ESRI, Redlands, CA.).

We collected physical, chemical, and biological data from each of the selected 26 stream sampling locations on a quarterly basis from August 2006 through June 2008. Locations were permanently marked reaches, defined as 40x the mean stream width, or a minimum of 150-m reach (TCEQ 2005). Selected reaches were required to include erosional (riffle/glide) habitats.

Surface-water nutrient samples (orthophosphate-P [SRP], total phosphorus-P [TP], ammonia-N [$NH_3$-N], nitrate-nitrite nitrogen [$NO_2$-$NO_3$-N], total nitrogen [TN]), chlorophyll-a, and turbidity were collected from each stream on a quarterly basis. Sampling periods were adjusted slightly to ensure sampling was conducted at least 2 weeks following storm flows. SRP, $NH_3$-N, and $NO_2$-$NO_3$-N samples were collected in triplicate and filtered immediately, whereas triplicate unfiltered TP and TN samples were only acidified. Single 1-L chlorophyll-a grab samples were collected and filtered in the laboratory within 24 h. Single 50 mL turbidity grab samples were collected and analyzed in the laboratory within 24 h. All samples were immediately placed on ice, stored in the dark, and transported back to the laboratory and stored at 4°C and analyzed in accordance with the approved project plan.

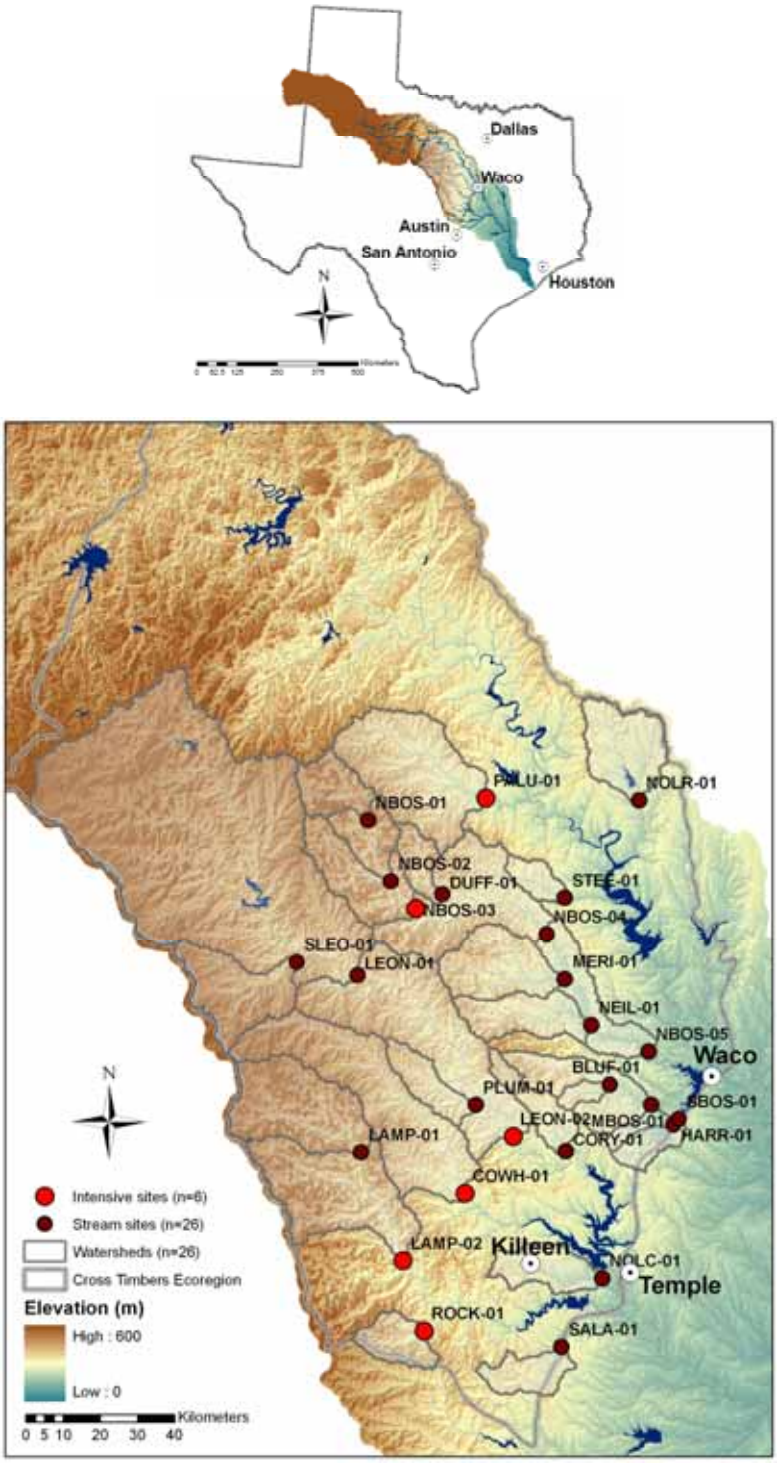



Figure 1. Map of Cross Timbers ecoregion and field study watersheds in relation to Texas and the Brazos River watershed. Twenty-six streams were selected for the field study, which entailed eight quarterly sampling events from August 2006 through June 2008. Six of the 26 streams were sampled for algal species composition during each of the 8 sampling events; these are shown as intensive sites. The remaining streams were sampled twice for algal species composition. All 26 sites were sampled during all 8 events for most other predictor and response variables (see Table 1).

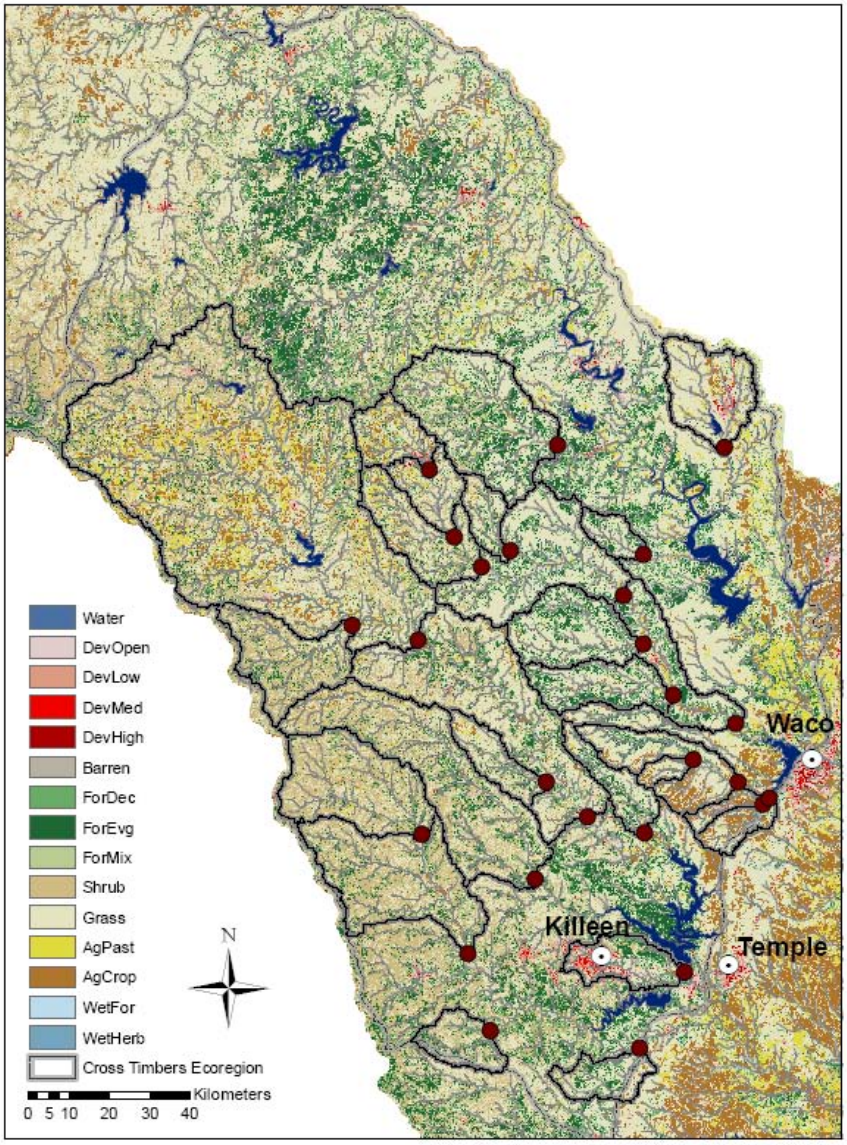



Figure 2. Spatial distribution of dominant land-cover classes among the 26 study watersheds (NLCD 2001). See Table 1 for percent cover of each land-cover class by watershed.

Table 2. Physiographic and land cover characteristics among the 26 field-study watersheds.

| SITE_ID | Precip. (in) | Elev (m) | Slope (%) | Area (km2) | Dams (n) | Outfalls (MGD) | Outfalls (n) | Water (%) | Dev. (%) | Forest (%) | Shrub (%) | Grass (%) | Pasture (%) | Crop (%) | Wetland (%) | Imp.. (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BLUF-01 | 33.4 | 216 | 2.83 | 68 | 0 | 0.00 | 0 | 0.1 | 0.6 | 8.8 | 0.0 | 69.1 | 1.4 | 17.2 | 2.9 | 0.0 |
| CORY-01 | 33.0 | 201 | 4.78 | 220 | 0 | 0.00 | 0 | 0.0 | 1.5 | 26.9 | 5.3 | 59.2 | 1.5 | 3.5 | 2.0 | 0.2 |
| COWH-01 | 31.4 | 232 | 4.53 | 1180 | 2 | 0.06 | 1 | 0.1 | 0.7 | 18.8 | 43.0 | 33.8 | 1.6 | 1.5 | 0.4 | 0.1 |
| DUFF-01 | 31.6 | 297 | 2.98 | 157 | 0 | 0.00 | 0 | 0.2 | 0.4 | 22.4 | 13.1 | 57.1 | 1.5 | 4.1 | 1.1 | 0.2 |
| HARR-01 | 34.4 | 153 | 1.48 | 77 | 0 | 0.00 | 0 | 0.1 | 12.0 | 1.6 | 0.0 | 42.6 | 3.0 | 38.6 | 2.1 | 2.0 |
| LAMP-01 | 30.3 | 331 | 3.01 | 720 | 20 | 0.00 | 0 | 0.3 | 0.6 | 10.9 | 56.6 | 28.8 | 1.5 | 0.9 | 0.3 | 0.1 |
| LAMP-02 | 31.1 | 271 | 3.08 | 1571 | 21 | 0.00 | 0 | 0.2 | 0.7 | 14.0 | 53.6 | 29.2 | 1.0 | 1.1 | 0.3 | 0.1 |
| LEON-01 | 30.5 | 304 | 3.13 | 4705 | 141 | 3.00 | 10 | 0.8 | 3.1 | 12.2 | 33.1 | 33.3 | 8.4 | 8.5 | 0.8 | 0.3 |
| LEON-02 | 32.2 | 226 | 3.31 | 6180 | 152 | 6.08 | 12 | 0.7 | 2.7 | 13.5 | 30.6 | 37.6 | 6.8 | 7.2 | 0.9 | 0.3 |
| MBOS-01 | 33.9 | 161 | 2.92 | 478 | 0 | 0.09 | 1 | 0.1 | 1.6 | 10.5 | 0.0 | 63.2 | 2.0 | 19.9 | 2.7 | 0.1 |
| MERI-01 | 32.6 | 198 | 5.14 | 480 | 4 | 0.04 | 1 | 0.4 | 0.3 | 32.3 | 0.4 | 63.7 | 0.5 | 1.0 | 1.4 | 0.2 |
| NBOS-01 | 31.3 | 366 | 2.75 | 257 | 16 | 3.50 | 2 | 0.6 | 8.0 | 11.0 | 17.6 | 36.8 | 12.1 | 12.0 | 1.9 | 1.6 |
| NBOS-02 | 31.0 | 324 | 2.94 | 489 | 30 | 3.50 | 2 | 0.6 | 6.5 | 10.7 | 20.1 | 43.9 | 8.5 | 7.8 | 1.9 | 1.3 |
| NBOS-03 | 31.2 | 302 | 3.18 | 925 | 49 | 3.50 | 2 | 0.5 | 4.3 | 13.3 | 22.6 | 44.6 | 7.1 | 6.0 | 1.6 | 0.8 |
| NBOS-04 | 32.1 | 220 | 3.60 | 1890 | 69 | 3.75 | 4 | 0.5 | 3.1 | 18.4 | 13.5 | 55.1 | 4.1 | 3.5 | 1.7 | 0.6 |
| NBOS-05 | 33.8 | 154 | 4.20 | 3097 | 82 | 5.28 | 8 | 0.5 | 2.5 | 23.8 | 8.5 | 56.2 | 3.4 | 3.1 | 2.0 | 0.5 |
| NEIL-01 | 32.9 | 177 | 5.20 | 357 | 1 | 0.00 | 0 | 0.2 | 0.2 | 35.6 | 1.4 | 57.7 | 0.7 | 2.2 | 1.8 | 0.2 |
| NOLC-01 | 33.6 | 167 | 3.27 | 275 | 17 | 33.77 | 9 | 0.5 | 32.4 | 24.0 | 7.9 | 30.2 | 1.4 | 1.3 | 1.6 | 11.7 |
| NOLR-01 | 33.6 | 200 | 2.35 | 451 | 2 | 6.73 | 5 | 1.6 | 12.3 | 3.2 | 0.1 | 65.7 | 8.8 | 5.9 | 2.3 | 2.5 |
| PALU-01 | 32.0 | 214 | 6.06 | 933 | 43 | 0.00 | 0 | 0.4 | 1.1 | 32.9 | 11.0 | 49.8 | 3.1 | 0.8 | 1.0 | 0.2 |
| PLUM-01 | 31.6 | 248 | 3.06 | 226 | 0 | 0.00 | 0 | 0.1 | 0.3 | 16.8 | 21.6 | 53.8 | 2.3 | 4.5 | 0.6 | 0.1 |
| ROCK-01 | 31.4 | 258 | 2.75 | 221 | 0 | 0.00 | 0 | 0.1 | 1.3 | 24.3 | 39.9 | 34.0 | 0.1 | 0.1 | 0.3 | 0.2 |
| SALA-01 | 33.0 | 198 | 1.94 | 215 | 0 | 0.25 | 1 | 0.1 | 2.1 | 27.9 | 5.2 | 59.7 | 0.6 | 3.1 | 0.9 | 0.4 |
| SBOS-01 | 34.5 | 144 | 2.40 | 220 | 1 | 1.10 | 1 | 0.2 | 6.7 | 3.7 | 0.0 | 48.5 | 4.9 | 33.9 | 2.2 | 0.9 |
| SLEO-01 | 30.1 | 335 | 4.75 | 518 | 4 | 0.08 | 1 | 0.4 | 0.5 | 14.7 | 52.4 | 26.8 | 2.6 | 2.1 | 0.5 | 0.1 |
| STEE-01 | 32.2 | 210 | 4.19 | 127 | 4 | 0.00 | 0 | 0.8 | 5.1 | 30.9 | 0.0 | 59.6 | 0.6 | 1.5 | 1.6 | 0.5 |

Diel dissolved oxygen (DO) concentrations were measured from each stream once during TCEQ's (2005) critical index period in 2006 and 2007 using YSI 6600 or 600 XLM Datasondes. One datasonde was deployed in shallow glide habitats in each stream reach for at least 48 consecutive hours in order to characterize diel variability in DO, particularly daily minimums, that could be indicative of important functional changes caused by nutrients. Diel dissolved oxygen sampling corresponded closely in space and time with surface-water nutrient and biological sampling.

Periphyton, filamentous macroalgae, and aquatic macrophytes were sampled concurrently with quarterly nutrient sampling. Two approaches were utilized to assess aquatic vegetation growth. The first involved a direct sampling of periphyton occurring on rock (epilithic) substrate for the analysis and calculation quantitative measures of biomass per unit area (chlorophyll-*a* and AFDM) and algal species composition. Quantitative periphyton sampling followed the protocols of the US Geological Survey's National Water Quality Assessment (NAWQA) program (Moulton et al. 2002) for epilithic substrates using the targeted sampling approach (riffle or glide habitat with gravel, cobble, or bedrock). In brief, 5 separate rock samples of attached periphyton were collected from each of 5 erosional habitats throughout the reach (n=25 rocks). Surfaces of rocks were quantitatively delineated and scraped to remove periphyton. Material from the rocks was composited and quantitatively subsampled (aliquots) to determine chlorophyll-a, AFDM, and number of cells per species per unit area. Additional subsamples were taken for elemental analysis to estimate percent carbon, nitrogen, and phosphorus and corresponding ratios of these three elements as indicators of nutrient availability and enrichment. Methods are detailed in Back et al. (2008) and Scott et al. (2008).

The second approach utilized the modified version of Hawkins et al. (2001) vegetation cover protocols, which yielded a reach-wide assessment of aquatic macrophyte, macroalgae, and periphyton percentage cover and thickness of biofilm cover. The method entailed walking a zig-zag transect from one end of the reach to the other and estimating vegetation cover at each of 100 equally spaced points located along the transect. The protocol was modified to include an index of sediment film thickness on stream substrate (sediment cover index), dominant substrate (percent of different particle sizes in the reach), an estimate of flow velocity (flow index), and water depth.

Periphyton taxonomic composition was measured at each of the 26 streams during August 2006 and September 2007. Taxonomic data were obtained from 10-ml subsamples from the quantatitive periphyton composite sample. Samples were collected, homogenized, preserved, and identified in accordance with taxonomic methods for non-diatom and diatom algae described in TCEQ (2005). One non-diatom and one diatom taxonomic sample was identified per stream per year. At least 500 diatom and 300 non-diatom cells per respective sample were identified (TCEQ 2005). Dr. Barbara Winsborough, an expert periphyton taxonomist from central Texas, performed all of the species identifications in accordance with the approved project plan.

Six of the 26 streams were sampled for algal species composition on each of the 8 quarterly sampling events (see Figure 1, "intensive" sites). These six sites were chose because they were widely separated throughout the study region and spanned a steep gradient of nutrient enrichment.

Benthic macroinvertebrate densities and taxonomic composition were measured in November 2006. Macroinvertebrates were sampled quantitatively using a Hess sampler from the same 5 erosional habitats sampled quantitatively for periphyton within the reach. Four Hess samples were collected from each of the 5 locations within the reach (n=20). Samples were composited, homogenized, and preserved in 5% (v/v) buffered formalin stained with rose bengal.

A two-phase macroinvertebrate subsampling approach (King and Richardson 2002) was employed in the laboratory. The first phase was a comprehensive removal of all large-bodied taxa (organisms easily seen with the naked eye). The second phase was quantitative removal of a minimum of 500 individuals (fixed count method) using a gridded, numbered pan and random numbers table. All organisms will be removed from each randomly selected grid until enough grids have been sorted to achieve >500 individuals (Barbour et al. 1999), or the entire sample contents have been sorted, whichever came first. Total number of grids subsampled will allow estimation of densities (no/$m^2$; King and Richardson 2002).

Macroinvertebrates were identified in the laboratory using operational taxonomic units, usually genus or species for most taxa. A voucher collection of specimens is maintained in the Center for Reservoir and Aquatic Systems Research (CRASR).

Physical habitat, flow, substrate, riparian attributes, and basic water chemistry of each reach were characterized according to methods outlined in TCEQ (2005). Cross-sectional sampling transects were established as described in TCEQ (2005). Discharge (cfs) was measured each quarter along a representative transect within each reach using a portable electromagnetic flow meter (Marsh-McBirney Flo-Mate Model 2000).

## DATA ANALYSES

We estimated potential threshold responses in the measurement endpoints to numerical levels of nutrients using nonparametric changepoint analysis (nCPA), a technique explicitly designed for detecting threshold responses using ecological data (King and Richardson 2003, Qian et al. 2003). This analysis is based on the fact that structural change in an ecosystem may result in a change in both the mean and the variance of an ecological response variable used to indicate a threshold. When observations are ordered along an environmental variable (gradient), a changepoint is a value that separates the data into the two groups that have the greatest difference in means and/or variances. This can also be thought of as the degree of within-group variance relative to the between group variance, or *deviance* (*D*). Analytically, the nCPA examines every point along the stressor gradient and seeks the point that maximizes the reduction in deviance.

There is one particular value of the predictor *y* (e.g, TP) that maximizes the reduction in deviance in the response data (in this case, the selected biological responses); however, there is uncertainty associated with that value. It is unlikely that any one value of the predictor (e.g., TP) is the only value that could represent a changepoint. In reality, depending on the acuteness of the biological change in response to TP, several observations of TP could represent the changepoint, each with varying probabilities. Thus, to assess the risk associated with particular levels of TP, nCPA

incorporates estimates of uncertainty in the changepoint (King and Richardson 2003). These estimates are calculated using a bootstrap simulation. This simulation resamples (with replacement) the original dataset and recalculates the changepoint with each simulation. Bootstrap simulations are repeated 1,000 times. The result is a distribution of changepoints that summarizes the uncertainty among multiple possible changepoints. This uncertainty is expressed as a cumulative threshold frequency based on the relative frequency of each changepoint value in the distribution.

Important gradients in algal and macroinvertebrate species compostion were identified using non-metric multidimensional scaling (nMDS). NMDS is a distance based procedure that ordinates study units based on rank dissimilarities (Minchin 1987, Clarke 1993, Legendre and Legendre 1998). We used Bray-Curtis dissimilarity (BCD) as the distance measure, a coefficient that has been repeatedly demonstrated to be robust for ecological community data (Faith and Norris 1989). A two-dimensional solution was used for all analyses as stress values (a measure of agreement between BCDs and the configuration of the ordination) were relatively low and did not substantially decrease when additional axes were included in ordinations. Before running ordinations on the data sets, algae or macroinvertebrate species occurring at only two sites within a data set were excluded, and abundances were log transformed. Variables from the watersheds and environmental measurements with high skewness (> 1) were also log transformed to improve linear relationships with the ordinations. Ordinations were performed in PC-Ord version 5.20 (MjM Software, Gleneden Beach, OR, U.S.A.).

We used rotational vector fitting to relate environmental and watershed variables to gradients in algal and macroinvertebrate community composition quantified by the NMS ordinations (Faith and Norris 1989). Vector fitting was used to find the direction of the maximum correlation for each environmental variable. Significance ($P \leq 0.05$) of each environmental vectors was estimated using 1,000 random permutations of the data. Vector fitting was performed using the ECODIST package in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing).

For species abundance thresholds, we employed a new analytical approach, Threshold Indicator Taxa ANalysis (TITAN; Matthew E. Baker and Ryan S. King), with the goals of (1) exploring and identifying abrupt changes in both the occurence frequency and relative abundance of individual taxa along nutrient gradients, (2) quantifying uncertainty associated with both observed distributions of each taxon and the broader sample, and (3) estimating the relative synchrony of those changes as a non-parametric assessment of a community threshold. Current statistical methods used for grouping samples and detecting community ecological thresholds are not developed for distinguishing responses of individual taxa with low occurrence frequencies or highly variable abundances (Dufrêne and Legendre 1997, Brenden et al. 2008, Andersen et al. 2008). Some methods assume a linear, univariate response along all or part of an environmental gradient (e.g., Toms and Lesperance 2003), whereas others focus solely on aggregate, community-level dissimilarity (e.g., De'Ath 2002, King et al. 2005) or species turnover between samples (i.e., beta-diversity). Noisy, non-linear, and poorly distributed occurrences are typical properties of the vast majority of taxa in multivariate community data matrices (McCune and Grace 2002). Multivariate or multi-metric analysis can obscure distinct responses of taxa subsets in a community data set, especially if both predominant and rare species do not respond in a

similar fashion or focal species do not respond as expected. TITAN circumvents most of these problems.

TITAN represents a combination and extension of change-point and indicator species analysis. In TITAN, we use normalized indicator species taxa scores (z) to identify the value of a continuous variable, x, resulting in the optimal partitioning of sample units, such that the indicator score is maximized either for individual taxa or the additive response of all normalized indicator z -scores at the community level. Negatively responding taxa (z–) are distinguished from those responding positively (z+) to yield taxa-specific change-point distributions as well as cumulative responses of declining [sum(z–)] and increasing [sum(z+)] subsets of the community. Resampling procedures are used to measure both indicator reliability and purity, and to estimate uncertainty surrounding the existence of community change-points.

TITAN analysis was performed on the same species data sets as in the ordinations using log-transformed abundance data. Predictors included important nutrient variables identified from the environmental vector fitting analysis. TITAN was conducted in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing) using the custom package TITAN written by M. E. Baker and R. S. King (Baker and King, accepted with revision; King and Baker, in review).

## RESULTS AND INTERPRETATION

*Temporal Patterns in Discharge and Nutrient Concentrations*

The 26 selected streams spanned a steep gradient of phosphorus enrichment, which is critical for identifying numerical thresholds. Sites with low, moderate, and high levels of P enrichment were widely distributed throughout the study area. This latter point was important because our goal was to avoid spatial "clusters" of sites that had similar levels of enrichment. Spatial clustering in a predictor variable can lead to erroneous conclusions about the effect of the predictor (e.g., TP) on biological responses because other factors such as biogeography, geology, or other unmeasured variables may coincidently correspond to the predictor variable (e.g., King et al. 2005). Our study design avoided such spatial problems, capturing the full range of natural variability in geology, drainage networks, stream size, etc., while still achieving a heterogeneous arrangement of P enrichment and its likely sources (WWTP outfalls, pasture).

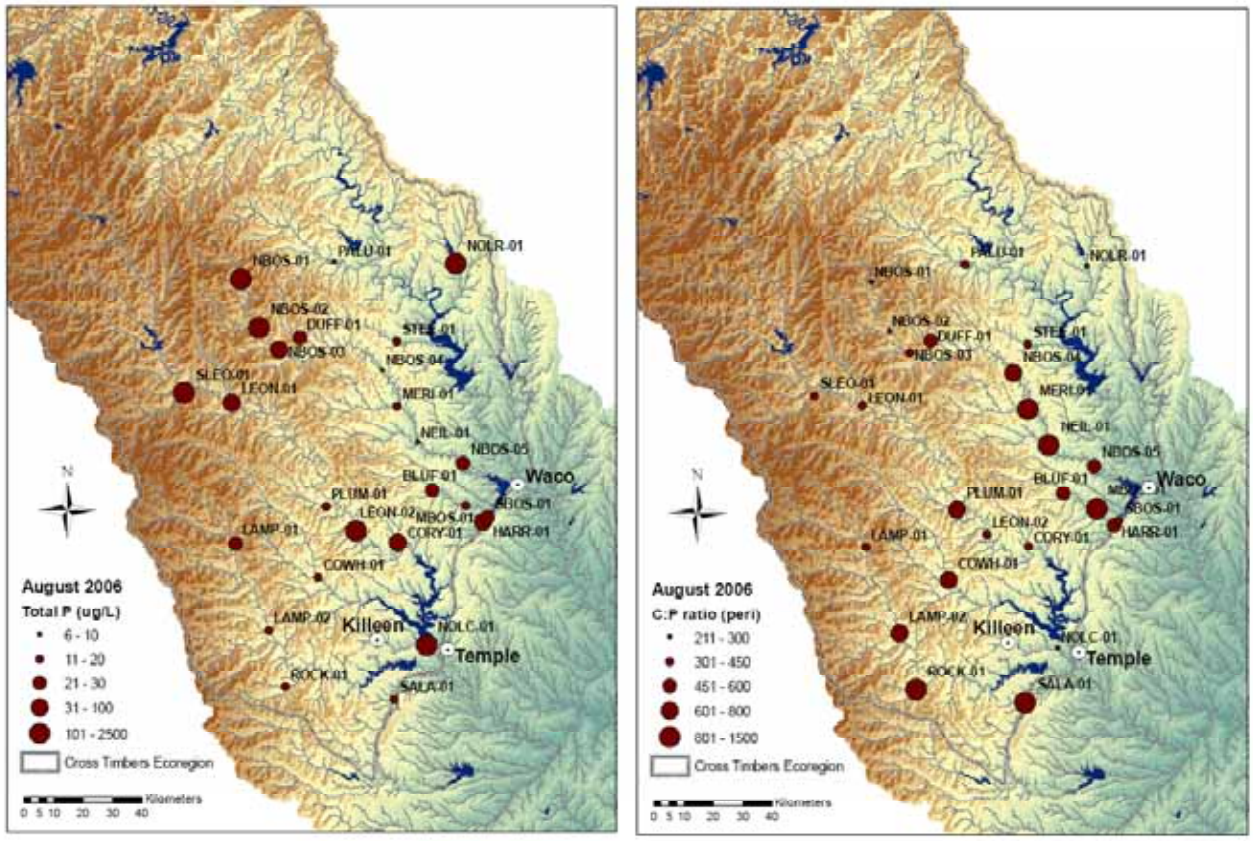



Figure 3. Spatial distribution of surface-water total phosphorus (TP, ug/L) and periphyton carbon-to-phosphorus (C:P, bulk) ratio during the first quarterly sampling event, August 2006.

Streams throughout the study area had similar discharge patterns during the study period (Figure 4). Differences in discharge were much greater among seasons and years than among the individual streams (also see Figure 6).

Stream discharge was very low in summer and fall 2006 and early winter 2007. This was a period of near-record drought, causing flow to cease or nearly so in most of the 26 streams during November 2006. This provided an excellent opportunity to evaluate the effects of P enrichment on biological responses under low-flow conditions.

Heavy precipitation in spring and summer 2007 led to very high discharges and numerous scouring events. May 2007 (#4 in Figure 4) was completed during a brief period of stream-flow recession. During this event, 8 of the 26 streams were unwadeable so some variables were not sampled at those sites on that date. More rain and flooding continued through August.

Streams finally began to recede in September 2007 (#6 in Figure 4), allowing us to reinstate our quarterly sampling schedule. However, baseflows remained much higher than 2006.

February 2008 (#7 in Figure 4) corresponded to an extended period of normal to above-normal winter baseflows, without any major scouring events in the preceding few months. However, rains and flooding came back in March through May, thus the final sampling event (June 2008) followed heavy flows and scouring.

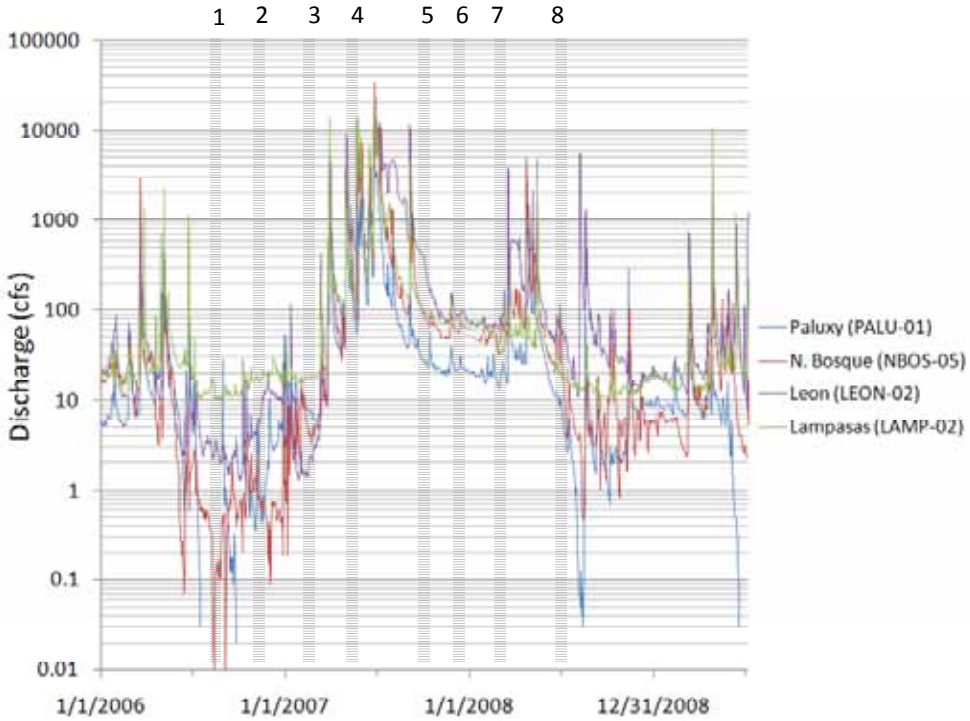



Figure 4. Hydrographs of four of the 26 study streams spanning a period before, during, and after the field study (data acquired from www.usgs.gov). The gray-shaded bars represent the sampling window corresponding to each of the 8 quarterly sampling events: 1=August 2006; 2=November 2006; 3=February 2007; 4=May 2007; 5=September 2007; 6=December 2007; 7=February 2008, and 8=June 2008.

Figure 5. Photograph of Salado Creek (SALA-01) during August 2006, illustrating the typical channel and bank morphology of the region.

All of the study streams had streambeds comprised predominantly of calcareous gravel, cobble, boulders, and bedrock (e.g, Figure 5). Streams had short reaches of erosional (riffle) habitat comprised mostly of larger substrate (gravel and cobble). Erosional areas were interspersed by typically longer sections of either shallow, low gradient glides underlain by bedrock, or deeper, depositional pools that often were associated with limestone bluffs on the erosional side of the stream channel. This particular photo reveals a moderate amount of calcareous periphyton and patches of bright green filamentous algae, a common pattern seen in sites with low nutrient levels during extended periods of low flow. Nutrient-enriched sites typically had a thinner films of dark-colored periphyton as well as dark-green filamentous algae (mostly *Cladophora*).

Instantaneous discharge measurements revealed that the hydrology of the 26 study streams was quite similar over time (Figure 6). All streams except for the 4 sites immediately downstream of significant effluent discharges (LEON-02, NOLC-01, NOLR-01, NBOS-01) exhibited a pattern of very low flow in August and November 2006, with a slight increase in February 2007 due to low evapotranspiration and increased groundwater discharge. However, the heavy rains and flooding of spring 2007 pushed discharge upward and maintained a relatively high baseflow through the end of the study in June 2008.

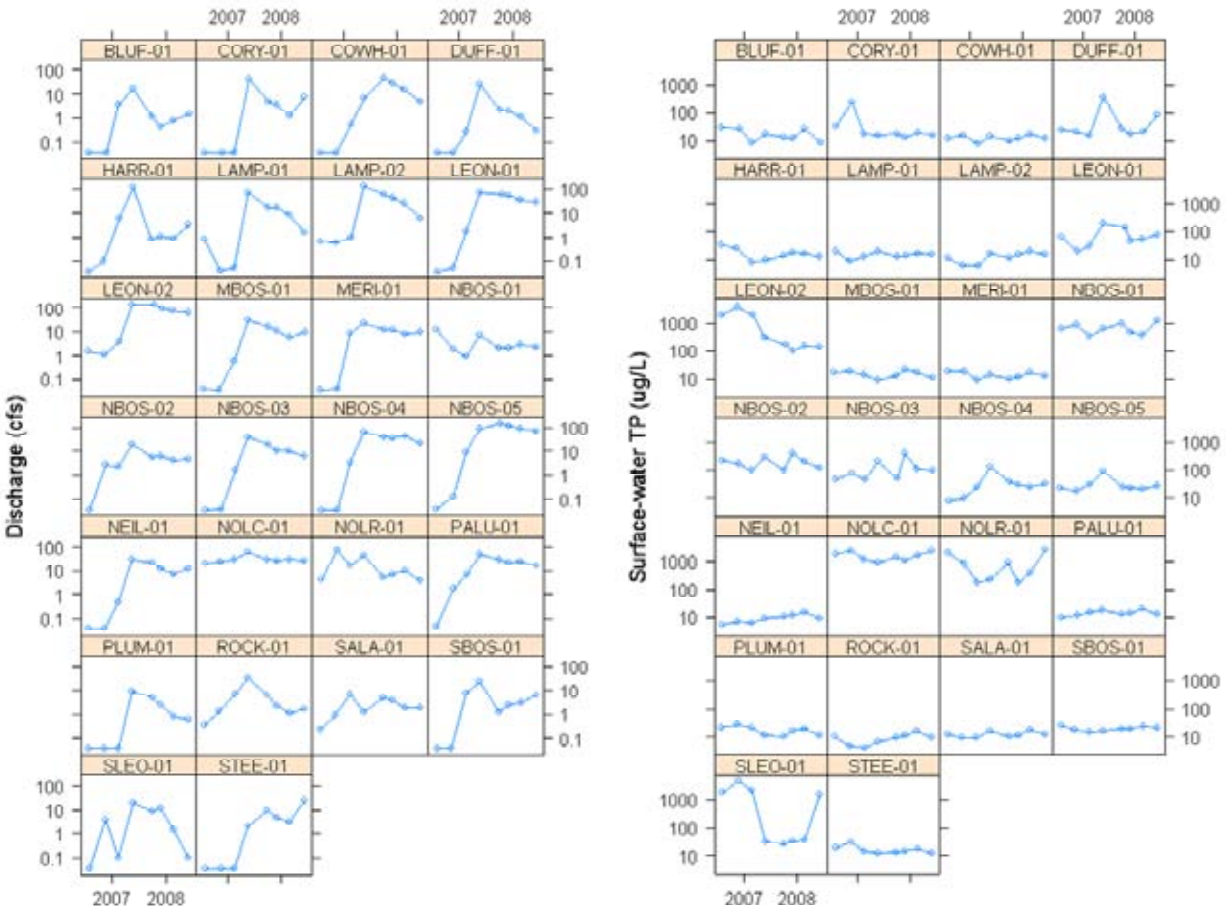



Figure 6. Temporal patterns of stream discharge (cfs) and surface water total phosphorus (TP, ug/L) among the 26 field sites, August 2006-June 2008. Both variables are plotted on a log-scale.

Surface-water TP, however, varied more by streams than by date of measurement. Streams with apparently few point or non-point sources of phosphorus (e.g., ROCK-01, NEIL-01, SALA-01, COWH-01, etc) exhibited consistently low TP, seen as relatively flat lines over time. Streams with predominantly non-point sources of phosphorus (watersheds with pasture or other sources) showed sharp increases in TP during high discharge, reflecting transport of P from the land from surface-water runoff. Streams with point sources (effluent discharges) either had consistently high TP (NOLC-01, NBOS-01) or were diluted by high surface-water runoff (LEON-02, NOLR-01, SLEO-01).

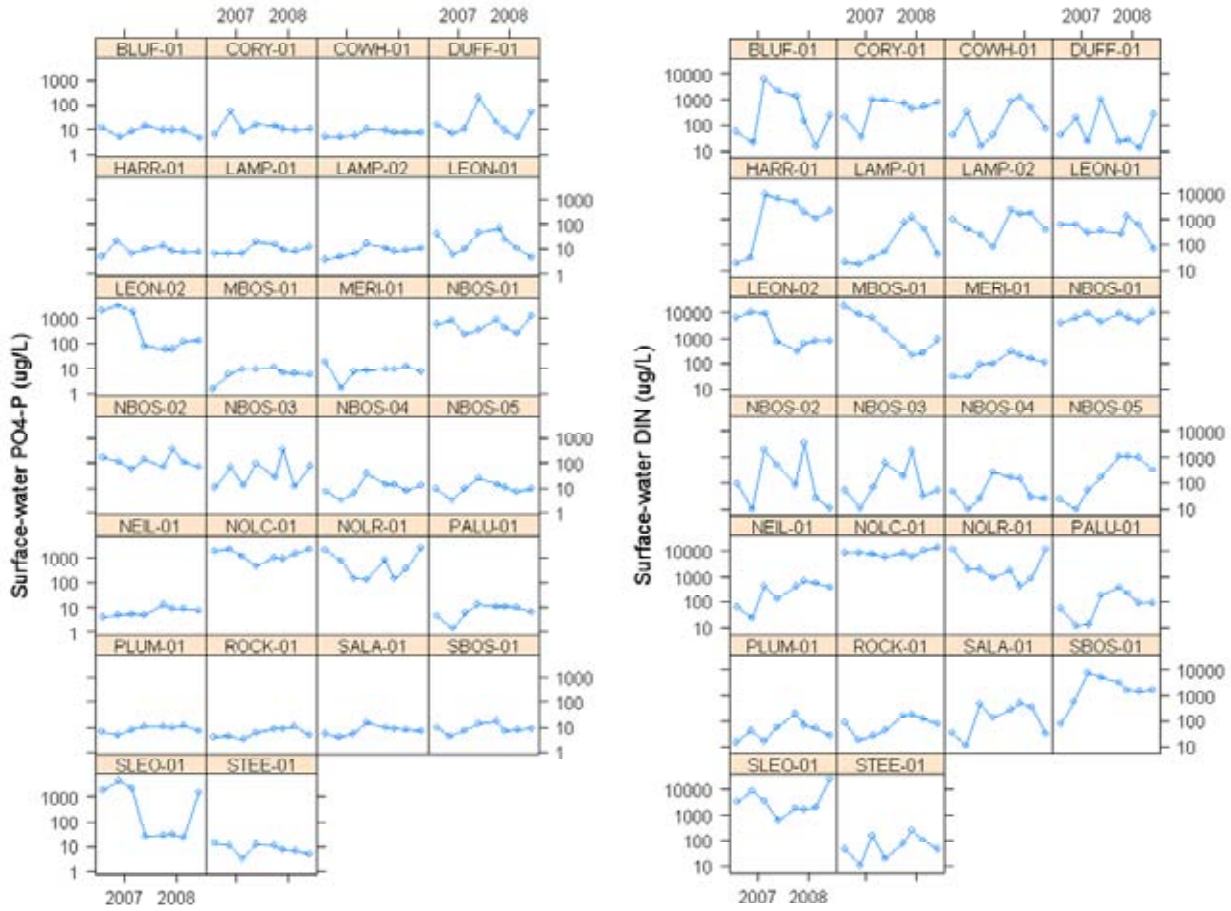



Figure 7. Temporal patterns of surface water orthophosphate (PO4-P, ug/L) and dissolved inorganic nitrogen (DIN) among the 26 field sites, August 2006-June 2008. Both variables are plotted on a log-scale.

Patterns in PO4-P among sites over time (Figure 7) were similar to that of TP, particularly for sites strongly influenced by point-source effluent discharges (NOLC-01, NOLR-01, NBOS-01, LEON-01, SLEO-01), as most of the TP at these sites was in the form of PO4-P. Orthophosphate comprised a smaller fraction (30-70%) of the TP at sites influence mostly by nonpoint sources (CORY-01, DUFF-01, PALU-01, LEON-01, etc). Orthophosphate represented a very small fraction of the TP at the sites with the fewest sources of phosphorus, and was often at or near the minimum detectable limit of 1-2 ug/L.

Dissolved inorganic nitrogen behaved much differently than PO4-P over time. During winter and spring of 2007, sites with rowcrop in their watersheds exhibited very high fluxes of DIN (mostly NO3-N). These sites tended to have consistently high DIN all year, but high concentrations were particularly evident as dissolved forms of N were transported from fields (mostly corn) via ground and surface water runoff to the streams (e.g., BLUF-01, CORY-01, HARR-01, MBOS-01, SBOS-01 NBOS-05). These high-DIN sites were important benchmarks because a few of them had low levels of TP (e.g., MBOS-01), revealing that biological variables did not respond to elevated DIN without a coincident subsidy of phosphorus (i.e., stream

ecosystem processes were strongly limited by P, and additions of N did not have a detectable effect on the streams unless additional P was available).

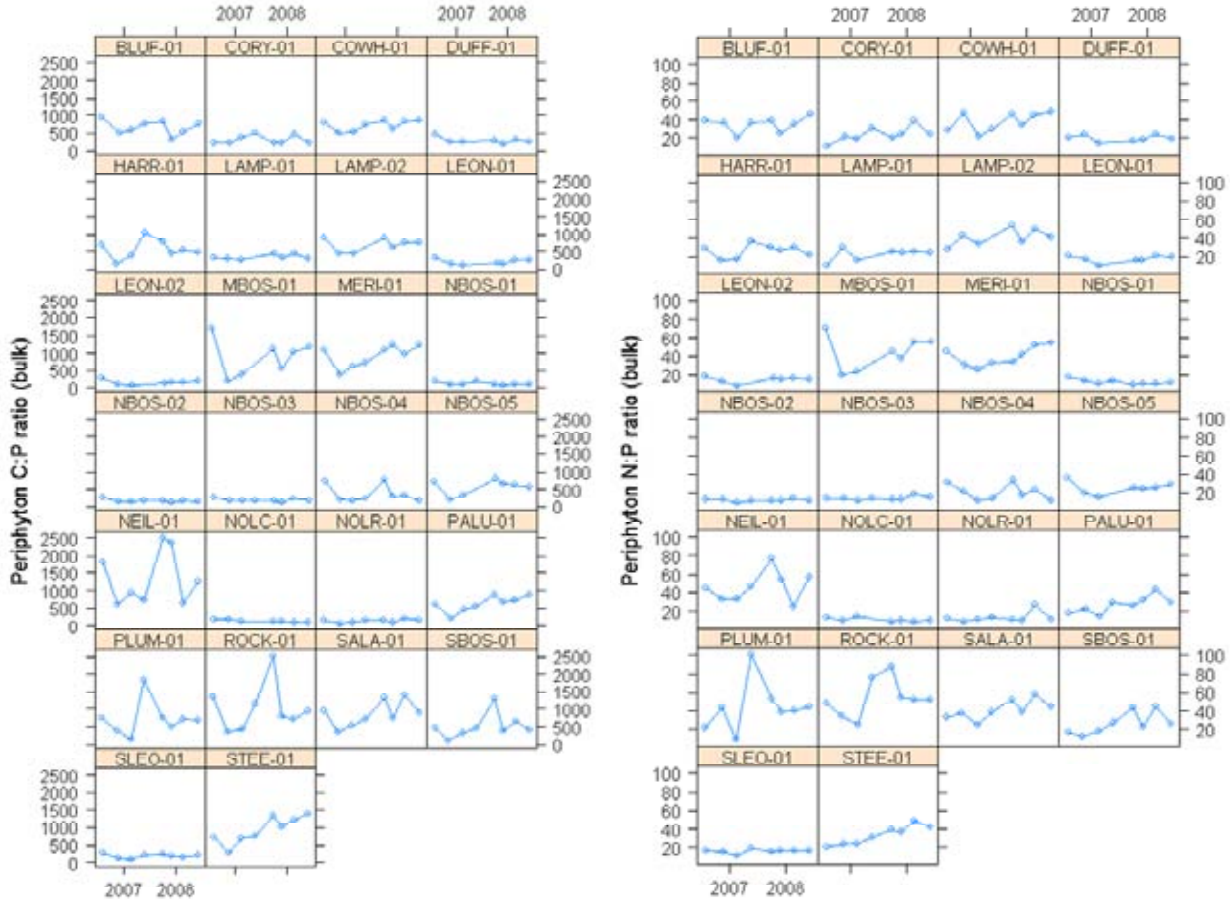



Figure 8. Temporal patterns of periphyton C:P ratio (bulk) and periphyton N:P ratio (bulk) among the 26 field sites, August 2006-June 2008.

We hypothesized that levels of enrichment of surface-water nutrients that were important would be reflected in enrichment of the tissues of the periphyton. Sites with elevated P, N or both should have more enriched periphyton P and/or N if either element was limiting its growth. Figure 8 illustrates that indeed sites differed markedly in their periphyton C:P and N:P ratios. Contrary to surface-water concentrations, *low values of C:P or N:P reflect greater enrichment* (i.e., more P per unit carbon or nitrogen in the periphyton = lower C:P or N:P ratio).

What is particularly compelling about these results is that sites with elevated surface-water TP had virtually constant C:P or N:P ratios in the periphyton. Moreover, despite the high variability of DIN in surface waters among sites over time, the periphyton N:P ratio was essentially identical to the C:P ratio, illustrating that P, not N, was driving differences in these ratios.

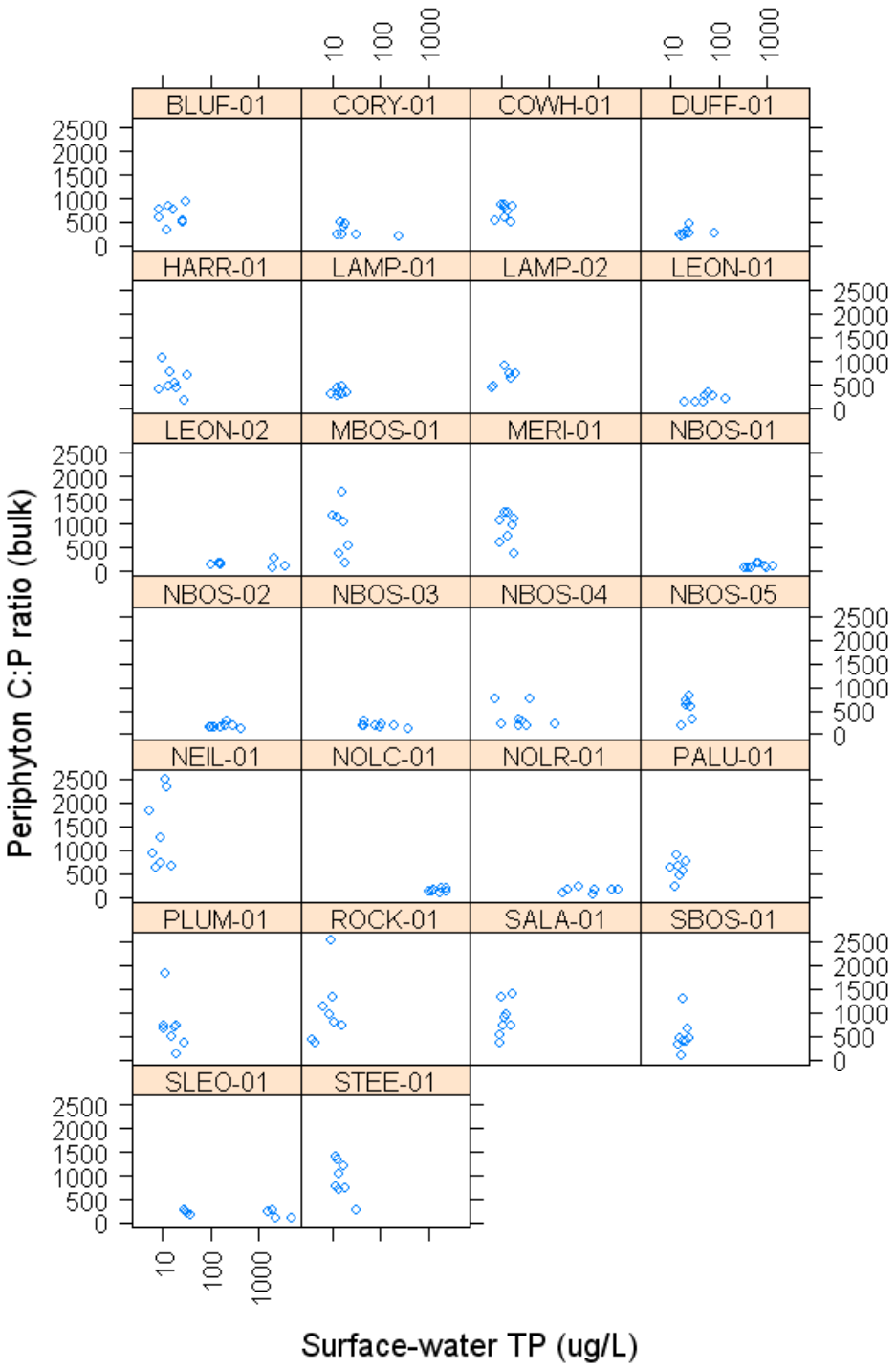



Figure 9. Scatterplot of surface-water TP (ug/L) versus periphyton C:P ratio (bulk) for each of the 8 sampling events, 2006-2008. Each point represents one of the eight dates of sampling. Surface-water TP is plotted on a log scale.

Figure 9 illustrates more directly the strong influence of surface-water TP on periphyton P content. There appear to be 3 types of relationships in this graph:

1. Sites with consistently low concentrations of surface-water TP (5-15 ug/L; e.g., NEIL-01, ROCK-01, SALA-01, STEE-01, etc) had highly variable periphyton C:P ratios. Importantly, these ratios almost always remained above 500. This showed that the periphyton was strongly limited by P, and variability in its P content was likely due to subtle seasonal differences in availability (either via recycling or pulses of low enrichment), taxonomic structure, and/or accumulation of biomass.

2. Sites with consistently moderate levels of TP (15-25 ug/L) had much less variability in the C:P ratios of the periphyton (e.g., BLUF-01, CORY-01, LAMP-01, HARR-01, SBOS-01) than sites with TP consistently < 15 ug/L. Moreover, these sites consisently had C:P ratios below 500, often below 200. This suggested that subtle increases in P availability resulted in a sharp, nonlinear response in the periphyton, with rapid uptake and storage of P.

3. Sites with consistently high levels of TP (e.g., effluent discharge sites such as NBOS-01, NOLC-01, NOLR-01), or with a wide range of levels of TP over time (e.g., SLEO-01, LEON-02, NBOS-02, NBOS-03) had consistently low (<200) periphyton C:P ratios, but these C:P ratios were not markedly lower than sites with consistently moderate levels of TP. This suggested that the periphyton was P-saturated (or nearly so) when exposed to low level P enrichment, and that temporal variability in surface-water TP did not correspond to variability in periphyton C:P because of its ability to store P during periods of high availability, and recycle P when it is less available.

In summary, these results imply that (1) periphyton was strongly limited by P, (2) it was very efficient at sequestering and recycling P, and (3) its C:P ratios were low and similar among sites ranging from subtle but consistent TP enrichment to consistently high levels of TP enrichment.

The next section of this report deals directly with quantification of levels of surface-water TP that result in thresholds, or nonlinear changes, in periphyton nutrient content and the implications of this enrichment on other biological response variables.

**RESULTS AND INTERPRETATION, CONTINUED**
*Biological Responses to TP Gradients*

The series of subfigures in Figure 10 effectively reveals the strikingly strong *nonlinear* relationship between surface-water TP and periphyton nutrient content. These figures use all of the data combined to show the remarkable consistency of the response, regardless of season or site. Threshold declines in periphyton C:P, N:P, and C:N ratios were highly likely between 15 and 25 ug/L TP, with 19-20 ug/L representing the most consistent concentration yielding a threshold response.

Note that C:N also declines with TP at the same threshold; this is important because it shows that as the periphyton is relaxed from its P limitation, it can also utilize the subsidy of nitrogen available in the streams. This pattern is not evident when C:P, N:P, and C:N are plotted against DIN or TN (see Figure 16).

Significant threshold changes were detected in biofilm thickness, filamentous macroalgal cover, and submersed macrophyte cover in response to TP, even when combining all of data from each site across the 8 sampling dates (Figure 11).

The thickness of microbial films (microalgae and other microbes; non-filamentous algae) growing on gravel, cobble, boulders, or bedrock showed consistently nonlinear declines in response to TP levels above 20 ug/L. This metric was computed as the percentage of observations in a reach (out of a maximum of 100 points along the reach-scale transect; see Methods) in which biofilms exceeded 1 mm in thickness on rocks. If no rocks were present at a point, or if the channel was too deep at a particular point to collect a rock, no measurement was recorded. Heavy accumulation of calcareous periphyton was a consistent trait of low-P streams (see Figure 12a). Streams above the threshold value of 20 ug/L usually had a thinner, darker layer of biofilms on the rocks (Figure 12b) but also tended to have more filamentous green algae (see next).

The percentage of points along the reach-scale transect that had filamentous macroalgae cover exceeding 75% of the substrate was also responsive to surface-water TP, but contrary to biofilm thickness, filamentous algae increased in response to TP (Figure 11). There were actually 2 distinct changepoints in filamentous algal cover: a consistent increase in cover among sites at about 20 ug/L TP, and a more diffuse but dramatic increase in cover at very high levels of TP (200-1000 ug/L). Because filamentous algal cover depends highly on seasonal changes in light and water temperature, as well as sloughing from overgrowth and scouring from high flows, it is not surprising that many of the highly enriched sites had little filamentous algal cover on some dates. However, when high filamentous algal cover occurred, it was almost always associated with levels of P enrichment > 20 ug/L, and particularly at >200 ug/L.

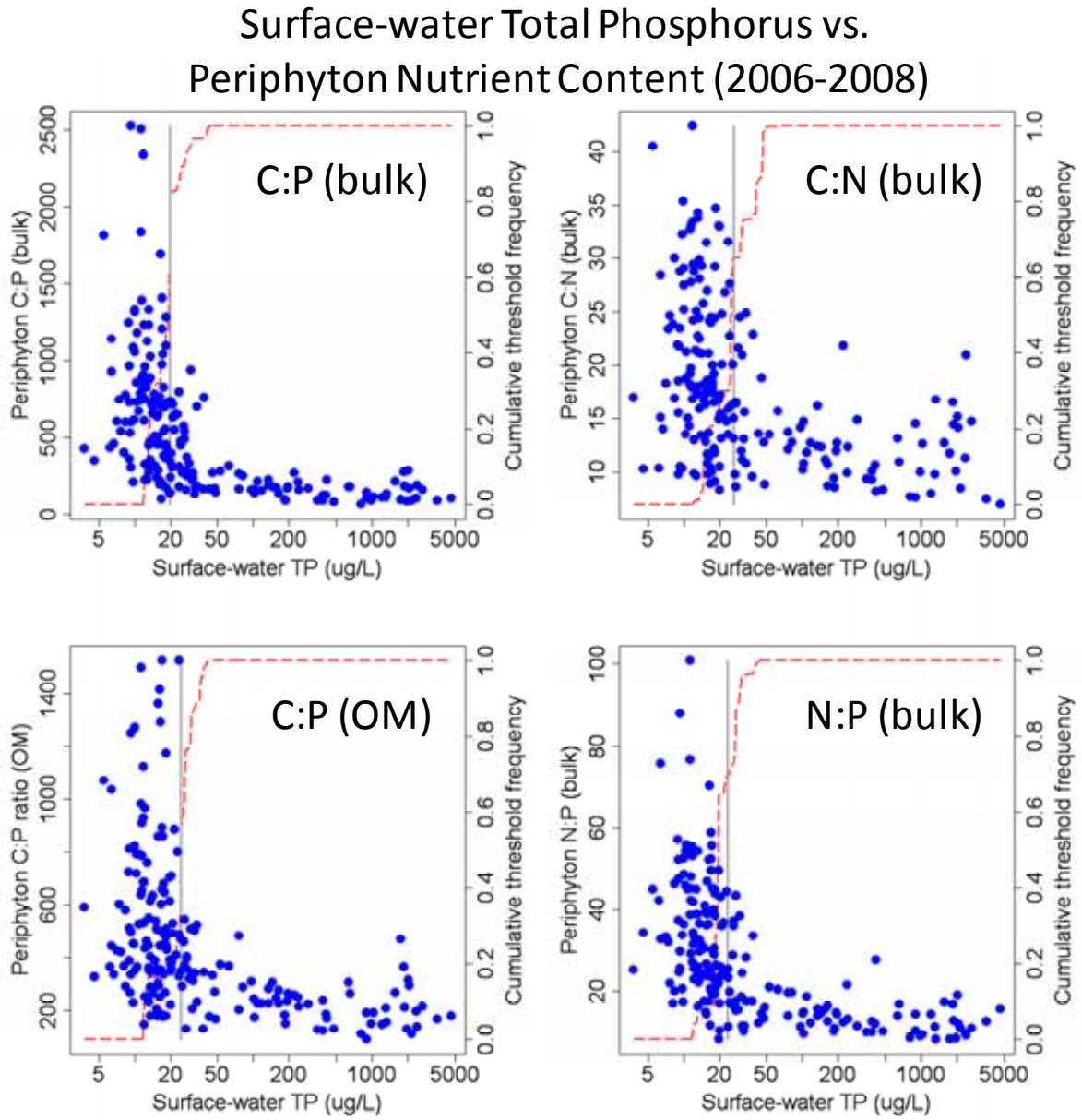



Figure 10. Results from nonparametric changepoint analysis using surface-water TP as a predictor of threshold changes in periphyton nutrient ratios across all 26 sites over the full study period. Bulk represents ratios of carbon, nitrogen, and phosphorus that were estimated using homogenized periphyton that was not subjected to the centrifugation separation. The OM (organic matter) ratio represents the fraction of homogenized periphyton that was separated from inorganic sediment using the centrifugation method described in Scott et al. (2008). Each blue dot represents one of the 26 sites, and each site is represented 8 times in the analysis, once for each sampling date. The gray vertical line is the observed TP threshold (the level of TP resulting in the greatest difference in the response variable to the left and right of that value). The dotted red line is the cumulative threshold frequency, an estimate of uncertainty based on 1,000 bootstrap samples of the data (see King and Richardson 2003). The cumulative threshold frequency illustrates the range of possible threshold values; different quantiles of this distribution can be interpreted as confidence intervals around the observed threshold. See Table 3 for summary of the corresponding statistical results.

One of the most important patterns that emerged from the field study was the sharp threshold declines in submersed macrophyte cover in response to TP >25-50 ug/L. The percentage of points along the reach-scale transect that had either some cover of macrophytes (top right panel, Figure 11) or cover exceeding 25% of the substrate (bottom right panel) both strongly declined in response to TP. The two dominant submersed macrophyte taxa were *Najas* (a vascular plant) and *Chara* (a nonvascular macroalga that is functionally equivalent to a vascular macrophyte). Other taxa less frequently encountered included *Potamogeton* and *Nitella*.

The mechanism for decline of these species may be related to filamentous algal growth on the plants themselves, which is a common problem in other systems experiencing eutrophication (e.g., Chesapeake Bay). *Chara* has also been shown to decline in response to TP levels above 30 ug/L in the Everglades (King et al. 2004, Richardson 2008), which is possibly related to changes in speciation of dissolved inorganic carbon with diel swings in pH associated with P-stimulated photosynthesis by algae. Regardless of mechanism, it is clear that P enrichment is associated with a significant negative change in an important structural component of these streams.

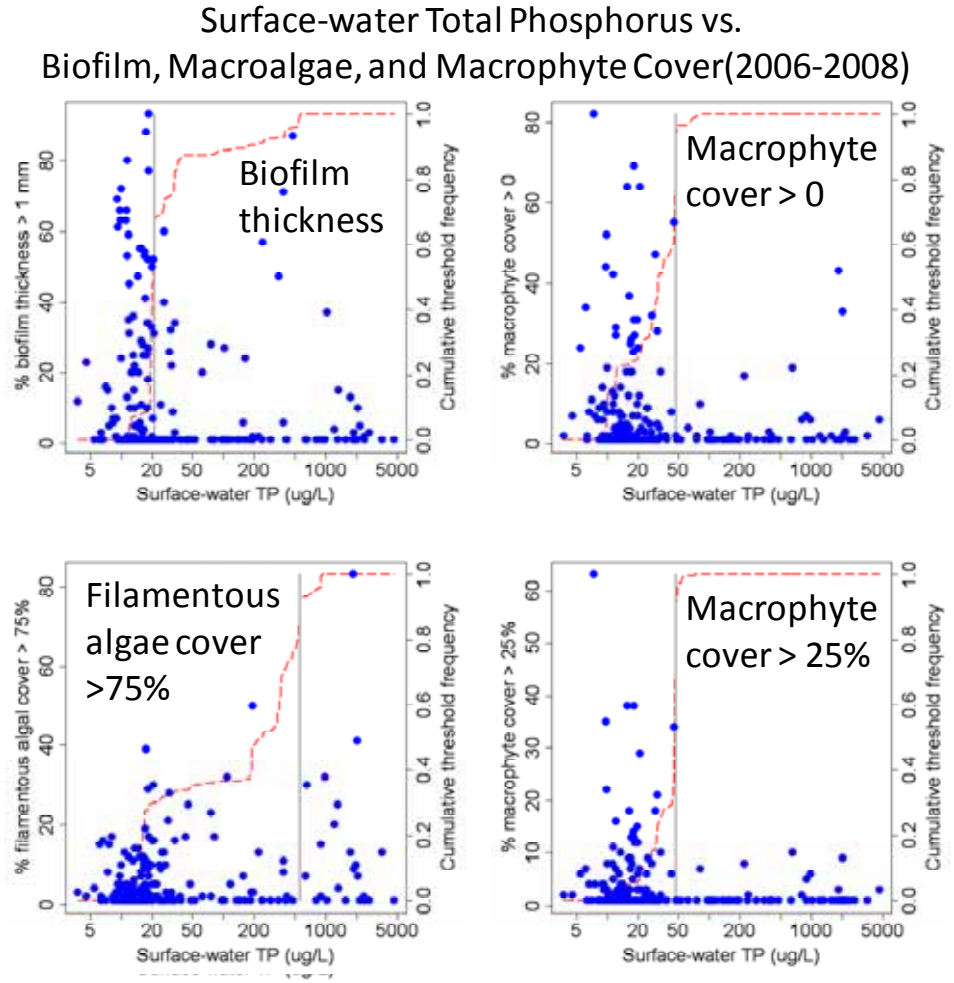



Figure 11. Results from nonparametric changepoint analysis using surface-water TP as a predictor of threshold changes in biofilm (non-filamentous periphyton) thickness on rocks, filamentous algae cover, and submersed macrophyte cover across all 26 sites over the full study period. See Figure 10 for other graphical details and Table 3 for statistical summaries corresponding to the figure.


Figure 12. Photographs of typical periphyton (biofilm) color and thickness from sites below the TP threshold of <15-20 ug/L (panel A) and above the threshold (panel B). In panel A, the periphyton is mostly diatoms, calcareous cyanobacteria, and other microbes (bacteria and fungi), with little obvious filamentous green algae. In panel B, the periphyton is comprised of a thinner veneer of mostly diatoms and bacteria, but the overall biomass does not change markedly because of the increase in colonial and filamentous green algae. This shift in structure becomes evident in the next series of figures.

The preceding pair of photographs in Figure 12 places the results presented in Figure 13 into context and aids in their interpretation. Periphyton ash-free dry mass was highly variable in response to TP, and showed a weak decline in response to TP levels ranging from as low as 10 ug/L to about 50 ug/L. Because total ash-free dry mass combines all types of attached periphyton (diatoms, cyanobacteria, other microbes and filamentous/colonial green algae), it is intuitive AFDM might not respond as sharply as biofilm thickness (decline) or filamentous algal cover (increase), because AFDM combines both. Nevertheless, the pattern of decreasing biofilm thickness does result in a significant decline in AFDM, although the threshold level of TP is relatively uncertain.

Periphyton chlorophyll-a tended to track the pattern in % filamentous algal cover shown in Figure 11, with possibly 2 changepoints (20 and 200 ug/L), each leading to higher levels of chlorophyll-a. However, this result is also relatively uncertain and noisy because diatoms and cyanobacteria characteristic of low-P sites also contain chlorophyll.

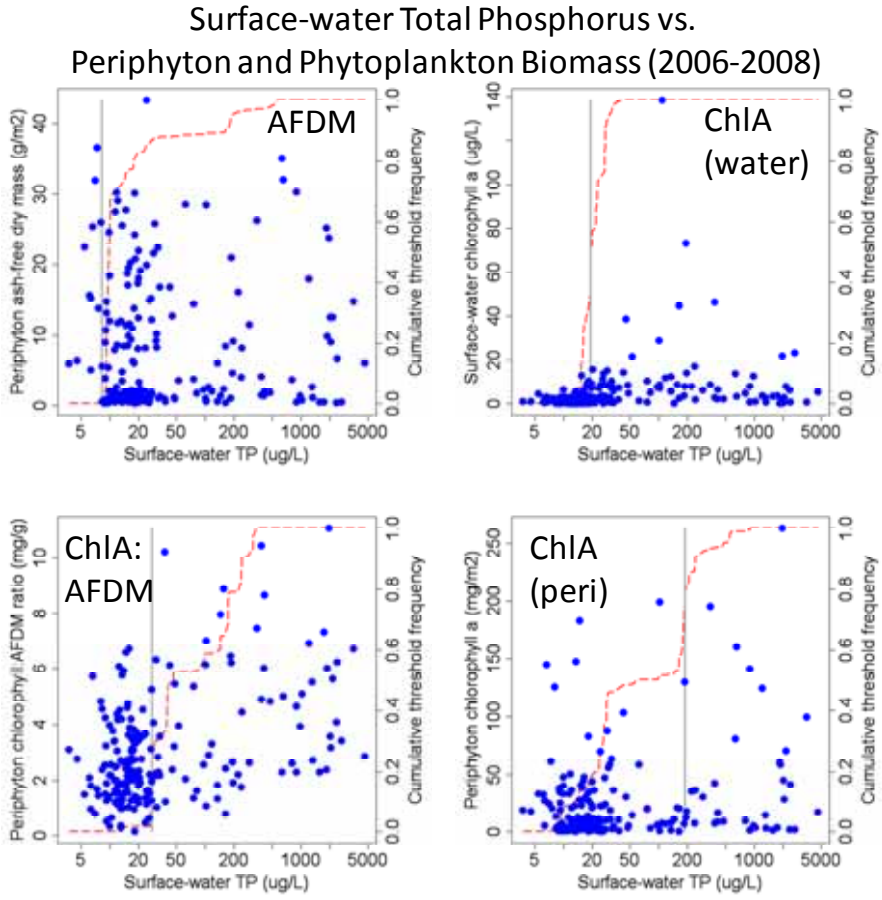



Figure 13. Results from nonparametric changepoint analysis using surface-water TP as a predictor of threshold changes in periphyton ash-free dry mass (AFDM, $g/m^2$), periphyton chlorophyll-a (ChlA (peri); $mg/m^2$), surface-water chlorophyll-a (ChlA (water); ug/L), and the ratio of periphyton ChlA to periphyton AFDM (ChlA:AFDM; mg/g) across all 26 sites over the full study period. See Figure 10 for other graphical details and Table 3 for statistical summaries corresponding to the figure.

The ratio of periphyton chlorphyll to AFDM did a much better job of revealing the shift in structure of the periphyton in response to P. This relationship (bottom left panel) reveals a clear, nonlinear shift toward a greater fraction of chlorophyll-bearing organisms in the periphyton in response to TP > 20-30 ug/L. A secondary threshold appears to be near 200 ug/L, coincident with the 2-tiered response of filamentous algae and chlorophyll-a.

Most primary production in wadeable streams is a result of periphyton and macroalgae rather than phytoplankton. However, wadeable streams in Texas typically have lower flows and thus longer residence times than wadeable streams in more mesic parts of North America. Longer residence times allow for some potential for phytoplankton blooms in response to TP. We observed a consistent nonlinear increase in surface-water chlorophyll-a at 20 ug/L TP. Streams with high TP did not consistently have high chlorophyll-a, but when high levels of chlorophyll were observed, TP was also elevated above 20 ug/L. This is somewhat circular, because TP is an unfiltered, digested sample that includes particulates such as phytoplankton. Thus, TP will necessarily increase if high chlorophyll is observed. It is also likely that much of the chlorophyll observed in the water column was from attached algal cells that had sloughed off of rocks.

Fine sediment runoff and deposition into streams may be an important contributor of non-point source phosphorus (Figure 14): C:P ratios in sediment that was separated from organic matter in the homogenized periphyton showed a strong nonlinear relationship to TP. It is likely that this relationship was at least partially caused by P-enriched organic matter (periphyton) that remained attached to sediment particles, but it also suggests that phosphorus bound to clay particles may be enriching the periphyton.

Streams with >20% of the channel dominated by silt or clay substrate were consistently associated with TP > 20 ug/L. Sediment-impacted streams often had high bank erosion, relatively high percentages of pasture in their watersheds, and usually had evidence of cattle activity in the stream channel and banks. However, many of the streams with TP>20 ug/L did not have obvious sedimentation problems, thus sedimentation alone was not the driving factor in these relationships. Rather, this suggests that sediment-bound P may have been an alternative source of enrichment that was driving the strong nonlinear biological responses.

The frequency of sediment cover classified as sufficiently heavy to obscure the color of periphyton (Sed>3) corresponded closely to the percentage of the reach dominated by fine silt-clay substrate. It also showed a sharp increase at 20 ug/L TP. This particular index was a direct measure of the potential effect of sediment on periphyton because it was a qualitative index of sediment film thickness on rock substrates. Again, not all streams with high TP had high sediment-index scores, suggesting that sediment alone was not the driver of threshold responses in biological endpoints.

Turbidity response to TP paralleled the responses of silt cover and the sediment index, sharply increasing at 20 ug/L TP. TP is partially dependent upon turbidity, however, because greater particulates in the water will necessarily lead to higher TP, assuming the particulates have P bound to them (clay) or the particulates are organic (detritus, phytoplankton).

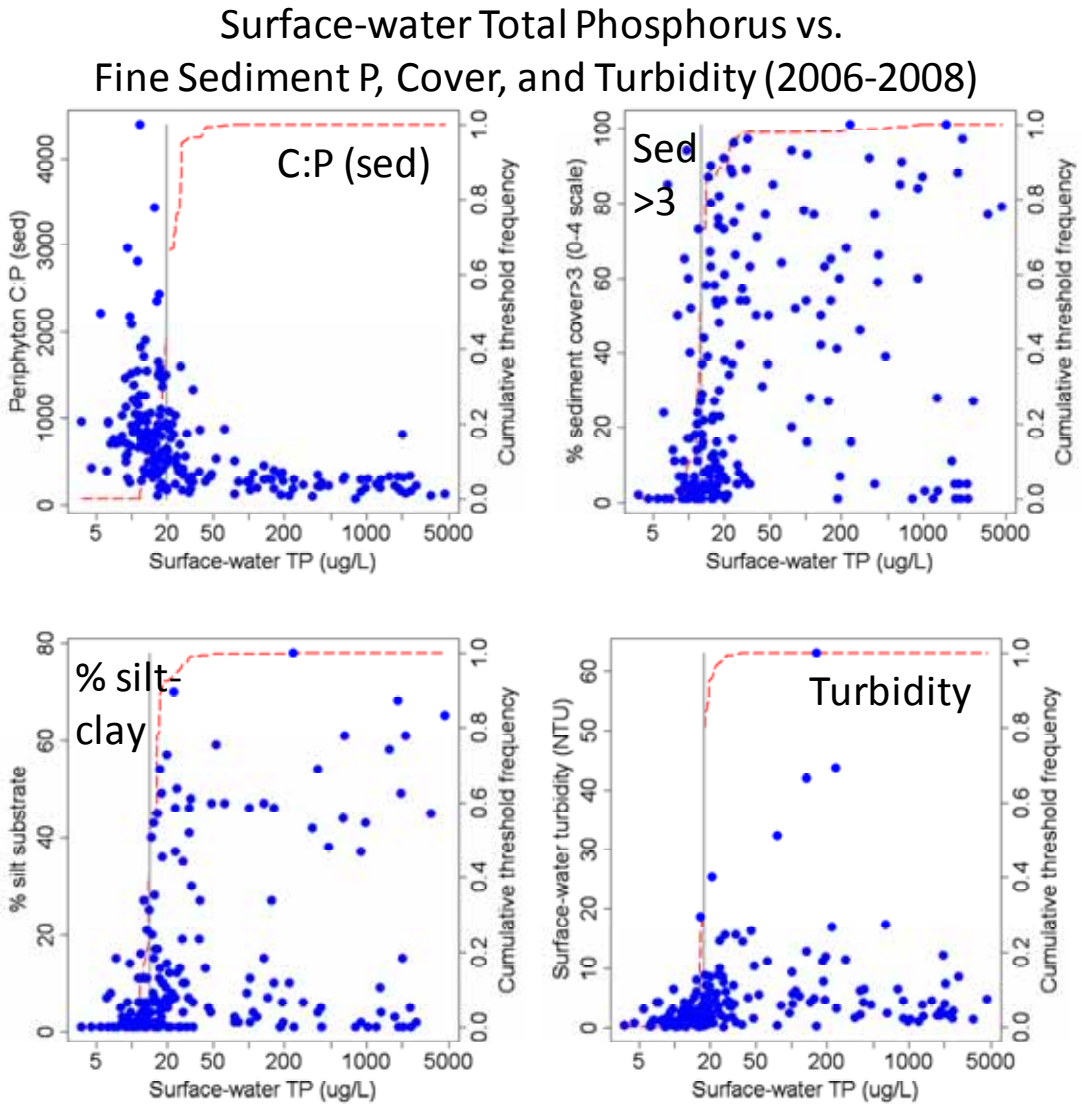



Figure 14. Results from nonparametric changepoint analysis using surface-water TP as a predictor of threshold changes in (1) periphyton C:P ratio (sediment fraction); (2) the percentage of points in the reach dominated by silt-clay substrate; (3) the percentage of points in the reach in which substrate had a heavy film of sediment (sediment index>3, where 3=sufficiently heavy to obscure color of periphyton); and (4) surface-water turbidity (NTU) across all 26 sites over the full study period. See Figure 10 for other graphical details and Table 3 for statistical summaries corresponding to the figure.

Numerical threshold responses of most of the measured water quality and biological endpoints from the the field study for all dates combined, 2006-2008 are presented in Table 3. The observed threshold value (shown as the gray vertical bar in Figures 10-14) is highlighted here in bold. The P-values represent the probabilities of Type I error (i.e., the probability of concluding that there is a threshold when in fact there is not) for each response to TP. Importantly, the cumulative threshold quantiles are narrow for most of the responses, with the 10% and 50% values corresponding closely to the observed value. The 90% quantile occasionally spiked up to near 200 ug/L for variables that were strongly influenced by filamentous algae, a response variable which likely had changepoints at ~20 and ~200 ug/L TP.

Table 3. Results of nonparametric changepoint analysis using **surface-water TP** as a predictor of threshold responses in selected biological and water quality response variables across 26 sites and 8 quarterly sampling events during 2006-2008. See Table 1 for full names and descriptions corresponding to variable IDs, and figures 10 through 14 for graphical display of most of these results.

| | | | | Cumulative Threshold Quantiles | | | Mean variable value | |
|---|---|---|---|---|---|---|---|---|
| Variable ID | **TP (ug/L)** | Response | P value | 10% | 50% | 90% | Below | Above |
| AFDM_M2 | **18.3** | Decline | 0.0027 | 9.4 | 10.2 | 163.9 | 5.30 | 3.30 |
| CHLA_UGL | **19.2** | Increase | <0.0001 | 15.5 | 19.2 | 28.3 | 0.81 | 3.86 |
| CHL:AFDM | **27.6** | Increase | <0.0001 | 27.5 | 40.7 | 239.8 | 2.05 | 3.57 |
| CHLA_M2 | **186.0** | Increase | 0.0042 | 12.0 | 57.6 | 239.8 | 7.53 | 19.88 |
| CN_ALG | **20.1** | Decline | 0.0001 | 12.0 | 20.1 | 46.5 | 14.98 | 12.18 |
| CN_BULK | **26.2** | Decline | <0.0001 | 15.6 | 25.4 | 46.5 | 20.43 | 12.91 |
| CP_ALG | **24.5** | Decline | <0.0001 | 12.1 | 24.5 | 32.9 | 564.95 | 259.96 |
| CP_BULK | **19.8** | Decline | <0.0001 | 12.2 | 19.8 | 26.8 | 768.59 | 260.21 |
| NP_ALG | **28.3** | Decline | <0.0001 | 22.7 | 32.9 | 174.5 | 34.59 | 19.60 |
| NP_BULK | **27.5** | Decline | <0.0001 | 19.8 | 27.5 | 40.7 | 31.49 | 14.01 |
| MACALG5+ | **554.7** | Increase | 0.0361 | 15.7 | 250.1 | 554.7 | 2.93 | 5.75 |
| MICALG3+ | **21.1** | Decline | 0.0005 | 18.2 | 21.0 | 174.7 | 6.24 | 2.48 |
| PLNT1+ | **46.5** | Decline | 0.0004 | 10.2 | 36.1 | 46.8 | 4.12 | 1.82 |
| PLNT3+ | **46.5** | Decline | 0.0014 | 25.9 | 46.5 | 46.8 | 2.39 | 1.31 |
| SED3+ | **12.8** | Increase | <0.0001 | 10.8 | 12.8 | 18.4 | 5.78 | 20.85 |
| TURB_NTU | **18.0** | Increase | <0.0001 | 15.5 | 17.8 | 19.8 | 1.08 | 4.24 |

Orthophosphate (PO4-P) was also a strong predictor of periphyton nutrient content. The relationships depicted in Figure 15 are very similar to the TP thresholds in Figure 10, but perhaps slightly more nonlinear. Virtually no sites with >18 ug/L PO4-P had bulk C:P ratios above 300 (upper left panel). This is further evidence for nonlinear uptake of P by periphyton, reaching a saturation point at the observed PO4-P threshold.

One disadvantage of PO4-P relative to TP as a predictor, however, is that it can be rapidly depleted during daylight when photosynthesis is active. Thus, some sites with low PO4-P are nevertheless highly enriched with P, shown by the low C:P ratios. TP is less variable and thus is a better indicator of P enrichment, but is still influenced by diel variability in photosynthesis and uptake. A major conclusion from this PO4-P result is that when there is a subsidy of bioavailable phosphorus (dissolved orthophosphate), it often indicates that the periphyton is near or beyond its point of saturation, thus excess dissolved P remains in the water column.

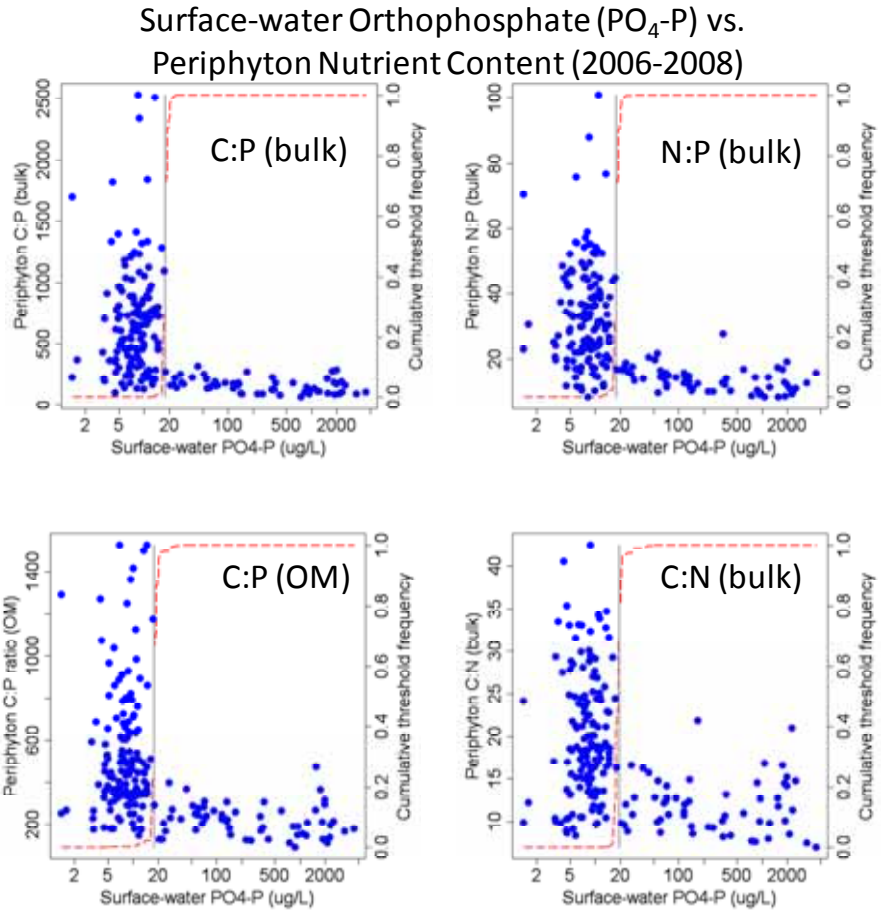



Figure 15. Results from nonparametric changepoint analysis using surface-water orthophosphate as a predictor of threshold changes in periphyton nutrient ratios across all 26 sites over the full study period. Bulk represents ratios of carbon, nitrogen, and phosphorus that were estimated using homogenized periphyton that was not subjected to the centrifugation separation. The OM (organic matter) ratio represents the fraction of homogenized periphyton that was separated from inorganic sediment using the previously described centrifugation method.

There were significant changes in periphyton C:P and N:P ratios in response to dissolved and total nitrogen, but the relationships were weak and noisy (Figure 16). Most of the signal in these relationships were driven by the covariation between P and N in effluent discharges (i.e., high P and N, but the periphyton was responding to the P), and the noise in the relationship is attributed to sites with high amounts of rowcrop in their watersheds but little pasture or effluent discharges (low P but high N, thus no response of the periphyton to the N because it was limited by P).

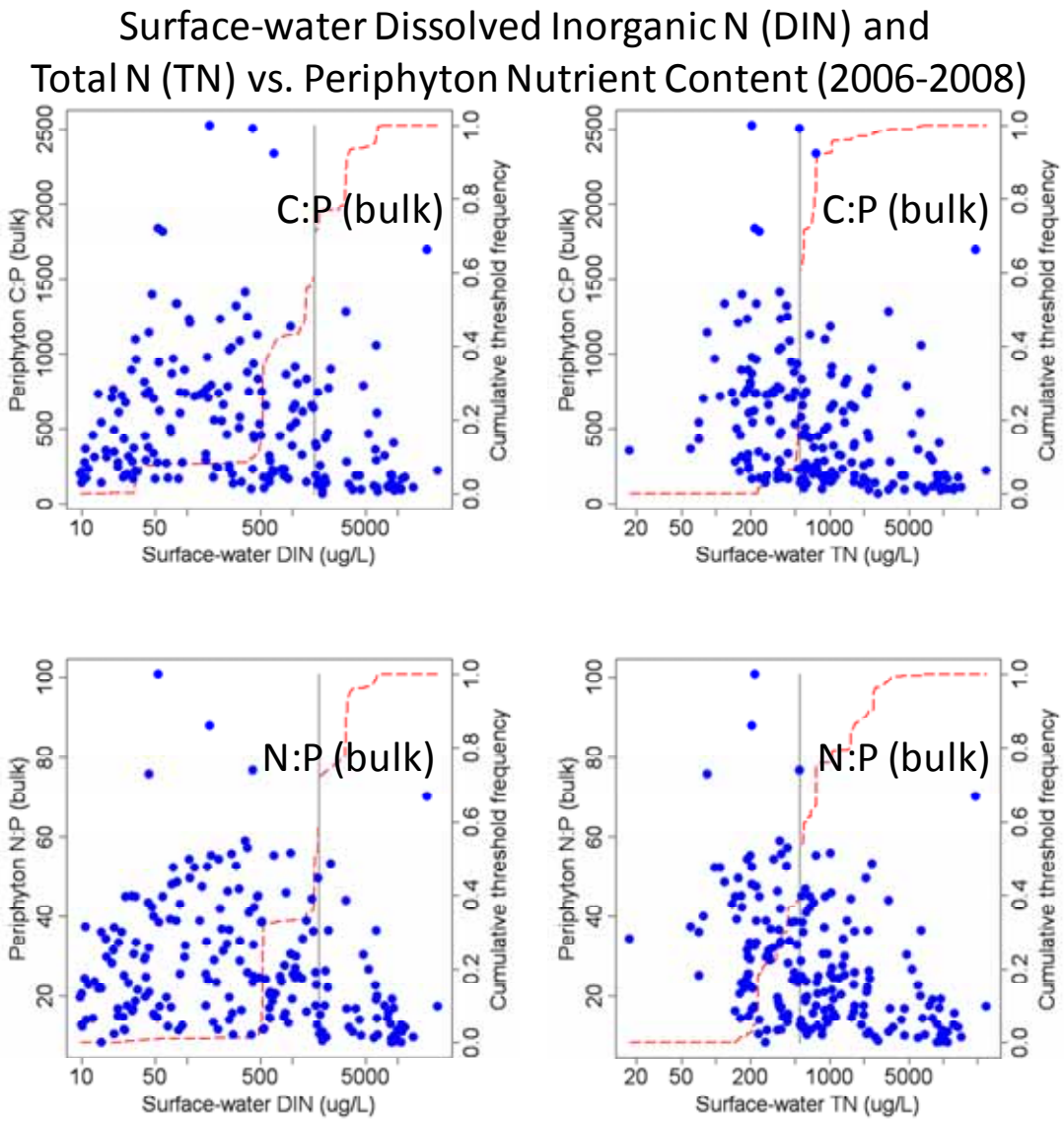



Figure 16. Results from nonparametric changepoint analysis using surface-water dissolved inorganic nitrogen (DIN, ug/L) and total N (TN, ug/L) as a predictor of threshold changes in periphyton nutrient ratios across all 26 sites over the full study period.

Table 4. Results of nonparametric changepoint analysis using **surface-water PO4-P, DIN, TN, and periphyton C:P ratio (bulk)** as a predictors of threshold responses in biological and water quality response variables from all 26 sites and 8 quarterly sampling events combined during 2006-2008. Significant thresholds are highlighted in bold. See Table 1 for full names and descriptions corresponding to variable IDs.

| | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|
| Predictor | Variable | Response | Obs. | P value | 10% | 50% | 90% |
| $PO_4$-P (ug/L) | **AFDM_M2** | **Decline** | 6.8 | <0.0001 | 6.8 | 7.4 | 7.9 |
| | **CHLA_UGL** | **Increase** | 28.9 | 0.0001 | 10.9 | 17.0 | 32.5 |
| | **CHL:AFDM** | **Increase** | 104.4 | <0.0001 | 19.4 | 113.5 | 139.3 |
| | **CHLA_M2** | **Increase** | 84.3 | <0.0001 | 6.8 | 7.7 | 145.2 |
| | **CN_ALG** | **Decline** | 7.0 | <0.0001 | 5.5 | 8.7 | 13.8 |
| | **CN_BULK** | **Decline** | 19.4 | <0.0001 | 17.3 | 19.2 | 20.7 |
| | **CP_ALG** | **Decline** | 17.9 | <0.0001 | 17.3 | 17.9 | 20.4 |
| | **CP_BULK** | **Decline** | 17.9 | <0.0001 | 16.5 | 17.9 | 19.9 |
| | **MACALG5+** | **Increase** | 509.0 | 0.0010 | 7.8 | 264.3 | 509.0 |
| | **MICALG3+** | **Decline** | 9.7 | 0.0015 | 5.7 | 10.0 | 235.1 |
| | **NP_ALG** | **Decline** | 17.9 | <0.0001 | 19.2 | 49.8 | 141.9 |
| | **NP_BULK** | **Decline** | 17.9 | <0.0001 | 17.3 | 17.9 | 21.0 |
| | **PLNT1+** | **Decline** | 6.8 | <0.0001 | 5.5 | 8.1 | 9.4 |
| | **PLNT3+** | **Decline** | 8.2 | <0.0001 | 5.1 | 7.7 | 11.7 |
| | **SED3+** | **Increase** | 9.8 | <0.0001 | 5.6 | 9.6 | 145.2 |
| | **TURB_NTU** | **Increase** | 13.7 | <0.0001 | 11.5 | 13.7 | 14.2 |
| Periphyton C:P (bulk) | **AFDM_M2** | **Increase** | 762.1 | 0.0159 | 431.6 | 736.2 | 963.6 |
| | **CHLA_UGL** | **Decline** | 496.1 | <0.0001 | 181.1 | 416.4 | 739.4 |
| | **CHL:AFDM** | **Decline** | 717.3 | <0.0001 | 141.6 | 442.3 | 869.1 |
| | **CHLA_M2** | **Decline** | 762.1 | <0.0001 | 454.4 | 731.6 | 834.5 |
| | **CN_ALG** | **Increase** | 317.5 | <0.0001 | 226.0 | 317.5 | 521.9 |
| | **CN_BULK** | **Increase** | 540.8 | <0.0001 | 337.6 | 459.6 | 554.9 |
| | **MACALG5+** | **Decline** | 1050.7 | <0.0001 | 129.3 | 272.6 | 1050.7 |
| | **MICALG3+** | **Increase** | 338.3 | <0.0001 | 301.3 | 338.3 | 491.3 |
| | **NP_ALG** | **Increase** | 623.9 | <0.0001 | 363.3 | 537.0 | 627.4 |
| | **NP_BULK** | **Increase** | 349.3 | <0.0001 | 301.3 | 349.3 | 454.9 |
| | **PLNT1+** | **Increase** | 195.6 | <0.0001 | 176.6 | 226.4 | 244.5 |
| | **PLNT3+** | **Increase** | 176.6 | <0.0001 | 175.1 | 184.0 | 234.7 |
| | **SED3+** | **Decline** | 482.9 | <0.0001 | 290.6 | 486.1 | 773.8 |
| | **TURB_NTU** | **Decline** | 403.5 | <0.0001 | 301.2 | 403.5 | 736.3 |
| TN (ug/L) | **AFDM_M2** | **Decline** | **2181.7** | **0.0234** | 212.6 | 2181.7 | 3973.3 |
| | **CHLA_UGL** | **Increase** | **351.8** | **<0.0001** | 243.0 | 350.3 | 368.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **CHL:AFDM** | **Increase** | **1060.7** | **<0.0001** | 987.6 | 1071.3 | 3965.0 |
| | **CHLA_M2** | **Increase** | **2181.7** | **0.0002** | 2075.0 | 2445.0 | 5083.4 |
| | **CN_ALG** | **Decline** | **285.4** | **<0.0001** | 261.4 | 285.4 | 580.8 |
| | **CN_BULK** | **Decline** | **548.9** | **<0.0001** | 524.9 | 574.4 | 1490.0 |
| | **CP_ALG** | **Decline** | **593.5** | **<0.0001** | 179.8 | 593.5 | 2445.0 |
| | **CP_BULK** | **Decline** | **548.9** | **<0.0001** | 511.2 | 548.9 | 771.7 |
| | **MACALG5+** | **Increase** | **3335.0** | **<0.0001** | 428.0 | 3071.3 | 7571.0 |
| | **MICALG3+** | **Decline** | **243.0** | **0.0013** | 204.4 | 240.5 | 587.4 |
| | **NP_ALG** | **Decline** | **2116.7** | **0.0001** | 1533.2 | 2116.7 | 2525.4 |
| | **NP_BULK** | **Decline** | **2445.0** | **0.0003** | 382.7 | 2145.0 | 3501.7 |
| | **PLNT1+** | **Decline** | **786.4** | **0.0001** | 258.1 | 786.4 | 939.0 |
| | **PLNT3+** | **Decline** | **922.2** | **0.0001** | 761.6 | 884.8 | 968.1 |
| | **SED3+** | **Increase** | **279.9** | **<0.0001** | 240.0 | 309.4 | 458.6 |
| | **TURB_NTU** | **Increase** | **160.2** | **<0.0001** | 160.2 | 208.2 | 587.4 |
| DIN (ug/L) | **AFDM_M2** | **Decline** | **67.6** | **0.0001** | 59.4 | 71.5 | 2092.4 |
| | CHLA_UGL | Increase | 9249.7 | 0.0754 | 64.0 | 69.1 | 3826.9 |
| | **CHL:AFDM** | **Increase** | **609.4** | **<0.0001** | 398.4 | 609.4 | 1939.6 |
| | **CHLA_M2** | **Increase** | **3263.8** | **<0.0001** | 1893.9 | 2912.5 | 3732.9 |
| | **CN_BULK** | **Decline** | **1372.4** | **<0.0001** | 323.6 | 1186.0 | 3525.3 |
| | **CP_ALG** | **Decline** | **1627.1** | **<0.0001** | 523.8 | 1660.1 | 3343.7 |
| | **CP_BULK** | **Decline** | **1627.1** | **<0.0001** | 436.2 | 1372.4 | 3343.7 |
| | MACALG5+ | Increase | 2147.6 | 0.0591 | 61.6 | 1007.5 | 3900.0 |
| | **MICALG3+** | **Decline** | **21.4** | **0.0147** | 21.4 | 82.9 | 2060.6 |
| | NP_ALG | Decline | 1783.9 | 0.1290 | 72.6 | 1783.9 | 3358.2 |
| | **NP_BULK** | **Decline** | **1783.9** | **<0.0001** | 1609.3 | 1783.9 | 6138.7 |
| | PLNT1+ | Decline | 71.5 | 0.0514 | 59.5 | 111.8 | 375.5 |
| | **PLNT3+** | **Decline** | **71.5** | **0.0002** | 61.6 | 101.4 | 492.4 |
| | **SED3+** | **Increase** | **523.8** | **0.0008** | 40.1 | 523.8 | 3202.8 |
| | **TURB_NTU** | **Increase** | **73.2** | **<0.0001** | 40.5 | 72.6 | 1372.4 |

TP and periphyton C:P were again used as predictors of threshold changes in each of the biological responses listed in Table 3 and 4, but for each of the 8 dates separately (Figures 17, 18; Tables 5, 6). This was done to evaluate seasonal changes and identify variables that responded consistently over the 2 year study.

The results of these date-specific analyses revealed several important conclusions:

- TP consistently predicted nonlinear declines in periphyton C:P ratios (Figure 17), and periphyton C:P ratios consistently predicted the same threshold responses in other variables that were predicted by TP, and even some that TP did not detect.

- The most consistent biological responses, aside from C:N:P ratios in the periphyton, were CHL_AFDM (ratio of periphyton chlorophyll to periphyton AFDM) and PLNT1 and/or PLNT3+ (percentage of the reach with > 0 or > 25% macrophyte cover). Both of these responses reflect fundamental changes to the structure and functioning of wadeable streams.

- Several biological variables were inconsistent responders to P enrichment, but usually this could be explained by seasonal patterns in light and/or discharge (scouring). The consistency of the direction of response (e.g., MACALG5+ always increasing, etc) supports these as reliable indices of excessive P enrichment, but also suggests that sites with TP levels above the reported thresholds will not always exhibit these responses (due to seasonal or hydrological limiting factors).

- Some metrics responded consistently among years within a particular season. In particular, MICALG3+ (% of reach with biofilm thickness > 1 mm) significantly declined only in the summer months (Aug 2006, Sep 2007, and June 2008), but this is perhaps the most critical time of the year in terms of primary production and other ecosystem processes. The consistent increase of CHLA:AFDM ratio also implies that biofilm structure changed with TP enrichment even when there was no effect on biofilm thickness (MICALG3+).

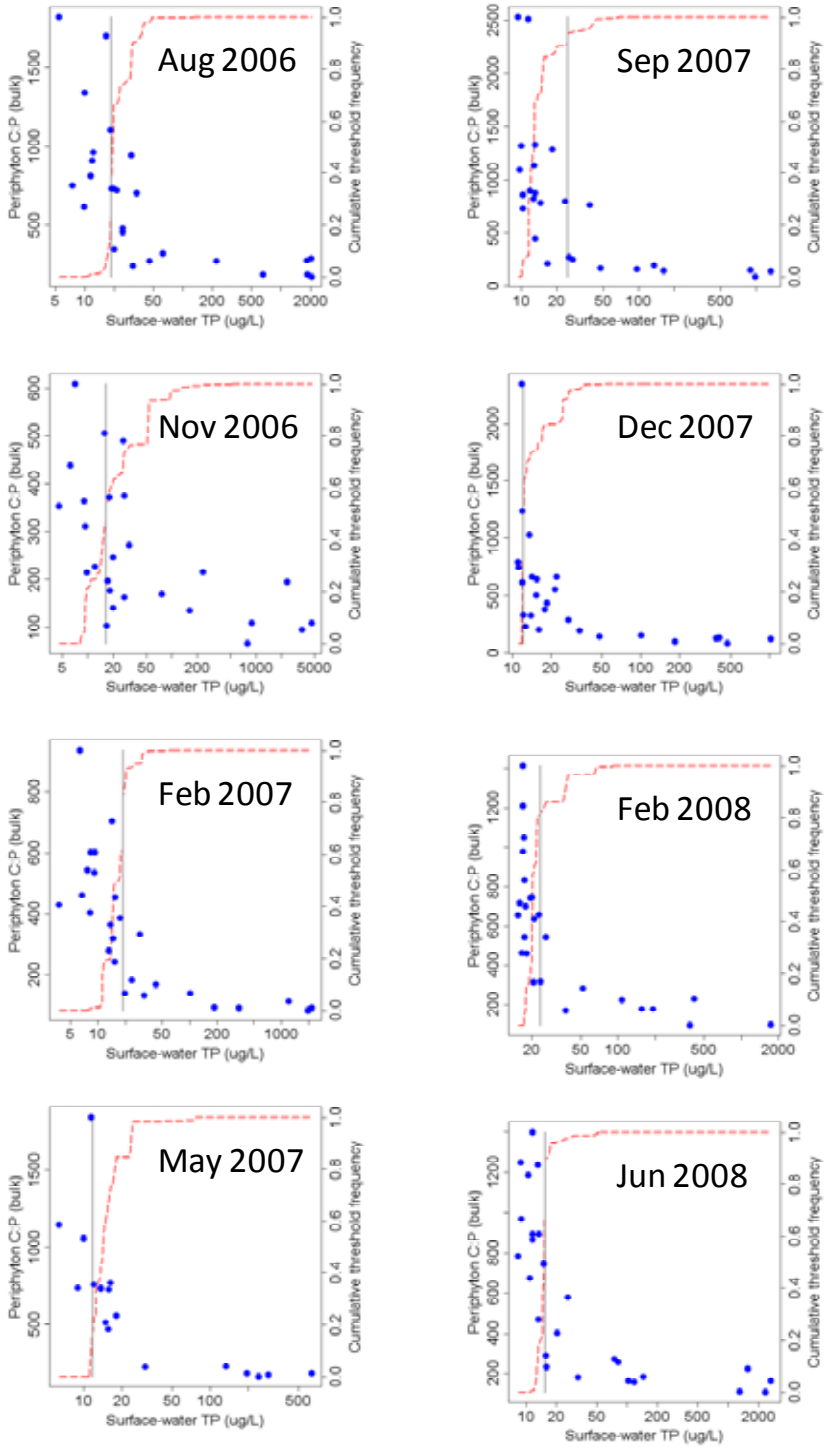



Figure 17. Results from nonparametric changepoint analysis using **surface-water TP (ug/L)** as a predictor of threshold changes in **periphyton C:P ratios (bulk)** across all 26 sites for each of the 8 quarterly sampling events separately, illustrating the consistency of the threshold decline of C:P (bulk), regardless of year, season, or discharge. All TP thresholds were significant, and ranged from a minimum of 11.7 ug/L (May 2007) and a maximum of 24.6 ug/L TP (September 2007). See Table 5 for detailed summaries of the statistical results.

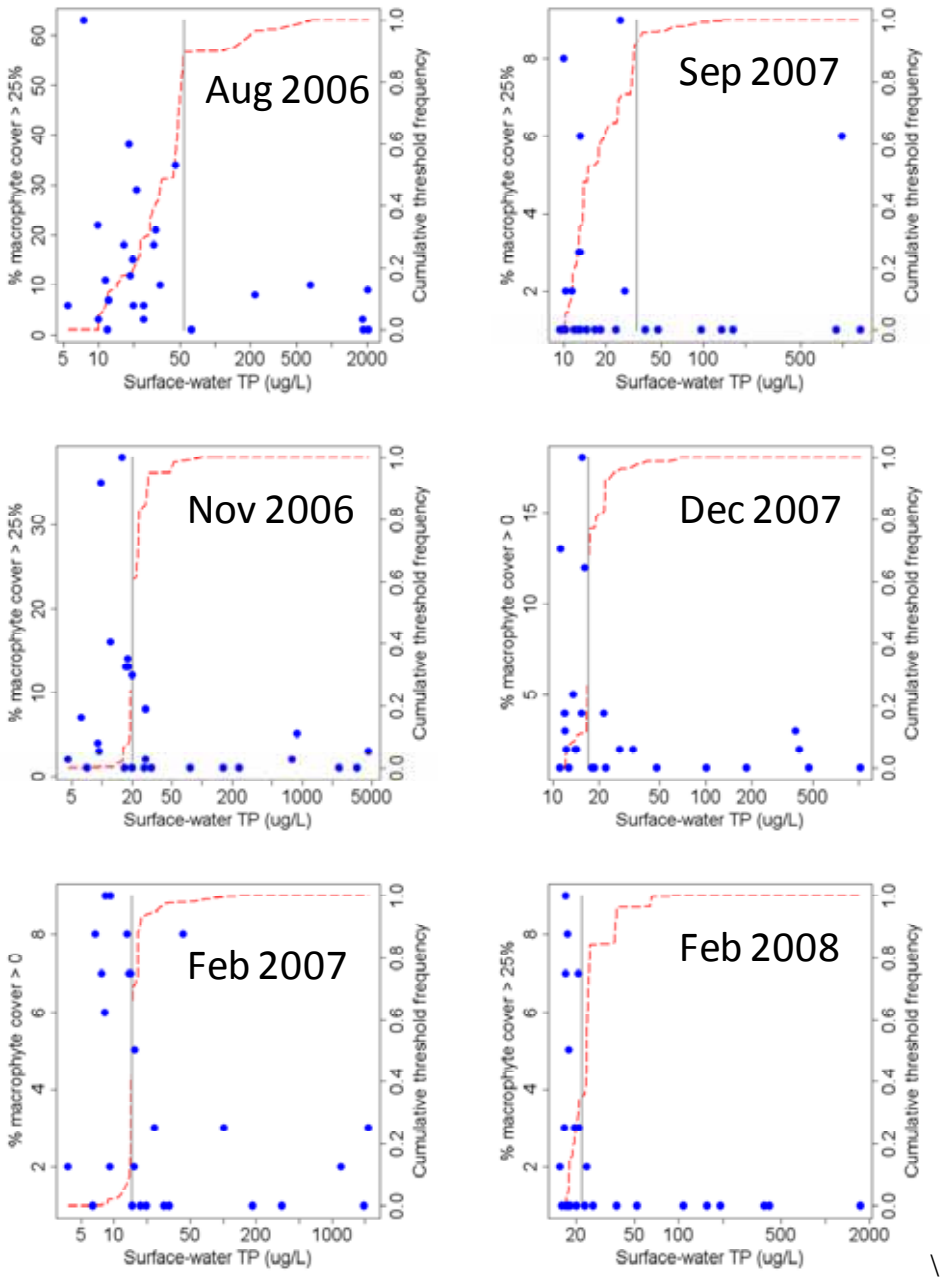



Figure 18. Results from nonparametric changepoint analysis using **surface-water TP (ug/L)** as a predictor of threshold changes in **macrophyte cover** across all 26 sites for 6 of the 8 quarterly sampling events separately, illustrating the consistency of the threshold decline of macrophyte cover regardless of year, season, or discharge. All TP thresholds were significant, and ranged from a minimum of 14.6 ug/L to a maximum of 54.2 ug/L TP (September 2007). See Table 5 for detailed summaries of the statistical results.

Table 5. Results from nonparamateric changepoint analysis using **surface-water TP (ug/L)** as a predictor of threshold responses in biological and water quality variables among each of the 8 quarterly sampling events from the 26 field study streams during 2006-2008. TP thresholds (Obs) and bootstrap quantiles are shown for each response variable and date combination. Direction (increase or decrease) of the response with increasing TP is shown for thresholds deemed significant (**bold**).

| | | | | Cumulative Threshold Quantiles | | | |
|---|---|---|---|---|---|---|---|
| Date | Variable | Response | Obs. | 10% | 50% | 90% | P value |
| **Aug-06** | **AFDM_M2** | **Decline** | **30.5** | **14.4** | **27.8** | **48.3** | **0.0357** |
| Nov-06 | AFDM_M2 | | 120.1 | 12.4 | 31.1 | 120.1 | 0.1596 |
| Feb-07 | AFDM_M2 | | 26.4 | 11.5 | 24.4 | 28.0 | 0.1046 |
| May-07 | AFDM_M2 | | 14.3 | 11.9 | 14.3 | 18.1 | 0.1593 |
| Sep-07 | AFDM_M2 | | 115.8 | 10.9 | 18.4 | 54.7 | 0.3967 |
| Dec-07 | AFDM_M2 | | 15.5 | 13.4 | 15.5 | 22.0 | 0.1587 |
| Feb-08 | AFDM_M2 | | 45.3 | 19.1 | 20.9 | 39.5 | 0.1532 |
| Jun-08 | AFDM_M2 | | 29.4 | 10.6 | 26.6 | 54.1 | 0.3157 |
| | | | | | | | |
| Aug-06 | CHLA_UGL | | 653.7 | 14.4 | 32.8 | 220.7 | 0.1326 |
| Nov-06 | CHLA_UGL | | 14.0 | 12.8 | 14.5 | 47.2 | 0.1156 |
| **Feb-07** | **CHLA_UGL** | **Increase** | **15.5** | **14.6** | **15.5** | **17.3** | **0.0016** |
| **May-07** | **CHLA_UGL** | **Increase** | **82.2** | **11.4** | **15.6** | **30.7** | **0.0503** |
| **Sep-07** | **CHLA_UGL** | **Increase** | **21.1** | **18.4** | **20.2** | **26.5** | **0.0050** |
| **Dec-07** | **CHLA_UGL** | **Increase** | **22.0** | **15.0** | **22.0** | **40.8** | **0.0105** |
| **Feb-08** | **CHLA_UGL** | **Increase** | **45.3** | **20.8** | **36.8** | **65.9** | **0.0037** |
| **Jun-08** | **CHLA_UGL** | **Increase** | **18.2** | **13.4** | **18.2** | **21.0** | **0.0087** |
| | | | | | | | |
| **Aug-06** | **CHL_AFDM** | **Increase** | **437.2** | **27.2** | **45.6** | **233.0** | **0.0028** |
| **Nov-06** | **CHL_AFDM** | **Increase** | **801.0** | **23.7** | **76.2** | **236.3** | **0.0074** |
| **Feb-07** | **CHL_AFDM** | **Increase** | **37.6** | **21.8** | **32.2** | **65.7** | **0.0044** |
| May-07 | CHL_AFDM | | 16.0 | 12.9 | 15.6 | 18.1 | 0.1849 |
| Sep-07 | CHL_AFDM | | 10.2 | 10.2 | 12.8 | 36.4 | 0.3732 |
| **Dec-07** | **CHL_AFDM** | **Increase** | **40.8** | **18.9** | **24.7** | **40.8** | **0.0170** |
| **Feb-08** | **CHL_AFDM** | **Increase** | **32.2** | **21.0** | **52.5** | **108.0** | **0.0082** |
| Jun-08 | CHL_AFDM | | 131.2 | 14.2 | 32.7 | 102.7 | 0.1321 |
| | | | | | | | |
| Aug-06 | CHLA_M2 | | 54.2 | 20.5 | 35.0 | 141.7 | 0.1179 |
| Nov-06 | CHLA_M2 | | 801.0 | 14.0 | 27.4 | 236.3 | 0.3263 |
| **Feb-07** | **CHLA_M2** | **Increase** | **26.4** | **16.5** | **26.4** | **37.6** | **0.0188** |
| **May-07** | **CHLA_M2** | **Increase** | **14.3** | **12.9** | **14.3** | **16.5** | **0.0438** |
| Sep-07 | CHLA_M2 | | 10.2 | 10.2 | 13.0 | 42.9 | 0.4436 |
| **Dec-07** | **CHLA_M2** | **Increase** | **22.0** | **14.2** | **22.0** | **24.7** | **0.0375** |
| **Feb-08** | **CHLA_M2** | **Increase** | **32.2** | **19.7** | **23.4** | **39.5** | **0.0178** |
| Jun-08 | CHLA_M2 | | 29.4 | 13.4 | 48.0 | 82.2 | 0.1926 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Aug-06** | **CP_ALG** | **Decline** | **18.5** | **17.6** | **18.5** | **20.5** | **0.0034** |
| **Nov-06** | **CP_ALG** | **Decline** | **9.6** | **9.6** | **16.3** | **97.6** | **0.0211** |
| **Feb-07** | **CP_ALG** | **Decline** | **37.6** | **8.5** | **26.4** | **37.6** | **0.0075** |
| **May-07** | **CP_ALG** | **Decline** | **11.7** | **10.7** | **12.5** | **17.0** | **0.0435** |
| **Sep-07** | **CP_ALG** | **Decline** | **12.3** | **12.3** | **13.0** | **31.1** | **0.0202** |
| **Dec-07** | **CP_ALG** | **Decline** | **30.6** | **12.2** | **22.0** | **35.1** | **0.0061** |
| **Feb-08** | **CP_ALG** | **Decline** | **17.5** | **16.9** | **17.5** | **23.6** | **0.0058** |
| **Jun-08** | **CP_ALG** | **Decline** | **13.3** | **12.5** | **13.3** | **45.7** | **0.0051** |
| | | | | | | | |
| **Aug-06** | **CP_BULK** | **Decline** | **18.5** | **17.6** | **19.3** | **30.5** | **0.0058** |
| **Nov-06** | **CP_BULK** | **Decline** | **16.3** | **9.4** | **16.6** | **53.7** | **0.0317** |
| **Feb-07** | **CP_BULK** | **Decline** | **18.7** | **11.3** | **17.1** | **20.7** | **0.0036** |
| **May-07** | **CP_BULK** | **Decline** | **11.7** | **11.7** | **14.3** | **23.5** | **0.0331** |
| **Sep-07** | **CP_BULK** | **Decline** | **24.6** | **11.6** | **13.0** | **24.6** | **0.0141** |
| **Dec-07** | **CP_BULK** | **Decline** | **12.2** | **12.0** | **12.3** | **24.9** | **0.0125** |
| **Feb-08** | **CP_BULK** | **Decline** | **23.5** | **18.2** | **20.6** | **39.5** | **0.0052** |
| **Jun-08** | **CP_BULK** | **Decline** | **15.4** | **13.3** | **15.4** | **15.5** | **0.0011** |
| | | | | | | | |
| **Aug-06** | **MACALG5+** | **Increase** | **141.7** | **18.5** | **29.2** | **141.7** | **0.0388** |
| Nov-06 | MACALG5+ | | 8.5 | 11.0 | 19.1 | 120.1 | 0.5798 |
| **Feb-07** | **MACALG5+** | **Increase** | **8.3** | **8.3** | **8.7** | **26.4** | **0.0420** |
| May-07 | MACALG5+ | | 15.7 | 11.7 | 15.6 | 17.2 | 0.4341 |
| Sep-07 | MACALG5+ | | 11.6 | 11.6 | 13.9 | 42.9 | 0.6199 |
| Dec-07 | MACALG5+ | | 30.6 | 13.6 | 22.9 | 35.1 | 0.3143 |
| Feb-08 | MACALG5+ | | 17.3 | 17.3 | 19.1 | 36.7 | 0.2957 |
| Jun-08 | MACALG5+ | | 10.8 | 10.8 | 15.9 | 75.5 | 0.6003 |
| | | | | | | | |
| **Aug-06** | **MICALG3+** | **Decline** | **20.6** | **12.1** | **20.6** | **22.3** | **0.0252** |
| Nov-06 | MICALG3+ | | 200.2 | 14.0 | 26.8 | 200.2 | 0.2573 |
| Feb-07 | MICALG3+ | | 9.3 | 9.3 | 13.5 | 72.6 | 0.5553 |
| May-07 | MICALG3+ | | 11.7 | 11.7 | 13.8 | 22.8 | 0.4841 |
| **Sep-07** | **MICALG3+** | **Decline** | **10.2** | **10.2** | **10.3** | **13.9** | **0.0013** |
| Dec-07 | MICALG3+ | | 11.9 | 11.9 | 15.4 | 58.4 | 0.1905 |
| Feb-08 | MICALG3+ | | 32.2 | 17.5 | 20.3 | 39.5 | 0.1097 |
| **Jun-08** | **MICALG3+** | **Decline** | **13.4** | **13.4** | **14.2** | **14.8** | **0.0368** |
| | | | | | | | |
| **Aug-06** | **PLNT1+** | **Decline** | **54.2** | **15.8** | **30.1** | **54.2** | **0.0484** |
| **Nov-06** | **PLNT1+** | **Decline** | **20.2** | **16.3** | **20.2** | **29.3** | **0.0153** |
| **Feb-07** | **PLNT1+** | **Decline** | **14.6** | **14.3** | **14.6** | **17.7** | **0.0267** |
| **May-07** | **PLNT1+** | **Decline** | **15.3** | **13.2** | **15.3** | **15.4** | **0.0860** |
| Sep-07 | PLNT1+ | | 13.9 | 10.3 | 13.9 | 31.9 | 0.3884 |
| **Dec-07** | **PLNT1+** | **Decline** | **17.1** | **14.3** | **17.1** | **22.0** | **0.0400** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Feb-08** | **PLNT1+** | **Decline** | **25.1** | **19.6** | **23.8** | **38.2** | **0.0269** |
| Jun-08 | | NA | | | | | |
| | | | | | | | |
| **Aug-06** | **PLNT3+** | **Decline** | **54.2** | **12.1** | **45.6** | **115.4** | **0.0643** |
| **Nov-06** | **PLNT3+** | **Decline** | **20.2** | **19.2** | **20.2** | **29.3** | **0.0227** |
| Feb-07 | PLNT3+ | | 72.6 | 9.3 | 14.9 | 42.9 | 0.1456 |
| May-07 | PLNT3+ | NA | | | | | |
| Sep-07 | PLNT3+ | | 33.0 | 11.4 | 14.9 | 31.9 | 0.5600 |
| **Dec-07** | **PLNT3+** | **Decline** | **17.1** | **12.0** | **17.1** | **19.0** | **0.0528** |
| **Feb-08** | **PLNT3+** | **Decline** | **22.1** | **18.2** | **23.8** | **38.2** | **0.0460** |
| Jun-08 | PLNT3+ | NA | | | | | |
| | | | | | | | |
| Aug-06 | SED3+ | | 22.8 | 14.4 | 22.2 | 41.0 | 0.1015 |
| **Nov-06** | **SED3+** | **Increase** | **9.6** | **9.4** | **11.4** | **17.1** | **0.0407** |
| **Feb-07** | **SED3+** | **Increase** | **8.0** | **7.6** | **9.3** | **42.9** | **0.0472** |
| May-07 | SED3+ | | 20.4 | 11.8 | 14.7 | 23.2 | 0.0699 |
| **Sep-07** | **SED3+** | **Increase** | **12.8** | **10.2** | **12.8** | **13.0** | **0.0128** |
| **Dec-07** | **SED3+** | **Increase** | **12.0** | **12.0** | **14.2** | **22.0** | **0.0429** |
| **Feb-08** | **SED3+** | **Increase** | **22.1** | **17.5** | **20.7** | **39.5** | **0.0279** |
| **Jun-08** | **SED3+** | **Increase** | **15.9** | **9.9** | **15.9** | **54.1** | **0.0183** |
| | | | | | | | |
| **Aug-06** | **TURB_NTU** | **Increase** | **17.4** | **15.3** | **17.4** | **19.9** | **0.0267** |
| Nov-06 | TURB_NTU | | 9.6 | 9.5 | 16.3 | 86.9 | 0.1315 |
| **Feb-07** | **TURB_NTU** | **Increase** | **9.3** | **8.9** | **9.3** | **13.5** | **0.0040** |
| **May-07** | **TURB_NTU** | **Increase** | **17.2** | **12.5** | **15.9** | **24.4** | **0.0360** |
| **Sep-07** | **TURB_NTU** | **Increase** | **17.6** | **13.9** | **20.2** | **31.9** | **0.0077** |
| **Dec-07** | **TURB_NTU** | **Increase** | **15.9** | **15.8** | **16.8** | **24.9** | **0.0066** |
| **Feb-08** | **TURB_NTU** | **Increase** | **45.3** | **19.4** | **22.4** | **39.5** | **0.0054** |
| **Jun-08** | **TURB_NTU** | **Increase** | **18.2** | **13.2** | **18.1** | **21.0** | **0.0027** |

Table 6. Results from nonparamateric changepoint analysis using **periphyton C:P ratio (bulk)** as a predictor of threshold responses in biological and water quality variables among each of the 8 quarterly sampling events from the 26 field study streams during 2006-2008. Periphyton C:P thresholds (*Obs.*) and bootstrap quantiles are shown for each response variable and date combination. Direction (increase or decrease) of the response with increasing periphyton C:P is shown for thresholds deemed significant (**bold**). **Note that an increasing value of C:P corresponds to a decline in P enrichment in the periphyton.*

| | | | | Cumulative Threshold Quantiles | | | |
|---|---|---|---|---|---|---|---|
| Date | Variable | Response* | Obs. | 10% | 50% | Date | Variable |
| **Aug-06** | **AFDM_M2** | **Increase** | **275.1** | **274.7** | **306.4** | **597.9** | **0.0386** |
| Nov-06 | AFDM_M2 | | 373.6 | 122.5 | 195.4 | 368.0 | 0.2509 |
| Feb-07 | AFDM_M2 | | 373.6 | 135.7 | 373.6 | 461.2 | 0.1407 |
| May-07 | AFDM_M2 | | 182.5 | 182.7 | 368.3 | 726.0 | 0.1484 |
| Sep-07 | AFDM_M2 | | 155.0 | 155.0 | 534.4 | 886.0 | 0.6122 |
| Dec-07 | AFDM_M2 | | 328.9 | 212.9 | 334.6 | 624.7 | 0.3682 |
| Feb-08 | AFDM_M2 | | 297.5 | 175.8 | 297.5 | 499.0 | 0.2741 |
| Jun-08 | AFDM_M2 | | 278.7 | 160.6 | 278.7 | 709.1 | 0.4895 |
| | | | | | | | |
| Aug-06 | CHLA_UGL | | 618.3 | 275.1 | 387.7 | 706.6 | 0.4483 |
| Nov-06 | CHLA_UGL | | 368.0 | 149.2 | 213.9 | 364.1 | 0.4072 |
| **Feb-07** | **CHLA_UGL** | **Decline** | **394.4** | **205.6** | **347.4** | **457.9** | **0.0191** |
| May-07 | CHLA_UGL | | 731.0 | 204.0 | 477.2 | 731.0 | 0.0520 |
| **Sep-07** | **CHLA_UGL** | **Decline** | **814.8** | **204.9** | **814.8** | **859.0** | **0.0086** |
| **Dec-07** | **CHLA_UGL** | **Decline** | **328.9** | **169.9** | **408.5** | **659.8** | **0.0158** |
| **Feb-08** | **CHLA_UGL** | **Decline** | **297.5** | **266.2** | **297.5** | **369.3** | **0.0068** |
| **Jun-08** | **CHLA_UGL** | **Decline** | **762.4** | **180.5** | **743.1** | **803.1** | **0.0108** |
| | | | | | | | |
| **Aug-06** | **CHL_AFDM** | **Decline** | **306.4** | **212.3** | **306.4** | **344.2** | **0.0052** |
| Nov-06 | CHL_AFDM | | 205.1 | 140.5 | 205.1 | 236.6 | 0.0514 |
| **Feb-07** | **CHL_AFDM** | **Decline** | **123.2** | **116.9** | **164.7** | **429.3** | **0.0280** |
| May-07 | CHL_AFDM | | 226.4 | 226.4 | 391.5 | 639.2 | 0.1566 |
| **Sep-07** | **CHL_AFDM** | **Decline** | **1207.6** | **501.2** | **867.0** | **1130.4** | **0.0220** |
| **Dec-07** | **CHL_AFDM** | **Decline** | **659.8** | **169.9** | **354.9** | **659.8** | **0.0225** |
| **Feb-08** | **CHL_AFDM** | **Decline** | **171.2** | **175.2** | **225.3** | **271.0** | **0.0086** |
| **Jun-08** | **CHL_AFDM** | **Decline** | **523.3** | **236.7** | **523.3** | **863.2** | **0.0294** |
| | | | | | | | |
| Aug-06 | CHLA_M2 | | 212.3 | 212.3 | 317.5 | 740.0 | 0.1763 |
| Nov-06 | CHLA_M2 | | 205.1 | 109.4 | 204.0 | 212.1 | 0.1650 |
| **Feb-07** | **CHLA_M2** | **Decline** | **123.2** | **123.2** | **300.3** | **429.3** | **0.0480** |
| May-07 | CHLA_M2 | | 226.4 | 204.0 | 345.9 | 616.1 | 0.0958 |
| **Sep-07** | **CHLA_M2** | **Decline** | **1207.6** | **510.8** | **851.5** | **993.1** | **0.0342** |
| **Dec-07** | **CHLA_M2** | **Decline** | **328.9** | **194.6** | **328.9** | **658.9** | **0.0460** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Feb-08** | **CHLA_M2** | **Decline** | **297.5** | **269.2** | **297.5** | **385.4** | **0.0178** |
| Jun-08 | CHLA_M2 | | 278.7 | 170.5 | 345.9 | 877.6 | 0.1525 |
| | | | | | | | |
| Aug-06 | MACALG5+ | | 306.4 | 299.4 | 306.4 | 658.6 | 0.1331 |
| Nov-06 | MACALG5+ | | 173.5 | 109.4 | 173.5 | 242.2 | 0.6289 |
| Feb-07 | MACALG5+ | | 537.6 | 123.2 | 262.2 | 457.9 | 0.7244 |
| May-07 | MACALG5+ | | 470.2 | 204.0 | 455.6 | 726.0 | 0.5601 |
| Sep-07 | MACALG5+ | | 143.5 | 143.5 | 352.5 | 867.0 | 0.1605 |
| Dec-07 | MACALG5+ | | 213.3 | 162.3 | 213.3 | 606.6 | 0.2077 |
| **Feb-08** | **MACALG5+** | **Decline** | **642.1** | **252.0** | **642.1** | **705.8** | **0.0481** |
| Jun-08 | MACALG5+ | | 158.4 | 158.4 | 241.6 | 709.1 | 0.3257 |
| | | | | | | | |
| **Aug-06** | **MICALG3+** | **Increase** | **306.4** | **299.4** | **317.5** | **724.5** | **0.0497** |
| Nov-06 | MICALG3+ | | 324.0 | 122.6 | 259.1 | 333.2 | 0.2077 |
| Feb-07 | MICALG3+ | | 354.6 | 114.4 | 277.7 | 441.6 | 0.6632 |
| May-07 | MICALG3+ | | 470.2 | 204.0 | 474.1 | 726.0 | 0.8096 |
| **Sep-07** | **MICALG3+** | **Increase** | **993.1** | **758.6** | **840.7** | **993.1** | **0.0208** |
| Dec-07 | MICALG3+ | | 328.9 | 127.5 | 328.9 | 528.8 | 0.2828 |
| Feb-08 | MICALG3+ | | 540.2 | 297.5 | 526.2 | 686.3 | 0.1434 |
| **Jun-08** | **MICALG3+** | **Increase** | **762.4** | **335.8** | **679.7** | **877.6** | **0.0264** |
| | | | | | | | |
| **Aug-06** | **PLNT1+** | **Increase** | **212.3** | **212.3** | **363.4** | **711.2** | **0.0273** |
| **Nov-06** | **PLNT1+** | **Increase** | **195.6** | **169.9** | **195.6** | **221.0** | **0.0234** |
| **Feb-07** | **PLNT1+** | **Increase** | **139.8** | **136.1** | **262.2** | **427.0** | **0.0563** |
| May-07 | PLNT1+ | | 486.6 | 368.3 | 486.6 | 727.9 | 0.2028 |
| Sep-07 | PLNT1+ | | 1207.6 | 233.5 | 843.5 | 1109.9 | 0.2403 |
| **Dec-07** | **PLNT1+** | **Increase** | **470.7** | **169.9** | **470.7** | **524.4** | **0.0591** |
| **Feb-08** | **PLNT1+** | **Increase** | **676.6** | **269.2** | **596.2** | **713.4** | **0.0331** |
| Jun-08 | PLNT1+ | NA | | | | | |
| | | | | | | | |
| Aug-06 | PLNT3+ | | 212.3 | 229.3 | 524.4 | 598.9 | 0.1627 |
| **Nov-06** | **PLNT3+** | **Increase** | **173.5** | **169.9** | **188.0** | **230.1** | **0.0546** |
| **Feb-07** | **PLNT3+** | **Increase** | **154.0** | **153.7** | **154.0** | **416.4** | **0.0436** |
| May-07 | PLNT3+ | | | | | | |
| Sep-07 | PLNT3+ | | 843.5 | 217.0 | 821.7 | 1013.6 | 0.3490 |
| Dec-07 | PLNT3+ | | 659.8 | 169.9 | 418.7 | 659.8 | 0.2043 |
| **Feb-08** | **PLNT3+** | **Increase** | **297.5** | **269.2** | **456.7** | **725.8** | **0.0572** |
| Jun-08 | PLNT3+ | | | | | | |
| | | | | | | | |
| **Aug-06** | **SED3+** | **Decline** | **779.8** | **484.6** | **749.6** | **908.4** | **0.0558** |
| **Nov-06** | **SED3+** | **Decline** | **292.3** | **177.2** | **292.3** | **313.4** | **0.0691** |
| Feb-07 | SED3+ | | 93.6 | 93.6 | 160.2 | 444.9 | 0.3262 |
| May-07 | SED3+ | | 486.6 | 205.1 | 486.6 | 726.0 | 0.1023 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sep-07** | **SED3+** | **Decline** | **793.0** | **767.2** | **806.2** | **993.1** | **0.0337** |
| Dec-07 | SED3+ | | 701.3 | 137.9 | 308.5 | 635.6 | 0.3543 |
| **Feb-08** | **SED3+** | **Decline** | **297.5** | **269.2** | **386.0** | **676.6** | **0.0692** |
| **Jun-08** | **SED3+** | **Decline** | **523.3** | **270.7** | **523.3** | **803.1** | **0.0187** |
| | | | | | | | |
| **Aug-06** | **TURB_NTU** | **Decline** | **779.8** | **724.5** | **770.2** | **829.0** | **0.0181** |
| Nov-06 | TURB_NTU | | 259.1 | 122.6 | 207.7 | 278.9 | 0.3126 |
| **Feb-07** | **TURB_NTU** | **Decline** | **373.6** | **352.2** | **394.4** | **457.9** | **0.0326** |
| **May-07** | **TURB_NTU** | **Decline** | **486.6** | **368.3** | **486.6** | **731.0** | **0.0307** |
| Sep-07 | TURB_NTU | | 814.8 | 188.3 | 754.5 | 830.3 | 0.0822 |
| **Dec-07** | **TURB_NTU** | **Decline** | **169.9** | **166.0** | **173.9** | **401.4** | **0.0197** |
| **Feb-08** | **TURB_NTU** | **Decline** | **385.4** | **297.5** | **385.4** | **642.1** | **0.0081** |
| **Jun-08** | **TURB_NTU** | **Decline** | **626.4** | **278.7** | **626.4** | **660.4** | **0.0054** |

**RESULTS AND INTERPRETATION, CONTINUED**

*Effects of TP on Diel Dissolved Oxygen*

Interannual differences in stream discharge strongly influenced the daily (diel) variation in dissolved oxygen, water temperature, and pH (Figure 19).

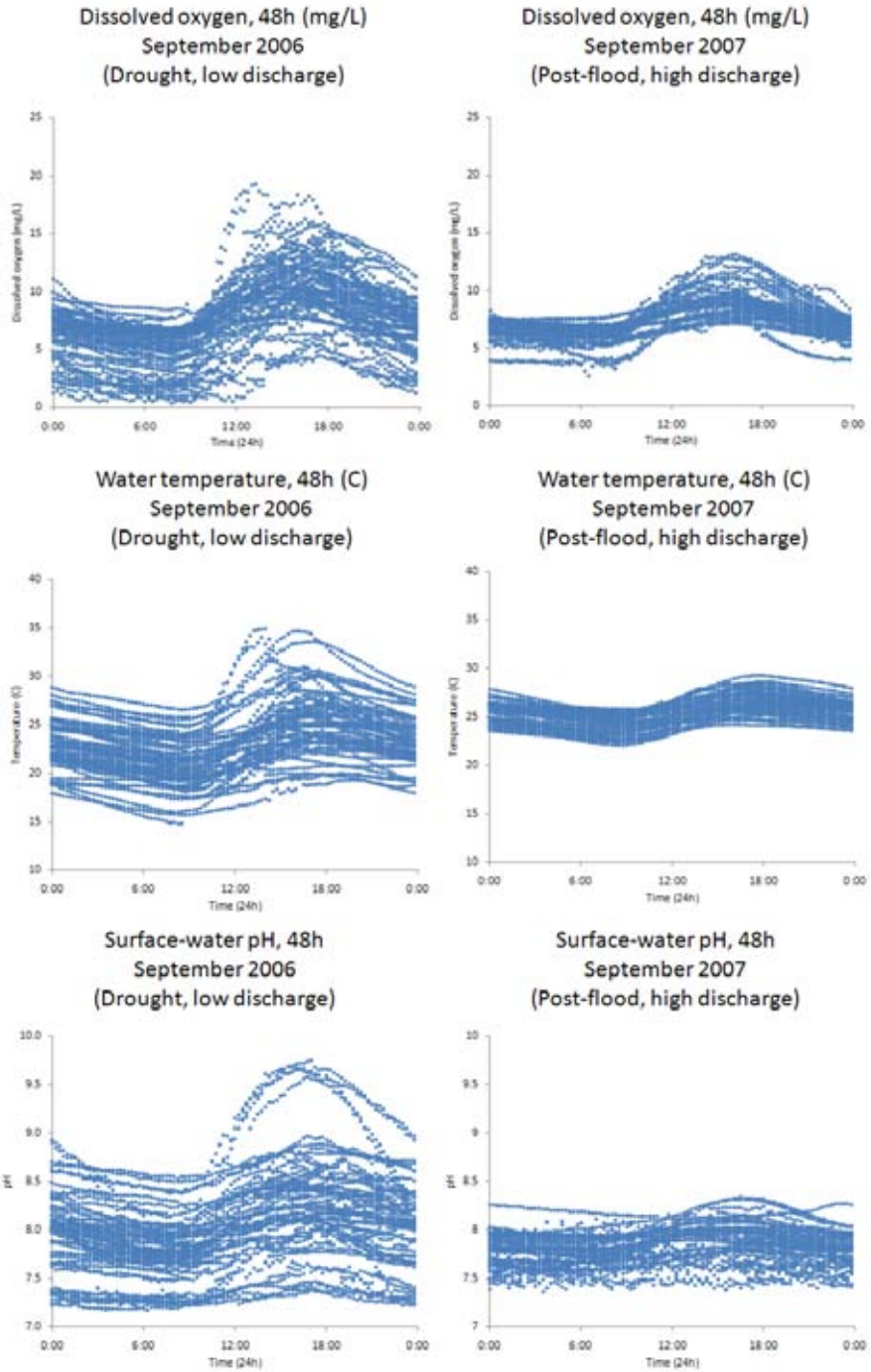



Figure 19. Patterns of dissolved oxygen, water temperature, and pH across 2-consecutive 24-hour periods at each of the 26 field study sites during a period of very low discharge (September 2006) and during a period of high flows following significant flooding (September 2007).

Figure 19 clearly illustrates the important influence of stream flow on diel water chemistry. In 2006, a drought year, most of the field-study streams had very low-to-no measureable discharge. This lack of flow limited the turbulent mixing of the water column. Without turbulence, gas exchange between air and water was limited to passive diffusion. During the day, this caused many of the streams to become supersaturated with dissolved oxygen (up to 20 mg/L DO; > 250% saturation) which concomitantly caused pH to increase to relatively high levels (8.5-9.5). At night, DO was rapidly consumed. Several streams had minimum DO levels near 0 mg/L, often for several hours (typically between 00:00-09:00, but in some cases until 12:00). Water temperature also varied widely at different hours of the day, reaching temperatures up to 30-35 C during late afternoon (Figure 19).

In September 2007, discharge was relatively high in all 26 study streams. Most of the streams were in flood stage for several of the preceding months, and had just recently subsided to wadeable flows. Even under these higher flows and turbulent mixing conditions, dissolved oxygen showed clear diel variation, peaking around 16:00 at concentrations up to 12 mg/L (120-150% saturation), and declining during the night to typically 6-8 mg/L DO. However, minimum DO levels were rarely below 5 mg/L, and no stream had minimum DO levels that would be considered detrimental to native fishes or macroinvertebrates in this region, especially in moving water.

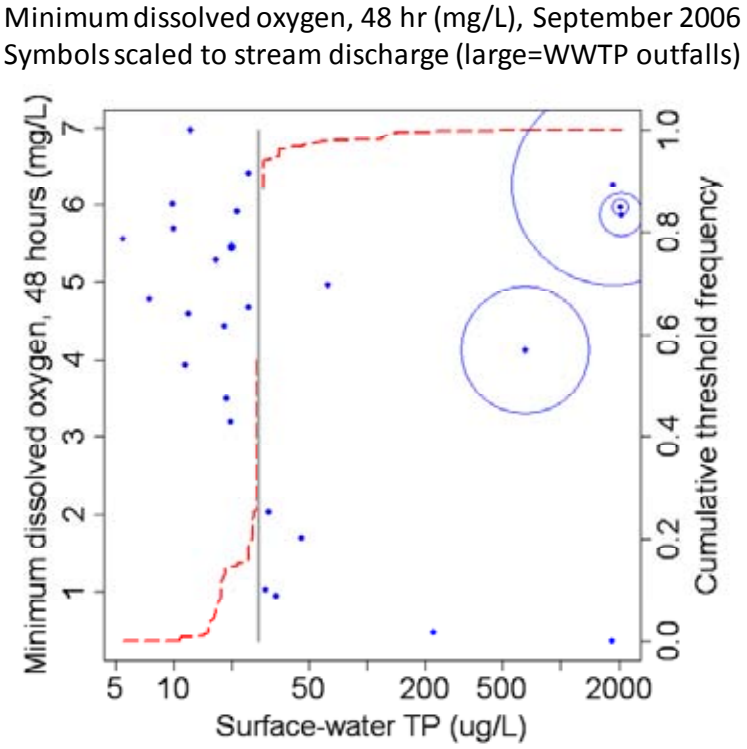



Figure 20. Results of nonparametric changepoint analysis using surface water TP (ug/L) as a predictor of threshold declines in minimum dissolved oxygen (DO, mg/L) during September 2006. The four points in the upper right corner of the plot correspond to streams immediately downstream of WWTP discharges; these were the only flowing streams during this sampling event.

The very low minimum DO values at several of the sites in 2006 were strongly related to surface-water TP, but also stream discharge (Figure 20). All 6 streams with no detectable flow and TP > 27.2 had minimum DO values < 2 mg/L, and two others with TP near 20 ug/L dropped below 3.5 mg/L DO. The four streams located immediately downstream of effluent discharges maintained relatively high minimum DO concentrations despite very high TP. This was related to reaeration associated with turbulent mixing. Despite these outliers, TP was nevertheless a significant predictor of threshold declines in minimum DO (Table 7).

The diel pattern of DO at NBOS-02 (North Bosque River - 02) is further evidence that WWTP discharge sites maintained relatively high minimum DO because of turbulent mixing associated with flow. NBOS-02 is located a few kilometers downstream of NBOS-01, one of the 4 WWTP discharge sites depicted in Figure 20. NBOS-02 had DO levels fall to near 0 mg/L the same night that NBOS-01 maintained DO above 5 mg/L. Because of the drought, the water table had dropped below the stream channel and discharge from NBOS-01 subsided to a slow trickle by the time it reached NBOS-02. Here, P enrichment stimulated photosynthesis during the day whereas microbial respiration, also fueled by P-rich organic matter, was even higher at night. Thus, the stream was essentially anoxic for several hours at night and early morning.

Minimum DO also corresponded to mean biofilm thickness, filamentous algal cover, and the ratio of periphyton chlorophyll to AFDM (Figure 21). As previously shown, thicker layers of calcareous periphyton comprised mostly of diatoms, cyanobacteria, and other microbes were associated with low-P streams, and as TP increased, these biofilms succeeded to filamentous and colonial greens and other types of algae. The results in Figure 21 explicitly link TP to sharp nonlinear responses of primary producers, which, in turn, cause sharp nonlinear declines in minimum DO. Thus, these metrics appear to not only be sensitive to TP enrichment, but also can be linked quantitatively to aquatic life use standards that rely on dissolved oxygen as a measure of biological integrity.

These flow-dependent results imply that studies on the effects of nutrients on DO will not adequately characterize risk to biota and associated aquatic life use designations without sampling during periods of low flow.

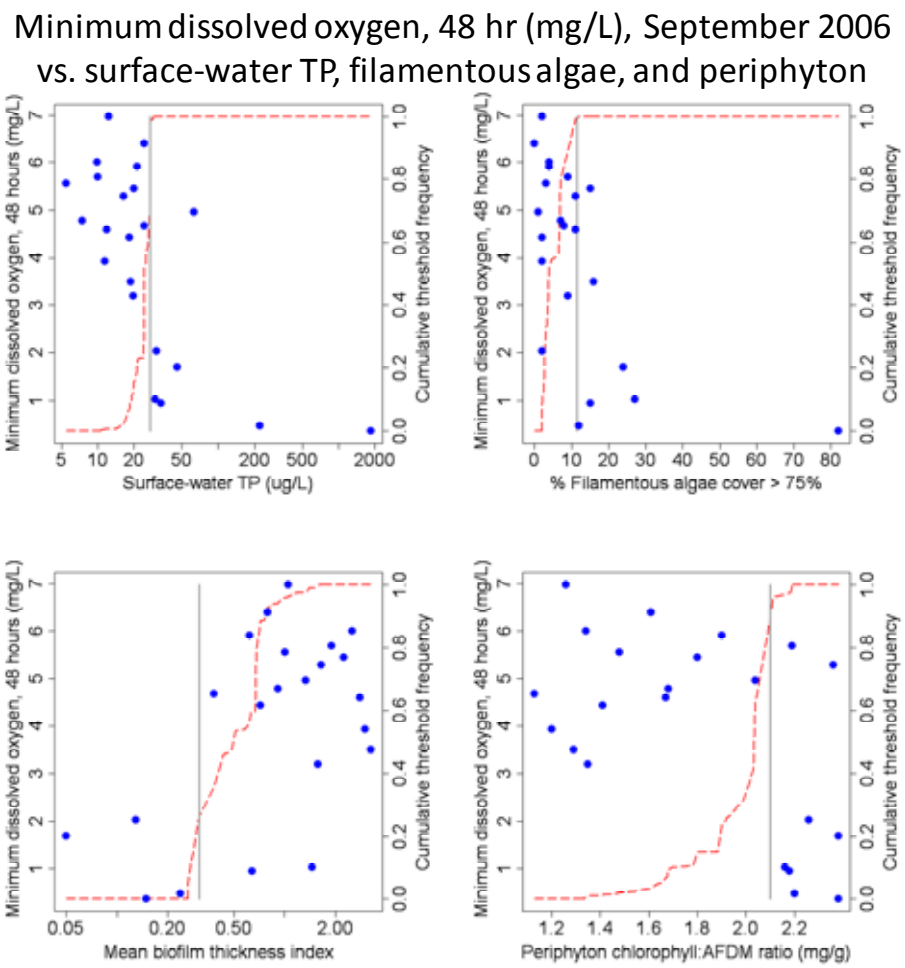


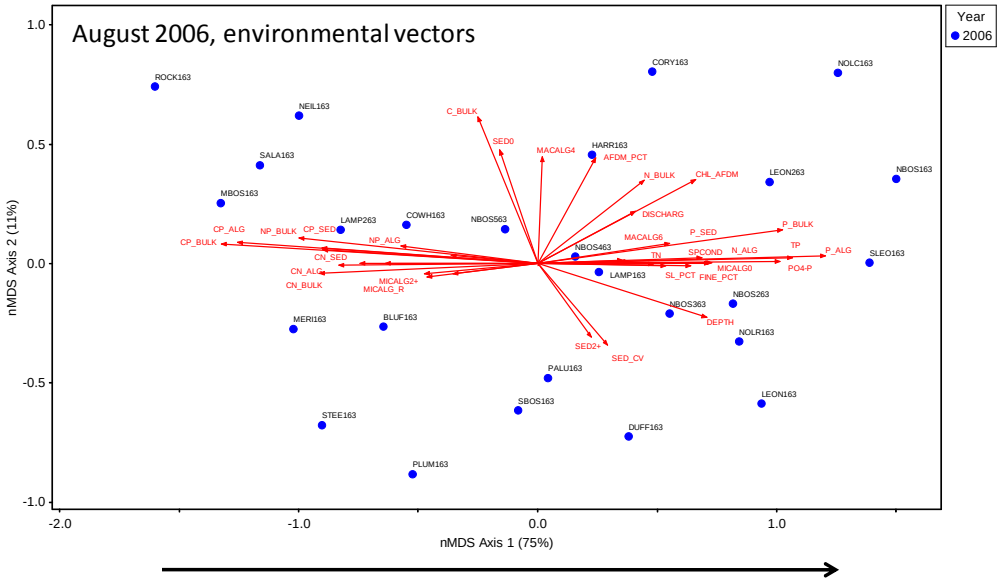

Figure 21. Results of nonparametric changepoint analysis using surface water TP (ug/L), biofilm thickness, filamentous algal cover, and periphyton chlorophyll-to-AFDM ratio as predictors of threshold declines in minimum dissolved oxygen (DO, mg/L) during September 2006. The four streams immediately downstream of WWTP discharges were excluded from the analysis because these were the only flowing streams during this sampling event.

Table 7. Results from nonparametric changepoint analysis using surface-water TP, periphyton C:P bulk, filamentous macroalgal cover > 75% (MACALG5+) and mean biofilm thickness (MICALG_R) as threshold predictors of **minimum dissolved oxygen** during 48 h among 26 stream sites in September 2006 and 2007. Significant thresholds in DO are highlighted in bold. *All 26 sites. **Four WWTP sites with high discharge and turbulent mixing removed from analysis. See Figures 20 and 21 for graphical display of results.

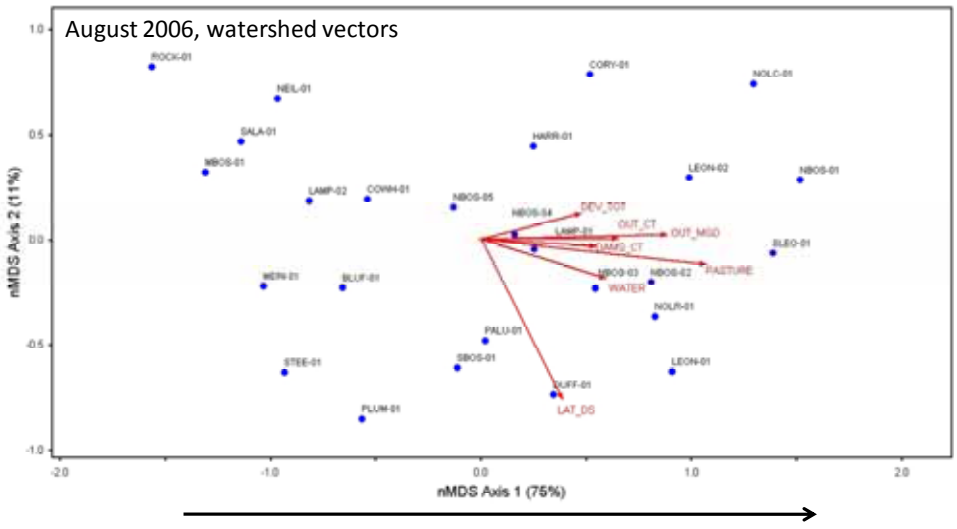
| | | | Cumulative Threshold Quantiles | | | | Mean DO 48 h min (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| | Predictor | Obs. | 10% | 50% | 90% | *P* value | Below | Above |
| September 2006 (drought, low flow) | | | | | | | | |
| | **TP*** | **27.2** | **17.6** | **27.2** | **29.2** | **0.0499** | **5.09** | **3.06** |
| | **TP**** | **27.2** | **19.4** | **24.5** | **27.2** | **0.0068** | **5.09** | **1.64** |
| | **CP_BULK**** | **299.4** | **299.4** | **321.9** | **698.8** | **0.0237** | **1.14** | **4.63** |
| | **MACALG5+**** | **11.5** | **2.5** | **4.0** | **11.0** | **0.0170** | **4.96** | **1.92** |
| | **MICALG_R**** | **0.31** | **0.31** | **0.51** | **0.775** | **0.0237** | **1.14** | **4.63** |
| September 2007 (post-flood, high flow) | | | | | | | | |
| | TP | 12.9 | 11.6 | 12.9 | 47.3 | 0.5489 | 5.59 | 6.13 |
| | CP_BULK | 143.4 | 143.4 | 517.8 | 885.9 | 0.1694 | 5.25 | 6.05 |

## RESULTS AND INTERPRETATION, CONTINUED

*Periphyton Taxonomic Responses to TP: Ordinations*

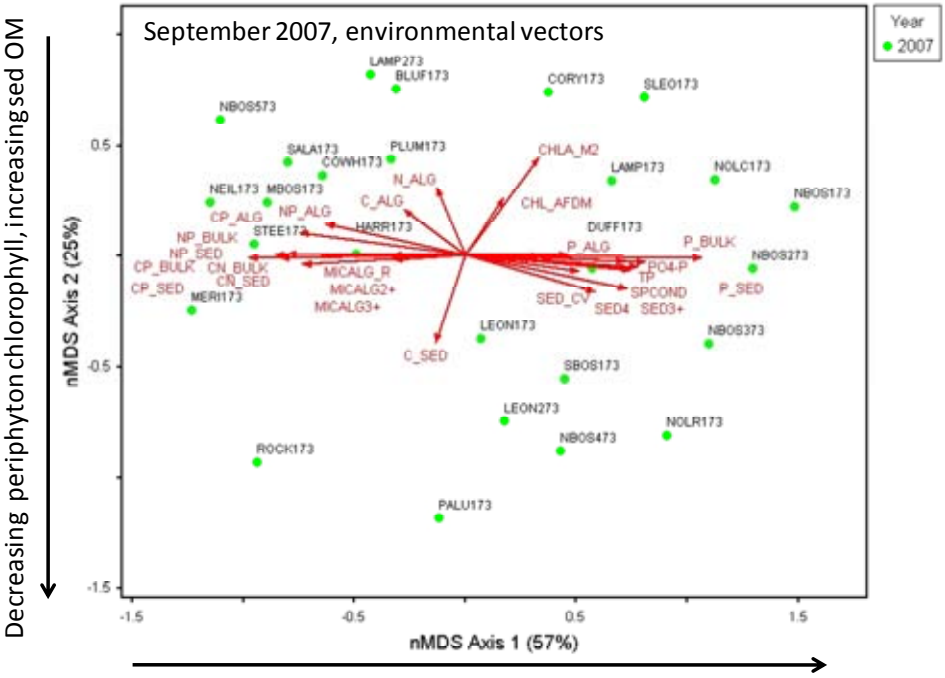



Increasing P enrichment, sedimentation, chlorophyll, filamentous macroalgae
Decreasing periphyton C:P, C:N ratios, biofilm thickness




Increasing WWTP outfall discharge, pasture, reservoirs, dams, and urban land

Figure 22. Nonmetric multidimensional scaling ordination of algal species composition among the 26 field study sites in August 2006. Abundance data (no. of cells/cm$^2$) was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and algal species composition. The upper panel shows the significant environmental predictors of algal species: nutrients (e.g., TP, periphyton C:P), sediment, biofilm thickness, and filamentous algae. The lower panel shows the significant watershed predictors of algae species: pasture, outfalls, and urban development in watersheds. See Table 1 and 2 for full variable names.



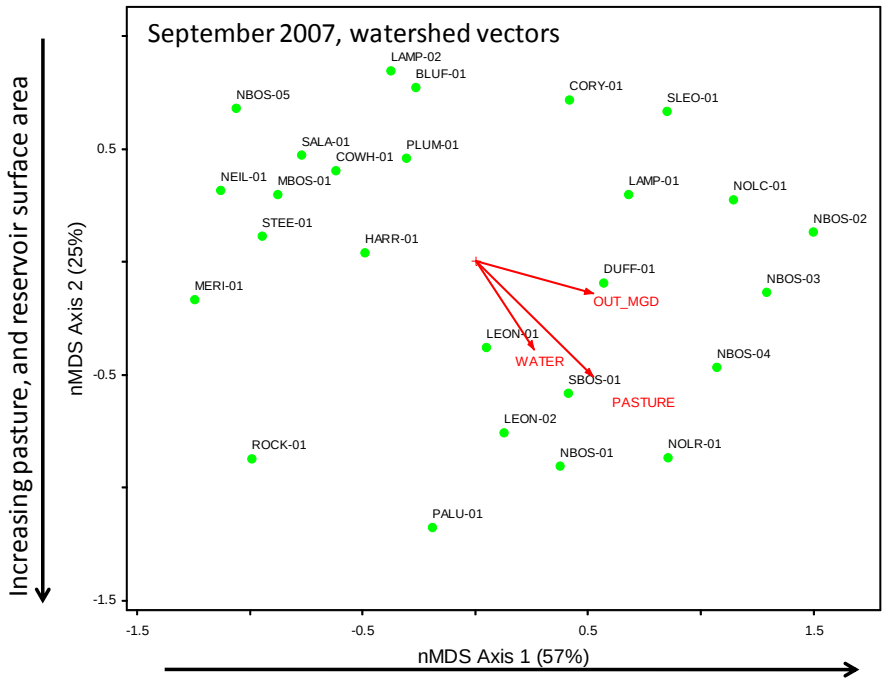




Figure 23. Nonmetric multidimensional scaling ordination of algal species composition among the 26 field study sites in September 2007. Most of the environmental and watershed variables that were strong predictors of algal species composition in 2006 were also significant predictors in these ordinations. See Figure 22 for details.

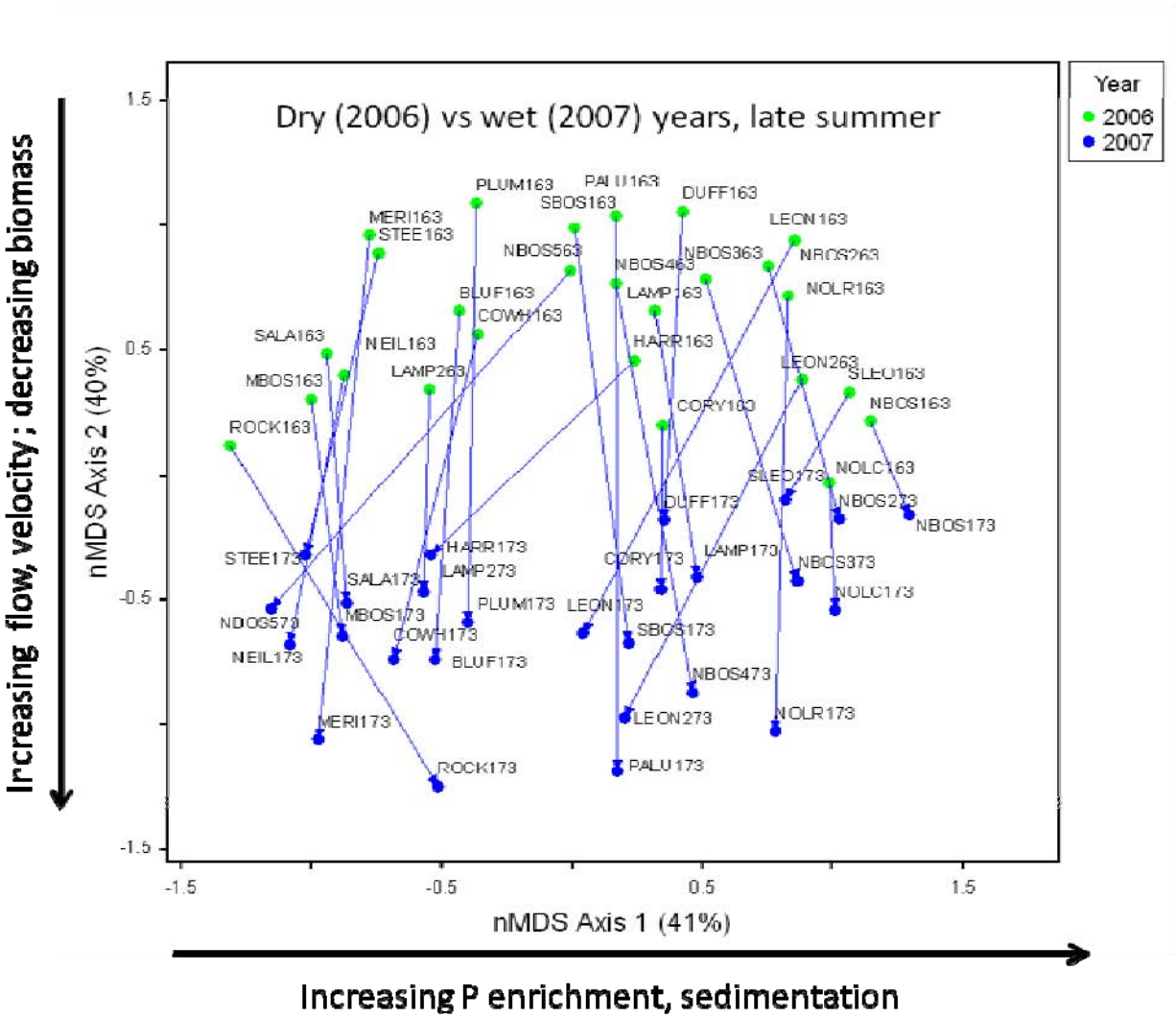



Figure 24. Nonmetric multidimensional scaling ordination of algal species composition among the 26 field study sites in August 2006 and September 2007. The arrows indicate directions and magnitude of change in algal species composition between years at each of the 26 sites.

The multivariate analyses of algal species composition during August 2006 and September 2007 continued to support the earlier conclusions that TP and related metrics had a strong influence on stream biota (Figures 22-24). In both years, most of the variance in algal species composition was related to TP. Sites with the lowest TP and highest periphyton C:P ratios were placed at the low end of axis 1 whereas sites with the highest TP and lowest C:P ratios were at the opposite end of axis 1. Figure 24 is particularly interesting because it showed that although algal species changed between years (illustrated by the shift in sites from 2006 to 2007 along axis 2), the relative order of sites from low P to high P remained intact on axis 1. This is compelling evidence that algal species composition was altered by nutrient enrichment, and the magnitude of its effect was similar regardless of interannual variation in stream discharge.

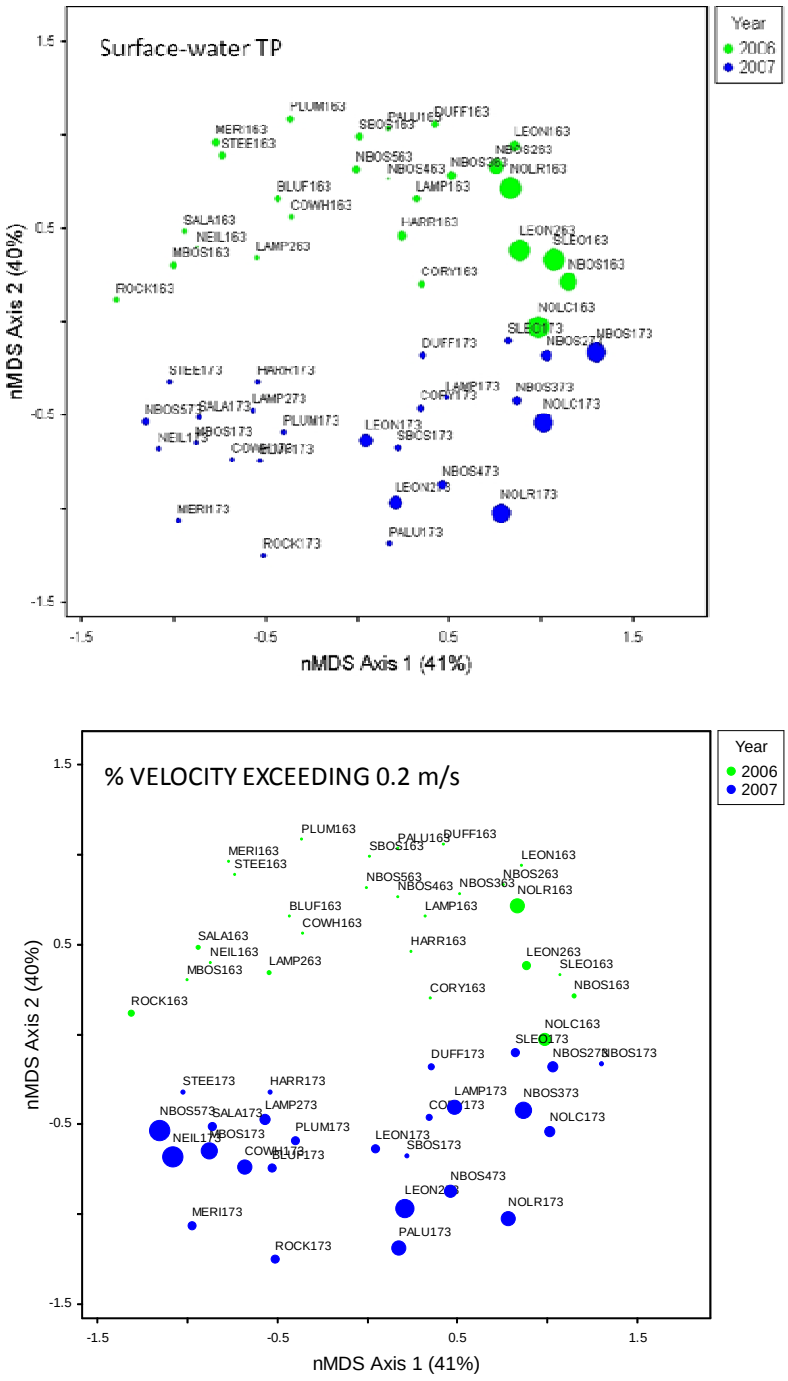



Figure 25. Nonmetric multidimensional scaling ordination of algal species composition among the 26 field study sites in August 2006 and September 2007. The ordination diagram is identical to Figure 24, except that site symbols are scaled in proportion to surface-water TP (upper panel) and the proportion of the reach with velocity > 0.2 m/s (lower panel).

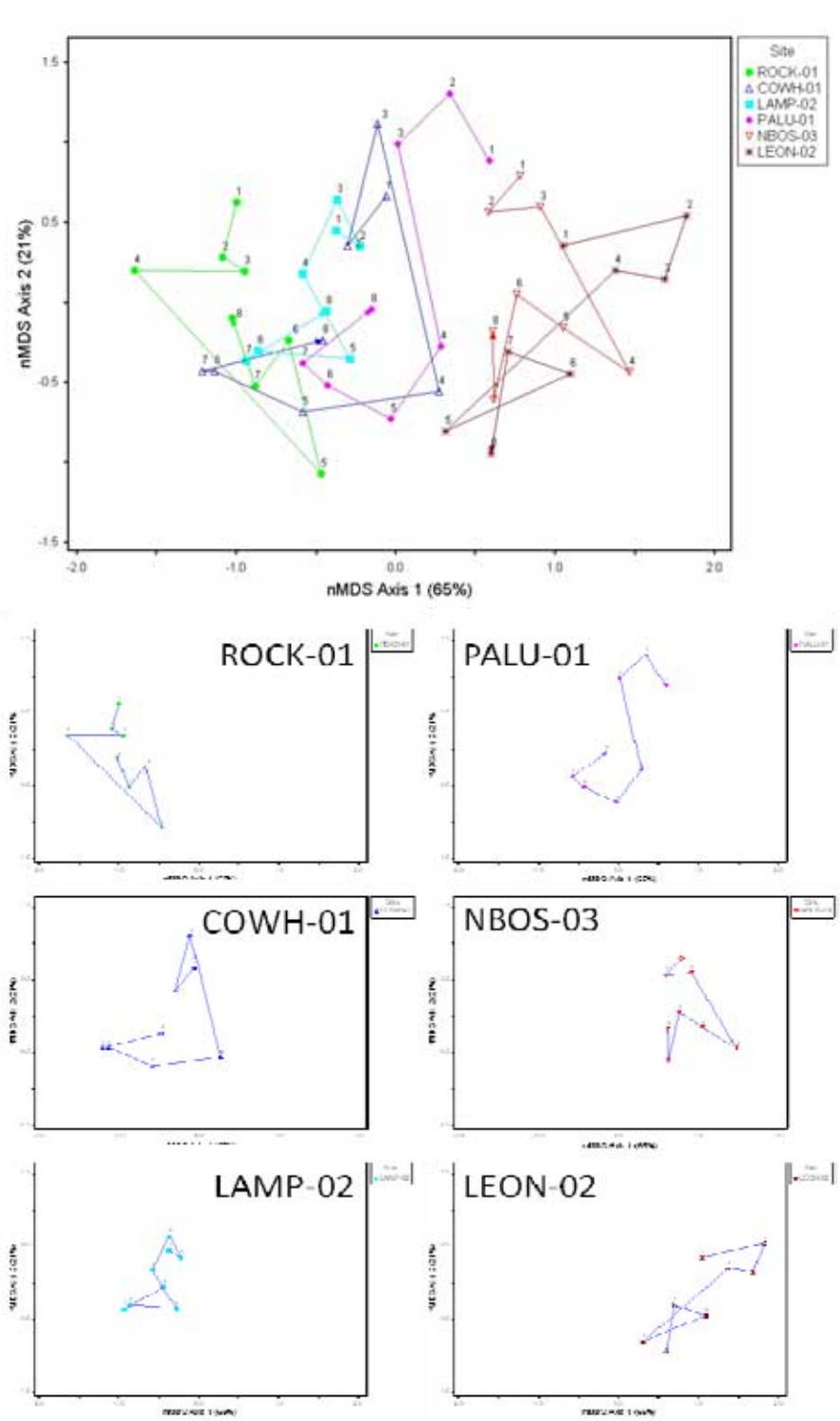



Figure 29. Nonmetric multidimensional scaling ordination of algal species composition over the 8 quarterly sampling events at the 6 intensive sampling sites (see Figure 1). Lines connect dates within each site in chronological order, starting with August 2006 (1) and ending with June 2008 (8). For clarity, patterns of community succession are shown for each site separately in the lower 6 panels.

As shown previously in the ordination of sites between years (2006 vs. 2007), algal species composition varied significantly over time (Figure 29). The 6 sites selected for species analysis on all 8 quarterly sampling events ("intensive sites") followed the same general trajectory of change from along nMDS Axis 2, shifting down axis 2 during the high flow periods of May and September 2007, but turning around and moving back up axis 2 toward their original positions in August 2006.

Importantly, each site stayed in the same approximate position along axis 1 (the nutrient axis) over time. ROCK-01, COWH-01, and LAMP-02 overlapped somewhat in their trajectories, but each of these sites was consistently below the observed TP thresholds reported in the preceding results. Thus, their similarity is to be expected.

PALU-01 remained consistently intermediate along Axis 1, distinctly separated from the low-P sites during the first few sampling events, but overlapping with COWH-01 near the end of the study. NBOS-03 and LEON-02, both highly enriched sites, consistently stayed on the right end of axis 1. NBOS-03 had high TP levels for most of the study (~100-200 ug/L). In 2006, LEON-02 had very high levels of TP (> 2 mg/L), but TP declined to ~100 ug/L in the latter half of the study (Figure 6). The decline in TP was also coincident with a slight shift to the left in algal species composition, possibly indicating a decline in the few taxa that were shown to proliferate mostly at the levels of TP > 200 ug/L (Tables 10-13).

**RESULTS AND INTERPRETATION, CONTINUED**

*Periphyton Taxonomic Responses to TP: Threshold Indicator Taxa Analysis (TITAN)*

TITAN analyses on algal species composition in 2006 and 2007 continued to add evidence to an already substantial case for a threshold at low levels of TP. Dozens of algal species essentially disappeared from streams between 12 and 30 ug/L TP, with the threshold of greatest overall decline (sum(z-)) at 19.2 and 21.6 ug/L in 2006 and 2007, respectively (Figure 26, Table 8). Simultaneously, numerous algal taxa showed sharp increases, either replacing taxa as they declined, or driving the declines via competitive exclusion (e.g., shading by *Cladophora*=CLAglome, a significant increasing taxon in both years; Table 10).

Although most taxa declined or increased at relatively low levels of TP, a few taxa responded only to much higher levels of TP (Figure 26, Table 10). These taxa were mostly associated with sites heavily influenced by effluent.

Fewer taxa declined in response to TP in 2007 than 2006, but as many or more increased in response to TP. Because of the protracted period of flooding prior to September 2007, many of the streams were in a period of rapid recolonization (community succession). Scouring of rocks reduced periphyton biomass substantially compared to 2006. It is likely that part of the explanation was that fast-responding "weedy" algal taxa proliferated with elevated TP, whereas slower responding, low-P indicator taxa had not become as consistently established across all streams as in 2006.

Another explanation for differences between 2006 and 2007 was that surface-water TP was not as strong of an indicator of P-enrichment status during higher and more variable flows. There is some support for this hypothesis given that more threshold indicator taxa were deemed significant in 2007 when using periphyton C:P ratios (bulk or OM) than TP (Table 9, 11; Figure 27, 28). This implies that exposure and availability (via recycling, sediment-bound P, etc) at these sites was better indicated by the P in the periphyton than in an instantaneous measurement from the water.

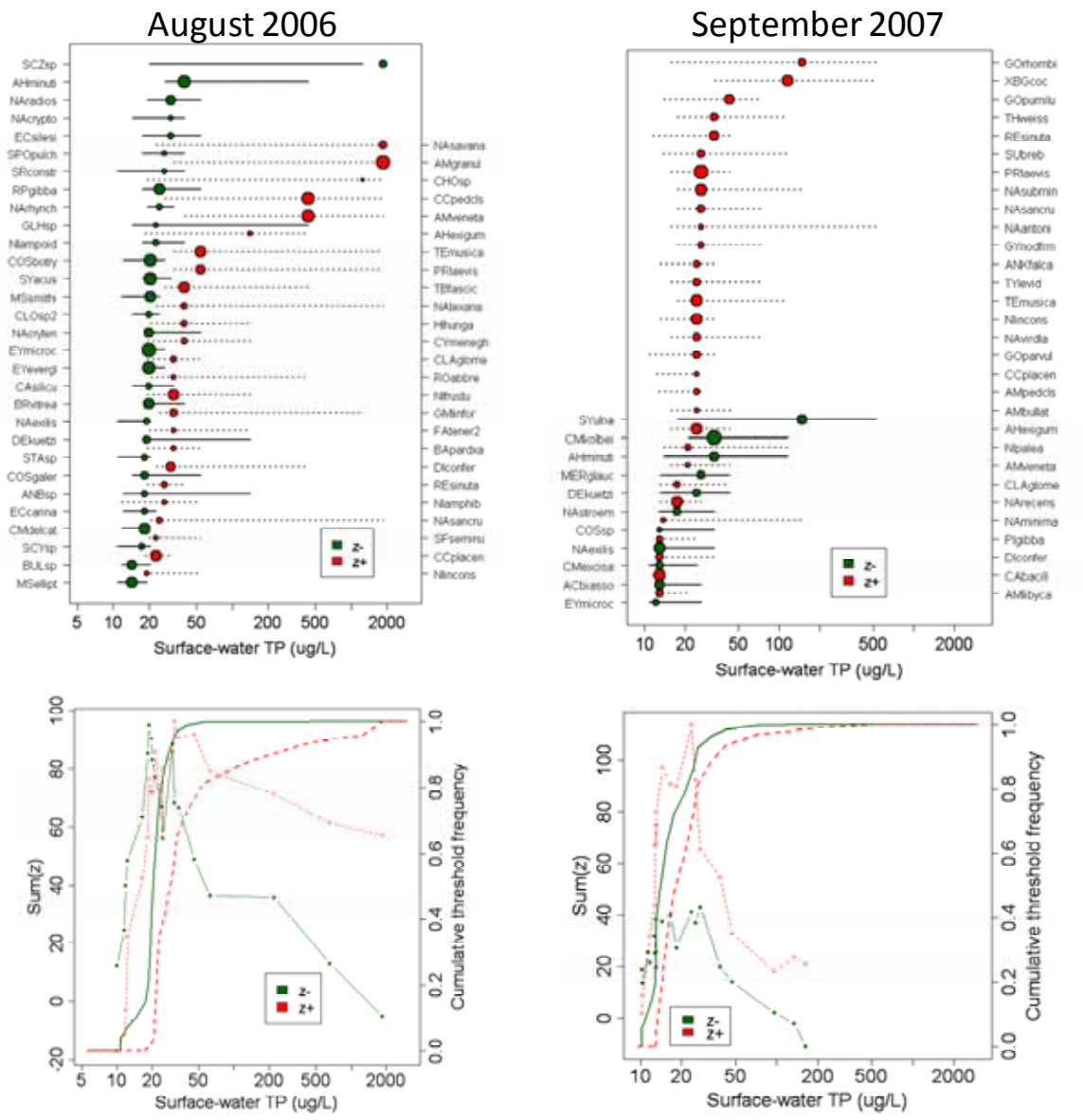



Figure 26. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TP a a predictor of threshold changes in individual algal species during August 2006 and September 2007. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to TP. The upper panels show the observed TP threshold value (colored symbols) for each taxon deemed to change significantly. Taxon IDs (see Appendix 1) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their TP thresholds. Line segments around each symbol are 90% confidence intervals around the TP threshold. The lower two panels show the aggregate response of negative (sum(z-)) and positive (sum(z+)) threshold indicator taxa. The TP value resulting in the highest sum(z) value is the point in which the greatest cumulative negative (z-) or positive (z+) occurs. Bootstrapping is used to estimate the cumulative threshold frequency for negative (green) and positive (red) responses, respectively. See Tables 8 and 9 for community level (sum(z)) thresholds, and Tables 10 and 12 for taxa-specific thresholds.

Table 8. Community-level results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to water and periphyton nutrient concentrations during **August 2006** (drought, n=26 sites). Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient conditions are shown in **bold.** The lower (*5%*, *10%*), middle (*50%*), and upper (*90%*, *95%*) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold.**Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that "decrease" sharply in response to increasing C:P are associated with higher levels of P-enrichment,, whereas "increaser" taxa are associated with lower levels of P enrichment.*. Figures 26-28 for details.

| August 2006 (n=26 streams) | | | Bootstrap Cumulative Distribution of Thresholds | | | | |
|---|---|---|---|---|---|---|---|
| Predictor | Obs. | Response > Obs Threshold Value | 5% | 10% | 50% | 90% | 95% |
| Surface-water TP (ug/L) | **19.2** | **Decline (z-)** | **11.7** | **17.1** | **20.6** | **30.4** | **32.4** |
| | 32.4 | Increase (z+) | 22.8 | 22.8 | 30.4 | 437.2 | 1250.2 |
| Surface-water PO4-P (ug/L) | **4.4** | **Decline (z-)** | **4.4** | **4.4** | **5.1** | **14.3** | **16.2** |
| | 30.8 | Increase (z+) | 9.6 | 9.6 | 14.3 | 1181.8 | 1808.3 |
| Periphyton C:P ratio (OM) | 369 | Decline *(z-) | 313 | 338 | 461 | 500 | 515 |
| | **628** | **Increase* (z+)** | **445** | **461** | **563** | **833** | **1017** |
| Periphyton C:P ratio (bulk) | 465 | Decline* (z-) | 284 | 306 | 546 | 711 | 725 |
| | **780** | **Increase* (z+)** | **659** | **659** | **729** | **925** | **926** |
| Surface-water DIN (ug/L) | **56.9** | **Decline (z-)** | **28.1** | **28.1** | **54.4** | **64.9** | **85.3** |
| | 85.3 | Increase (z+) | 56.9 | 56.9 | 834.3 | 7411.5 | 7411.5 |
| Surface-water TN (ug/L) | **280.6** | **Decline (z-)** | **222.6** | **222.6** | **280.6** | **663.0** | **1162.5** |
| | 1162.5 | Increase (z+) | 613.0 | 663.0 | 1852.5 | 8510.0 | 8510.0 |

Table 9. Community-level results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to water and periphyton nutrient concentrations during **September 2007** (post-flood, n=26 sites). Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient conditions are shown in **bold.** See Table 8 and Figures 26-28 for details.

| September 2007 (n=26 streams) | | | Bootstrap Cumulative Distribution of Thresholds | | | | |
|---|---|---|---|---|---|---|---|
| Predictor | Obs. | Response > Obs. Threshold Value | 5% | 10% | 50% | 90% | 95% |
| Surface-water TP (ug/L) | **21.6** | **Decline (z-)** | **10.3** | **11.6** | **15.6** | **26.5** | **33.0** |
| | 24.5 | Increase (z+) | 13.1 | 13.8 | 21.1 | 42.8 | 71.9 |
| Surface-water PO4-P (ug/L) | **13.5** | **Decline (z-)** | **10.0** | **10.9** | **13.5** | **14.8** | **17.3** |
| | 13.5 | Increase (z+) | 12.1 | 13.2 | 14.3 | 28.4 | 45.1 |
| Periphyton C:P ratio (OM) | 421 | Decline *(z-) | 279 | 283 | 379 | 457 | 485 |
| | **737** | **Increase* (z+)** | **356** | **379** | **612** | **973** | **1187** |
| Periphyton C:P ratio (bulk) | 775 | Decline* (z-) | 182 | 235 | 749 | 815 | 844 |
| | **775** | **Increase* (z+)** | **362** | **749** | **844** | **1328** | **1328** |
| Surface-water DIN (ug/L) | **189.4** | **Decline (z-)** | **159.3** | **159.3** | **189.4** | **446.5** | **446.5** |
| | 1827.1 | Increase (z+) | 446.5 | 446.5 | 1827.1 | 3981.5 | 3981.5 |
| Surface-water TN (ug/L) | **435.5** | **Decline (z-)** | **362.0** | **362.0** | **423.0** | **753.0** | **753.0** |
| | 1720.0 | Increase (z+) | 753.0 | 753.0 | 2080.0 | 4080.0 | 4080.0 |

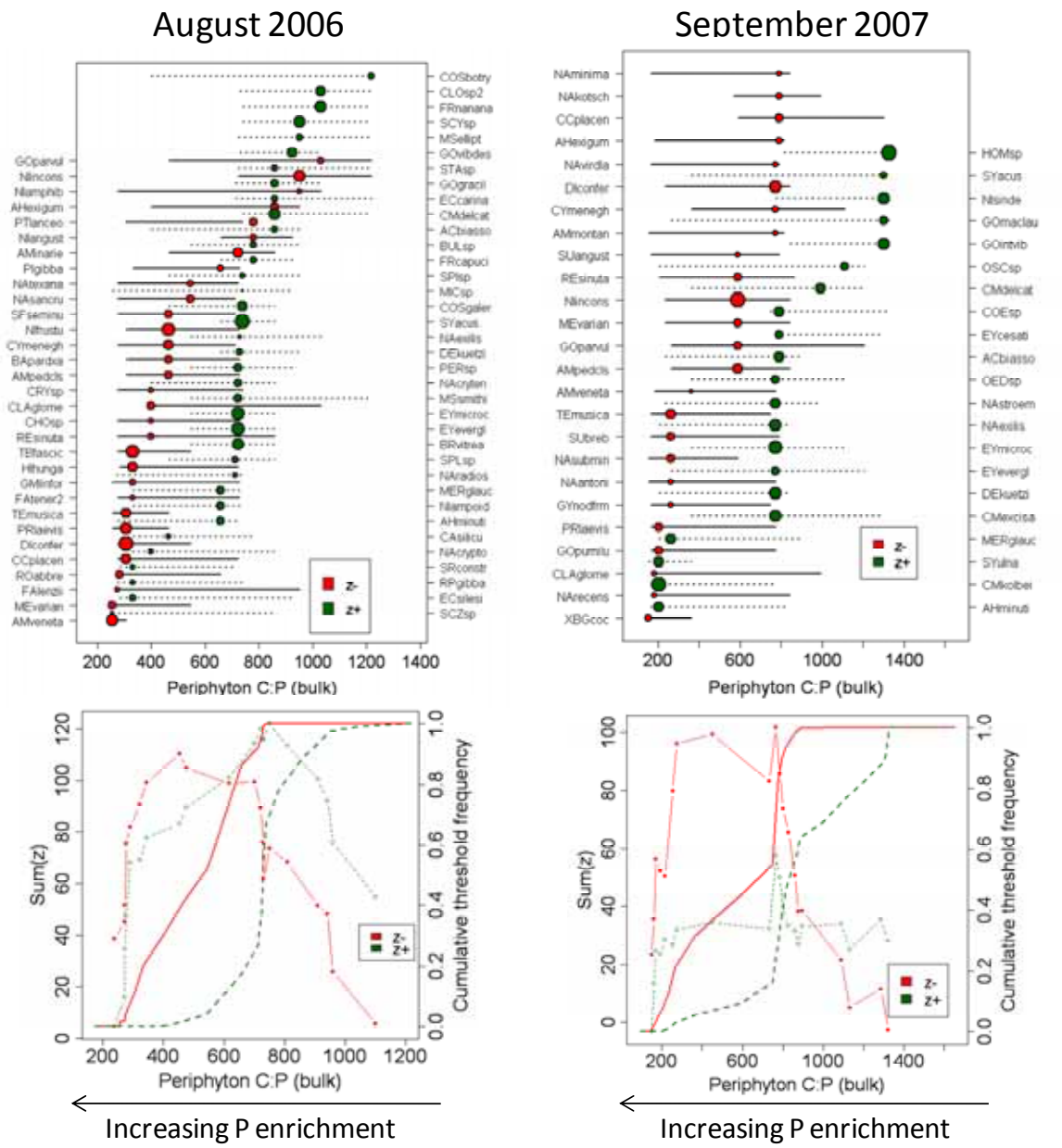



Figure 27. Results of Threshold Indicator Taxa ANalysis (TITAN) using periphyton C:P ratio (bulk) as a predictor of threshold changes in individual algal species during August 2006 and September 2007. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to TP. *Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that are negative indicators (z-) in these results are associated with higher levels of P-enrichment,, whereas positive threshold taxa are associated with lower levels of P enrichment (high C:P ratios).* See Figure 26 for other graphical details. See Tables 8 and 9 for community level (sum(z)) thresholds, and Tables 11 and 13 for taxa-specific thresholds.

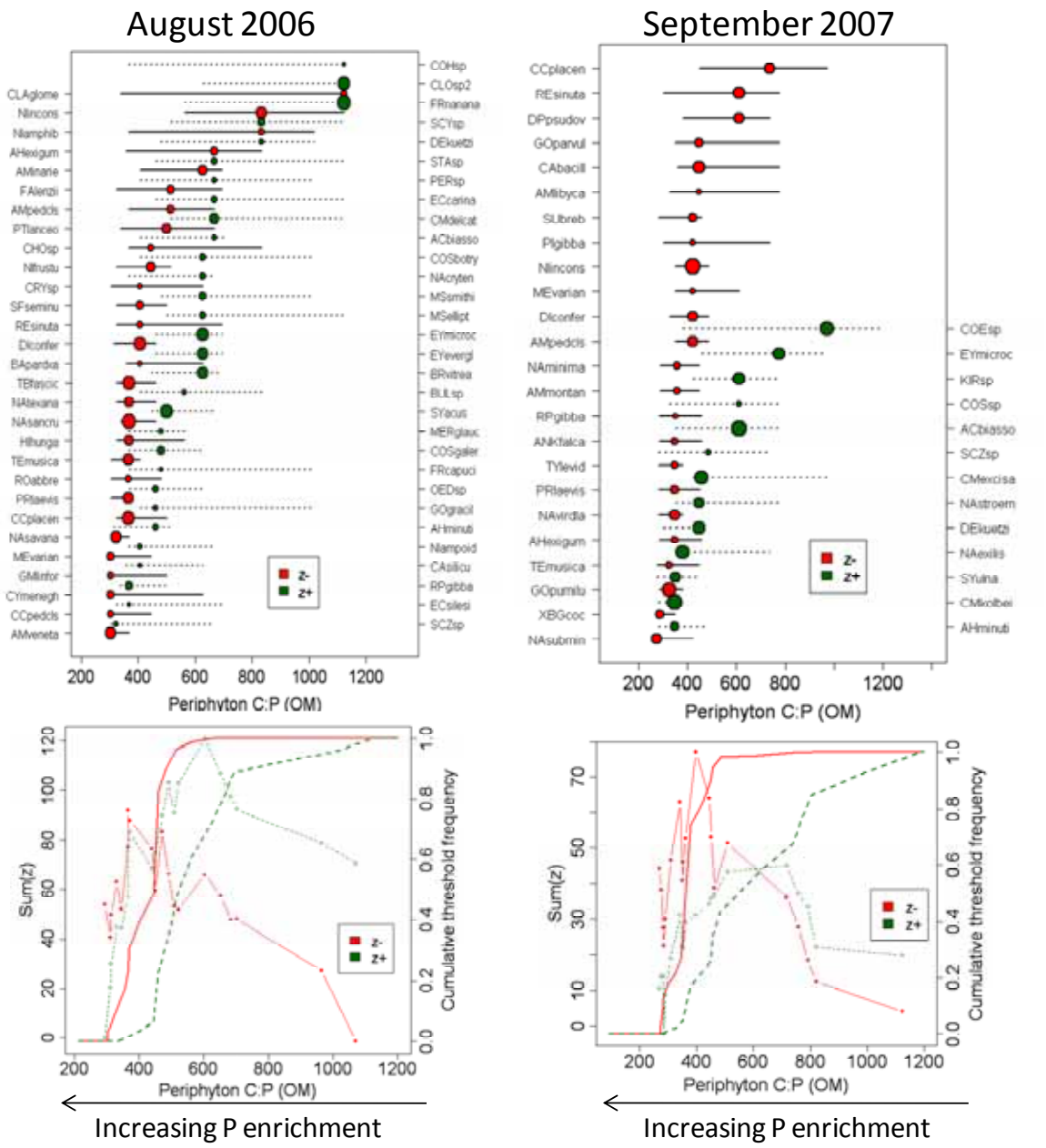



Figure 28. Results of Threshold Indicator Taxa ANalysis (TITAN) using periphyton C:P ratio (organic fraction, sediment removed) as a predictor of threshold changes in individual algal species during August 2006 and September 2007. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to TP. *Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that are negative indicators (z-) in these results are associated with higher levels of P-enrichment,, whereas positive threshold taxa are associated with lower levels of P enrichment (high C:P ratios).* See Figure 26 for other graphical details. See Tables 8 and 9 for community level (sum(z)) thresholds, and Tables 11 and 13 for taxa-specific thresholds.

Table 10. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to **surface-water total phosphorus (TP, ug/L)** in **August 2006** (n=26 sites). Only species that showed significant threshold declines or increases to **surface-water (TP)** are included in this table. The observed (*Obs*) threshold value of TP for each taxon is shown in bold, whereas lower (*10%*), middle (*50%*), and upper (*90%*) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. *Z* represents the standardized indicator score from TITAN (larger numbers = stronger threshold response), *IndVal* is the unstandardized indicator score (scaled from 0-100%, with 100=perfect indicator). *Purity* is the relative consistency of the response direction among the 1,000 bootstraps (*purity* > 0.95 is significant). *P-value* is the likelihood of getting an equal or larger IndVal if the score were computed with random shuffling of the observed data (*P*<0.05 is significant). See Appendix 1 for full species names corresponding to *Taxon IDs*.

| | | | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon ID | **Obs.** | Response > obs. value | *z* | IndVal | Purity | *P* value | 10% | 50% | 90% |
| AHminuti | **39.7** | Decline | 5.97 | 87.76 | 0.994 | <0.004 | 27.2 | 39.7 | 437.2 |
| BRvitrea | **19.9** | Decline | 5.37 | 70.39 | 0.998 | <0.004 | 18.5 | 22.8 | 39.7 |
| CAsilicu | **19.9** | Decline | 2.93 | 39.70 | 0.960 | 0.044 | 14.4 | 20.6 | 32.4 |
| CMdelcat | **18.5** | Decline | 5.41 | 55.56 | 0.998 | 0.008 | 11.7 | 17.4 | 19.9 |
| DEkuetzi | **19.2** | Decline | 3.95 | 68.49 | 0.998 | 0.012 | 17.4 | 20.6 | 141.7 |
| ECcarina | **18.5** | Decline | 3.01 | 33.33 | 0.958 | 0.040 | 12.1 | 18.5 | 22.8 |
| ECsilesi | **30.4** | Decline | 3.40 | 60.53 | 0.982 | 0.012 | 17.4 | 27.2 | 54.2 |
| EYevergl | **19.9** | Decline | 5.94 | 75.95 | 1.000 | <0.004 | 17.4 | 20.6 | 27.2 |
| EYmicroc | **19.9** | Decline | 6.22 | 77.91 | 1.000 | <0.004 | 17.4 | 20.6 | 27.2 |
| MSellipt | **14.4** | Decline | 5.02 | 57.14 | 0.982 | <0.004 | 10.8 | 14.4 | 19.2 |
| MSsmithi | **20.6** | Decline | 5.09 | 50.00 | 0.998 | <0.004 | 11.7 | 19.9 | 24.4 |
| NAcrypto | **30.4** | Decline | 2.69 | 43.75 | 0.982 | 0.028 | 14.4 | 22.8 | 39.7 |
| NAcryten | **19.9** | Decline | 4.35 | 65.68 | 1.000 | 0.008 | 19.2 | 27.2 | 54.2 |
| NAexilis | **19.2** | Decline | 3.05 | 30.00 | 0.962 | 0.024 | 10.8 | 14.4 | 20.6 |
| NAradios | **30.4** | Decline | 4.54 | 67.68 | 0.984 | <0.004 | 19.2 | 27.2 | 54.2 |
| NArhynch | **24.4** | Decline | 2.92 | 42.86 | 0.962 | 0.012 | 19.2 | 24.4 | 32.4 |
| NIampoid | **22.8** | Decline | 3.42 | 48.77 | 0.990 | 0.016 | 17.4 | 22.8 | 39.7 |
| RPgibba | **24.4** | Decline | 4.97 | 71.87 | 1.000 | <0.004 | 17.4 | 27.2 | 54.2 |
| SRconstr | **27.2** | Decline | 2.87 | 47.38 | 0.994 | 0.028 | 10.8 | 19.9 | 39.7 |
| SYacus | **20.6** | Decline | 5.60 | 75.66 | 1.000 | <0.004 | 17.4 | 20.6 | 30.4 |
| ANBsp | **18.5** | Decline | 3.13 | 57.14 | 0.984 | 0.012 | 12.1 | 19.2 | 141.7 |
| BULsp | **14.4** | Decline | 4.30 | 62.60 | 0.978 | <0.004 | 11.7 | 17.4 | 20.6 |
| CLOsp2 | **19.9** | Decline | 3.03 | 36.36 | 0.974 | 0.040 | 14.4 | 19.9 | 24.4 |
| COSbotry | **20.6** | Decline | 5.73 | 66.67 | 1.000 | <0.004 | 12.1 | 19.2 | 27.2 |
| COSgaler | **18.5** | Decline | 4.01 | 65.53 | 0.988 | 0.008 | 14.4 | 19.2 | 54.2 |
| GLHsp | **22.8** | Decline | 2.38 | 57.28 | 0.964 | 0.024 | 14.4 | 22.8 | 437.2 |
| SCYsp | **17.4** | Decline | 3.24 | 37.50 | 0.954 | 0.036 | 10.8 | 17.4 | 20.6 |
| SCZsp | **1853.3** | Decline | 3.71 | 53.86 | 0.996 | <0.004 | 19.9 | 32.4 | 1250.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SPOpulch | **27.2** | Decline | 2.77 | 40.00 | 0.956 | 0.020 | 17.4 | 24.4 | 39.7 |
| STAsp | **18.5** | Decline | 3.30 | 33.33 | 0.962 | 0.024 | 10.8 | 17.4 | 20.6 |
| | | | | | | | | | |
| AHexigum | **141.7** | Increase | 3.40 | 72.59 | 0.994 | 0.012 | 18.5 | 32.4 | 437.2 |
| AMgranul | **1853.3** | Increase | 8.83 | 92.88 | 0.964 | <0.004 | 32.4 | 1250.2 | 1853.3 |
| AMveneta | **437.2** | Increase | 8.45 | 80.00 | 0.992 | <0.004 | 39.7 | 437.2 | 1853.3 |
| BApardxa | **32.4** | Increase | 4.08 | 58.27 | 0.998 | 0.012 | 19.2 | 27.2 | 54.2 |
| CCpedcls | **437.2** | Increase | 8.34 | 60.00 | 0.954 | <0.004 | 27.2 | 437.2 | 1853.3 |
| CCplacen | **22.8** | Increase | 7.53 | 83.85 | 1.000 | <0.004 | 18.5 | 22.8 | 30.4 |
| CYmenegh | **39.7** | Increase | 4.80 | 73.39 | 1.000 | <0.004 | 19.2 | 30.4 | 141.7 |
| DIconfer | **30.4** | Increase | 6.32 | 82.53 | 1.000 | <0.004 | 22.8 | 32.4 | 437.2 |
| FAtener2 | **32.4** | Increase | 4.01 | 49.79 | 0.992 | <0.004 | 19.9 | 32.4 | 141.7 |
| GMlinfor | **32.4** | Increase | 5.21 | 62.98 | 0.974 | <0.004 | 24.4 | 39.7 | 1250.2 |
| HIhunga | **39.7** | Increase | 4.83 | 71.10 | 0.982 | <0.004 | 20.6 | 30.4 | 141.7 |
| NAsancru | **24.4** | Increase | 4.32 | 50.00 | 1.000 | <0.004 | 20.6 | 32.4 | 1853.3 |
| NAsavana | **1853.3** | Increase | 5.80 | 62.68 | 0.958 | 0.008 | 22.8 | 1250.2 | 1853.3 |
| NAtexana | **39.7** | Increase | 4.23 | 44.83 | 0.998 | 0.008 | 22.8 | 36.1 | 1853.3 |
| NIamphib | **27.2** | Increase | 4.05 | 54.51 | 1.000 | <0.004 | 11.7 | 19.2 | 54.2 |
| NIfrustu | **32.4** | Increase | 7.08 | 87.20 | 1.000 | <0.004 | 19.2 | 30.4 | 141.7 |
| NIincons | **19.2** | Increase | 3.55 | 68.12 | 0.998 | <0.004 | 18.5 | 24.4 | 54.2 |
| PRlaevis | **54.2** | Increase | 6.75 | 71.43 | 0.998 | <0.004 | 32.4 | 141.7 | 1853.3 |
| REsinuta | **27.2** | Increase | 4.63 | 66.23 | 0.966 | <0.004 | 19.2 | 27.2 | 41.2 |
| ROabbre | **32.4** | Increase | 3.73 | 40.42 | 0.990 | 0.008 | 20.6 | 32.4 | 437.2 |
| SFseminu | **22.8** | Increase | 3.72 | 46.15 | 0.994 | 0.020 | 19.9 | 27.2 | 54.2 |
| TBfascic | **39.7** | Increase | 7.63 | 82.70 | 0.992 | <0.004 | 27.2 | 39.7 | 437.2 |
| TEmusica | **54.2** | Increase | 6.98 | 71.43 | 0.994 | <0.004 | 32.4 | 141.7 | 1853.3 |
| CHOsp | **1250.2** | Increase | 2.57 | 53.57 | 0.976 | 0.016 | 19.2 | 32.4 | 1853.3 |
| CLAglome | **32.4** | Increase | 4.34 | 67.04 | 0.986 | <0.004 | 19.2 | 30.4 | 54.2 |

Table 11. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to **C:P ratio in the periphyton** (bulk, no separation from sediment) in **August 2006** (drought, n=26 sites). Only species that showed significant threshold declines or increases to **periphyton C:P (bulk)** are included in this table. The observed (*Obs*) threshold value of **C:P** for each taxon is shown in bold. **Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that "decrease" sharply in response to increasing C:P are associated with higher levels of P-enrichment,, whereas "increaser" taxa are associated with lower levels of P enrichment.*

| | | | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon ID | **Obs.** | Response > obs. value | *z* | IndVal | Purity | *P* value | 10% | 50% | 90% |
| ACbiasso | **859.2** | Increase* | 3.94 | 66.74 | 0.972 | <0.004 | 398.8 | 724.5 | 950.8 |
| AHminuti | **658.6** | Increase* | 5.35 | 76.65 | 1.000 | <0.004 | 275.1 | 398.8 | 724.5 |
| BRvitrea | **724.5** | Increase* | 7.25 | 85.66 | 1.000 | <0.004 | 546.4 | 729.1 | 859.2 |
| BULsp | **779.8** | Increase* | 3.90 | 53.41 | 0.992 | 0.008 | 546.4 | 740.0 | 950.8 |
| CAsilicu | **465.1** | Increase* | 3.18 | 40.00 | 0.974 | 0.028 | 333.1 | 658.6 | 779.8 |
| CLOsp2 | **1030.0** | Increase* | 6.01 | 70.87 | 0.990 | <0.004 | 729.1 | 950.8 | 1218.3 |
| CMdelcat | **859.2** | Increase* | 6.73 | 71.43 | 1.000 | <0.004 | 740.0 | 925.2 | 1218.3 |
| COSbotry | **1218.3** | Increase* | 3.98 | 83.30 | 0.998 | <0.004 | 398.8 | 779.8 | 1218.3 |
| COSgaler | **740.0** | Increase* | 5.86 | 79.48 | 1.000 | <0.004 | 465.1 | 729.1 | 859.2 |
| DEkuetzi | **729.1** | Increase* | 3.95 | 68.49 | 0.990 | 0.008 | 658.6 | 740.0 | 950.8 |
| ECcarina | **859.2** | Increase* | 4.07 | 42.86 | 0.966 | 0.028 | 711.2 | 925.2 | 1218.3 |
| ECsilesi | **333.1** | Increase* | 3.92 | 66.93 | 0.996 | <0.004 | 283.8 | 546.4 | 925.2 |
| EYevergl | **724.5** | Increase* | 8.11 | 90.91 | 1.000 | <0.004 | 546.4 | 729.1 | 859.2 |
| EYmicroc | **724.5** | Increase* | 8.18 | 90.91 | 1.000 | <0.004 | 546.4 | 729.1 | 859.2 |
| FRcapuci | **779.8** | Increase* | 4.40 | 64.66 | 0.978 | <0.004 | 658.6 | 740.0 | 925.2 |
| FRnanana | **1030.0** | Increase* | 6.70 | 70.64 | 0.994 | <0.004 | 740.0 | 950.8 | 1218.3 |
| GOgracil | **859.2** | Increase* | 4.96 | 74.19 | 0.992 | <0.004 | 711.2 | 859.2 | 1030.0 |
| GOvibdes | **925.2** | Increase* | 6.33 | 50.00 | 0.960 | <0.004 | 729.1 | 925.2 | 1030.0 |
| MERglauc | **658.6** | Increase* | 4.79 | 71.00 | 0.980 | <0.004 | 333.1 | 658.6 | 729.1 |
| MICsp | **740.0** | Increase* | 1.69 | 57.43 | 0.960 | 0.044 | 255.8 | 658.6 | 925.2 |
| MSellipt | **950.8** | Increase* | 4.58 | 55.96 | 0.986 | 0.008 | 724.5 | 925.2 | 1218.3 |
| MSsmithi | **724.5** | Increase* | 4.86 | 54.55 | 0.998 | 0.012 | 546.4 | 729.1 | 1218.3 |
| NAcrypto | **398.8** | Increase* | 3.07 | 43.75 | 0.974 | 0.016 | 330.4 | 658.6 | 859.2 |
| NAcryten | **724.5** | Increase* | 4.86 | 68.32 | 1.000 | <0.004 | 398.8 | 724.5 | 859.2 |
| NAexilis | **729.1** | Increase* | 2.75 | 30.00 | 0.950 | 0.048 | 546.4 | 740.0 | 1030.0 |
| NAradios | **711.2** | Increase* | 3.37 | 61.58 | 0.994 | 0.008 | 273.0 | 465.1 | 740.0 |
| NIampoid | **658.6** | Increase* | 4.84 | 61.54 | 0.994 | 0.008 | 333.1 | 658.6 | 740.0 |
| PERsp | **724.5** | Increase* | 5.52 | 54.55 | 0.998 | <0.004 | 546.4 | 729.1 | 925.2 |
| RPgibba | **333.1** | Increase* | 3.88 | 66.94 | 0.994 | <0.004 | 275.1 | 465.1 | 740.0 |
| SCYsp | **950.8** | Increase* | 6.80 | 60.00 | 0.956 | <0.004 | 740.0 | 990.4 | 1218.3 |
| SCZsp | **255.8** | Increase* | 2.80 | 52.84 | 0.978 | 0.012 | 255.8 | 398.8 | 859.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SPIsp | **740.0** | Increase* | 3.85 | 60.21 | 0.980 | <0.004 | 465.1 | 779.8 | 950.8 |
| SPLsp | **711.2** | Increase* | 3.91 | 56.36 | 0.956 | 0.008 | 465.1 | 724.5 | 859.2 |
| SRconstr | **333.1** | Increase* | 3.30 | 52.94 | 0.958 | 0.016 | 283.8 | 398.8 | 711.2 |
| STAsp | **859.2** | Increase* | 4.68 | 42.86 | 0.958 | 0.012 | 724.5 | 950.8 | 1218.3 |
| SYacus | **740.0** | Increase* | 8.28 | 92.42 | 1.000 | <0.004 | 658.6 | 729.1 | 859.2 |
| | | | | | | | | | |
| AHexigum | **859.2** | Decrease* | 4.62 | 78.95 | 1.000 | <0.004 | 398.8 | 779.8 | 950.8 |
| AMinarie | **724.5** | Decrease* | 5.18 | 75.36 | 0.980 | <0.004 | 465.1 | 724.5 | 859.2 |
| AMpedcls | **465.1** | Decrease* | 4.23 | 60.72 | 0.988 | <0.004 | 306.4 | 546.4 | 729.1 |
| AMveneta | **255.8** | Decrease* | 5.86 | 70.99 | 0.984 | 0.008 | 255.8 | 273.0 | 306.4 |
| BApardxa | **465.1** | Decrease* | 4.45 | 57.57 | 0.998 | <0.004 | 306.4 | 546.4 | 729.1 |
| CCplacen | **306.4** | Decrease* | 5.22 | 81.46 | 0.998 | <0.004 | 275.1 | 333.1 | 724.5 |
| CHOsp | **398.8** | Decrease* | 3.05 | 52.60 | 0.976 | <0.004 | 275.1 | 398.8 | 729.1 |
| CLAglome | **398.8** | Decrease* | 4.31 | 67.96 | 1.000 | <0.004 | 398.8 | 740.0 | 1030.0 |
| CRYsp | **398.8** | Decrease* | 3.30 | 49.39 | 0.958 | 0.016 | 274.9 | 398.8 | 740.0 |
| CYmenegh | **465.1** | Decrease* | 5.55 | 73.04 | 0.998 | <0.004 | 275.1 | 398.8 | 711.2 |
| DIconfer | **306.4** | Decrease* | 7.31 | 91.22 | 1.000 | <0.004 | 275.1 | 333.1 | 546.4 |
| FAlenzii | **275.1** | Decrease* | 3.40 | 71.86 | 0.986 | 0.008 | 273.0 | 333.1 | 950.8 |
| FAtener2 | **333.1** | Decrease* | 3.29 | 49.79 | 0.992 | 0.008 | 275.1 | 398.8 | 729.1 |
| GMlinfor | **333.1** | Decrease* | 3.86 | 48.26 | 0.986 | <0.004 | 255.8 | 333.1 | 729.1 |
| GOparvul | **1030.0** | Decrease* | 3.65 | 77.92 | 0.950 | <0.004 | 465.1 | 950.8 | 1218.3 |
| HIhunga | **333.1** | Decrease* | 5.60 | 74.27 | 0.998 | <0.004 | 283.8 | 398.8 | 724.5 |
| MEvarian | **255.8** | Decrease* | 4.69 | 46.93 | 0.952 | 0.012 | 255.8 | 273.0 | 546.4 |
| NAsancru | **546.4** | Decrease* | 4.42 | 50.00 | 0.998 | <0.004 | 275.1 | 398.8 | 711.2 |
| NAtexana | **546.4** | Decrease* | 3.57 | 41.67 | 0.992 | <0.004 | 275.1 | 465.1 | 724.5 |
| NIamphib | **950.8** | Decrease* | 3.01 | 54.67 | 0.996 | 0.012 | 275.1 | 724.5 | 1030.0 |
| NIangust | **779.8** | Decrease* | 3.97 | 66.67 | 0.962 | 0.008 | 658.6 | 779.8 | 925.2 |
| NIfrustu | **465.1** | Decrease* | 6.62 | 83.23 | 1.000 | <0.004 | 306.4 | 546.4 | 729.1 |
| NIincons | **950.8** | Decrease* | 5.95 | 90.48 | 0.996 | <0.004 | 724.5 | 925.2 | 1218.3 |
| PIgibba | **658.6** | Decrease* | 3.73 | 38.46 | 0.954 | 0.028 | 333.1 | 658.6 | 729.1 |
| PRlaevis | **306.4** | Decrease* | 5.83 | 62.50 | 0.994 | <0.004 | 255.8 | 306.4 | 465.1 |
| PTlanceo | **779.8** | Decrease* | 4.47 | 66.67 | 0.998 | 0.008 | 304.1 | 658.6 | 740.0 |
| REsinuta | **398.8** | Decrease* | 3.90 | 63.69 | 0.996 | 0.008 | 275.1 | 465.1 | 859.2 |
| ROabbre | **283.8** | Decrease* | 4.62 | 53.44 | 0.996 | 0.008 | 273.0 | 306.4 | 658.6 |
| SFseminu | **465.1** | Decrease* | 4.43 | 54.55 | 0.998 | 0.008 | 275.1 | 398.8 | 711.2 |
| TBfascic | **333.1** | Decrease* | 6.56 | 72.75 | 0.998 | <0.004 | 275.1 | 333.1 | 546.4 |
| TEmusica | **306.4** | Decrease* | 5.23 | 62.50 | 0.990 | <0.004 | 255.8 | 283.8 | 465.1 |

Table 12. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to **surface-water total phosphorus (TP, ug/L)** in **September 2007** (post-flood, n=26 sites). Only species that showed significant threshold declines or increases to **surface-water (TP)** are included in this table. The observed (*Obs*) threshold value of TP for each taxon is shown in bold.

| | | | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon ID | **Obs.** | Response > obs. value | Z | IndVal | Purity | Pr. (Type I) | 10% | 50% | 90% |
| ACbiasso | **12.9** | Decrease | 4.45 | 63.21 | 1.000 | <0.004 | 12.3 | 15.6 | 26.5 |
| AHminuti | **32.9** | Decrease | 4.56 | 76.14 | 1.000 | <0.004 | 13.8 | 26.5 | 115.8 |
| CMexcisa | **12.9** | Decrease | 3.57 | 55.34 | 0.982 | 0.008 | 10.9 | 12.9 | 24.5 |
| CMkolbei | **32.9** | Decrease | 6.15 | 88.52 | 0.998 | <0.004 | 21.0 | 32.9 | 115.8 |
| DEkuetzi | **24.5** | Decrease | 4.08 | 71.96 | 1.000 | <0.004 | 12.9 | 21.0 | 42.8 |
| EYmicroc | **12.3** | Decrease | 3.12 | 56.18 | 0.976 | 0.020 | 10.9 | 12.9 | 26.5 |
| NAexilis | **12.9** | Decrease | 5.02 | 72.15 | 1.000 | <0.004 | 12.8 | 13.8 | 32.9 |
| NAstroem | **17.6** | Decrease | 4.12 | 64.13 | 0.988 | 0.008 | 12.8 | 15.6 | 32.9 |
| SYulna | **149.3** | Decrease | 4.17 | 66.36 | 0.978 | <0.004 | 17.6 | 42.8 | 529.3 |
| COSsp | **13.1** | Decrease | 2.60 | 61.08 | 0.984 | 0.016 | 12.3 | 13.1 | 32.9 |
| MERglauc | **26.5** | Decrease | 3.83 | 58.82 | 0.968 | 0.016 | 13.1 | 21.0 | 42.8 |
| | | | | | | | | | |
| AHexigum | **24.5** | Increase | 5.56 | 60.00 | 0.996 | <0.004 | 15.6 | 24.5 | 42.8 |
| AMbullat | **24.5** | Increase | 3.08 | 30.00 | 0.956 | 0.028 | 15.6 | 26.5 | 45.7 |
| AMlibyca | **12.9** | Increase | 4.00 | 58.82 | 0.984 | 0.012 | 12.3 | 13.1 | 21.0 |
| AMpedcls | **24.5** | Increase | 3.63 | 54.59 | 0.998 | 0.008 | 12.8 | 13.8 | 26.5 |
| AMveneta | **21.0** | Increase | 2.73 | 27.27 | 0.960 | <0.004 | 15.6 | 24.5 | 42.8 |
| CAbacill | **12.9** | Increase | 6.03 | 82.35 | 1.000 | <0.004 | 11.6 | 12.9 | 13.8 |
| CCplacen | **24.5** | Increase | 3.23 | 64.12 | 0.992 | 0.012 | 12.3 | 13.8 | 26.5 |
| DIconfer | **13.1** | Increase | 4.14 | 56.25 | 0.998 | 0.008 | 12.9 | 15.6 | 32.9 |
| GOparvul | **24.5** | Increase | 4.05 | 68.06 | 0.994 | 0.012 | 10.9 | 17.6 | 32.9 |
| GOpumilu | **42.8** | Increase | 4.74 | 74.47 | 0.978 | <0.004 | 13.8 | 24.5 | 71.9 |
| GOrhombi | **149.3** | Increase | 4.34 | 45.34 | 0.954 | 0.048 | 15.6 | 42.8 | 529.3 |
| GYnodfrm | **26.5** | Increase | 3.46 | 33.33 | 0.964 | 0.020 | 17.6 | 26.5 | 71.9 |
| NAantoni | **26.5** | Increase | 3.08 | 33.33 | 0.952 | 0.032 | 15.6 | 26.5 | 529.3 |
| NAminima | **13.8** | Increase | 2.87 | 35.71 | 0.966 | 0.008 | 13.1 | 17.6 | 149.3 |
| NArecens | **17.6** | Increase | 6.05 | 80.36 | 0.992 | <0.004 | 13.1 | 17.6 | 26.5 |
| NAsancru | **26.5** | Increase | 4.35 | 44.44 | 0.992 | 0.012 | 17.6 | 26.5 | 71.9 |
| NAsubmin | **26.5** | Increase | 5.50 | 55.56 | 0.998 | <0.004 | 17.6 | 32.9 | 149.3 |
| NAvirdla | **24.5** | Increase | 4.19 | 50.00 | 0.994 | 0.008 | 15.6 | 24.5 | 76.3 |
| NIincons | **24.5** | Increase | 5.62 | 76.31 | 1.000 | <0.004 | 12.9 | 17.6 | 32.9 |
| NIpalea | **21.0** | Increase | 3.72 | 45.45 | 0.994 | 0.016 | 13.8 | 24.5 | 115.8 |
| PIgibba | **13.1** | Increase | 3.67 | 53.33 | 0.988 | <0.004 | 12.8 | 13.1 | 24.5 |
| PRlaevis | **26.5** | Increase | 6.96 | 85.44 | 1.000 | <0.004 | 15.6 | 24.5 | 42.8 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REsinuta | **32.9** | Increase | 4.76 | 72.10 | 0.998 | <0.004 | 11.6 | 13.8 | 42.8 |
| SUbreb | **26.5** | Increase | 4.61 | 60.28 | 1.000 | <0.004 | 13.8 | 24.5 | 115.8 |
| TEmusica | **24.5** | Increase | 5.66 | 60.00 | 1.000 | <0.004 | 17.6 | 26.5 | 115.8 |
| THweiss | **32.9** | Increase | 4.19 | 37.50 | 0.966 | 0.012 | 17.6 | 32.9 | 115.8 |
| TYlevid | **24.5** | Increase | 4.03 | 40.00 | 0.984 | 0.012 | 15.6 | 24.5 | 71.9 |
| ANKfalca | **24.5** | Increase | 3.71 | 59.42 | 0.980 | 0.016 | 12.9 | 17.6 | 32.9 |
| CLAglome | **17.6** | Increase | 3.79 | 64.41 | 0.980 | <0.004 | 12.9 | 17.6 | 42.8 |
| XBGcoc | **115.8** | Increase | 5.64 | 76.12 | 0.978 | <0.004 | 32.9 | 115.8 | 529.3 |

Table 13. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on algal species composition in response to **C:P ratio in the periphyton** (bulk, no separation from sediment) in **September 2007** (drought, n=26 sites). Only species that showed significant threshold declines or increases to **periphyton C:P (bulk)** are included in this table. The observed (*Obs*) threshold value of **C:P** for each taxon is shown in bold. Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that "decrease" sharply in response to increasing C:P are associated with higher levels of P-enrichment,, whereas "increaser" taxa are associated with lower levels of P enrichment.

| | | | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon ID | **Obs.** | Response > obs. value | Z | IndVal | Purity | *P* value | 10% | 50% | 90% |
| ACbiasso | **793.0** | Increase* | 3.70 | 54.99 | 0.994 | 0.016 | 234.9 | 775.2 | 886.0 |
| AHminuti | **204.9** | Increase* | 3.51 | 69.35 | 0.972 | 0.004 | 165.7 | 234.9 | 817.7 |
| CMdelcat | **993.1** | Increase* | 3.47 | 65.32 | 0.972 | 0.012 | 362.3 | 843.5 | 1207.6 |
| CMexcisa | **775.2** | Increase* | 4.36 | 57.94 | 0.990 | 0.004 | 362.3 | 793.0 | 1303.4 |
| CMkolbei | **204.9** | Increase* | 4.94 | 81.92 | 1.000 | 0.004 | 165.7 | 204.9 | 775.2 |
| DEkuetzi | **775.2** | Increase* | 4.26 | 71.73 | 1.000 | 0.008 | 204.9 | 749.4 | 843.5 |
| EYcesati | **793.0** | Increase* | 2.96 | 33.33 | 0.984 | 0.020 | 772.6 | 886.0 | 1303.4 |
| EYevergl | **775.2** | Increase* | 3.05 | 50.34 | 0.952 | 0.016 | 263.5 | 793.0 | 1207.6 |
| EYmicroc | **775.2** | Increase* | 4.92 | 58.10 | 0.996 | 0.004 | 362.3 | 793.0 | 1109.9 |
| GOintvib | **1303.4** | Increase* | 4.16 | 45.86 | 0.964 | 0.024 | 843.5 | 1303.4 | 1327.6 |
| GOmaclau | **1303.4** | Increase* | 3.36 | 61.24 | 0.956 | 0.024 | 263.5 | 1109.9 | 1327.6 |
| NAexilis | **775.2** | Increase* | 4.35 | 64.18 | 1.000 | 0.004 | 204.9 | 592.0 | 843.5 |
| NAstroem | **775.2** | Increase* | 3.66 | 59.11 | 0.950 | 0.012 | 234.9 | 775.2 | 993.1 |
| NIsinde | **1303.4** | Increase* | 4.26 | 67.35 | 0.984 | 0.008 | 775.2 | 1109.9 | 1327.6 |
| SYacus | **1303.4** | Increase* | 2.54 | 60.60 | 0.950 | 0.020 | 362.3 | 886.0 | 1327.6 |
| SYulna | **204.9** | Increase* | 3.58 | 61.58 | 0.954 | 0.008 | 155.0 | 182.3 | 362.3 |
| COEsp | **793.0** | Increase* | 3.75 | 41.67 | 0.994 | 0.004 | 749.4 | 843.5 | 1327.6 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HOMsp | **1327.6** | Increase* | 4.98 | 63.39 | 0.958 | 0.012 | 814.8 | 1255.5 | 1327.6 |
| MERglauc | **263.5** | Increase* | 3.82 | 58.82 | 1.000 | 0.016 | 204.9 | 775.2 | 896.7 |
| OEDsp | **775.2** | Increase* | 3.08 | 38.46 | 0.980 | 0.048 | 362.3 | 814.8 | 1109.9 |
| OSCsp | **1109.9** | Increase* | 3.24 | 68.82 | 0.990 | 0.004 | 204.9 | 886.0 | 1207.6 |
| | | | | | | | | | |
| AHexigum | **793.0** | Decrease* | 4.06 | 42.86 | 0.996 | 0.008 | 182.3 | 592.0 | 814.8 |
| AMmontan | **775.2** | Decrease* | 3.05 | 38.46 | 0.974 | 0.040 | 155.0 | 592.0 | 814.8 |
| AMpedcls | **592.0** | Decrease* | 5.57 | 70.16 | 1.000 | 0.004 | 263.5 | 749.4 | 843.5 |
| AMveneta | **362.3** | Decrease* | 2.78 | 30.00 | 0.958 | 0.048 | 182.3 | 362.3 | 775.2 |
| CCplacen | **793.0** | Decrease* | 5.14 | 72.90 | 0.998 | 0.004 | 592.0 | 814.8 | 1303.4 |
| CYmenegh | **775.2** | Decrease* | 4.04 | 65.60 | 0.992 | 0.008 | 362.3 | 814.8 | 1109.9 |
| DIconfer | **775.2** | Decrease* | 6.82 | 69.23 | 1.000 | 0.004 | 234.9 | 749.4 | 843.5 |
| GOparvul | **592.0** | Decrease* | 5.11 | 72.24 | 1.000 | 0.004 | 263.5 | 793.0 | 1207.6 |
| GOpumilu | **204.9** | Decrease* | 5.12 | 74.47 | 0.990 | 0.004 | 165.7 | 234.9 | 775.2 |
| GYnodfrm | **263.5** | Decrease* | 3.11 | 33.33 | 0.972 | 0.036 | 165.7 | 234.9 | 749.4 |
| MEvarian | **592.0** | Decrease* | 5.33 | 58.63 | 0.990 | 0.004 | 234.9 | 749.4 | 843.5 |
| NAantoni | **263.5** | Decrease* | 3.00 | 33.33 | 0.962 | 0.036 | 155.0 | 234.9 | 775.2 |
| NAkotsch | **793.0** | Decrease* | 4.08 | 62.24 | 0.956 | 0.004 | 569.0 | 793.0 | 993.1 |
| NAminima | **793.0** | Decrease* | 3.00 | 35.71 | 0.978 | 0.008 | 165.7 | 592.0 | 843.5 |
| NArecens | **182.3** | Decrease* | 3.38 | 69.06 | 0.982 | 0.012 | 165.7 | 263.5 | 843.5 |
| NAsubmin | **263.5** | Decrease* | 4.84 | 55.56 | 0.998 | 0.004 | 155.0 | 234.9 | 592.0 |
| NAvirdla | **775.2** | Decrease* | 3.17 | 38.46 | 0.994 | 0.032 | 165.7 | 263.5 | 793.0 |
| NIincons | **592.0** | Decrease* | 8.00 | 86.56 | 1.000 | 0.004 | 234.9 | 749.4 | 843.5 |
| PRlaevis | **204.9** | Decrease* | 4.98 | 72.21 | 0.998 | 0.004 | 165.7 | 263.5 | 775.2 |
| REsinuta | **592.0** | Decrease* | 5.25 | 71.84 | 0.998 | 0.004 | 204.9 | 362.3 | 867.0 |
| SUangust | **592.0** | Decrease* | 3.57 | 36.36 | 0.986 | 0.020 | 165.7 | 362.3 | 793.0 |
| SUbreb | **263.5** | Decrease* | 5.20 | 64.36 | 1.000 | 0.004 | 165.7 | 263.5 | 793.0 |
| TEmusica | **263.5** | Decrease* | 5.66 | 66.67 | 1.000 | 0.004 | 165.7 | 263.5 | 749.4 |
| CLAglome | **182.3** | Decrease* | 2.94 | 68.42 | 0.988 | 0.016 | 165.7 | 592.0 | 993.1 |
| XBGcoc | **155.0** | Decrease* | 4.55 | 67.72 | 0.958 | 0.004 | 155.0 | 182.3 | 362.3 |

**RESULTS AND INTERPRETATION, CONTINUED**

*Macroinvertebrate Taxonomic Responses to TP*

Results of ordinations and Threshold Indicator Taxa Analysis (TITAN) on macroinvertebrate taxonomic composition during November 2006 showed that nutrients, specifically phosphorus, corresponded to shifts in community structure (Figure 30, 31). Because composition differed substantially between sites with and without flow, and because of established relationship between flow and other water chemistry variables (e.g., DO), we separated sites into groups by flow status prior to analysis.

Sites with no flow yielded more reliable relationships to TP than sites with flow, but this result should be interpreted with the caveat that there were only 9 sites in the group with flow. Ordinations clearly showed that in both sets of analyses, the sites with low TP and high periphyton C:P sorted on the left side of axis 1, whereas high TP and low C:P sites fell out on the right side of axis 1 (Figure 30).

In both sets of TITAN analyses, more taxa were classified as negative threshold indicators (decline in response to TP) than as positive ones (increase). A few of the negative indicators of note included the stoneflies *Zealeuctra* and *Perlesta*, the caddisflies *Chimarra*, and *Hydroptila*, the mayfly *Neochoroterpes nanita*, and the dryopid beetle *Postelichus.* Notable increasers included the filter-feeding caddisfly *Cheumatopsyche*, the amphipod *Hyallela azteca*, the fingernail clam Sphaeriidae, and aquatic worms (Oligochaeta).

Because macroinvertebrates are so highly tied to flow, these results likely represent a weak representation of the potential influence of nutrient enrichment on macroinvertebrates. Many of the sites had gone dry or nearly so between August and October 2006, so some of the sites with flow during this event had been reduced to tiny disconnected pools in weeks prior to sampling. We anticipate producing a larger macroinvertebrate data set (J. A. Back, Ph. D dissertation) spanning more flow conditions, and therein more completely evaluate macroinvertebrate responses to TP enrichment.

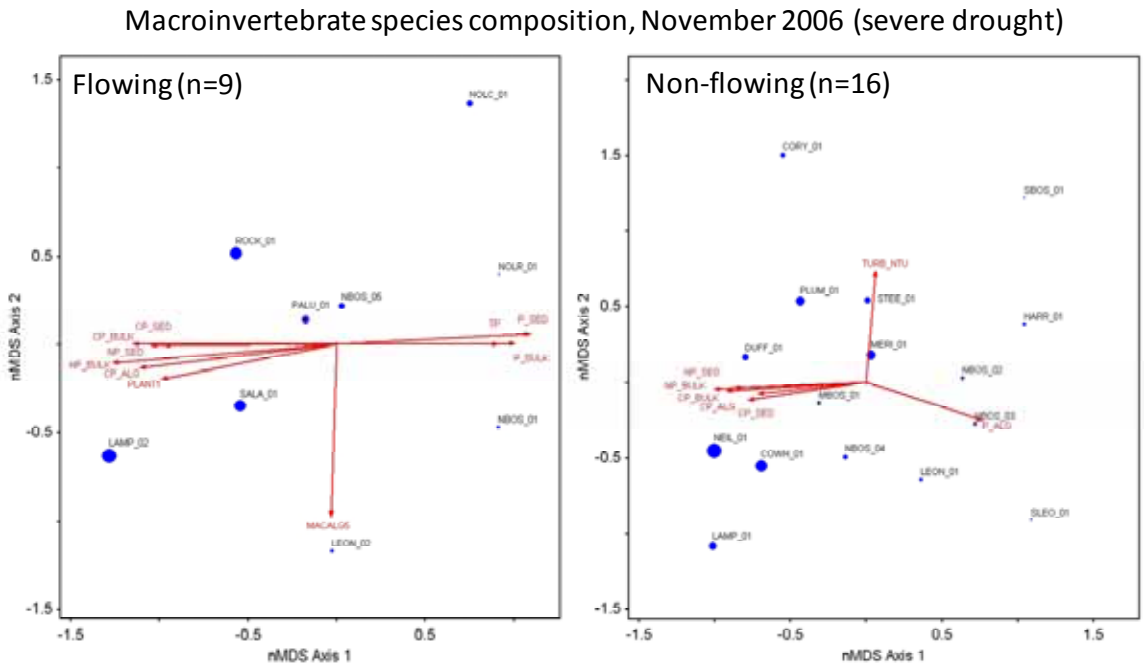



Figure 30. Nonmetric multidimensional scaling ordination of macroinvertebrate species composition during a period of drought in November 2006. Dissimilarity (Bray-Curtis) was computed using log-transformed densities (no./m2) per taxon. Ordinations were conducted separately for sites with detectable flow (n=9) and those without flow (n=16; one site had insufficient surface water for macroinvertebrate sampling and was removed). Red arrows (vectors) correspond to significant predictors of taxonomic composition.

Table 14. Community-level results from Threshold Indicator Taxa Analysis (TITAN) on **macroinvertebrate species composition** in response to **surface-water TP and periphyton C:P ratio (bulk)** during November 2006 (severe drought, n=26 sites). Analyses were conducted separately for sites that had measureable flow preceding and during the sampling (flow, n=9), and those that were not flowing (no flow, n=16). Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community**.** See Figure 31 for details.

| | | | | Bootstrap Cumulative Distribution of Thresholds | | | | |
|---|---|---|---|---|---|---|---|---|
| Data set | Predictor | Method | **Obs.** | 5% | 10% | 50% | 90% | 95% |
| No flow (n=16) | Surface-water TP (ug/L) | Decline (sumz-) | **16.3** | 16.3 | 16.3 | 18.3 | 20.2 | 20.2 |
| | | Increase (sumz+) | **17.5** | 18.3 | 19.3 | 27.3 | 120.1 | 120.1 |
| No flow (n=16) | Periphyton C:P ratio (bulk) | Decline (sumz-) | **230.1** | 152.1 | 152.1 | 195.4 | 230.1 | 259.1 |
| | | Increase (sumz+) | **373.6** | 173.5 | 195.4 | 230.1 | 373.6 | 373.6 |
| Flow (n=9) | Surface-water TP (ug/L) | Decline (sumz-) | **10.7** | 10.7 | 10.7 | 14.8 | 850.4 | 1636.5 |
| | | Increase (sumz+) | **409.2** | 10.7 | 14.8 | 850.4 | 1636.5 | 1636.5 |
| Flow (n=9) | Periphyton C:P ratio (bulk) | Decline (sumz-) | **195.6** | 151.8 | 151.8 | 195.6 | 290.9 | 359.2 |
| | | Increase (sumz+) | **359.2** | 195.6 | 195.6 | 290.9 | 359.2 | 359.2 |

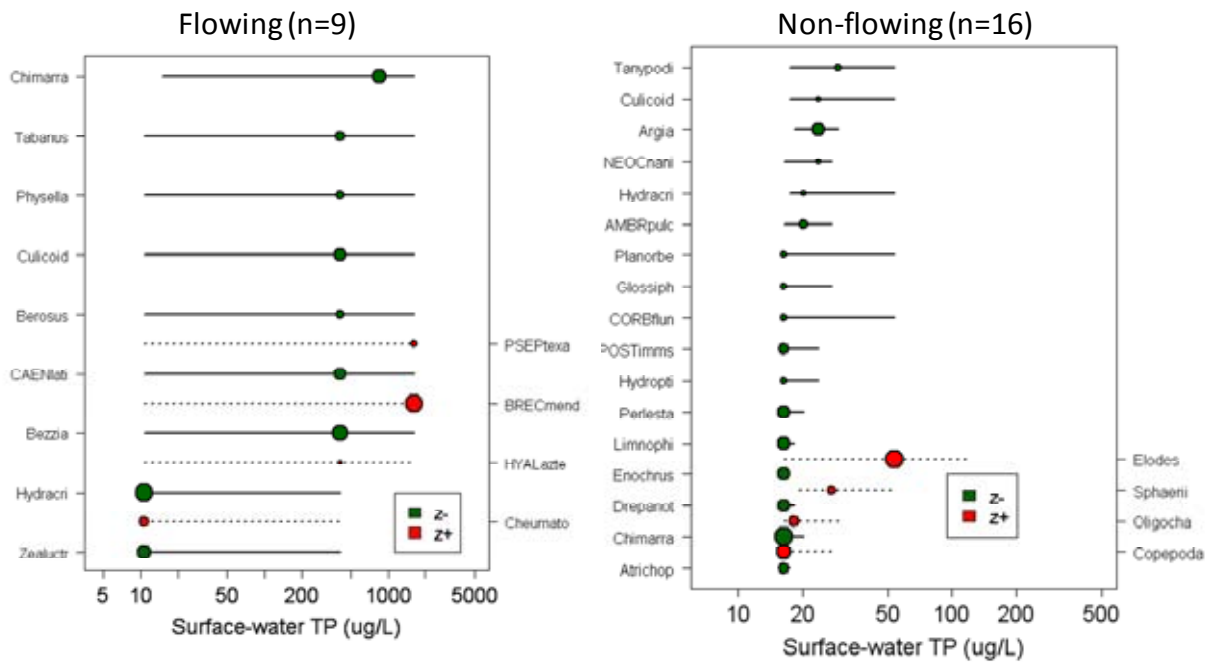



Figure 31. Results of TITAN using surface-water TP as a predictor of threshold changes in macroinvertebrate taxa abundances from sites with flow (n=9) and those without flow (n=16) in November 2006.

Table 15. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on **macroinvertebrate species composition** in response to **surface-water TP** during November 2006 (severe drought, n=26 sites). Analyses were conducted separately for sites that had measureable flow preceding and during the sampling (flow, n=9), and those that were not flowing (no flow, n=16). Only species that showed significant threshold declines or increases to **surface-water (TP)** are included in this table. The observed (*Obs*) threshold value of TP for each taxon is shown in bold. See Table 14 and Figure 31 for details.

| | | | | | | | Cumulative Threshold Quantiles | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon ID | **Obs.** | Response > Obs. Value | z | IndVal | Purity | P-value | 10% | 50% | 90% |
| **Non-flowing sites (n=16)** | | | | | | | | | |
| AMBRpulc | **20.2** | Decline | 3.82 | 75.00 | 0.990 | 0.020 | 16.3 | 20.2 | 27.3 |
| Argia | **23.7** | Decline | 5.63 | 95.20 | 1.000 | <0.004 | 18.3 | 23.7 | 29.3 |
| Atrichop | **16.3** | Decline | 4.60 | 50.00 | 0.872 | 0.048 | 16.3 | 16.3 | 17.5 |
| Chimarra | **16.3** | Decline | 7.29 | 50.00 | 0.878 | 0.016 | 16.3 | 16.3 | 20.2 |
| CORBflun | **16.3** | Decline | 3.19 | 80.09 | 0.966 | 0.012 | 16.3 | 19.3 | 53.7 |
| Culicoid | **23.7** | Decline | 2.64 | 66.48 | 0.942 | 0.024 | 17.5 | 23.7 | 53.7 |
| Drepanot | **16.3** | Decline | 5.09 | 50.00 | 0.880 | 0.040 | 16.3 | 16.3 | 18.3 |
| Enochrus | **16.3** | Decline | 5.16 | 50.00 | 0.872 | 0.036 | 16.3 | 16.3 | 17.5 |
| Glossiph | **16.3** | Decline | 3.40 | 81.85 | 0.980 | <0.004 | 16.3 | 19.3 | 27.3 |
| Hydracri | **20.2** | Decline | 2.63 | 63.98 | 0.872 | 0.024 | 17.5 | 20.2 | 53.7 |
| Hydropti | **16.3** | Decline | 3.17 | 70.72 | 0.950 | 0.012 | 16.3 | 17.5 | 23.7 |
| Limnophi | **16.3** | Decline | 4.98 | 50.00 | 0.880 | 0.040 | 16.3 | 16.3 | 18.3 |
| NEOCnani | **23.7** | Decline | 2.61 | 55.56 | 0.984 | 0.044 | 16.3 | 19.3 | 27.3 |
| Perlesta | **16.3** | Decline | 5.29 | 50.00 | 0.872 | 0.036 | 16.3 | 16.3 | 20.2 |
| Planorbe | **16.3** | Decline | 2.75 | 74.33 | 0.938 | 0.032 | 16.3 | 19.3 | 53.7 |
| POSTimms | **16.3** | Decline | 4.83 | 89.02 | 0.988 | <0.004 | 16.3 | 17.5 | 23.7 |
| Tanypodi | **29.3** | Decline | 3.13 | 62.64 | 0.982 | 0.008 | 17.5 | 23.7 | 53.7 |
| | | | | | | | | | |
| Copepoda | **16.3** | Increase | 4.05 | 56.40 | 0.884 | <0.004 | 16.3 | 17.5 | 27.3 |
| Elodes | **53.7** | Increase | 4.98 | 50.00 | 0.884 | 0.040 | 16.3 | 53.7 | 120.1 |
| Oligocha | **18.3** | Increase | 2.96 | 53.86 | 0.982 | 0.024 | 16.3 | 19.3 | 29.3 |
| Sphaerii | **27.3** | Increase | 2.60 | 67.64 | 0.876 | 0.024 | 19.3 | 27.3 | 53.7 |
| | | | | | | | | | |
| **Flowing sites (n=9)** | | | | | | | | | |
| Berosus | **409.2** | Decline | 1.82 | 75.40 | 0.904 | 0.032 | 10.7 | 409.2 | 1636.5 |
| Bezzia | **409.2** | Decline | 3.14 | 91.83 | 0.994 | 0.020 | 10.7 | 409.2 | 1636.5 |
| CAENlati | **409.2** | Decline | 2.57 | 82.76 | 0.982 | 0.040 | 10.7 | 409.2 | 1636.5 |
| Chimarra | **850.4** | Decline | 2.76 | 76.34 | 0.894 | <0.004 | 14.8 | 850.4 | 1636.5 |
| Culicoid | **409.2** | Decline | 2.62 | 86.52 | 0.988 | 0.020 | 10.7 | 409.2 | 1636.5 |
| Hydracri | **10.7** | Decline | 3.48 | 100.00 | 0.964 | 0.024 | 10.7 | 10.7 | 409.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Physella | **409.2** | Decline | 1.97 | 75.05 | 0.930 | 0.028 | 10.7 | 409.2 | 1636.5 |
| Tabanus | **409.2** | Decline | 2.26 | 82.85 | 0.972 | 0.040 | 10.7 | 409.2 | 1636.5 |
| Zealuctr | **10.7** | Decline | 3.03 | 66.67 | 0.880 | 0.084 | 10.7 | 10.7 | 409.2 |
| | | | | | | | | | |
| BRECmend | **1636.5** | Increase | 4.82 | 100.00 | 0.894 | <0.004 | 10.7 | 1636.5 | 1636.5 |
| Cheumato | **10.7** | Increase | 3.08 | 100.00 | 0.960 | <0.004 | 10.7 | 10.7 | 409.2 |
| HYALazte | **409.2** | Increase | 1.71 | 72.75 | 0.894 | 0.100 | 10.7 | 409.2 | 1636.5 |
| PSEPtexa | **1636.5** | Increase | 2.05 | 82.98 | 0.910 | 0.100 | 10.7 | 850.4 | 1636.5 |

## SECTION 2: EXPERIMENTAL STREAM STUDY

## SITE DESCRIPTION

Experimental evidence derived from controlled studies is important for the development of scientifically defensible water quality standards, yet most studies are limited only to field observations from natural lakes, wetlands, and streams because these habitats are too large and complex to manipulate experimentally. The intention of the Baylor Experimental Aquatic Research (BEAR) facility in Waco, TX, is to bridge the gap between field observations, which represent the habitat of interest but may be influenced by many interacting chemicals or other aquatic stressors simultaneously, and laboratory or small field experiments, which allow for control of environmental variables yet are too small and unrepresentative of natural conditions to be realistic. Because of its size (over 30,000 square feet), outdoor location, and close proximity to natural aquatic habitats the BEAR facility is a unique, state-of-the-art resource for conducting controlled yet realistic water research studies (Figure 32).

The BEAR facility supports 12 experimental stream mesocosms. The facility is located at the Lake Waco Wetlands, an 80-hectare constructed wetland. Water is pumped from the outflow of cell 2 of the 5-cell wetland to the BEAR facility. Here, the water has relatively low levels of dissolved N and P and thus provides high-quality source water for a nutrient addition experiment.

Water from the wetland is delivered to the BEAR facility via a 2500 L/min pump. Water is pumped into a 160,000 L water tower and delivered in controlled volumes to the streams via a series of 12 valves, each adjusted to regulate flow equivalently among all 12 streams (180 L/min). Before entering the streams, water is pumped into a mixing tank where chemicals representing experimental treatments can be dosed at a fixed rate using peristaltic pumps connected to a second, adjacent tank of chemical stock solution. Once dosed with the treatment levels of non-toxic chemicals, water is released into the streams and is discharged back into the Lake Waco Wetlands.

The experimental streams are approximately 0.6 m wide and 20 m in length. The streams are stratified into riffle, glide, and pool sections and are designed mimic natural habitat in central Texas streams. A riffle is a high-gradient habitat that is usually fast-flowing, quite shallow, and usually composed of gravel or cobble substrate. Glides are also shallow but usually much slower in water velocity. Pools are deep, slow areas in streams. Each of these habitats tends to support different species with specific adaptations to those particular environments.

The BEAR stream facility was covered by a canopy that excluded 60% of incoming solar radiation to approximate riparian canopies of a typical stream in the field study (800-1,000 μE, RSK, unpublished data), and reduce variability of sunlight across experimental units.

Figure 32. Aerial view of the Lake Waco Wetland and the location of the Baylor Experimental Aquatic Research (BEAR) stream facility (red arrow, upper panel). The lower panel is an aerial view the BEAR experimental stream facility (80' x 100', covered in 60% shade cloth), adjacent pond mesocosms, and a 40K gallon water tower used to store water pumped from the Lake Waco Wetlands en route to the experimental streams. See http://www.baylor.edu/aquaticlab/index.php?id=45868 for a video tour of the BEAR streams.

## EXPERIMENTAL DESIGN

Clean river cobble and gravel were placed in streams in January 2008 to simulate erosional habitats found in natural streams in our field study. Stream flow was initiated on 31 January 2008 and calibrated to 182 L/min (+/- 2 L/min) for each stream.

Streams were seeded with organic matter, periphyton, and macroinvertebrates collected from ROCK-01 and NBOS-03, two of the intensive field sites. These sites were chosen because their P concentrations were representative of the background (control) and high P treatments to be employed in the experiment. Inoculating the streams with organisms only from the low-P streams would bias the study because of the reduced probability of introducing taxa that proliferate under high P conditions.

Streams were seeded twice: 1 February and 15 February 2008. The first event was to primarily introduce algae, bacteria, fungi, and detritus, as we anticipated high mortality of macroinvertebrates due to the lack of food in the streams at the beginning of the colonization period. The second seeding was done to facilitate establishment of macroinvertebrates found in both low and high P streams in the region.

Seeding was accomplished by collecting five 1-$m^2$ kick screen samples from ROCK-01 and NBOS-03 for each of the 12 experimental streams. Samples were taken from 5 different locations in the field study sites, with one sample collected for each of the 12 streams at each of the 5 different locations. A composite of the five samples was placed in one 20-L bucket per experimental stream. Each bucket was aerated during transport from the field back to the BEAR facility. One bucket per stream was mixed and gently dumped into the top of the riffle section to permit drifting organisms to colonize downstream areas in the reach.

The streams were allowed to run without any nutrient additions from 31 January to 10 March 2008 in an effort to allow growth of periphyton at low nutrient concentrations. However, we were also interested the potential effect of nutrients on established periphyton communities from real streams. To this end, we transplanted 600 cobbles with established periphyton communities from riffle habitat in ROCK-01 and NBOS-03. Cobbles from ROCK-01 and NBOS-03 were placed in random cross sections throughout the reach of each experimental stream on 7 and 8 March 2008, respectively, a few days prior to initiation of the experiment.

Experimental dosing of phosphorus began on 11 March 2008. Background concentration of PO4-P during the colonization phase of the study was approximately 6 ug/L, slightly higher than our lowest field sites, but nevertheless relatively low. Four streams were randomly assigned no additional phosphorus, and thus a target concentration of **6 ug/L** (**Control**). Because our field study had identified consistent nonlinear changes in numerous biological response variables between 10 and 20 ug/L PO4-P (usually corresponding to 15-25 ug/L TP), we selected a target PO4-P concentration of 20 ug/L for our lower nominal P treatment. Four streams were assigned a treatment of +15 ug/L PO4-P to achieve a target PO4-P concentration of **20 ug/L (Low P treatment**). The remaining four streams received +95 ug/L PO4-P to achieve a target concentration of **100 ug/L (High P treatment).** The higher P concentration was selected

because it was clearly above the level of P enrichment that was correlated with significant biological changes in the field study.

Streams were dosed with $NaH_2PO_4$ using peristaltic pumps. Pumps were calibrated to deliver phosphorus stock solutions from 50-L carboys to mixing tanks located at the head of each experimental stream.

Figure 33. Collage of BEAR stream photographs illustrating the layout of the stream facility (top left), a view from within a glide section of a stream channel (top right), the substrate of a typical riffle section during the colonization phase of the study (bottom left), and a lateral view of the streams from the far left side of the facility, looking toward the adjacent Lake Waco Wetlands.

**SAMPLING AND DATA ANALYSES**

Dissolved nutrients (PO4-P, NH3-N, and NO2NO3-N) were sampled in triplicate biweekly from the mixing tank and pool (terminal outlet) of the streams. Because of the relatively short length of the streams and insufficient amount of time for phytoplankton growth before water is emptied from the streams, turbidity and chlorophyll-a in surface water were not monitored. Because of the effect of turbulent mixing on dissolved oxygen in streams and lack of response of DO to TP in field streams during periods of high flow, diel dissolved oxygen was not monitored in this study.

Biotic sampling was stratified into riffle, and glide habitats. Each section was partitioned into approximately forty 20-cm wide cross-sections. Each cross section was assigned a number for random sampling location for biota sampling.

Quantitative periphyton samples from rocks that were exclusively colonized in the experimental streams ("bare rock", non-transplant periphyton) were collected on Day 0 (day before dosing commenced) and Day 28 (4 weeks of dosing) of the study. On each date, four random cross-sections from the riffle sections were sampled. Within each of the four cross sections, 5 cobbles were removed quantitatively sampled in accordance with the field study protocol. The composite of 20 rocks was subsampled for chlorophyll-a, AFDM, and CNP chemical analysis following the QAPP (Appendix B). On Day 28, filamentous algae (*Cladophora*) was gently removed from the rocks (most of it was loosely attached) and quantified separately for AFDM, chlorophyll-a and CNP chemical analysis. The remaining attached periphyton was processed as previously described.

Quantitative periphyton samples from transplanted cobbles (ROCK-01 and NBOS-03) were processed on Day -2 and -1 (two and one days prior to dosing, the same day rocks were collected from the field streams) to characterize the baseline AFDM, chlorphyll a, and CNP content prior to transplanting. Four separate composite samples were collected from each site (four sets of 5 rocks). Five transplanted cobbles per field stream were collected from each experimental stream on Day 7 (17 March 2008) and 28 (7 April 2008). Each set of five rocks represented a single composite sample (following Day -1 and -2) from each of the 2 field streams. AFDM, chlorophyll-a, and CNP content of the periphyton was quantified for each composite sample. Additionally, algal species composition was measured from samples from Day -2/-1 (hereafter, Day 0) and Day 28.

An additional set of quantitative periphyton samples was collected from 5 x 5 cm ceramic tiles. Clean tiles were placed in the glide section of streams on Day -14. A set of 10 tiles was randomly removed from each stream on Day 0, 14, and 28 for analysis of chlorophyll-a and AFDM.

Quantitative macroinvertebrate sampling was conducted on Day 0 and 28 and was coordinated with periphyton sampling so that the same cross-sections were sampled on the same dates. Samples were collected with a custom-made quantitative sampler developed specifically for the BEAR facility. The sampler, called a BEAR Trap, is functionally similar in design and function to a Hess sampler, but has dimensions that fit within the 20-cm cross-sections of the BEAR

streams (52 x 20 cm; area=0.104 $m^2$). Four quantitative samples were collected per stream per riffle and run sections per date and were processed as described in the QAPP (Appendix B).

Depending upon the response variable, most data were analyzed as a one-way ANOVA with P treatment as a 3-level main effect (Control, Low P, High P). Individual streams were replicates (n=4 per treatment). Appropriate transformations were applied when necessary. Multiple comparison tests were done when ANOVA deemed the main effect significant ($p<0.05$).

Algal and macroinvertebrate species composition were analyzed using nonmetric multidimensional scaling ordination (see Section I) and analysis of successional vectors. Tests for diffrerences in treatments were done using Multi-Response Permutation Procedure (MRPP). Indicator Species Analysis was conducted to identify taxa that changed significantly in response to P treatments if the overall MRPP test of community differences was deemed significant. Multivariate analyses were conducted in PC-ORD v.5.20.

## RESULTS AND INTERPRETATION

### *Nutrient Concentrations among the Experimental Streams*

Experimental PO4-P additions yielded concentrations among treatments that were very close to target nominal concentrations of 6, 20, and 100 ug/L PO4-P (Figure 34, Table 16). The 4-week PO4-P average from the control streams ranged between 7.7 and 8.1 ug/L PO4-P, slightly higher than anticipated due to slight increases in nutrients in the wetland during the study period. The average PO4-P for the Low P treatment ranged from 17.7 to 19.3 among streams, slightly lower than the target of 20 ug/L. High P treatments ranged from 102 to 112 ug/L PO4-P, slightly higher than the 100 ug/L target concentration.

DIN and total N were very similar among all 12 study streams (Table 16). DIN and TN were within the range of the least impacted field study streams. TP was higher than expected, largely due to wind re-suspension of fine sediment in the wetland. Mean TP ranged from 19.9-20.5 among the Control streams during the 4 week dosing period. This TP level was close to the threshold observed from the field study.

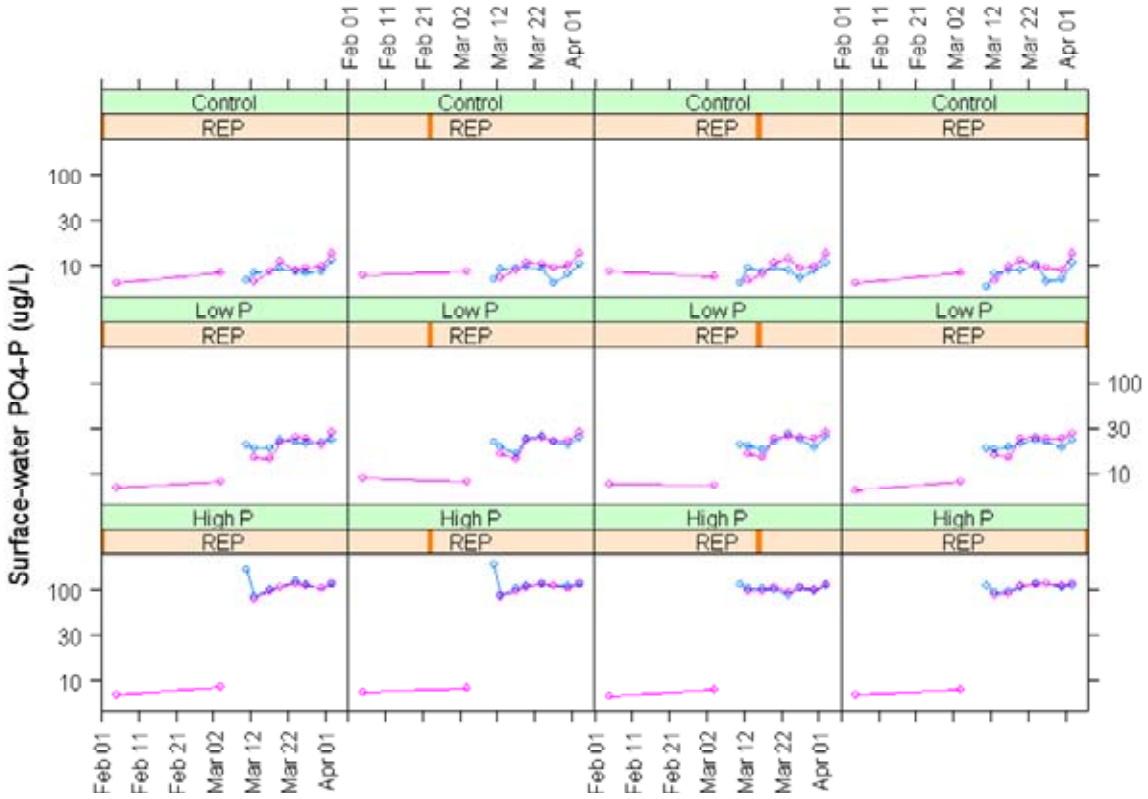



Figure 34. Time series multi-panel plot showing concentrations of PO4-P among the 12 experimental streams during the colonization (pre-dosing; 1 February – 10 March 2008) and dosing (11 March – 7 April 2008) phases of the experiment. The magenta points correspond to the samples collected from the outlet of each stream, whereas the blue points are samples from the mixing tank. No mixing tank samples were collected prior to dosing. Control = no P addition (6 ug/L), Low P = 20 ug/L and high P = 100 ug/L. REP = stream 1 -4 per treatment.

Table 16. Mean dissolved and total P and N concentrations during the 28-days of PO4-P dosing in the experimental streams. Dissolved nutrients (PO4-P, DIN) were sampled in triplicate per location per stream, twice weekly during the study period (11 March – 7 April 2008; n=9 sampling events). Total N and P were sampled once per week during the dosing period (n=4 dates). See figure 34 for temporal patterns in PO4-P prior to and during dosing.

| Treatment | Stream rep. | Location | **$PO_4$-P** | DIN | $NH_3$-N | $NO_2NO_3$-N | TN | TP |
|---|---|---|---|---|---|---|---|---|
| Control | 1 | Mixing tank | **8.1** | 264.9 | 15.7 | 249.1 | 419.3 | 19.9 |
| Control | 2 | Mixing tank | **7.9** | 272.1 | 15.6 | 256.6 | 468.5 | 20.2 |
| Control | 3 | Mixing tank | **7.8** | 288.1 | 15.7 | 272.4 | 472.5 | 20.5 |
| Control | 4 | Mixing tank | **7.7** | 267.2 | 15.0 | 252.3 | 501.3 | 20.5 |
| Low | 1 | Mixing tank | **18.3** | 258.5 | 15.4 | 243.1 | 452.0 | 37.8 |
| Low | 2 | Mixing tank | **19.3** | 273.2 | 14.9 | 258.2 | 449.3 | 39.9 |
| Low | 3 | Mixing tank | **19.3** | 273.2 | 14.7 | 258.6 | 389.8 | 40.3 |
| Low | 4 | Mixing tank | **17.7** | 250.9 | 14.6 | 236.4 | 432.5 | 37.8 |
| High | 1 | Mixing tank | **109.8** | 267.2 | 15.6 | 251.6 | 469.5 | 136.2 |
| High | 2 | Mixing tank | **112.7** | 273.3 | 16.2 | 257.2 | 464.3 | 137.2 |
| High | 3 | Mixing tank | **102.7** | 265.4 | 14.7 | 250.8 | 447.0 | 127.4 |
| High | 4 | Mixing tank | **109.5** | 277.6 | 14.2 | 263.4 | 421.9 | 136.0 |
| Control | 1 | Outlet | 8.5 | 259.2 | 18.0 | 241.2 | 463.1 | 19.4 |
| Control | 2 | Outlet | 8.8 | 259.7 | 16.2 | 243.5 | 459.3 | 19.1 |
| Control | 3 | Outlet | 9.1 | 272.3 | 16.6 | 255.6 | 521.0 | 19.5 |
| Control | 4 | Outlet | 8.9 | 251.5 | 14.6 | 236.9 | 488.3 | 19.2 |
| Low | 1 | Outlet | 17.8 | 226.8 | 15.5 | 211.3 | 411.0 | 37.7 |
| Low | 2 | Outlet | 19.5 | 262.8 | 15.0 | 247.8 | 396.5 | 39.3 |
| Low | 3 | Outlet | 18.4 | 245.4 | 15.5 | 229.9 | 426.5 | 40.3 |
| Low | 4 | Outlet | 17.4 | 224.2 | 15.5 | 208.7 | 457.3 | 38.5 |
| High | 1 | Outlet | 96.6 | 216.6 | 16.1 | 200.5 | 431.4 | 128.7 |
| High | 2 | Outlet | 98.7 | 214.9 | 15.6 | 199.3 | 441.8 | 134.4 |
| High | 3 | Outlet | 102.8 | 250.9 | 15.7 | 235.2 | 466.8 | 127.2 |
| High | 4 | Outlet | 103.1 | 225.9 | 18.0 | 207.9 | 420.8 | 134.5 |

**RESULTS AND INTERPRETATION, CONTINUED**
***Periphyton and Filamentous Algal Biomass Response to P Dosing***

Periphyton growing on the ceramic tiles responded rapidly to experimental P dosing (Figure 35). There were no differences in treatments on Day 0 (Figure 36). High P treatments had significantly more chlorophyll than Low-P and Controls on Day 14, although Low-P chlorophyll-a was trending higher than the Control. By Day 28, Low P and High P treatments were not different, but had significantly more chlorophyll-a than Controls (Figure 35, 36). The lack of difference between low and high P treatments is consistent with the threshold response observed in the field study.


Figure 35. Comparison of periphyton growing on ceramic tiles among the 3 P treatments on Day 14 (left) and Day 28 (right). Note the obvious difference in color between Conrol and Low/High P treatments, even on Day 14. By Day 28, Low/High P tiles were covered in green algae.

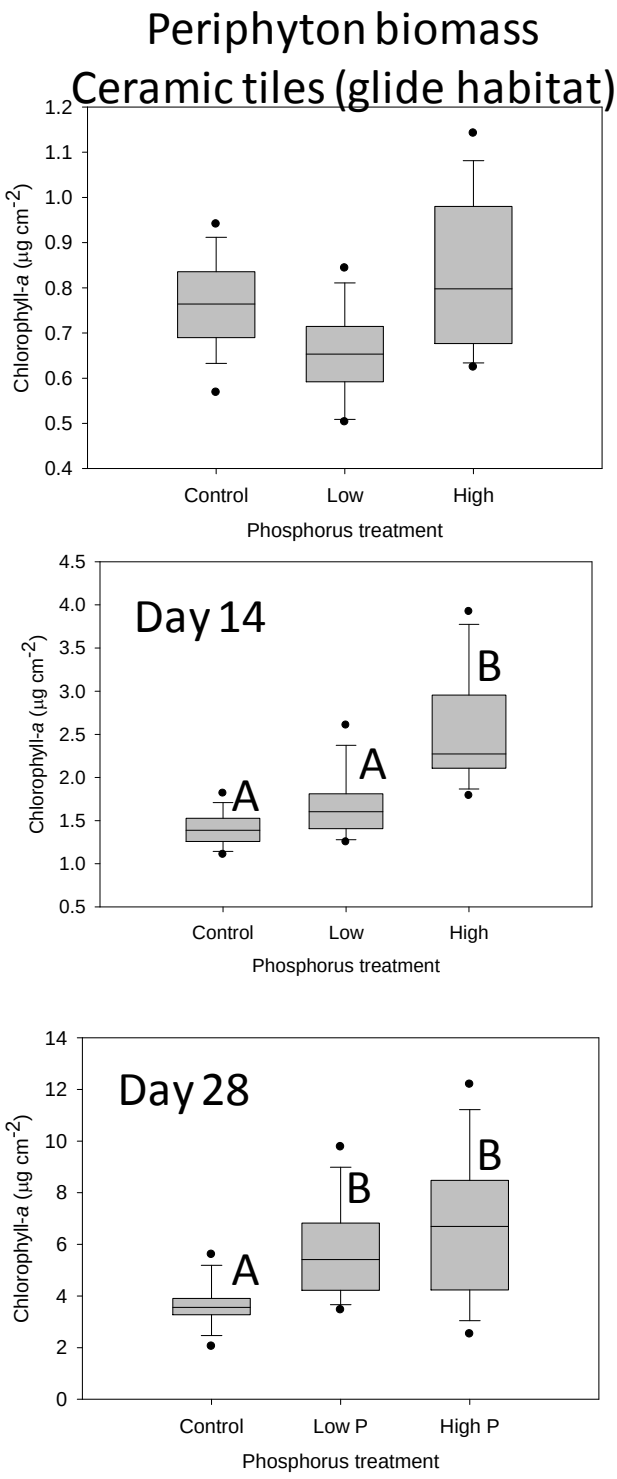



Figure 36. Results of ANOVA comparing mean chlorophyll-a (ug/cm2) among P treatments on Day 0, 14, and 28. Means significantly differed on Day 14 and 28, but not Day 0. Means with the same letter are not different ($p>0.05$).

Periphyton AFDM and chlorophyll-a from the bare rocks and transplanted rocks was highly variable among P treatments and did not differ significantly. However, this was largely due to the very strong response of *Cladophora* (filamentous green algae) in the low and high P treatments. *Cladophora* biomass exploded near the end of the study, and was significantly higher in the Low and High P streams than the Controls on the bare rocks and transplant rocks (ROCK-01, NBOS-03) on Day 28 (Figures 37-40).

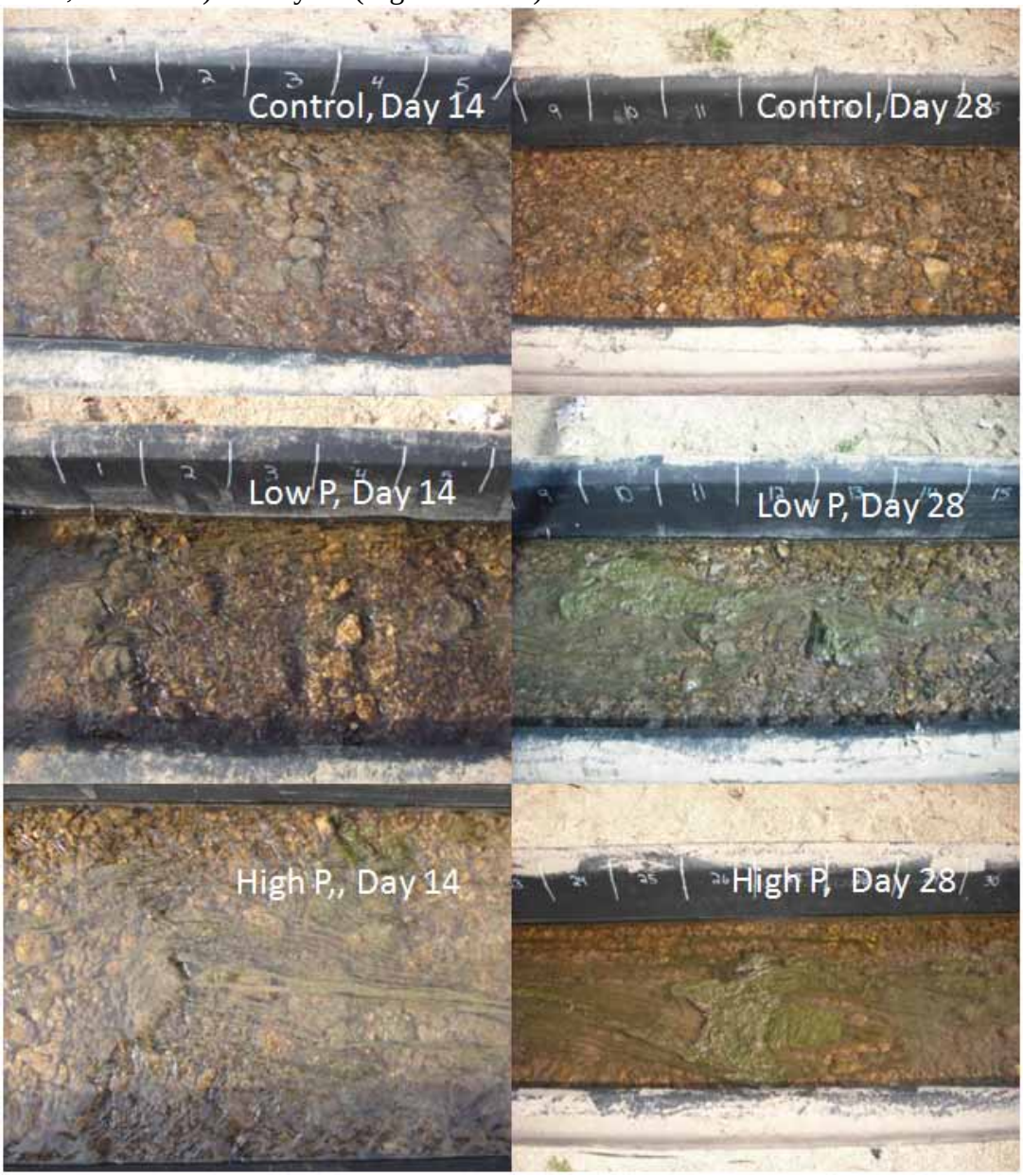



Figure 37. Photographs of riffle sections of Control, Low P, and High P treated experimental streams on Day 14 (left) and Day 28 (right). On Day 14, patches of *Cladophora* had begun to appear in Low and High P streams, but was more visually abundant in the High P streams. By Day 28, Low and High P streams were virtually indistinguishable, both supporting high biomass of *Cladophora*. Controls maintained a golden-brown coating of diatoms on rocks thorughout the study, although some *Cladophora* had begun to appear by Day 28.

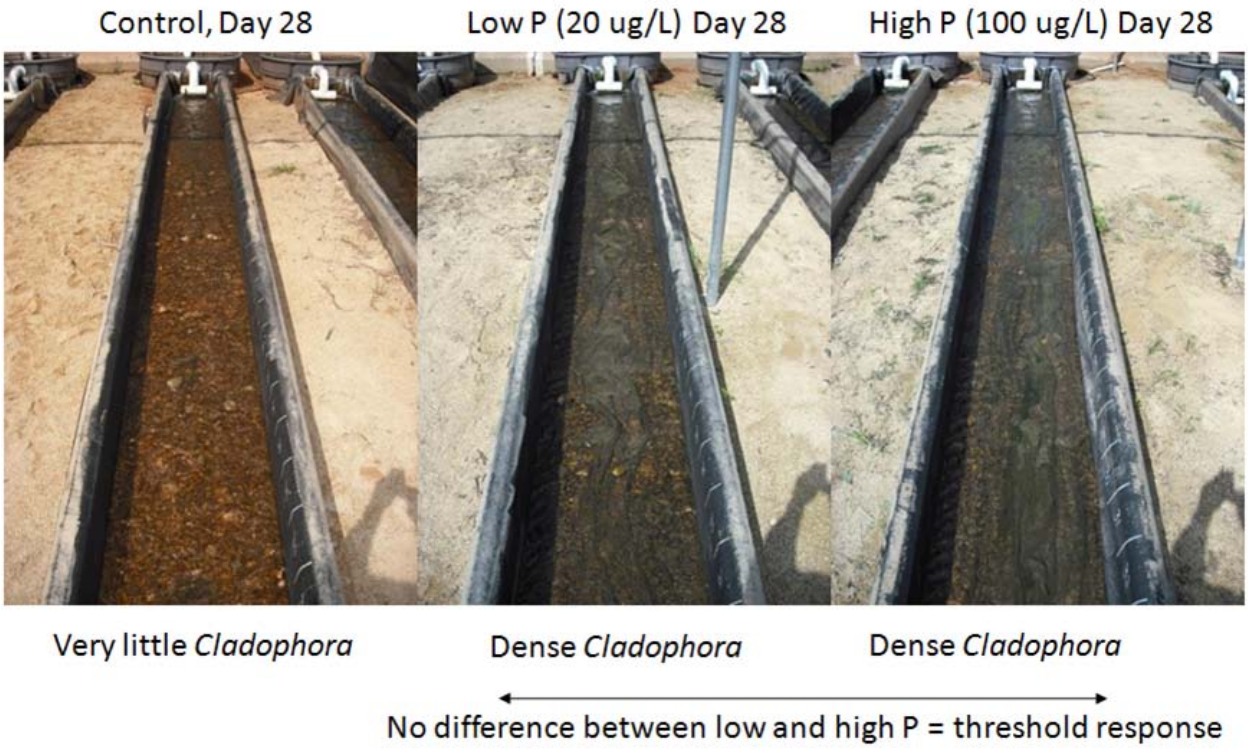



Figure 38. A longitudinal view of Control, Low-P, and High-P streams on Day 28.

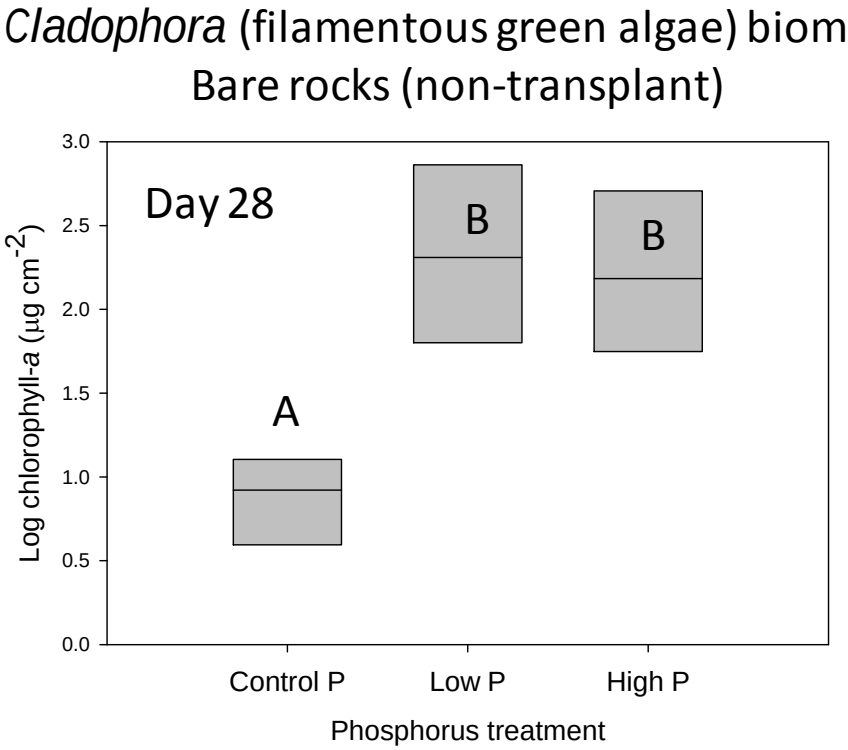



Figure 39. Results of ANOVA comparing mean *Cladophora* chlorophyll-a (ug/cm$^2$) among P treatments on Day 28. Means with the same letter are not different ($p>0.05$).

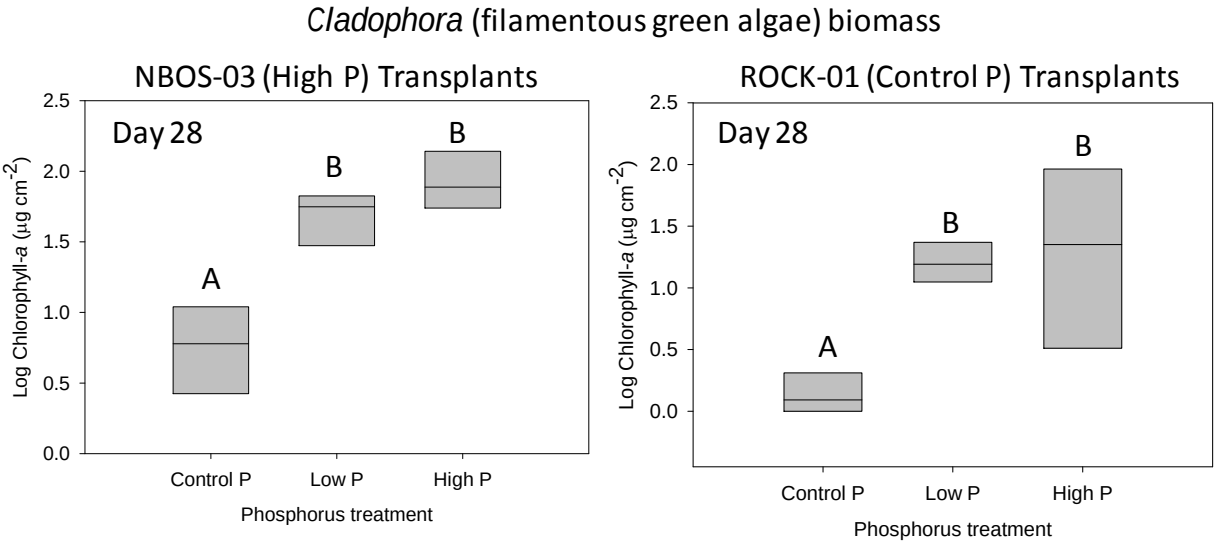



Figure 40. Results of ANOVA comparing mean *Cladophora* chlorophyll-a (ug/cm2) on transplanted rocks (ROCK-01=very low nutrient site, NBOS-03=high nutrient site) among P treatments on Day 28. Means with the same letter are not different ($p>0.05$).

## RESULTS AND INTERPRETATION, CONTINUED

### *Periphyton Nutrient Content Response to Experimental P Dosing*

Periphyton C:P, C:N, and N:P ratios from non-transplant bare rock samples did not differ among streams on Day 0, but differed significantly by treatment on Day 28 (Figure 41). High-P treated streams had the lowest C:P ratio (~150), Control streams had the highest (~320), whereas Low-P streams were intermediate (~230). Even the control C:P ratios were approaching levels deemed to be near or below a C:P threshold in the field study, suggesting that the background PO4-P concentrations were high enough to allow periphyton to sequester much higher amounts of P than typical of the lowest nutrient field sites.

Periphyton C:P ratios from the ROCK-01 transplant rocks responded very strongly to all 3 experimental treatments (Figure 42). On Day 0, ROCK-01 periphyton had C:P ratios above 2,000. After transplanting these rocks into the BEAR streams for just 7 days, mean C:P ratios had dropped to 689, 346, and 215 among the Control, Low P and High P treatments, respectively. By Day 28, ROCK-01-Control C:P ratios had dropped to 250, whereas Low and High P values were near 150 (Figure 43). This illustrated the remarkable affinity of stream periphyton for phosphorus, and provided additional evidence in support of nonlinear uptake of P as the explanation for sharp declines in periphyton C:P with small increases in surface-water TP.

In contrast to the ROCK-01 transplants, the NBOS-03 transplants did not respond to any of the experimental P treatments on Day 7 or 28 (Figure 42, 43), supporting the hypothesis that recycling of P within the periphyton is an important mechanism for maintaining low C:P ratios over time in streams with highly variable surface-water TP. Moreover, it also showed that periphyton from NBOS-03 was already saturated with P and thus did not sequester more P per unit carbon in the low or high P treatments.

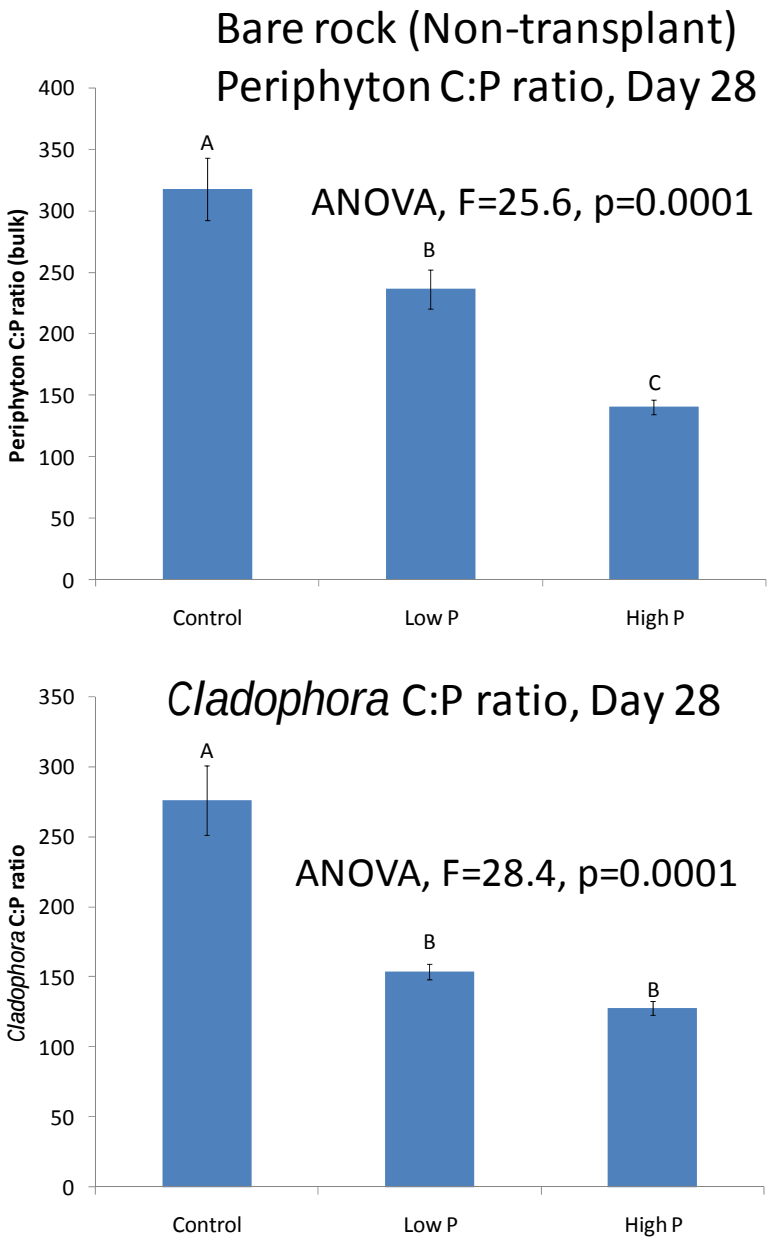



Figure 41. Results of ANOVA comparing mean periphyton C:P ratio (upper panel) and *Cladophora* C:P ratio (lower panel) among P treatments. Means with the same letter are not different (P>0.05).

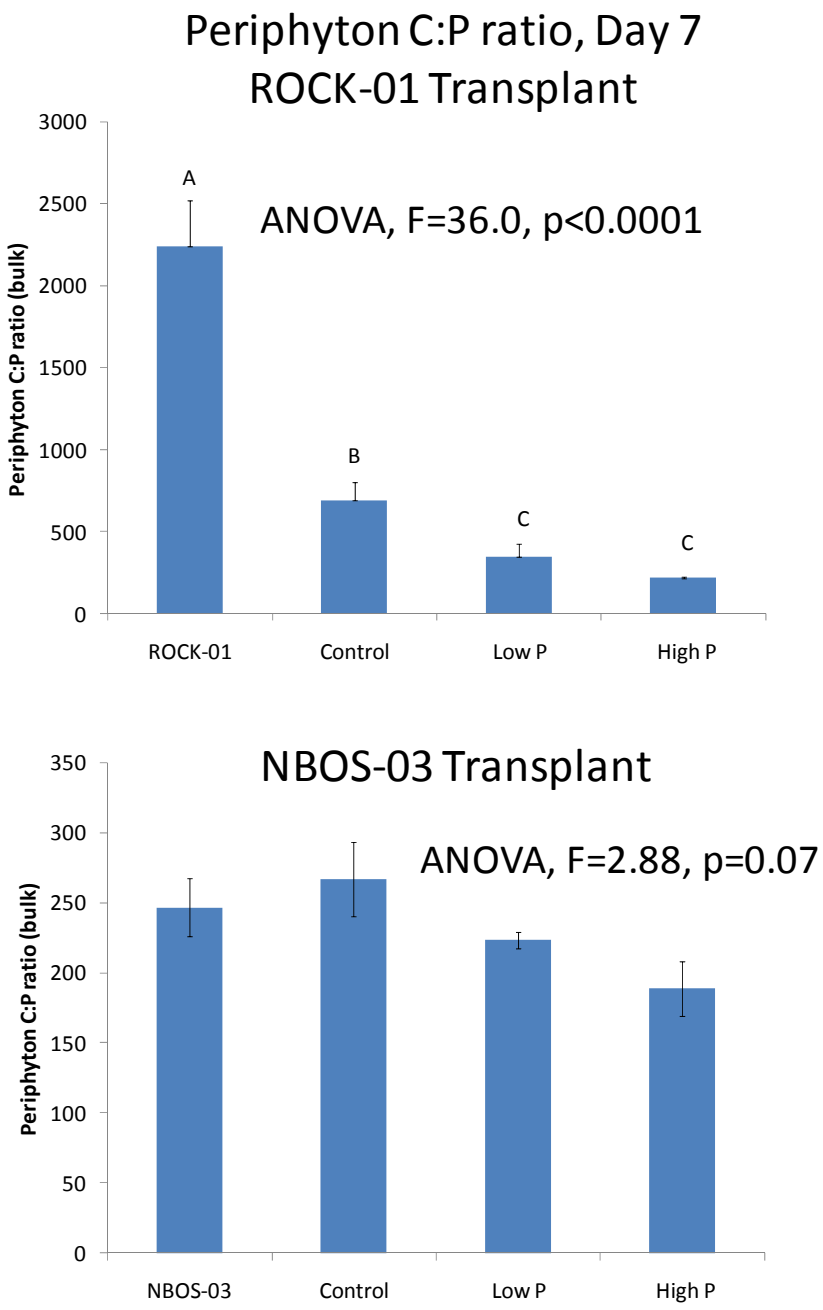



Figure 42. Results from ANOVA comparing periphyton C:P ratios from transplant rocks from ROCK-01 (top panel) and NBOS-03 (lower panel) after 7 days of exposure in the experimental streams. The ROCK-01 (top panel) and NBOS-03 (bottom panel) column corresponds to the mean from 4 composite samples collected on Day 0 of the study (prior to transplanting into the streams). Means with the same letter are not significantly different.

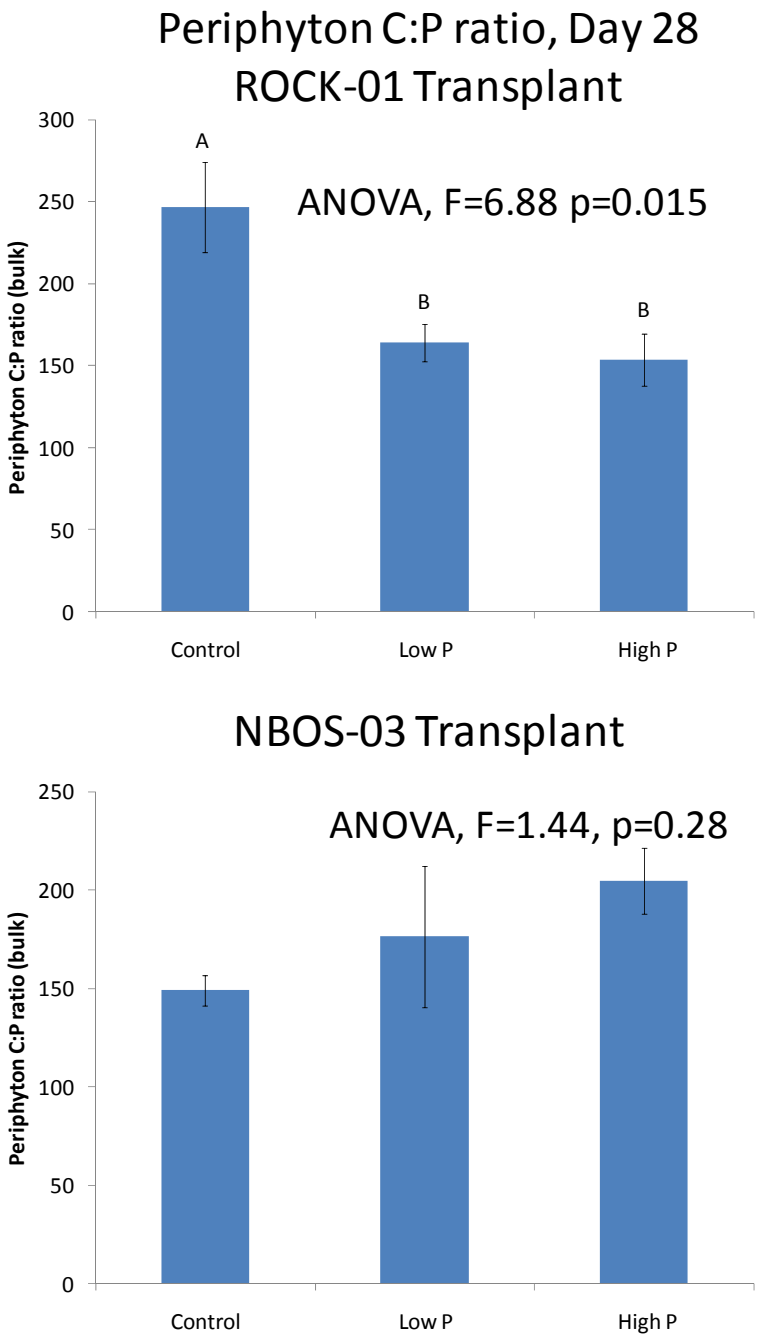



Figure 43. Results from ANOVA comparing periphyton C:P ratios from transplant rocks from ROCK-01 (top panel) and NBOS-03 (lower panel) after 28 days of exposure in the experimental streams. Means with the same letter are not significantly different.

**RESULTS AND INTERPRETATION, CONTINUED**
***Algae Species Responses to Experimental P Dosing***

Nonmetric multidimensional scaling ordination of algal species composition from the 6 intensive field sites in February 2008 and June 2008, and the ROCK-01 and NBOS-03 transplant rocks on Day 0 and Day 28 revealed several interesting responses (Figure 44). ROCK-01 samples from Day 0 were significantly different than the Control, Low P or High P samples on Day 28 (MRPP, $p<0.0001$). Algal species composition at ROCK-01 in February 2008 (prior to the BEAR study) and in June 2008 (after the BEAR study) remained clearly separated from any of the ROCK-01 samples that were transplanted in the BEAR streams.

ROCK-01 Control species composition shifted significantly to the right along nMDS Axis 1 by Day 28, moving in the direction of the NBOS-03 samples and toward field sites with species indicative of moderate to high TP (PALU-01, LEON-02). However, ROCK-01 Low and High P samples shifted significantly farther along this axis (MRPP, $P<0.0032$), indicating changes in species composition in Low and High P treatments were significantly greater than Controls. Consistent with a threshold response, algal species composition in Low and High P treatments did not differ (Figure 44).

In contrast, NBOS-03 rocks shifted in the same direction as the June 2008 NBOS-03 sample, remaining on the high-P end of Axis 1, regardless of P treatment. Algal species composition did not differ among treatments after 28 days of exposure (Figure 44).

Indicator Species Analysis of ROCK-01 transplants from Day 28 revealed that at least 5 diatom species were significantly less abundant (or absent) from Low P and High P samples than Controls, and 2 diatoms were significant indicators of the Low/High P treatments (Table 17). Five of these 7 taxa were identified as significant threshold indicators in response to TP gradients in the field study. All five responded in the same direction (decline, increase) in the BEAR study as in the field study.

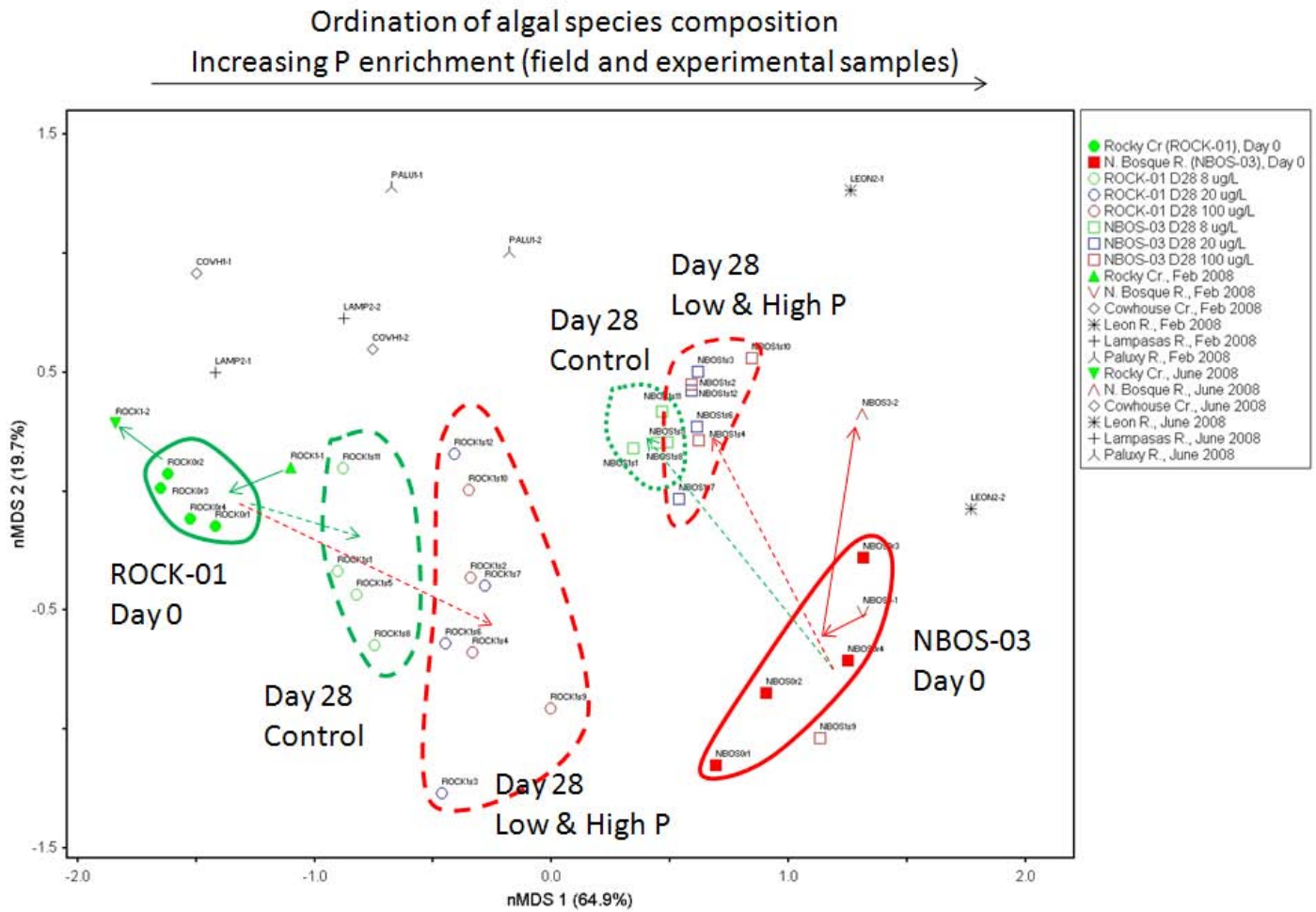



Figure 44. Nonmetric multidimensional scaling ordination of algal species composition from ROCK-01 and NBOS-03 transplant rocks on Day 0 and Day 28. Algal species composition from the 6 intensive field sites during February (before) and June 2008 (after) are also ordinated with the BEAR samples for a frame of reference. Sites sorted from low P sites (left end of Axis 1) to high P sites (right end of Axis 1). All ROCK-01 samples shifted toward NBOS-03 samples after 28 days of exposure in the BEAR facility, but Low and High P treated rocks shifted farther away from the natural stream and were significantly different than Controls. In contrast, NBOS-03 rocks shifted in the same direction as the June 2008 NBOS-03 sample, and algal species composition did not differ among treatments after 28 days of exposure.

Table 17. Results of Indicator Species Analysis (IndVal) using **Control vs. Low/High P** treatments as a predictor of algal species composition growing on ROCK-01 transplanted cobbles at Day 28 of the BEAR experimental stream study. Only taxa with significant IndVal scores are shown below. Taxa that showed significant declines or increases in response to P in both the experimental dosing study and in the field are highlighted in **bold.**

| Taxon ID | Indicator group | Response to experimental PO4-P enrichment | Response to field study TP gradients | IndVal | p |
|---|---|---|---|---|---|
| ACbiasso | Control | **Decline** | **Decline** | 51.8 | 0.0408 |
| AHminuti | Control | **Decline** | **Decline** | 77.6 | 0.0034 |
| CMaffins | Control | Decline | | 88.2 | 0.0022 |
| EYevergl | Control | **Decline** | **Decline** | 100 | 0.0022 |
| MDcircul | Control | Decline | | 100 | 0.0022 |
| CCpedcls | Low/High P | **Increase** | **Increase** | 58.9 | 0.0022 |
| REsinuta | Low/High P | **Increase** | **Increase** | 87.5 | 0.0102 |

**RESULTS AND INTERPRETATION, CONTINUED**
***Macroinvertebrate Community Response to Experimental P Dosing***

Macroinvertebrate taxonomic composition did not differ among treatments on Day 0 or Day 28, although composition shifted significantly between dates (Figure 45). Forty-five taxa were identified from the experimental streams, including 17 genera from the orders Ephemeroptera, Plecoptera, and Trichoptera (Appendix A). The relatively short dosing period, in contrast to the relatively long life cycle of these taxa, necessarily limited the potential response of this diverse group of organisms to P treatments. Future experiments spanning a longer dosing period or focused on the effect of low flow and P interactions may produce more meaningful results in the context of animal responses to experimental P enrichment.

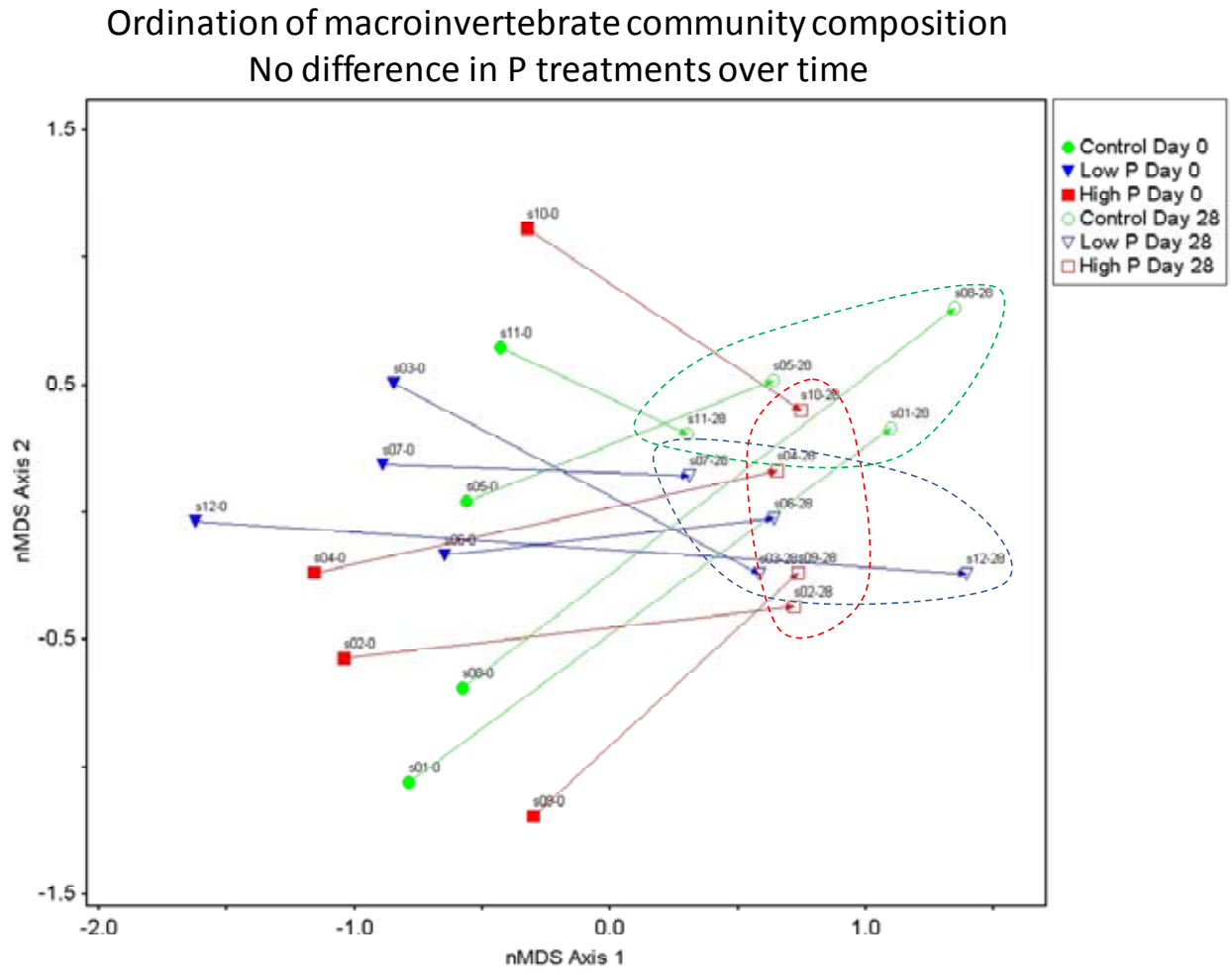



Figure 45. Nonmetric multidimensional scaling ordination of macroinvertebrate community responses to experimental P additions at the BEAR facility, Day 0 and Day 28. Arrows indicate the direction and magnitude of community change within each stream between Day 0 and 28. Ellipses are drawn to envelop all 4 streams by treatment on Day 28. There was extensive overlap among treatments, and community composition did not differ (MRPP, p=0.13).

**SECTION III.**
**CONCLUSIONS AND RECOMMENDATIONS**

Shifts from periphyton communities comprised of sensitive diatoms, calcareous cyanobacteria, and other non-chlorophyll bearing microbes to communities with higher chlorophyll content and more filamentous green algae is highly likely at concentrations of surface-water TP above 20 ug/L. Streams with TP > 200-1000 ug/L likely represent a second tier of degradation, and appear at greater risk for nuisance algal growth based on our threshold analyses. However, results from the P dosing experiment suggest that concentrations as low as 20 ug/L PO4-P can lead to high levels of *Cladophora* biomass in as little as 28 days. Adding more PO4-P (100 ug/L) did not result in more *Cladophora* in a 4-week period. However, faster growth rates were observed in the 100 ug/L treatment, which may explain why field streams with very high levels of TP (>200 ug/L) had greater frequency of heavy growth of filamentous algae.

Aquatic macrophyte cover consistently declined in streams with TP > 25-50 ug/L. These submersed plants serve as important refugia for juvenile fishes and macroinvertebrates, and provide a source of dissolved oxygen during low flows. Their decline likely represents a key structural and functional change in these stream ecosystems.

Minimum dissolved oxygen levels are highly dependent upon an interaction between flow and nutrient enrichment. Our study suggests that TP levels > 20-30 ug/L, coupled with low flows, will cause detrimental declines in minimum dissolved oxygen levels. This is particularly important in the context of minimum flows, a contentious issue in the southwestern USA. It is unlikely that studies geared toward detecting effects of nutrients on DO will adequately characterize this relationship without sampling during periods of low flow when gas exchange is with the atmosphere is very slow. Distance downstream and flow status are two very important considerations when evaluating the influence of WWTP discharges on wadeable streams in semi-arid regions. Future research is needed to better quantify the interaction between minimum flows and biological integrity in streams.

Based on the weight of evidence from the coupling of the field stream study and experimental stream study suggests that streams of the study area are very sensitive to phosphorus enrichment. There is a very high probability that streams exposed to surface-water TP levels exceeding 20 ug/L, and possibly 15 ug/L, will experience a sharp decline in biological integrity, including loss of characteristic structure (periphyton and macrophytes), loss of numerous species (algae and macroinvertebrates), minimum dissolved oxygen levels unsuitable for supporting native fauna during low flows, and increase likelihood of nuisance algal growth that limits recreational use of strreams. Streams exceeding 200 ug/L may represent a second tier of degradation, with more consistent nuisance algal growth and additional losses of algal and macroinvertebrate species.

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Appendix A. List of algal taxa identified in the field and experimental dosing studies. Only taxa that occurred >2 times are included in the table (n=295). Taxonomic identifications were conducted exclusively by Dr. Barbara Winsborough, Winsborough Consulting, Leander, TX.

| TYPE | SPECIES | TAXON_ID |
|---|---|---|
| Diatom | *Achnanthes biassolettiana* | ACbiasso |
| Diatom | *Achnanthes brevipes* | ACbrevip |
| Diatom | *Achnanthes hungarica* | AChungar |
| Diatom | *Adlafia bryophila* | ADbryoph |
| Diatom | *Achnanthidium exiguum* | AHexigum |
| Diatom | *Achnanthidium minutissimum* | AHminuti |
| Diatom | *Amphipleura pellucida* | ALpelluc |
| Diatom | *Amphora bullatoides* | AMbullat |
| Diatom | *Amphora coffeaeformis* | AMcoffea |
| Diatom | *Amphora granulata* | AMgranul |
| Diatom | *Amphora inariensis* | AMinarie |
| Diatom | *Amphora libyca* | AMlibyca |
| Diatom | *Amphora montana* | AMmontan |
| Diatom | *Amphora ovalis* | AMovalis |
| Diatom | *Amphora pediculus* | AMpedcls |
| Diatom | *Amphora veneta* | AMveneta |
| Diatom | *Anomoeoneis sphaerophora* | ANsphaer |
| Diatom | *Aulacoseira ambigua* | AUambig |
| Diatom | *Aulacoseira granulata* | AUgranlt |
| Diatom | *Aulacoseira granulata var. angustissima* | AUgrnang |
| Diatom | *Bacillaria paradoxa* | BApardxa |
| Diatom | *Brachyseira vitrea* | BRvitrea |
| Diatom | *Caloneis bacillum* | CAbacill |
| Diatom | *Campylodiscus clypeus* | CAclypeu |
| Diatom | *Caloneis molaris* | CAmolars |
| Diatom | *Caloneis schumanniana* | CAschuma |
| Diatom | *Caloneis silicula* | CAsilicu |
| Diatom | *Cocconeis pediculus* | CCpedcls |
| Diatom | *Cocconeis placentula* | CCplacen |
| Diatom | *Cymbella cistula* | CMcistul |
| Diatom | *Cymbella cymbiformis* | CMcymbis |
| Diatom | *Encyonema delicatula* | CMdelcat |
| Diatom | *Encyonema elginensis* | CMelgine |
| Diatom | *Cymbella excisa* | CMexcisa |
| Diatom | *Cymbella falaisensis* | CMfalais |
| Diatom | *Cymbella hustedtii* | CMhusted |
| Diatom | *Cymbella kolbei* | CMkolbei |
| Diatom | *Cymbella laevis* | CMlaevis |
| Diatom | *Cymbella tumida* | CMtumida |
| Diatom | *Cyclostephanos tholiformis* | CStholif |

| | | |
|---|---|---|
| Diatom | *Cymatopleura elliptica* | CTellipt |
| Diatom | *Cymatopleura solea* | CTsolea |
| Diatom | *Cyclotella cf. stelligera* | CYcfstel |
| Diatom | *Cyclotella meneghiniana* | CYmenegh |
| Diatom | *Denticula kuetzingii* | DEkuetzi |
| Diatom | *Denticula subtilis* | DEsubtil |
| Diatom | *Diadesmis (Navicula) confervacea* | DIconfer |
| Diatom | *Diploneis ovalis* | DPovalis |
| Diatom | *Diploneis pseudovalis* | DPpsudov |
| Diatom | *Diploneis puella* | DPpuella |
| Diatom | *Encyonema carina* | ECcarina |
| Diatom | *Encyonema minutum* | ECminutu |
| Diatom | *Encyonema silesiacum* | ECsilesi |
| Diatom | *Encyonema triangulum* | ECtriang |
| Diatom | *Epithemia adnata* | EPadnata |
| Diatom | *Epithemia sorex* | EPsorex |
| Diatom | *Epithemia turgida* | EPturgid |
| Diatom | *Eucocconeis (Achnanthes) flexella* | ESflexel |
| Diatom | *Eunotia pectinalis* | EUpectin |
| Diatom | *Eunotia arcus* | EUarcus |
| Diatom | *Encyonopsis cesatii* | EYcesati |
| Diatom | *Eunotia bilunaris* | EUbilun |
| Diatom | *Encyonema (Encyonopsis) evergladianum* | EYevergl |
| Diatom | *Encyonema (Encyonopsis) microcephala* | EYmicroc |
| Diatom | *Navicula (Fallatia) lenzii* | FAlenzii |
| Diatom | *Fallacia litoricola* | FAlitori |
| Diatom | *Fallacia monoculata* | FAmonoc |
| Diatom | *Fallacia pygmaea* | FApygmae |
| Diatom | *Fallatia tenera* | FAtener2 |
| Diatom | *Fragilaria capucina* | FRcapuci |
| Diatom | *Fragilaria elliptica* | FRellptc |
| Diatom | *Fragilaria famelica* | FRfameli |
| Diatom | *Fragilaria nanana* | FRnanana |
| Diatom | *Fragilaria pinnata var. lancettula* | FRpinlan |
| Diatom | *Fragilaria tenera* | FRtenera |
| Diatom | *Geissleria decussis* | GEdecu |
| Diatom | *Gomphosphenia (Gomphonema) lingulatiformis* | GMlinfor |
| Diatom | *Gomphosphenia reicheltii* | GMreicht |
| Diatom | *Gomphonema acuminatum* | GOacumin |
| Diatom | *Gomphonema affine* | GOaffine |
| Diatom | *Gomphonema angustatum (micropus)* | GOangstt |
| Diatom | *Gomphonema angustum* | GOangust |
| Diatom | *Gomphonema clavatum* | GOclavat |
| Diatom | *Gomphonema gracile* | GOgracil |
| Diatom | *Gomphonema insigne* | GOinsign |

| | | |
|---|---|---|
| Diatom | *Gomphonema intricatum* | GOintric |
| Diatom | *Gomphonema intricatum var vibrio* | GOintvib |
| Diatom | *Gomphonema mclaughlinii* | GOmaclau |
| Diatom | *Gomphonema parvulum* | GOparvul |
| Diatom | *Gomphonema pumilum* | GOpumilu |
| Diatom | *Gomphonema rhombicum* | GOrhombi |
| Diatom | *Gomphonema truncatum* | GOtrunca |
| Diatom | *Gomphonema vibrioides* | GOvibdes |
| Diatom | *Gyrosigma nodiferum* | GYnodfrm |
| Diatom | *Gyrosigma obscurum* | GYobscur |
| Diatom | *Gyrosigma spencerii* | GYspence |
| Diatom | *Hantzschia amphioxys* | HAamphio |
| Diatom | *Hippodonta (Navicula) hungarica* | HIhunga |
| Diatom | *Craticula cuspidata* | KCcuspid |
| Diatom | *Craticula (Navicula) halophila* | KChaloph |
| Diatom | *Achnanthes (Lemnicola) hungarica* | LEhungar |
| Diatom | *Luticola goeppertiana* | LUgoept2 |
| Diatom | *Luticola mutica* | LUmutica |
| Diatom | *Meridion circulare* | MDcircul |
| Diatom | *Melosira varians* | MEvarian |
| Diatom | *Mastogloia elliptica* | MSellipt |
| Diatom | *Mastogloia smithii* | MSsmithi |
| Diatom | *Navicula (Mayamaea) atomus* | MYatomus |
| Diatom | *Navicula antonii* | NAantoni |
| Diatom | *Navicula cf. pseudanglica* | NAcfpsan |
| Diatom | *Navicula circumtexta* | NAcirctx |
| Diatom | *Navicula constans* | NAconstn |
| Diatom | *Navicula cryptocephala* | NAcrypto |
| Diatom | *Navicula cryptotenella* | NAcryten |
| Diatom | *Navicula erifuga* | NAerifga |
| Diatom | *Brachyseira neoexilis (Navicula exilis)* | NAexilis |
| Diatom | *Navicula gregaria* | NAgregar |
| Diatom | *Navicula ingenua* | NAingua |
| Diatom | *Navicula integra* | NAintgra |
| Diatom | *Navicula kotschii (texana)* | NAkotsch |
| Diatom | *Navicula leptostriata* | NAleptos |
| Diatom | *Navicula libonensis* | NAlibone |
| Diatom | *Navicula menisculus* | NAmenscl |
| Diatom | *Navicula (Eolima) minima* | NAminima |
| Diatom | *Navicula oblonga* | NAoblong |
| Diatom | *Navicula perminuta* | NApermnt |
| Diatom | *Navicula phyllepta* | NAphylpt |
| Diatom | *Navicula radiosa* | NAradios |
| Diatom | *Navicula recens* | NArecens |
| Diatom | *Navicula rhynchocephala* | NArhynch |

| | | |
|---|---|---|
| Diatom | *Navicula sanctaecrucis* | NAsancru |
| Diatom | *Navicula savannahiana* | NAsavana |
| Diatom | *Navicula schroeteri var escambia* | NAschesc |
| Diatom | *Sellaphora (Navicula) stroemii* | NAstroem |
| Diatom | *Fallatia (Navicula) subminuscula* | NAsubmin |
| Diatom | *Navicula symmetrica* | NAsymtrc |
| Diatom | *Navicula texana (Grimmei)* | NAtexana |
| Diatom | *Navicula tridentula* | NAtriden |
| Diatom | *Navicula trivialis* | NAtrivis |
| Diatom | *Navicula veneta* | NAveneta |
| Diatom | *Navicula viridula var. rostellata* | NAvirdla |
| Diatom | *Neidium iridis* | NEiridis |
| Diatom | *Nitzschia acuminata* | NIacuata |
| Diatom | *Nitzschia amphibia* | NIamphib |
| Diatom | *Nitzschia amphibioides* | NIampoid |
| Diatom | *Nitzschia angustatula* | NIangtu |
| Diatom | *Nitzschia angustata* | NIangust |
| Diatom | *Nitzschia brevissima* | NIbrevis |
| Diatom | *Nitzschia calida* | NIcalida |
| Diatom | *Nitzschia compressa var. balatonis* | NIcombal |
| Diatom | *Nitzschia constricta* | NIcstric |
| Diatom | *Nitzschia debilis* | NIdibili |
| Diatom | *Nitzschia dissipata* | NIdissip |
| Diatom | *Nitzschia filiformis* | NIfilifr |
| Diatom | *Nitzschia fonticola* | NIfontic |
| Diatom | *Nitzschia frustulum* | NIfrustu |
| Diatom | *Nitzschia inconspicua* | NIincons |
| Diatom | *Nitzschia linearis* | NIlinear |
| Diatom | *Nitzschia obtusa* | NIobtusa |
| Diatom | *Nitzschia palea* | NIpalea |
| Diatom | *Nitzschia serpentiraphe* | NIserpen |
| Diatom | *Nitzschia sigmoidea* | NIsigdea |
| Diatom | *Nitzschia sigma* | NIsigma |
| Diatom | *Nitzschia sinuata v delognii* | NIsinde |
| Diatom | *Nitzschia solita* | NIsolita |
| Diatom | *Nitzschia subacicularis* | NIsubaci |
| Diatom | *Nitzschia tropica* | NItropic |
| Diatom | *Nitzschia tryblionella* | NItrybla |
| Diatom | *Nitzschia vitrea* | NIvitrea |
| Diatom | *Pseudostaurosira brevistriata* | PDbrevis |
| Diatom | *Pseudostaurosira parasitica var. subconstricta* | PDparsub |
| Diatom | *Plagiotropis lepidoptera* | PGlepidp |
| Diatom | *Pinnularia acrosphaeria* | PIacro |
| Diatom | *Pinnularia gibba* | PIgibba |
| Diatom | *Pinnularia microstauron* | PImicros |

| | | |
|---|---|---|
| Diatom | *Pinnularia streptoraphe* | PIstrept |
| Diatom | *Pleurosira (Ceratulina) laevis* | PRlaevis |
| Diatom | *Achnanthes (Planothidium) lanceolata* | PTlanceo |
| Diatom | *Reimeria sinuata* | REsinuta |
| Diatom | *Rhoicosphenia abbreviata* | ROabbre |
| Diatom | *Rhopalodia brebissonii* | RPbrebsn |
| Diatom | *Rhopalodia gibba* | RPgibba |
| Diatom | *Rhopalodia musculus* | RPmuscul |
| Diatom | *Sellaphora laevissima* | SFlaevis |
| Diatom | *Sellaphora pupula* | SFpupula |
| Diatom | *Sellaphora seminulum* | SFseminu |
| Diatom | *Fragilaria (Staurosira) construens* | SRconstr |
| Diatom | *Fragilaria construens var. venter* | SRconven |
| Diatom | *Stauroneis phoenicentron* | SSphoeni |
| Diatom | *Stephanodiscus sp1* | ST1 |
| Diatom | *Surirella angusta* | SUangust |
| Diatom | *Surirella bifrons* | SUbifron |
| Diatom | *Surirella brebissonii* | SUbreb |
| Diatom | *Surirella elegans* | SUelegan |
| Diatom | *Surirella minuta* | SUminuta |
| Diatom | *Surirella ovalis* | SUovalis |
| Diatom | *Surirella spiralis* | SUspiral |
| Diatom | *Surirella splendida* | SUsplen |
| Diatom | *Synedra (Fragilaria) acus* | SYacus |
| Diatom | *Synedra goulardi* | SYgoular |
| Diatom | *Synedra (Fragilaria) ulna* | SYulna |
| Diatom | *Fragilaria (Tabularia) fasciculata* | TBfascic |
| Diatom | *Terpsinoe musica* | TEmusica |
| Diatom | *Thalassiosira weissflogii* | THweiss |
| Diatom | *Tryblionella apiculata* | TYapicul |
| Diatom | *Tryblionella (Nitzschia) calida* | TYcaldid |
| Diatom | *Nitzschia (Tryblionella) levidensis* | TYlevid |
| Soft | *Aphanothece sp.* | AFCsp |
| Soft | *Aphanizomenon sp.* | AFNsp |
| Soft | *Aphanocapsa sp.* | AFPsp |
| Soft | *Aphanochaete sp.* | AFTsp |
| Soft | *Anabaena sp.* | ANBsp |
| Soft | *Ankistrodesmus falcatus* | ANKfalca |
| Soft | *Audouinella hermannii* | AUDhernn |
| Soft | *Blennothrix sp.* | BLNsp |
| Soft | *Bulbochaete sp.* | BULsp |
| Soft | *Calothrix sp.* | CALsp |
| Soft | *Chlorococcum sp.* | CHCOAUL |
| Soft | *Chlamydomonas sp.* | CHLsp |
| Soft | *Chlorophytan zoospores* | CHLzoo |

| | | |
|---|---|---|
| Soft | *Chroococcus sp.* | CHOsp |
| Soft | *Characium sp.* | CHRsp |
| Soft | *Chaetophora sp.* | CHTsp |
| Soft | *Cladophora glomerata* | CLAglomer |
| Soft | *Chlorobotrys simplex* | CLBsimp |
| Soft | *Closterium sp* | CLOsp2 |
| Soft | *Coelastrum sp.* | COEsp |
| Soft | *Coelosphaerium sp.* | COHsp |
| Soft | *Cosmarium botrytis* | COSbotry |
| Soft | *Cosmarium galeritum* | COSgaler |
| Soft | *Cosmarium sp.* | COSsp |
| Soft | *Crucigenia sp.* | CRUsp |
| Soft | *Cryptomonas sp.* | CRYsp |
| Soft | *Dictyosphaerium sp.* | DICsp |
| Soft | Unidentified dinoflagellates | DINuid |
| Soft | *Euastrum binale* | EUAbinal |
| Soft | *Euglena sp.* | EUGsp |
| Soft | *Gloeocystis sp.* | GLCsp |
| Soft | *Gloeothece sp.* | GLHsp |
| Soft | *Gloeoskene turfosa* | GLKturf |
| Soft | *Gloeocapsa sp.* | GLOsp |
| Soft | *Gongrosira sp.* | GOGsp |
| Soft | *Golenkinia sp.* | GOLsp |
| Soft | *Gomphosphaeria sp.* | GOMsp |
| Soft | *Homoeothrix sp.* | HOMsp |
| Soft | *Hormidium sp.* | HORsp |
| Soft | *Kirchneriella sp.* | KIRsp |
| Soft | *Kirchneriella obesa* | KIRobesa |
| Soft | *Merismopedia glauca* | MERglauc |
| Soft | *Micractinium sp.* | MIAsp |
| Soft | *Microcystis sp* | MICsp |
| Soft | *Mougeotia sp.* | MOUsp |
| Soft | *Oedogonium sp.* | OEDsp |
| Soft | *Oocystis sp.* | OOCsp |
| Soft | *Ophiocytium capitatum* | OPHcapit |
| Soft | *Oscillatoria sp.* | OSCsp |
| Soft | *Pandorina morum* | PANmorum |
| Soft | *Pediastrum boryanum* | PEDboryn |
| Soft | *Pediastrum duplex* | PEDduplx |
| Soft | *Pediastrum simplex* | PEDsimpl |
| Soft | *Peridinium sp.* | PERsp |
| Soft | *Phacus sp.* | PHAsp |
| Soft | *Phormidium sp* | PHOsp |
| Soft | *Raphidiopsis curvata* | RAPcurvt |
| Soft | *Scenedesmus abundans* | SCEabund |

| | | |
|---|---|---|
| Soft | *Scenedesmus acuminatus* | SCEacumn |
| Soft | *Scenedesmus acutiformis* | SCEacutf |
| Soft | *Scenedesmus armatus* | SCEarmat |
| Soft | *Scenedesmus bijuga var. alternans* | SCEbialt |
| Soft | *Scenedesmus bijuga* | SCEbijug |
| Soft | *Scenedesmus dimorphus* | SCEdimor |
| Soft | *Scenedesmus quadricauda* | SCEquadr |
| Soft | *Scenedesmus sp.* | SCEsp |
| Soft | *Schroderia setigera* | SCRsetig |
| Soft | *Scytonema sp.* | SCYsp |
| Soft | *Schizothrix sp.* | SCZsp |
| Soft | *Sphaerocystis schroeteri* | SPHschro |
| Soft | *Sphaerocystis sp.* | SPHsp |
| Soft | *Spirogyra sp.* | SPIsp |
| Soft | *Spirulina sp.* | SPLsp |
| Soft | *Spondylosium pulchrum* | SPOpulch |
| Soft | *Staurastrum sp.* | STAsp |
| Soft | *Synechococcus sp.* | SYCsp |
| Soft | *Tetraedron caudatum* | TETcaudt |
| Soft | *Tetraedron minimum* | TETminum |
| Soft | *Tetraedron trigonum* | TETtrign |
| Soft | *Trachelomonas sp.* | TRAsp |
| Soft | Centric diatoms | Uncent |
| Soft | Pennate diatoms | Unpennt |
| Soft | Unknown Cyanophyte coccoid | XBGcoc |
| Soft | Unknown Cyanophyte filament | XBGfilam |
| Soft | Unidentified dinoflagellates | XDFalga |
| Soft | Unknown alga sp. | XXAsp |
| Soft | *Zygnema sp.* | ZYGsp |

Appendix A,continued List of dominant macroinvertebrate taxa identified in the BEAR experimental streams, Day 0 and 28, including several **EPT (Ephemeroptera, Trichoptera, Plecoptera) taxa (bold).**

*Argia*
***Baetis***
***Baetodes***
*Berosus*
*Brechmorhoga*
***Caenis***
***Camelobaetidius***
***Cheumatopsyche***
***Chimarra***
Chironomidae
*Chironomus*
*Coptotmus*
*Erpetogomphus*
Erpobdellidae
*Gammarus*
*Gyrinus*
*Haematopota*
***Helicopsyche***
*Hetaerina*
Hydracarina
*Hydrocanthus*
***Hydroperla***
***Hydropsyche***
***Isonychia***
*Lutrochus*
***Macaffertium***
*Micromenetus*
Naididae
***Neochoroterpes***
***Oecetis***
***Perlesta***
*Petrophila*
*Physella*
*Simulium*
Sphaeriidae
*Stenelmis*
***Stenonema***
***Thraulodes***
*Trichocorixa*
***Tricorythodes***
Turbellaria
Veliidae

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# Integrating Bioassessment and Ecological Risk Assessment: An Approach to Developing Numerical Water-Quality Criteria

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ABSTRACT / ioassessment is used worldwide to monitor aquatic health but is infrequently used with risk-assessment objectives, such as supporting the development of defensible, numerical water-quality criteria. To this end, we present a generalized approach for detecting potential ecological thresholds using assemblage-level attributes and a multimetric index (Index of Biological Integrity—IBI) as endpoints in response to numerical changes in water quality. To illustrate the approach, we used existing macroinvertebrate and surface-water total phosphorus (TP) datasets from an observed P gradient and a P-dosing experiment in wetlands of the south Florida coastal plain nutrient ecoregion. Ten assemblage attributes were identified as potential metrics using the observational data, and five were validated in the experiment. These five core metrics were subjected individually and as an aggregated Nutrient–IBI to nonparametric changepoint analysis (nCPA) to estimate cumulative probabilities of a threshold response to TP. Threshold responses were evident for all metrics and the IBI, and were repeatable through time. Results from the observed gradient indicated that a threshold was ≥50% probable between 12.6 and 19.4 μg/L TP for individual metrics and 14.8 μg/L TP for the IBI. Results from the P-dosing experiment revealed ≥50% probability of a response between 11.2 and 13.0 μg/L TP for the metrics and 12.3 μg/L TP for the IBI. Uncertainty analysis indicated a low (typically ≥5%) probability that an IBI threshold occurred at ≤ 10 μg/L TP, while there was ≥95% certainty that the threshold was ≤ 17 μg/L TP. The weight-of-evidence produced from these analyses implies that a TP concentration > 12–15 μg/L is likely to cause degradation of macroinvertebrate assemblage structure and function, a reflection of biological integrity, in the study area. This finding may assist in the development of a numerical water-quality criterion for TP in this ecoregion, and illustrates the utility of bioassessment to environmental decision-making.

Bioassessment has become a widely accepted technique for monitoring aquatic health in streams, lakes, and wetlands throughout the world (Rosenberg and Resh 1993). Bioassessment has a long history in Europe (reviewed by Cairns and Pratt 1993) and has more recently become popular in North America, largely in response to the mandate of §101(a) of the Clean Water Act (CWA) to restore and maintain the biological integrity of the USA's waters (Karr 1981). One bioassessment approach that has received considerable attention in the USA is the multimetric approach (sensu Karr 1981). Multimetric indices, such as the Index of Biological Integrity (e.g., Karr and Chu 1997), are an aggregation of a suite of biological attributes that represent key elements of structure or function of an aquatic assemblage and show a consistent, predictable response to human influence. The strength of multimetric assessments lies in their ability to integrate multiple facets of biological condition (Barbour and others 1995), and thus provide an overall indication of biological integrity (Karr and Dudley 1981; Angermeier and Karr 1994).

One potentially important but underutilized application of multimetric bioassessment is supporting the development of numerical water-quality criteria (Miltner and Rankin 1998; Dodds and Welch 2000). The premise of bioassessment is that resident biota in a water body are natural integrators of environmental conditions and thus can reveal the effects of episodic changes in water quality as well as cumulative pollution (Rosenberg and Resh 1993). Nevertheless, development of water-quality criteria has historically been based on laboratory tests on individual species or solely on chemical endpoints without accounting for the assemblage-level consequences (Barbour and others 2000). The United States Environmental Protection

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Agency (USEPA) has recognized the shortcomings of this former approach and its inconsistency with goals of the CWA (USEPA 1998a). In response, the USEPA has issued a comprehensive plan for the development of scientifically defensible, numerical water-quality criteria. The plan emphasizes the need for the inclusion of assemblage-level endpoints in criteria development, and that the criteria need to be stratified into different regions and types of water bodies (USEPA 1998a). Metrics used in bioassessment may be well suited for this purpose.

Here we extend the multimetric bioassessment concept to directly supporting the development of numerical water quality criteria (Barbour and others 1995). Unlike traditional multimetric approaches, which are based primarily on observational data, our approach relies on a coupling of observational and experimental datasets to elucidate potential cause-effect linkages (e.g., Daehler and Strong 1996; Lemly and Richardson 1997; Beyers 1998; Adams and Greeley 2000). This approach allows the development of metrics that are diagnostic and stressor-specific, a limitation of most bioassessment techniques in use today. For example, multimetric indexes have historically been developed along gradients of general types of human influence (e.g., urban land-use) over a broad geographic area (Karr and Chu 1997). While the description of biotic responses to general disturbance is useful for assessing status and trends of aquatic health, these assessments were not developed to characterize the effects of specific stressors on biological endpoints (Norton and others 2000; USEPA 2000b; Griffith and others 2001). Thus, traditional multimetric indexes have a limited capacity to diagnose causes of impairment or estimate the risk associated with a stressor (Suter 2001). Therefore, our goal was to identify biological attributes that responded to a specific stressor in a specific region and water body type. These attributes would serve as measurement endpoints to estimate levels of a stressor that may result in a high risk of degradation to biological integrity (USEPA 1998b).

To illustrate the approach, we estimated levels of surface-water total phosphorus (TP) that affected macroinvertebrate assemblages in wetlands of a nutrient-sensitive ecoregion using existing, published datasets (King and Richardson 2002; Qian and others, in press; King and Richardson, in press). We defined macroinvertebrate structure and function as our assessment endpoint, assemblage attributes as measurement endpoints, and TP as the stressor—however, any biological endpoint or stressor of concern could be substituted. Ultimately, the broad objective of this paper is to show how assemblage-level data can be used in a risk-based framework to quantify potential ecological thresholds, which, in turn, can be used to support environmental decision-making.

## Methods

### Study Area

Data used for this study were collected in Water Conservation Area 2A (WCA-2A) in the northern Everglades of Florida, USA (26° 15' N, 80° 23' W). WCA-2A is located in the south Florida coastal plain nutrient ecoregion, an area considered P-sensitive by USEPA (2000a). WCA-2A is a 43,280 ha diked wetland landscape, with water-control structures governing the inflow and outflow of surface water. Inflow primarily occurs along the northern levee through three water-control structures (S10-A, C, and D) on the Hillsboro Canal, a conduit for outflow from Lake Okeechobee and P-enriched runoff from the Everglades Agricultural Area (EAA). Inflow from the Hillsboro Canal has induced a steep longitudinal eutrophication gradient in WCA-2A due primarily to excessive inputs of P (SFWMD 1992). Surface-water and soil P has been shown to be elevated above natural, background concentrations up to 7 km into the interior of WCA-2A (e.g., DeBusk and others 1994; McCormick and others 1996; SFWMD 2000). TP in these interior, reference areas of WCA-2A typically ranges between 5–10 μg/L, while often exceeding 100 μg/L in areas near inflow structures on the Hillsboro Canal (Vaithiyanathan and Richardson 1998; SFWMD 2000). Maps and greater detail about physical and chemical characteristics of the study area are provided in Davis and Ogden (1994), Richardson and others (1999), SFWMD (2000), and King and Richardson (2002).

### Observational Data

The first dataset was observational and collected along a 10-km TP gradient in WCA-2A by King and Richardson (2002). In this study, 126 stations were sampled for surface-water TP (μg/L) and macroinvertebrate assemblage composition (density of taxa). Sampling stations extended from a highly impacted region near the canal inflow structures into the interior of WCA-2A, which was defined as a reference area (e.g., SFWMD 2000; King and Richardson 2002). For this analysis, we only used stations located in open-water sloughs ($n$ = 37) to reduce variability associated with different habitats and because the experimental data also were limited to sloughs.

Surface-water TP sample collection, sample storage, and analysis (external standards, blanks, spikes) were in

accordance with QA/QC protocols mandated by the Florida Department of Environmental Protection and standard methods (APHA 1992). Due to the large spatial extent of this study, TP was collected only once during October 1998. TP concentrations at slough stations ranged from 4.5 to 50.4 μg/L, consistent with long-term observations for sloughs along this P gradient (SFWMD 2000).

Macroinvertebrate sampling and sample processing were based on a slight modification of protocols used by FDEP (1996; SOP #BA-7, 8) and USEPA (1997b; Barbour and others 1999). A D-framed dip net was used to collect a 1.5-$m^2$ composite sample from each station. Sampling was conducted in October of 1998, simultaneous with TP collections. Macroinvertebrates were identified to the lowest practical taxonomic level (usually species), and data were expressed as number of individuals/$m^2$. A total of 202 taxa from 37 samples were included in the slough-station dataset. Greater detail on methods is presented in King and Richardson (2002).

### Experimental Data

The experimental dataset was obtained from a P-dosing study in the interior, reference area of WCA-2A where TP concentrations average $< 10$ μg/L (Vaithiyanathan and Richardson 1998; Richardson and others 2000; King and Richardson, in press; Qian and others, in press). Two P-dosing sites, each with six mesocosms (12 mesocosms in total), were constructed in adjacent open-water sloughs in 1992. Mesocosms were 2-m wide and 8-m long flumes and were constructed around natural, undisturbed slough habitat. Mesocosms were oriented parallel to surface-water flow and closed at the upstream end. P was dosed from the closed end of each mesocosm downstream toward the open end. P was dosed in the form of soluble reactive phosphate (SRP) continuously from 1992–1998. Each flume was assigned one of six P treatments, ranging from walled and unwalled control treatments (no P added above background concentrations; 0.25 g/$m^2$/y TP) up to 8.2 g/$m^2$/y P. This design created experimental P gradients both among and within mesocosms (i.e., gradients in concentrations down the length of each flume due to uptake and dilution).

Sampling stations were established at positions 2, 4, and 6 m down the length of each mesocosm (36 sampling stations in total). Thus, measured P concentrations at stations were a product of physical, biogeochemical, and biological factors that resulted from different, controlled input concentrations, just as along the P gradient (Richardson and others 2000). For this analysis, this was desirable because our research question specifically dealt with estimating an ecological threshold based on a measured concentration of TP in surface waters, as mandated by the Everglades Forever Act (1994) and USEPA (2000a). Because each station had unique TP and macroinvertebrate data associated with it and spatial autocorrelation among stations was minimal (King and Richardson, in press), stations could validly be considered independent observations (Hurlbert 1984).

Surface-water TP was collected biweekly at each sampling station throughout the majority of the six-year experiment following QA/QC protocols used in the observational gradient study. Because there were many observations from each station, TP data were expressed as geometric means in accordance with the Everglades Forever Act (EFA 1994) and USEPA guidelines (2000a). Geometric means were calculated for a six-month period prior to each macroinvertebrate collection, and provided an integrated estimate of long-term TP exposure at each station (note, however, that USEPA [1998b] recommends arithmetic means for describing chronic exposure, thus geometric means were conservative estimates of TP exposure). Geometric means of TP ranged from 5.8 to 60.9 μg/L among stations, very similar to the range of values observed along the P gradient.

Macroinvertebrates were collected four times during 1996–1998 (September 1996, January 1997, February 1998, September 1998) at each station using FDEP protocols (1996; SOP #BA-13). Macroinvertebrates were collected using Hester-Dendy (HD) artificial substrates because active sampling methods (e.g., dip nets) would have significantly disturbed the habitat in the experimental mesocosms. King (2001) demonstrated that HD samples were effective for characterizing the macroinvertebrate assemblage of Everglades sloughs. In addition, assemblage attributes evaluated in the experiment were required to respond in the same manner (increase or decrease and temporal repeatability) to TP as those measured along the observed gradient to be considered metrics. Moreover, we were not interested in comparing the absolute values of attributes between the two studies; rather, we were interested in the levels of TP that elicited changes in attribute values, which is completely independent of any potential differences in the magnitudes of attribute values. Thus, any biases associated with differences in sampling methods between the two studies were eliminated because attributes selected as metrics were demonstrated to show the same response using both methods.

A composite of three HD samples were collected from each station ($n = 36$) on each date ($n = 4$). Macroinvertebrates were identified to the lowest prac-

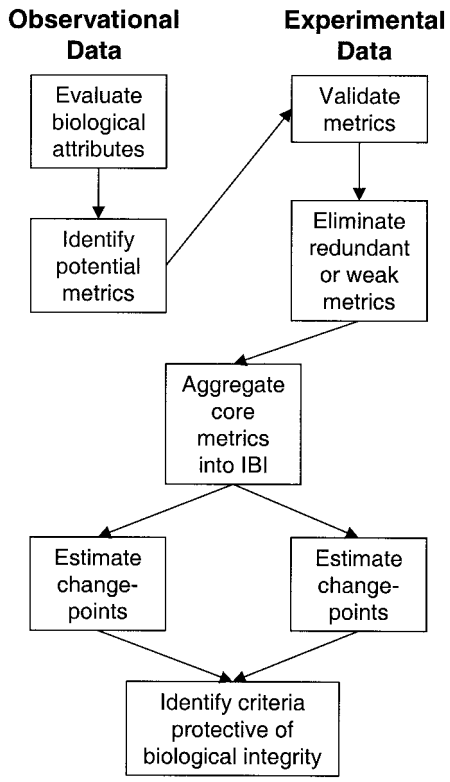


**Figure 1.** Conceptual framework for developing numerical water-quality criteria using bioassessment.

tical taxonomic level (usually species), and expressed as number of individuals/$m^2$. A total of 123 taxa from 144 samples were included in the experimental dataset. Comparisons of macroinvertebrate assemblage composition between unwalled and walled control mesocosms showed that composition in the walled controls was not different than the unwalled controls (King and Richardson, in press). Thus, the experiment was representative of the reference condition. Greater detail on the P-dosing experiment is provided in Richardson and others (2000), King (2001), and King and Richardson (in press).

### Metric Development and Analytical Approach

Our approach was patterned after the conceptual framework of multimetric development outlined by Barbour and others (1995). Our initial step (Step 1) was to select a suite of assemblage-level attributes and use the observational data to evaluate the response of these attributes to TP (Figure 1). We supposed that if attributes did not exhibit a response in the "real" world, then these should not be tested experimentally (Daehler and Strong 1996; Lemly and Richardson 1997; Adams and Greeley 2000). Thus, Step 2 was the identification of a suite of *candidate* metrics, which would then be scrutinized more fully using the experimental data. Attributes that met several selection criteria using the experimental data were subsequently validated as TP metrics (Steps 3 and 4). Selected metrics were aggregated into an IBI-type multimetric index, which we termed a *Nutrient-IBI*, in addition to being assigned as individual biological endpoints for analysis (Step 5). Data from both the observational and experimental studies were then analyzed using changepoint analysis to estimate levels of TP that could be expected to change biological condition (Step 6). We defined a detectable change in the mean and/or variance of an attribute of macroinvertebrate structure and function, coupled with uncertainty estimates, as an indication of an ecological threshold response to TP. Because our data spanned observed and experimental gradients from reference conditions (TP $< 10$ μg/L) to highly P-enriched conditions, we argued that such changes represented a significant deflection from the reference condition, and consequently, degradation of biological integrity. This argument was also consistent with the Everglades Forever Act (1994), which mandated that a TP criterion for this region should not result in an imbalance of flora and fauna representative of the natural Everglades. Thus, in the final step (Step 7) we synthesized results from the changepoint analysis to identify levels of TP that were likely to be protective of biological integrity, as reflected by the metrics of macroinvertebrate structure and function.

*Step 1. Select assemblage attributes.* The first step toward metric evaluation was to select a variety of attributes that represented key elements of the structure and function of macroinvertebrate assemblages found in the reference area of the observed P gradient. Attributes were selected from four general classes: (1) taxonomic composition, (2) species richness and diversity, (3) tolerance/intolerance, and (4) trophic structure (Barbour and others 1999). In all, over 50 attributes were selected, with the majority falling under the taxonomic composition category. As recommended by Barbour and others (1995), we used relative (percent) rather than absolute abundance in calculating attributes except those of richness/diversity because percentage metrics have been shown to be more robust and reliable and were more likely to reflect structural changes resulting from nutrients. Barbour and others (1999) provided a summary of potential benthic metrics, which helped direct our selection process.

Composition attributes, expressed as percent of total numerical abundance, were selected according to the dominant major taxonomic groups present in the study, which corresponded to families (e.g., percent

Chironomidae), orders or classes (e.g., percent Odonata), or a combination of higher groups with relatively similar habits or food preferences (e.g., percent Microcrustacea).

An additional composition attribute was Bray-Curtis dissimilarity (BCD, percent dissimilarity), a coefficient shown to be a robust and ecologically interpretable index of changes in taxonomic composition (Faith and others 1987; Legendre and Legendre 1998). BCD was calculated using the taxa ($n$ = 202) abundance data (standardized using $\log_{10}(x + 1)$ transformation; Legendre and Legendre 1998). Because it is multivariate, BCD was ordinated using nonmetric multidimensional scaling (nMDS), rotated using varimax rotation, and extracted as univariate scores along nMDS Axis 1 (McCune and others 1997; Legendre and Legendre 1998). The objective in the use of nMDS was to recover a multivariate assemblage pattern that corresponded to a gradient in TP concentration, and to produce individual sample scores that could be used for analysis.

Richness and diversity attributes included total number of taxa (richness per unit area, or areal richness; Larsen and Herlihy 1998), numerical richness (total number of taxa per 300 individuals, or NR300; Larsen and Herlihy 1998), Shannon-Wiener diversity ($H'$), and number of taxa belonging to several major taxonomic groups (e.g., number of taxa of Chironomidae).

Tolerance/intolerance attributes were derived using a list of taxa (species) shown to either disappear at low levels of P enrichment or proliferate with high levels of P enrichment in the Everglades (King 2001). A relatively small proportion (< 20%) of taxa were considered either highly tolerant or highly sensitive. These attributes were expressed as the percent of total numerical abundance comprised of taxa shown to be tolerant or sensitive to P enrichment.

Trophic-structure attributes were selected according to the predominant functional feeding groups in the study area, which were predators, filterers, scrapers, and gatherers (Merritt and Cummins 1996; Barbour and others 1999). Trophic attributes were expressed as percent of total numerical abundance.

*Step 2: Identify potential metrics.* As recommended by several authors who have developed multimetric indexes (Barbour and others 1996; Fore and others 1996; Karr and Chu 1997), we graphically evaluated the response of assemblage attributes to TP concentrations along the observed gradient. Attributes with values that either increased or decreased monotonically with TP were identified as potential metrics. Attributes that either did not respond or showed very weak responses were eliminated from consideration. Attributes that responded unimodally were also discarded because values were similar at low and high concentrations of TP.

*Step 3: Validate metrics.* The suite of potential metrics identified from the observed gradient were further evaluated using the experimental data. We graphically examined each attribute separately for each of the four sampling dates. Attributes were discarded if they did not respond, showed very weak responses, or showed unimodal responses to TP on more than one sampling date. Attributes that responded in a different direction than the observed gradient (e.g., an increase with TP in the experiment while a decrease with TP along the observed gradient) were deemed too variable and also discarded. Thus, attributes that met all criteria as metrics responded to TP (1) in the real world, (2) in an experimental setting, (3) in the same direction in both studies, and (4) repeatedly over time.

*Step 4: Eliminate redundant metrics.* Metrics used in a multimetric index are intended to individually capture some variation not explained by other metrics. Collinear metrics do not add new information to an index, and may weight it too heavily toward one facet of biological condition. Thus, it was important to cull metrics that were excessively redundant before proceeding. A Pearson product-moment correlation matrix was used to evaluate collinearity among metrics ($r > 0.90$; Kleinbaum and others 1988; Barbour and others 1996). When pairs or sets of metrics were deemed collinear, the metric that showed the strongest, most consistent response to TP was retained.

*Step 5: Aggregate core metrics into IBI.* Metrics that met all selection criteria formed a core set to construct a multimetric index, which we termed a Nutrient Index of Biological Integrity (Nutrient-IBI). Typically, metric values are assigned a tiered score of 1, 3, or 5, ranging from poor to good, based on an arbitrary cutoff for each of the three tiers (Barbour and others 1995; Karr and Chu 1997). While this approach has been shown to be effective, we chose to scale the continuous metric values from 0 to 1 (low to high condition) to avoid making value judgments about tiers of condition (Suter 2001). This scaling procedure gave each metric continuous values and equal weight in the IBI. Metrics with low values at low TP were first inverted so that the raw minimum value was scaled to highest condition. The IBI score was the sum of all metric values for each observation, scaled from 0 to 5 (5 = highest condition).

*Step 6: Estimate changepoints.* We estimated potential threshold responses in the measurement endpoints to numerical levels of TP using nonparametric changepoint analysis (nCPA), a technique explicitly designed for detecting threshold responses using ecological data (Qian and others, in press). Nonparametric change-

point analysis is a derivative of a family of techniques historically used in classification and divisive partitioning of ecological data (e.g., Pielou 1984). This analysis is based on the idea that a structural change in an ecosystem may result in a change in both the mean and the variance of an ecological response variable used to indicate a threshold. When observations are ordered along a stressor gradient, a changepoint is a value that separates the data into the two groups that have the greatest difference in means and/or variances. This can also be thought of as the degree of within-group variance relative to the between group variance, or *deviance* (*D*) (see Venables and Ripley 1994 and Qian and others, in press, for details). Analytically, the nCPA examines every point along the stressor gradient and seeks the point that maximizes the reduction in deviance. Thus, each stressor value is a potential changepoint and is associated with a deviance reduction:

$$\Delta_i = D - (D_{\leq i} + D_{>i}), \quad (1)$$

where $D$ is the deviance of the entire data set $y_1, \cdots, y_n$, $D_{\leq i}$ is the deviance of the sequence $y_1, \cdots, y_i$, and $D_{>i}$ is the deviance of the sequence $y_{i+1}, \cdots, y_n$, where $i = 1, \cdots, n$. The changepoint $r$ is the $i$ value that maximizes $\Delta_i : r = \max_i \Delta_i$.

There is one particular value of the predictor $y$ (in this case, TP) that maximizes the reduction in deviance in the response data (in this case, the selected metrics); however, there is uncertainty associated with that value. It is unlikely that any one value of TP is the only value that could represent a changepoint. In reality, depending on the acuteness of the biological change in response to TP, several observations of TP could represent the changepoint, each with varying probabilities. Thus, to assess the risk associated with particular levels of TP, nCPA incorporates estimates of uncertainty in the changepoint (Qian and others, in press). These estimates are calculated using a bootstrap simulation (Efron and Tibshirani 1993). This simulation resamples (with replacement) the original dataset and recalculates the changepoint with each simulation. Bootstrap simulations are repeated 1,000 times. The result is a distribution of changepoints that summarizes the uncertainty among multiple possible changepoints. This uncertainty is expressed as a cumulative probability of a changepoint based on the relative frequency of each changepoint value in the distribution. To illustrate, a cumulative probability curve is shown in Figure 2 for the percent sensitive taxa metric in response to TP from the observed P gradient. Here, there is at least a 5% cumulative probability, or risk, that a detectable change in the percentage of sensitive taxa occurs at or below 13.3 μg/L TP. In other words, 5% of the bootstrap simulations resulted in a changepoint that was ≤ 13.3 μg/L TP. To fully visualize the range of uncertainty, the cumulative probability curve is extended to the highest level of TP that resulted in a changepoint in at least one of the simulations (Figure 2). Thus, the cumulative probability curve depicts the range of TP values that could potentially represent a changepoint and illustrates a cumulative level of risk associated with each TP value.

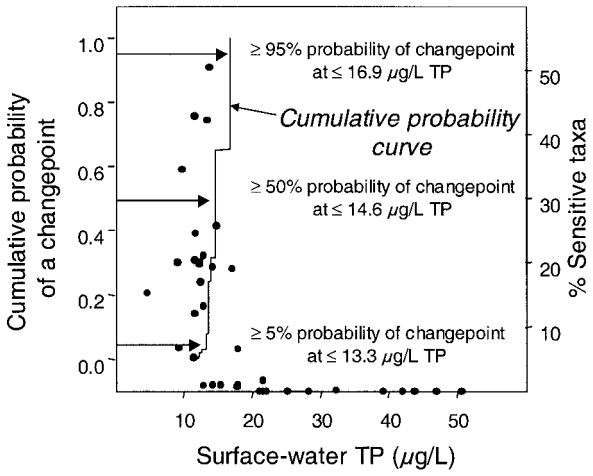


**Figure 2.** Illustration of the cumulative probability of a changepoint estimated for an individual metric in response to surface-water TP. The cumulative probability curve describes the cumulative risk of a change in a response variable (% sensitive taxa, y-axis [right side]; depicted by filled circles) associated with a range of stressor values. Cumulative probabilities are calculated using 1,000 bootstrap simulations. Any given location along the curve corresponds to a specific cumulative probability of a changepoint (y-axis [left side]) at a specific level of TP (x-axis). In this example, there was at least a 5% cumulative probability, or risk, that a detectable change in the mean and/or variance of the % sensitive taxa metric occurred at or below 13.3 μg/L TP. In other words, ≥5% of the bootstrap simulations resulted in a changepoint that was ≤ 13.3 μg/L TP. Similarly, there was ≥50% risk of a changepoint ≤ 14.6 μg/L TP, while there was ≥95% probability that a changepoint occurred ≤ 16.9 μg/L TP. Data are from the observed P-gradient study.

An additional factor to consider when using nCPA is an estimate of the probability of Type I error. A $\chi^2$ test statistic (1 df) can be used to evaluate the likelihood that an observed changepoint is real (Qian and others, in press). However, we only used this statistic to help assess the likelihood that changepoints with relatively wide cumulative probability distributions represented real biological changes, as uncertainty around the changepoint was a much more relevant issue (Suter 1996; Germano 1999; Johnson 1999).

Changepoint analysis works best when stressor-response relationships are nonlinear or heteroscedastic, properties very common to ecological data. For strong linear relationships, the analysis will find a significant changepoint but uncertainty will be high. Preliminary examination of the observational and experimental data revealed that all relationships were nonlinear and/or heteroscedastic, thus were well suited for nCPA. We estimated changepoints for individual metrics and the IBI using the observational and experimental datasets. Analyses were conducted for each date separately using the experimental data to better evaluate temporal variability in threshold responses. Analyses were conducted using the custom function "chngp.nonpar" (Qian and others, in press) in S-Plus 2000 (Mathsoft, Inc., Seattle, WA, USA).

*Step 7: Identify criteria protective of biological integrity.* We graphically concatenated the results from the observational and experimental studies to help identify levels of TP that were protective of biological integrity, as reflected by the metrics of macroinvertebrate structure and function. We interpreted a cumulative probability of a changepoint ≥50% to imply that a threshold response for a certain endpoint was more likely than not to occur at the respective predictor level of TP. We evaluated the range of TP levels that resulted in a ≥50% likelihood of a threshold response for individual metrics and the IBI, and contrasted this range of values between the observational and experimental data. Similarly, we contrasted the range of TP levels that had low (5%) and high (95%) probabilities of resulting in a threshold response to better characterize the risk to macroinvertebrate structure and function. However, it is important to note that the level of risk that scientists, managers, and decision-makers may be willing to accept will most certainly depend on a variety of ecological, economic, and social factors. Thus, our evaluation of cumulative probabilities of a changepoint at 5%, 50%, and 95% should not be implied to be an endorsement of these levels as the only levels of risk that should be evaluated in the criteria development process. Our approach provides a continuum of risk for each level of a stressor, and our focus on these three levels was largely necessitated by the limitation in presenting levels of risk for every possible changepoint.

## Results

Ten of the metrics evaluated using the observational P-gradient data showed clear responses to TP and were identified as potential metrics. Of these 10 candidate metrics, five exhibited consistent responses to TP in the P-dosing experiment: BCD, percent tolerant taxa, percent sensitive taxa, percent Oligochaeta (aquatic worms), and percent predators. Results from correlation analysis among these five metrics indicated that no pair was collinear ($r < 0.90$), thus each metric was sufficiently unique to retain as core metrics. These five metrics were subsequently analyzed individually and as an aggregated Nutrient-IBI using nCPA.

Changepoints were detected for all selected metrics and the IBI using the observational P-gradient data (Table 1). Probabilities of Type I error ($P$ in Table 1) were all quite low, indicating that it was highly likely that changepoints were real and represented a threshold response. The cumulative probability distributions generated from nCPA indicated that a changepoint was ≥50% probable between 12.6 and 19.4 μg/L TP for individual metrics and 14.8 μg/L TP for the IBI (Table 1, Figures 2–5 ). These changepoints represented biologically significant shifts in assemblage structure and function. Sensitive taxa dropped from a mean of over 21% to only 1.3% above 14.6 μg/L TP (Table 1, Figure 2). Conversely, tolerant taxa increased from only 2.2% to nearly 20% above 17.7 μg/L TP (Table 1, Figure 3). Percent Oligochaeta, a group of aquatic worms, nearly doubled when TP exceeded 13 μg/L. Mean BCD values (nMDS Axis 1 scores) were highly negative to the left of the 50% probability of a changepoint, while highly positive to the right, indicating a markedly different species assemblage once a cumulative probability of 50% had been exceeded (Table 1). Elevated TP also resulted in functional changes, reducing the proportion of predators in the assemblages from a mean of 9.2% to only 3.4% at TP levels above 12.6 μg/L. Finally, mean IBI scores above 14.8 μg/L were reduced by one-half when compared to IBI scores below that concentration (Table 1, Figure 4). In addition to these changes in means, all of these metrics exhibited distinct changes in variances that corresponded to TP changepoints (e.g., Figures 2–4 ).

Results from the P-dosing experiment mirrored those of the observed P gradient. Changepoints were evident for all metrics and the IBI, and were repeatable through time. Overall, median threshold responses from the four dates were ≥50% probable between 11.2 and 13.0 μg/L TP for individual metrics and 12.3 for the IBI (Table 1, Figures 3–5 ). Means and variances of metric values above and below the 50% level of risk were very similar to the biologically significant changes observed along the P gradient, and highly suggested that the changepoints represented threshold responses to TP (Table 1).

The cumulative probability distributions of changepoints indicated that there was a relatively tight range of TP levels likely to result in degradation in biological

Table 1. Results from nonparametric changepoint analysis showing cumulative probabilities of a threshold response for individual metrics and the aggregated IBI at specific levels of TP from the experimental and observational studies

| Metric | Study (Date) | Cumulative Probability of a Changepoint (TP, μg/L) | | | | Mean Metric Value (± 1 SE)[a] | |
|---|---|---|---|---|---|---|---|
| | | 5% | 50% | 95% | *P** | Left | Right |
| Bray-Curtis Dissimilarity (BCD)[b] | Experimental (Sep 1996) | 10.1 | 12.3 | 18.4 | 0.0012 | −0.75 (0.16) | 0.44(0.15) |
| | Experimental (Jan 1997) | 11.1 | 11.6 | 12.8 | 0.0001 | −0.95 (0.14) | 0.48(0.09) |
| | Experimental (Feb 1998) | 10.1 | 10.5 | 10.7 | 0.0007 | −0.80 (0.20) | 0.54(0.10) |
| | Experimental (Sep 1998) | 8.3 | 10.8 | 13.9 | 0.0006 | −0.79 (0.23) | 0.46(0.12) |
| | Observational (Oct 1998) | 15.2 | 19.4 | 21.4 | 0.0002 | −0.98 (0.13) | 0.61(0.25) |
| % Sensitive Taxa | Experimental (Sep 1996) | 7.4 | 14.5 | 25.7 | 0.1207 | 9.2 (3.8) | 3.4 (2.3) |
| | Experimental (Jan 1997) | 8.7 | 11.3 | 11.8 | 0.0032 | 21.3 (4.8) | 8.0 (1.5) |
| | Experimental (Feb 1998) | 7.1 | 11.4 | 18.7 | 0.0122 | 7.9 (1.6) | 3.6 (1.1) |
| | Experimental (Sep 1998) | 6.8 | 9.8 | 11.6 | 0.0414 | 4.7 (1.7) | 0.9 (0.4) |
| | Observational (Oct 1998) | 13.3 | 14.6 | 16.9 | 0.0013 | 21.2 (3.1) | 1.3 (1.1) |
| % Tolerant Taxa | Experimental (Sep 1996) | 10.2 | 12.1 | 19.0 | 0.0016 | 5.6 (2.4) | 24.2 (3.3) |
| | Experimental (Jan 1997) | 11.3 | 14.0 | 16.4 | 0.0008 | 7.5 (1.8) | 23.3 (3.4) |
| | Experimental (Feb 1998) | 9.1 | 10.7 | 12.1 | 0.0162 | 3.3 (1.5) | 18.5 (4.1) |
| | Experimental (Sep 1998) | 7.1 | 10.7 | 14.4 | 0.0098 | 7.2 (3.0) | 20.9 (3.8) |
| | Observational (Oct 1998) | 14.6 | 17.7 | 25.5 | 0.0002 | 2.2 (1.2) | 20.0 (5.0) |
| % Oligochaeta | Experimental (Sep 1996) | 9.6 | 13.3 | 25.7 | 0.0026 | 7.3 (6.2) | 41.6 (8.4) |
| | Experimental (Jan 1997) | 8.8 | 12.7 | 18.1 | 0.0154 | 17.6 (5.0) | 38.5 (7.0) |
| | Experimental (Feb 1998) | 9.0 | 18.4 | 21.6 | 0.0262 | 22.2 (4.2) | 40.0 (7.2) |
| | Experimental (Sep 1998) | 8.3 | 12.6 | 13.9 | 0.0035 | 7.7 (4.4) | 41.5 (6.0) |
| | Observational (Oct 1998) | 11.4 | 13.0 | 16.9 | 0.0212 | 33.9 (5.3) | 57.9 (4.2) |
| % Predators | Experimental (Sep 1996) | 7.6 | 11.1 | 18.1 | 0.0162 | 21.0 (12.2) | 4.7 (1.4) |
| | Experimental (Jan 1997) | 7.9 | 11.7 | 12.8 | 0.0006 | 18.3 (4.5) | 5.0 (1.1) |
| | Experimental (Feb 1998) | 7.1 | 10.2 | 10.6 | 0.0221 | 8.7 (1.2) | 4.4 (0.6) |
| | Experimental (Sep 1998) | 8.3 | 14.5 | 21.2 | 0.1694 | 9.4 (3.3) | 5.6 (2.6) |
| | Observational (Oct 1998) | 8.9 | 12.6 | 16.4 | 0.0419 | 9.2 (3.7) | 3.4 (1.2) |
| Nutrient Index of Biological Integrity (Nutrient–IBI) | Experimental (Sep 1996) | 11.9 | 13.6 | 13.8 | 0.0003 | 3.4 (0.2) | 1.9 (0.1) |
| | Experimental (Jan 1997) | 9.7 | 11.7 | 12.8 | 0.0002 | 3.9 (0.2) | 2.2 (0.1) |
| | Experimental (Feb 1998) | 9.1 | 10.6 | 12.1 | 0.0122 | 2.9 (0.2) | 2.1 (0.1) |
| | Experimental (Sep 1998) | 10.0 | 13.0 | 14.2 | 0.0020 | 2.9 (0.1) | 1.8 (0.1) |
| | Observational (Oct 1998) | 12.3 | 14.8 | 16.9 | 0.0004 | 3.0 (0.2) | 1.5 (0.2) |

*$P$ = Probability of Type I error, indicating the likelihood that there was no changepoint in the response data.
[a]Mean (± 1 SE) metric values to the left and right of the level of TP corresponding to ≥50% cumulative probability of a changepoint.
[b]BCD values were expressed as standardized nMDS Axis 1 scores (see Methods for greater detail).

condition (Table 1, Figures 3–5). Both the observational and experimental data revealed that there was a low (5%) probability that a threshold response occurred ≤ 10 μg/L TP for some metrics. There was high (≥95%) certainty that the threshold was ≤ 20 μg/L TP for the majority of individual metrics. Aggregating the individual metrics into the IBI reduced this range of variability, however. Results indicated a 5% probability that a threshold response for the IBI occurred at or below 9 μg/L TP (experimental) and 12.3 μg/L TP (observational), whereas there was ≥95% certainty that a threshold response occurred ≤ 15 μg/L TP (experimental) and ≤ 17 μg/L TP (observational) (Table 1, Figures 4 and 5). Although these differences were relatively small, the lower changepoints from the P-dosing experiment than the observed P gradient implied that changepoints from the P-dosing experiment might have been conservative estimates of TP levels that may pose a risk to macroinvertebrate structure and function.

## Discussion

### Can Bioassessment Be Used To Develop Numerical Water-Quality Criteria?

Bioassessments generally are performed with the intent of detecting impairment in an aquatic ecosystem, which usually implies degraded water quality. Despite the fundamental linkage between bioassessment and water quality, there are surprisingly few examples of

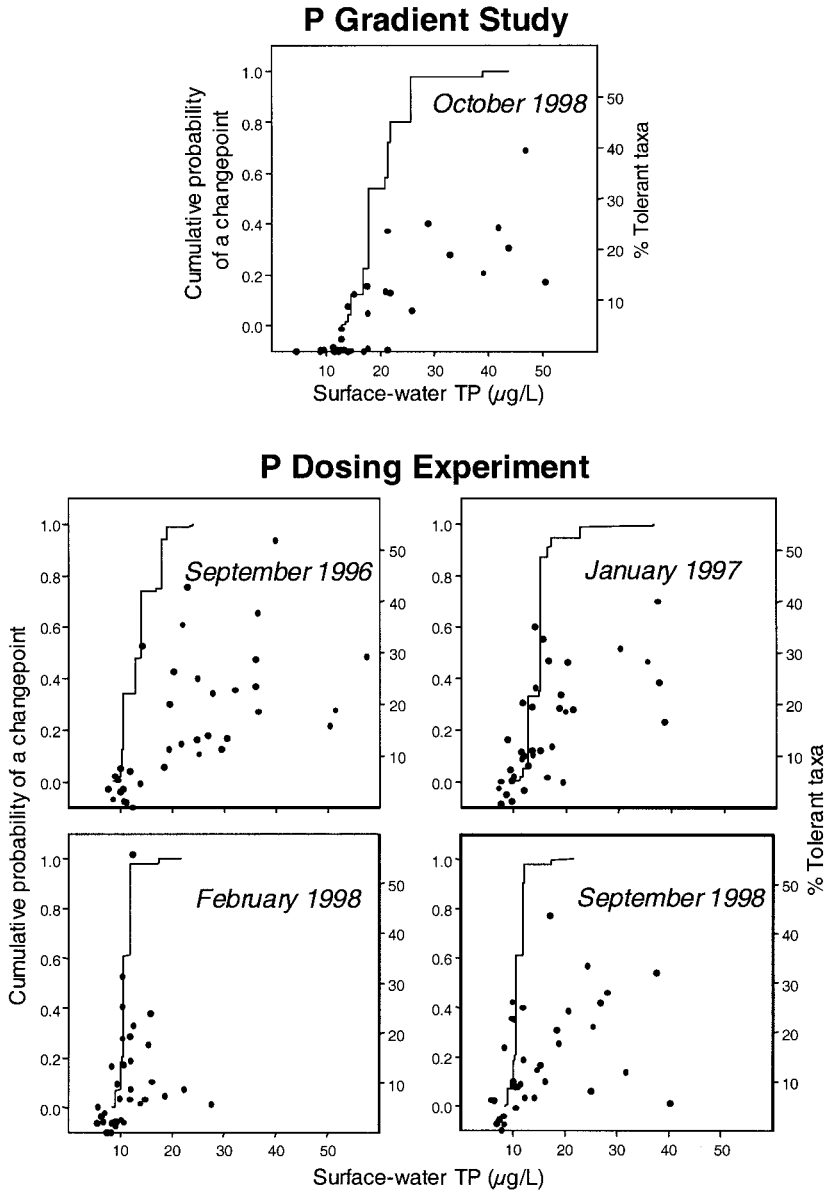


**Figure 3.** Cumulative probabilities of changepoints for the percent of tolerant taxa metric in response to surface-water TP. Results are shown for the observational P-gradient study and the four dates from the P-dosing experiment.

bioassessment used explicitly to support the development of numerical water-quality criteria (see Dodds and Welch 2000). One of the primary reasons for this is that traditional bioassessments, such as multimetric approaches, are intentionally developed to capture the effect of a wide range of stressors to biological integrity. This lack of specificity results in ambiguity about the potential cause(s) of impairment and, consequently, the levels of a stressor that may result in a threshold response. However, the results of our study provide evidence the multimetric approach to bioassessment is robust and appears to be easily adaptable to a particular stressor, such as nutrients. We identified several attributes of macroinvertebrate assemblages that responded to surface-water TP using observational, real-world data. Experimental data provided evidence that changes observed in the observational study were indeed due to P enrichment. Temporal replication from the experiment also indicated that, despite seasonal variation, attributes responded in a consistent direction (increase or decrease) to TP. These core metrics also responded repeatedly over time to TP. Finally, threshold responses were detected at similar levels of TP among different metrics and across several dates. Thus, this approach was consistent with the water-quality criteria development strategy proposed by the USEPA

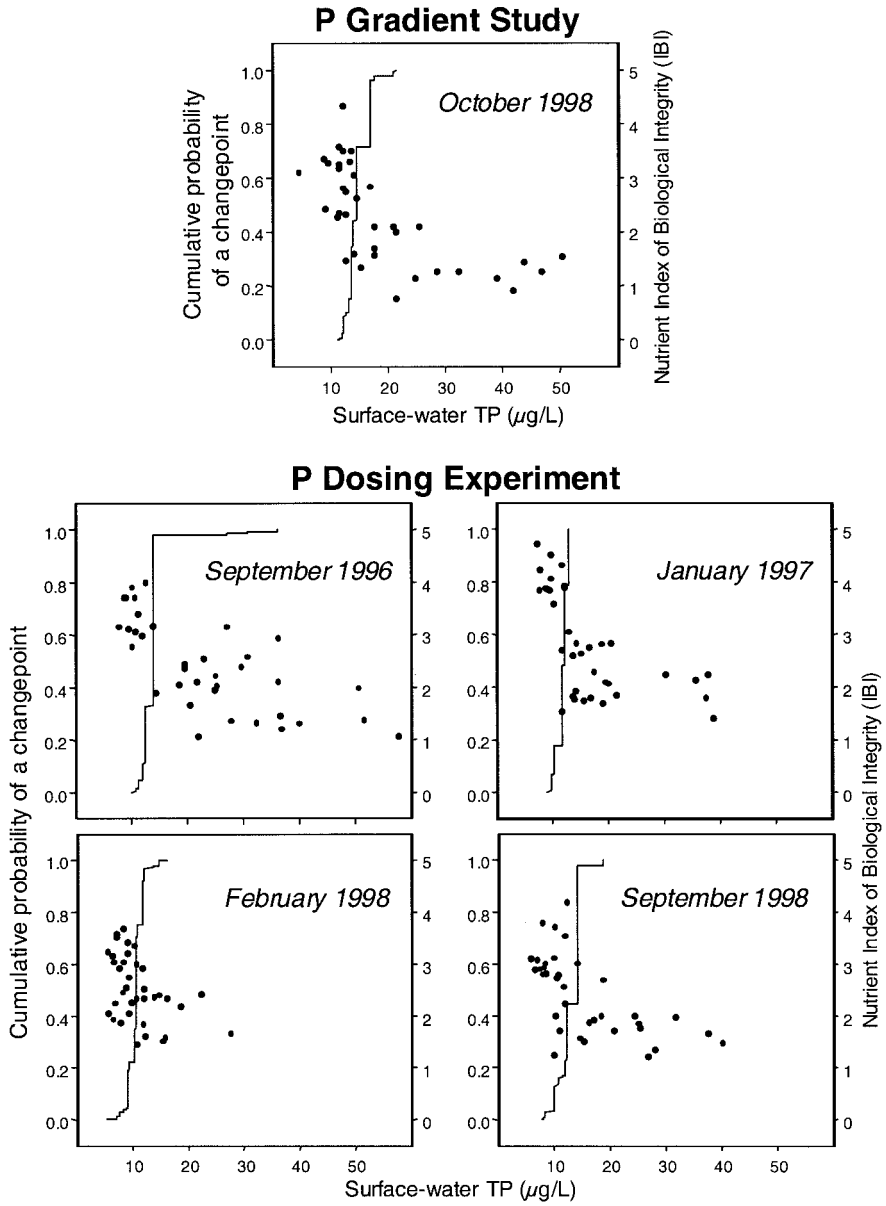


**Figure 4.** Cumulative probabilities of changepoints for the Nutrient-IBI in response to surface-water TP. Results are shown for the observational P-gradient study, and the four dates from the P-dosing experiment.

(1998a), as our findings (1) established a cause-effect linkage between TP and biological attributes within a given nutrient ecoregion, (2) estimated levels of TP that may cause biological changes, and (3) estimated uncertainty in TP levels that may lead to degradation of biological integrity.

The use of ecological experiments may be the most critical step in the validation of numerical criteria using bioassessment. Descriptive, correlative studies are often very useful for generating hypotheses but often are insufficient for establishing cause-effect linkages (e.g., Beyers 1998). A number of recent studies have shown creative ways to use descriptive biomonitoring data to ascribe causation using a stressor-identification framework (e.g., Norton and others 2000; Griffith and others 2001; Cormier and others 2002). However, without experimental evidence, it is still very difficult to eliminate other potential causes of an observed biological response to a candidate stressor (USEPA 2000b). Moreover, it is nearly impossible to quantify the uncertainty associated with additive or synergistic effects of multiple stressors in an aquatic ecosystem without first isolating a single stressor using an experiment. This latter point is particularly critical in the context of numerical criteria development because the level of a stressor that apparently results in an observed threshold response

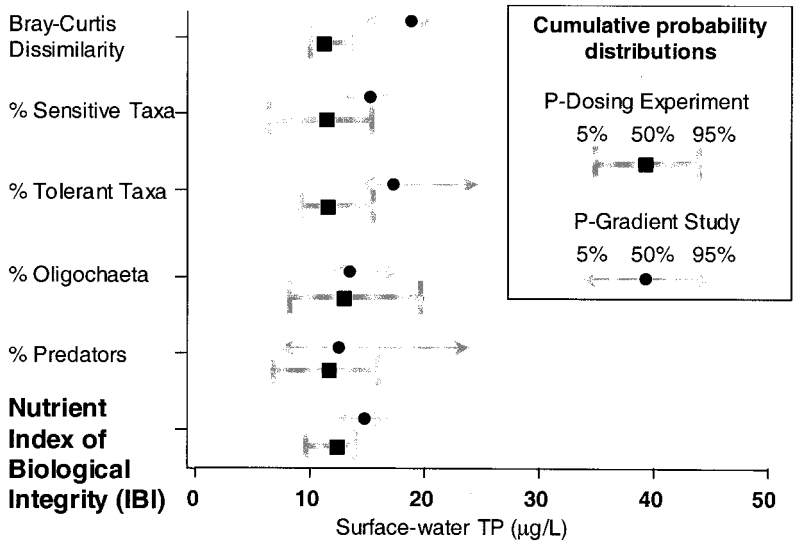


**Figure 5.** Synthesis of results from the P-gradient study and P-dosing study for the identification of a TP criterion protective of biological integrity. Median values from the four dates in the P-dosing experiment were used for the ≥5%, 50%, and 95% cumulative probabilities.

may be confounded by another, perhaps unmeasured, factor (Suter 2001). For these reasons, we highly recommend the collection of experimental data to support observed assessments used for numerical criteria development.

Conversely, experimental studies suffer from some important limitations. Most are not conducted at the appropriate scale (e.g., watershed) and need to be coupled with observational research to help validate the applicability of experimental findings to the real world (e.g., Daehler and Strong 1996; Lemly and Richardson 1997; Adams and Greeley 2000). In our approach, we relied on a descriptive study to identify biological attributes that may have been affected by TP. Once identified, these biological attributes were further examined in a long-term P-dosing experiment to corroborate their P sensitivity and estimate TP changepoints. Without the observational study, however, it would have been difficult to extrapolate the experimental findings to the much larger scale of the study area. By coupling the two studies, each provided evidence that the other study could not, which made for a much stronger case about the levels of TP that were likely to degrade macroinvertebrate structure and function.

One potential criticism of our approach is that it may be impractical for state, tribal, other regulatory agencies that have limited funding to conduct long-term exposure studies such as the P-dosing experiment we illustrated here. While large-scale (spatial, temporal or both) experiments are probably too costly in most situations, small in situ microcosm or mesocosm studies may provide sufficient evidence to support an observational finding. For example, Clements and others (2002) provided an excellent illustration of the coupling of small experiments with descriptive data. Here, lab experiments, small in situ exposure experiments, and large-scale observational studies afforded strong inference about the levels of heavy metals that affected biota in Rocky Mountain streams. Similar examples also exist for nutrients (e.g., Hart and Robinson 1990; Perrin and Richardson 1997; Lemly and King 2000). Thus, it seems that experiments can be a practical addition to the criteria development process if efficiently and purposefully designed.

### Detecting Threshold Responses with Changepoint Analysis

Estimation of risk should be a critical step in developing numerical water-quality criteria. Risk analysis requires a tangible, numerical estimate of the levels of a stressor that are likely to result in an effect on an assessment endpoint. However, the most commonly employed types of data analyses—hypothesis-testing statistics—are insufficient and possibly misleading when used for this purpose (e.g. Germano 1999; Johnson 1999). Suter (1996) provided a thorough review of the problems with hypothesis testing in ecological risk assessment, most notably the inability of the approach to provide a clear estimate of expected or observed effects and associated uncertainties related to a predictor variable. In contrast, our results suggest that nCPA has potential to be a useful analytical tool in the development of criteria because of the easily interpretable, numerical estimates it affords. Rather than asking the question "is there a statistically significant relationship between predictor $x$ and response $y$?" as implied with most hypothesis-testing statistics, this risk-based analysis more explicitly asks "what level of predictor $x$ results in

a threshold response of *y*, and how uncertain is this threshold?" Using this analysis, we were able to identify levels of TP that were likely to result in a threshold response in the macroinvertebrate assemblage as well as provide an estimate of the cumulative probability that a particular level of TP would elicit a threshold response. Although we included a $\chi^2$ significance test (1 df) to assess the likelihood that changepoint was real, this test was of limited value because such tests provide little information about the risk of a threshold response at various levels of TP. Thus, we contend that results from hypothesis testing fail to provide enough information to decision-makers, and generally be avoided for supporting the development of numerical criteria.

Another advantage of nCPA is that it is particularly appropriate for ecological data analysis because it makes few assumptions about the distributional properties of data (Qian and others, in press). A deviance reduction algorithm, nCPA considers both the mean and the variance of response variables, contrary to most parametric techniques that focus only on the mean (Breiman and others 1984; Sokal and Rohlf 1995). Most parametric techniques require that data meet the assumptions of homogeneity of variances (e.g., ANOVA) or homoscedasticity (regression) despite the fact that changes in the variance may be equally informative as changes in the mean (e.g., Palmer and others 1997). For example, in ecological risk assessment, a fitted function that describes the dose-response relationship between a measurement endpoint and level of exposure to a contaminant is often used to estimate the magnitude of effect on the endpoint at a particular contaminant concentration (effective concentration, or EC) (Suter 1993). However, distributional properties of most metrics used in bioassessment are not conducive for these types of fitted models and, we argue, are not appropriate. In our study, many threshold responses were detected due to dramatic changes in variance in metric values with increasing levels of TP (e.g., Figure 2). This change in variance would have violated the assumptions of commonly employed parametric statistics but was paramount to the detection of levels of TP that resulted in a changepoint in our study.

While nCPA was effective in this study and has advantages over other many other methods for this application, one potential criticisms of nCPA is that it may not detect a low-level changepoint if a second, competing changepoint occurs at a higher concentration. First, we recommend that all data be examined graphically before any analysis is conducted so that the shape of the response can be evaluated (e.g., Karr and Chu 1997). If multiple changepoints are evident, a tree-based, recursive approach (i.e., tree regression; Breiman and others 1984) can be used to help isolate the lower changepoint. Here, the model splits the data into multiple subsets rather than just two. The subset of data above the upper changepoint can be discarded, and nCPA conducted on the lower subset of data. In this study, all primary changepoints occurred at low concentrations, although bootstrapping revealed that, in a few instances, a second, slightly weaker change also occurred at a higher concentration and subsequently skewed the upper range of the cumulative probabilities (e.g., Figure 3). Because nCPA is an extension of recursive-partitioning techniques such as tree regression, they are compatible and may provide a tactical, conservative means of detecting secondary changes at low concentrations if a primary response occurs at a greater concentration.

In our study, we defined macroinvertebrate structure and function as our assessment endpoint, and used a stressor-identification process to select five individual biological metrics and a multimetric index, the Nutrient-IBI, as measurement endpoints. We analyzed the individual metrics separately because we were concerned about the effect of blending metrics into one score on our threshold estimates. Of particular concern was that some metrics might have responded at different levels of TP, thus the IBI would have found the middle of this response range and possibly underestimated the risk posed by lower levels of TP. Conversely, we recognized that aggregating the individual metrics into the IBI might have increased the signal-to-noise ratio and allowed us to detect assemblage-levels changes that may have been clouded by variability at the individual-metric level. In reality, most of the individual attributes responded at a relatively similar levels of TP as the IBI, but the IBI overall had a tighter range of cumulative probabilities of a threshold response to TP than the individual metrics. However, there was modest deviation in the TP changepoints between the IBI and some metrics, suggesting that aggregating the responses into one index may have masked the variation in responses among individual attributes of the macroinvertebrate assemblage. Considering that biological responses to other stressors in other regions could lead to a wider range of changepoints than observed in this study, it is important to recognize this potential artifact of multimetric indexes. Moreover, the reduction in variance of individual metric values that invariably results from aggregating them into a multimetric index may actually eliminate biologically relevant changes in variance that could be detected using nCPA. Thus, we highly recommend the analysis of individual metrics in addition to an aggregated multim-

etric index to better characterize the range of levels of a candidate stressor that pose a risk to different facets of biological condition.

A final consideration when using nCPA is that it is a just a statistical tool, and any tool can be used inappropriately. We used nCPA for quantifying the cumulative probability that a particular level of TP resulted in a biologically significant change in macroinvertebrate structure and function, as expressed by the selected metrics (measurement endpoints). However, as with any statistical technique, nCPA may detect a statistical change in the data that may not represent a biologically significant change—clearly, the definition of biological significance is a subjective one and will vary among scientists and decision-makers. However, our results indicated that means and variances of assemblage attributes to the left and right of the ≥50% cumulative probabilities of changepoints differed markedly, sometimes by a factor > 10. We contend that these changepoints represented TP levels that resulted in a qualitatively different biological community, as expressed by various attributes of assemblage structure and function, and were indicative of biologically significant changes.

## Conclusions and Recommendations

Bioassessment and ecological risk assessment are inherently complementary in nature (Pittinger and others 2000). We presented a generalized approach for integrating these two assessment systems for the purpose of supporting numerical water-quality criteria. The strengths of the approach are the establishment of cause-effect linkages and the estimation of numerical thresholds. Moreover, the results are easy to interpret and communicate to environmental decision-makers and the public (Schiller and others 2001).

In this study, the weight-of-evidence produced from these analyses implied that a TP criterion > 12–15 μg/L is likely to cause degradation of macroinvertebrate structure and function, a reflection of biological integrity, in at least this area of the south Florida coastal plain nutrient ecoregion. Our results also indicated that there is a very low (typically ≤5%) probability that an IBI threshold response would occur at ≤ 10 μg/L TP, while there is ≥95% certainty that a threshold would occur at ≤ 17 μg/L TP. However, this study only considers the macroinvertebrate component of biological integrity. The purpose of this study is not to imply that macroinvertebrate attributes should be the only endpoints used to assign a water-quality criterion to a region and water body. On the contrary, we highly recommend the evaluation of the responses of multiple biological endpoints from a variety of indicator groups across multiple trophic levels to better identify criteria protective of biological integrity. It is also important to recognize that the establishment of numerical criteria is ultimately a societal decision that will be based on a host of factors. However, these results do provide some compelling evidence that bioassessment can be used in a risk-assessment framework to identify critical levels of pollution, and ultimately guide environmental decision-making. Although the approach seems promising, it remains to be seen how well it will perform in different geographic regions and water bodies of the USA and other parts of the world.

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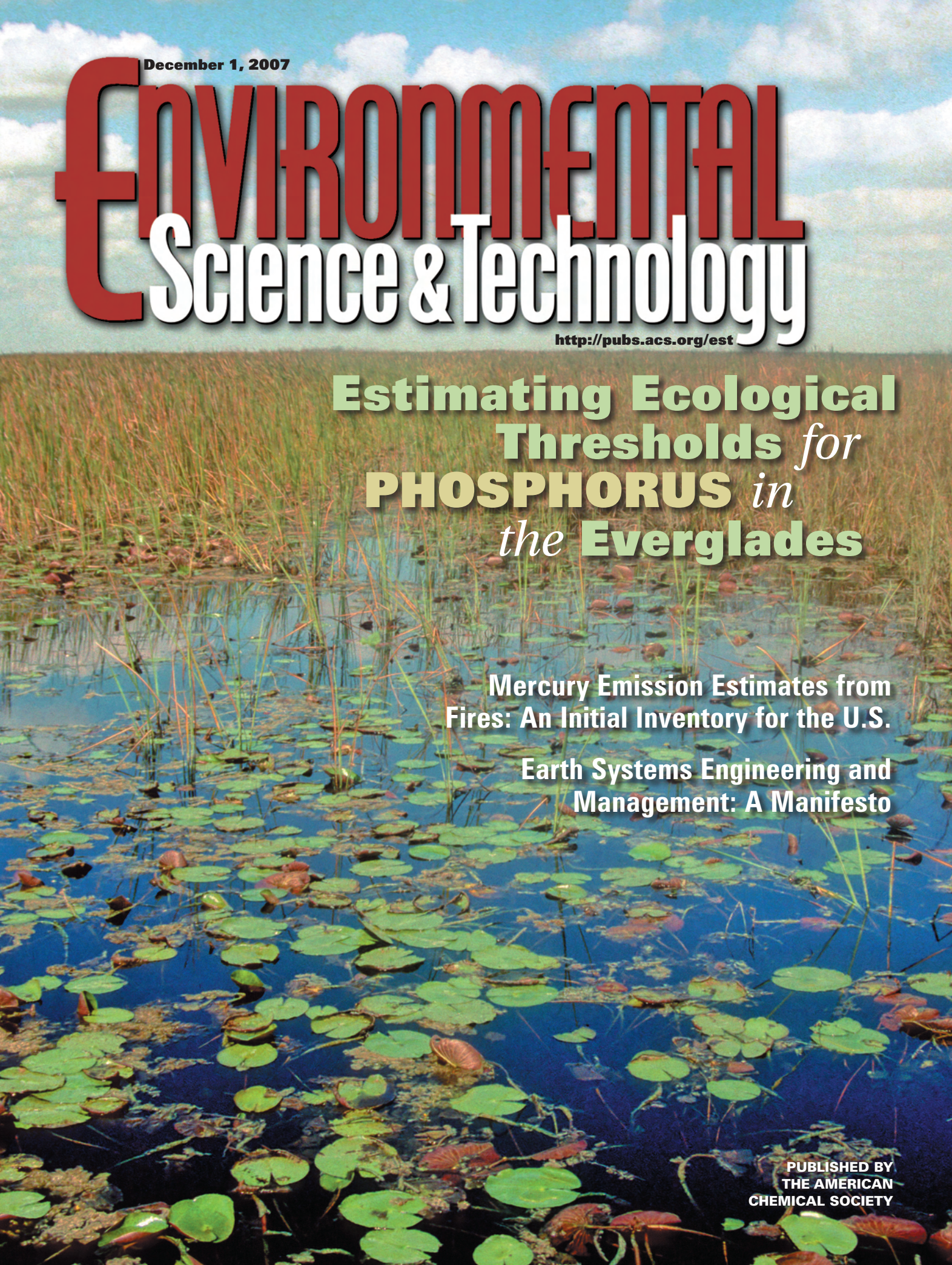

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# Estimating Ecological Thresholds for Phosphorus in the Everglades

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The Florida Everglades, a wetland of international importance, has been undergoing a significant shift in its native flora and fauna due to excessive total phosphorus (TP) loadings (an average of 147 t per annum from 1995 to 2004) and an elevated mean TP concentration (69 $\mu$g $L^{-1}$ of TP in 2004) from agricultural runoff and Lake Okeechobee outflow despite the use of 16000 ha of stormwater treatment areas. Here, we present a Bayesian changepoint analysis of long-term experimental research and show that exceeding a surface water geometric mean TP threshold concentration of 15 $\mu$g $L^{-1}$ causes an ecological imbalance in algal, macrophyte, and macroinvertebrate assemblages as well as slough community structure. A phosphorus threshold for all trophic levels may be more realistic and protective when presented as a threshold zone (12–15 $\mu$g $L^{-1}$) because estimates of uncertainty must be utilized to accurately define TP thresholds, which change with seasons and water depths. Most interior areas of the Everglades are currently at or below this threshold zone, but the exterior areas near inflow structures (except for the Everglades National Park) are presently receiving double or triple the proposed threshold. Our Bayesian approach, used here to address ecological imbalance along nutrient gradients, is applicable to determining thresholds and stable states in other aquatic ecosystems.

## Introduction

Only 51% of its original size, the Everglades remains the largest and most ecologically important subtropical wetland in the United States. This 618000 ha fen, mostly classified as an alkaline peatland, is highly impacted by drainage canals, nutrient additions, and altered hydrology (*1*–*5*). Its resilient flora and fauna have evolved to survive in an ecosystem disturbed by hurricanes, flooding, seasonal droughts, and fire (*1, 6*–*8*). Primary productivity is restricted by severe P limitations (*9*), a condition found in similar limestone-based marshes covering extensive areas on the Yucatan Peninsula, northern Belize, and many Caribbean islands (*10*). One prominent hypothesis proposes that the Everglades is unique with respect to the multiple factors causing a high degree of P limitation and organism sensitivity to small additions of P (*11*).

The problem of P-induced eutrophication in the last 30 years has occurred mainly in the northern Everglades due to increased runoff from agricultural lands and eutrophic Lake Okeechobee (*12*–*14*). A series of outflow structures have created a P-enrichment gradient that often extends 5–8 km into the interior of the wetlands as a result of total phosphorus (TP) loadings averaging 229 t from 1978 to 1988 and 147 t from 1995 to 2004 from agricultural runoff and Lake Okeechobee outflow despite the use agricultural Best Management Practices (BMPs) since 1996 and the construction of 16000 ha of stormwater treatment areas (*14, 15*). In 2004 BMPs had resulted in a 55% cumulative P load reduction to the northern Everglades and a reduction in the three-year flow weighted mean P concentration to 71 $\mu$g $L^{-1}$ P.

Although 2004 TP loadings were reduced to 83 t, loading rates per unit area have often exceeded 4 g $m^{-2}$ $yr^{-1}$ at the edge of the fens (*14, 16*). We had earlier shown that these excessive TP loadings would only reach nonimpact levels in the downstream water column when P loading inputs decreased below the ecosystem's P assimilative capacity of 1 g $m^{-2}$ $yr^{-1}$ (*16*). Surface water total phosphorus (TP) input concentrations along these gradients during the 1980s ranged from 173 $\mu$g $L^{-1}$ near agricultural outflow gates flowing into the fen to a 2004 stormwater treatment area (STA) mean output concentration of 41 $\mu$g $L^{-1}$ of TP, a 76% reduction. However, these values still remain in excess of the 10 $\mu$g $L^{-1}$ marsh TP criterion passed by the State of Florida in 2003 and approved by EPA in 2005 (*15*). Specifically the criterion for the Everglades indicates that all measured sites must meet a five-year geometric mean criterion of less than or equal to 10 $\mu$g $L^{-1}$ TP in three of five years, have annual concentrations less than or equal to 11$\mu$g $L^{-1}$ P across all stations, and have concentrations less than or equal to 15 $\mu$g $L^{-1}$ P annually at all individual stations.

Importantly, 2004 inner concentrations range from 5 $\mu$g $L^{-1}$ in the Everglades National Park (ENP) to near 17 $\mu$g $L^{-1}$ in the core background areas of Water Conservation Area 2A (WCA-2A) in the northern Everglades (*15*). This suggests that many areas of the Everglades fen are already meeting standards. However, the interior of the most impacted areas of the northern Everglades WCA-2A and most wetland borders adjacent to input canals have TP values two to three times the criterion (*15*). The past two decades of increased P nutrient loadings have resulted in elevated water and soil P concentrations in large portions of the Everglades, typically extending nutrient gradients into the interior of the fen communities (*9, 15*–*18*). This elevated nutrient gradient has resulted in extensive shifts in algal, macroinvertebrate, and macrophyte species. It has also altered community structure, especially in the highly and moderately P-enriched areas (*19*–*24*).

These observations of biotic change immediately downstream of the inputs do not elucidate the threshold surface water TP concentration that causes an imbalance of flora and fauna in the Everglades nor do they provide insights into the level of TP that causes changes in both structure and function of aquatic ecosystems resulting in a shift between alternate stable states (*11, 25, 26*). Most importantly, the northern Everglades is now undergoing a reduction in external inputs, and therefore, the Everglades restoration efforts provide us with a unique opportunity to establish and confirm the environmental thresholds that will trigger a reversal from impaired states.

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The recently promulgated EPA TP criterion concentration of 10 $\mu g\ L^{-1}$ was selected because it was considered necessary to maintain the ecological balance and integrity of the ecological community (*14, 15, 27, 28*). If true, then the Everglades has no capacity to assimilate phosphorus much above background concentrations without significant changes occurring in the flora and fauna. Here we present a Bayesian changepoint analysis of long-term experimental research to support the hypothesis that the Everglades has the capacity to assimilate phosphorus above background TP concentrations without causing imbalances in flora and fauna or a shift in stable states. To test this hypothesis, we applied five different concentrations of phosphate (as soluble reactive phosphorus (SRP)) for 6 years at 2 P-limited slough field sites in the northern Everglades (*29*).

## Materials and Methods

**Field Methods and P Dosing Treatments.** We used a mesocosm-scale experiment at two sites to assess the biotic responses of the algal–plant–macroinvertebrate complex in a six-year P dosing in a slough community located in an undisturbed area of the northern Everglades. Two P dosing facilities (hereafter called "flumes") were constructed in the unimpacted interior of WCA-2A (26° 15′ N, 80° 23′ W) in fall of 1990 and then calibrated for over one year. The two sites were almost identical in macrophyte species composition and structure in the calibration year prior to treatment (*29*). Flumes were constructed in open-water sloughs, a habitat that has been shown to be sensitive to P-enrichment (*30, 31*) and ecologically important (*32*). Each site had five walled flumes, 2 m wide × 8 m long with walls approximately 120 cm in height above the slough substrate. An additional unwalled control area of identical size to that of flumes was established on the west side of both sites to concurrently monitor the potential effects of placing walls around slough habitat. Dye and bromide tracers were used to confirm that flumes were isolated and that dilution could be accounted for. Wall effects proved minimal and were also further reduced by only measuring responses in the center meter of the channels. Flumes were oriented N-S and separated by 2 m, where permanent boardwalks were built to allow investigators access for sampling. Each flume was randomly named and assigned one of five soluble reactive phosphate (SRP as $Na_2HPO_4$) treatments: ~5 $\mu g\ L^{-1}$ (mean background concentration [walled and unwalled controls]; 0.25 g $m^{-2}\ y^{-1}$), 30 channels, ~22 $\mu g\ L^{-1}$ (1.5 g $m^{-2}\ y^{-1}$), 50 channels, ~39 $\mu g\ L^{-1}$ (2.75 g $m^{-2}\ y^{-1}$), 75 channels, ~57 $\mu g\ L^{-1}$ (3.5 g $m^{-2}\ y^{-1}$), and 150 channels, ~126 $\mu g\ L^{-1}$ (8.2 g $m^{-2}\ y^{-1}$). SRP was dosed from the northern end of flumes via large mixing tanks. Flumes were dosed on a continuous schedule except during low- or high-water shutdowns or periodic maintenance to specific flumes. Dosing was applied from 30 November 1992 to 21 September 1998. Greater detail on the design and operation of the P dosing study is described by Richardson et al. (*33*). The dosing system created a total phosphorus (TP) and SRP gradient down each flume. Water was sampled every two weeks for SRP, and TP analyses at each meter (1–8) down the length of each channel. Between March 1993 and September 1998, once each month, additional water samples were sampled for a suite of chemical analyses. This includes not only SRP, but TP, dissolved organic P, particulate P, ammonia, nitrate+nitrite, dissolved organic N, particulate N, calcium, potassium, and pH at 2, 4, and 6 m distances down the channel length. Sample collection methods, storage, analysis, and QA/QC procedures were in accordance with standard methods (*34*). A detailed presentation of methods as well as procedures for laboratory and field protocols using blanks, spikes and standards are given in detail in our FDEP QA/QC approved plan (*29*). Biotic responses and water chemistry were measured at each meter location, then selected metrics were compared to the geometric mean water column TP concentrations for either the previous 3 month (algal responses) or 6 month period (macrophytes, macroinvertebrates, and community metrics). Other time periods (1, 2, 8, and 12 month means) were also tested, but the 3 and 6 month periods were chosen because they most closely represent the biological life of each trophic level and because values among other tested time periods proved to be more variable. Daily variations in water depth and temperature were measured at both sites using an Omni Data automated data logger water level system coupled with a Metri-Tape unit and a thermocouple probe placed 10 cm below the water surface. Standard methods and EPA approved methods were used for all analyses.

**Ecological Indicators.** We initially selected over fifty potential ecological and biological attributes of imbalance for the Everglades study (*35*). Final attributes were selected based on ecological importance and statistical analyses (*35*) at key trophic levels in the Everglades. The attributes tested are categorized as follows: (units shown in parentheses)

- Fast response time: algae–diatom relative abundance (%), diatom density (cell/$m^2$), diatom biovolume ($cm^3$/cell); and blue green algae biovolume ($cm^3$/cell),
- Intermediate response time: Macrophytes- *Utricularia spp* (stem densities), *Utricularia purpurea* (stem densities); Macroinvertebrates–biomass (mg), abundance (number) Oligochaeta (number), microcrustacea (number), sensitive species (%), predators (%), Gastropoda (%);
- Slow response time: Community-macroinvertebrate taxa (number), Bray–Curtis dissimilarity (scaled index), calcareous mat cover (%).

**Statistical Modeling.** To first evaluate which abiotic (e.g., water depth, year) and ecological (e.g., algal, macrophyte, macroinvertebrate) variables were the best indicators of TP concentration, we used classification and regression tree (CART) models, an analysis that recursively partitions observations into groups using the best indicators of TP (*36*). The analysis used data collected over more than 6 years, spanning all seasons and a wide range of water depths (15–120 cm).

We then used a Bayesian hierarchical changepoint model, which was specifically designed for detecting changes along a gradient (*17, 37*). Specifically, we assume that the response variable values, $y_1,\ldots, y_n$, collected from the $n$ sites along the P gradient of interest, are random samples from the sequence of random variables $Y_1, \ldots, Y_n$. The corresponding P concentration values are $x_1, \ldots, x_n$, where $x_1 < x_2 < \ldots < x_n$. Assuming that variables $Y_1, \ldots, Y_n$ belong to the same family of distributions with parameter $\theta$. The random variables $Y_1, \ldots, Y_n$ have a changepoint $r$ ($1 \le r \le n$) if the parameter value changes at $r$:

$$Y_1, \ldots, Y_r \sim \pi(Y_i|\ \theta_1)$$
$$Y_{r+1}, \ldots, Y_n \sim \pi(Y_i|\ \theta_2)$$

The corresponding P concentration $x_r$ is the P threshold or changepoint. A uniform prior is used for $r$ and noninformative or vague priors were used for $\theta_1$ and $\theta_2$. The results of the model is presented in the form of a probability distribution of the $n$ P concentration values $x_1,\ldots, x_n$ being the changepoint, or $p(x_i)$, where $\sum_{i=1}^{n} p(x_i) = 1$.

## Results and Discussion

**Phosphorus Treatments.** Six experimental treatment flumes at each site created a surface water TP concentration gradient that ranged from 10 to 75 $\mu g\ L^{-1}$ TP along which to test biotic responses (ecological attributes) across several trophic levels (Figure 1). In a P-limited aquatic system such as the Florida Everglades, the biotic assimilation of SRP is rapid, and water column concentrations are reduced to uniformly low concentrations throughout most of the flumes, which negates the formation of a measurable SRP gradient

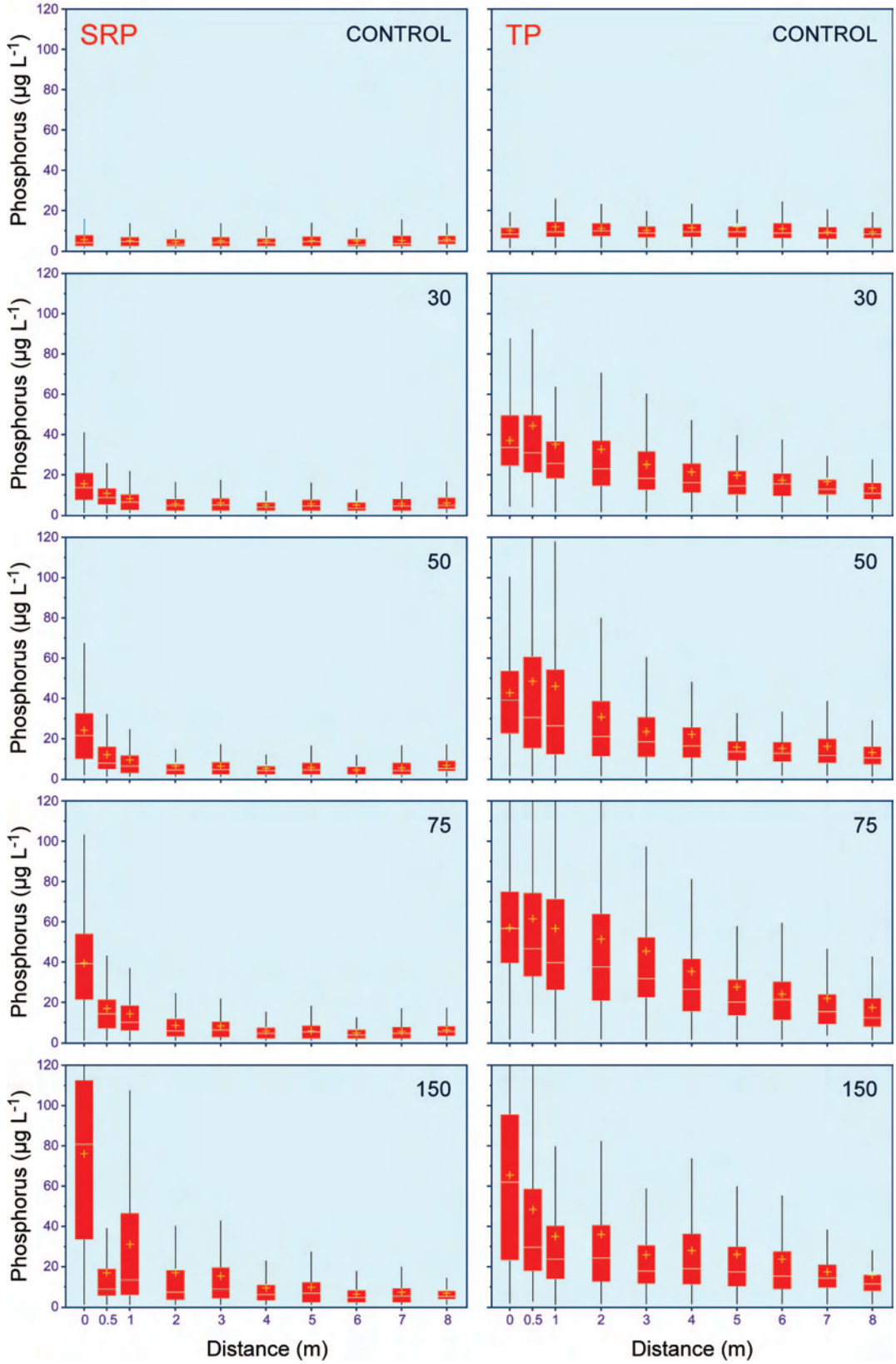


**FIGURE 1. The mean water column soluble reactive phosphorus (SRP) and total phosphorus (TP) concentrations in the dosing flume channels at one-meter intervals downstream of the influent head boxes. The flume channels were labeled unwalled controls (UC), walled controls (WC), and 30, 50, 75, and 150 to represent P treatment status (see channel label numbers in upper right corner). Actual SRP dosing concentrations are given in the methods section. The top, midline, and bottom of each box represents the 75th, 50th (median), and 25th percentile of the data, respectively. The means (+) represent the TP found at dosing sites 1 and 2 during the dosing period (1992–1998). The vertical lines represent the 10th and 90th percentiles, respectively. Water samples were replicated at each date and measurements were taken at midcolumn water depths. Water depths ranged from >120 cm during the wet season to <10 cm during the dry season. Error bars represent the standard error of the mean. ($n = 1117$).**

(Figure 1). Therefore, TP serves as a more reliable measure of P-enrichment and is typically used by agencies to set water quality standards. The long-term data set of TP concentrations in the experiment allowed us to test responses over each season and at different water depths, which seasonally ranged from <15 cm to over 120 cm. This experimental TP gradient closely matched the concentration range of existing edge-to-interior TP gradients found in the Everglades (*15, 16, 38*). However, ecological attributes often show little change along a gradient until a critical environmental value (threshold) is reached (*39*), and quantitative description of such exposure-response relationships (*40, 41*) are very difficult to obtain. To address this problem, we utilized a Bayesian changepoint analysis (*17*) to estimate TP thresholds for selected biological response variables across multiple trophic levels. Here we define ecological imbalance as a significant alteration in distribution parameters (e.g., mean and/or variance) of the biological response variable (attribute) found above versus below a gradient changepoint. The significance is determined by a small (<0.05) probability of no changepoint.

**Ecological Responses.** The density (stems $m^{-2}$) of *Utricularia purpurea* (bladderwort), a floating aquatic macrophyte, was selected as the best indicator of TP using CART (Figure 2A). Almost no stems per $m^{-2}$ of *U. purpurea* were found at TP concentrations averaging ≥21 $\mu$g $L^{-1}$ (Figure 2B). By contrast we found up to 63 stems $m^{-2}$ in channel locations with TP concentrations near 12 $\mu$g $L^{-1}$. The second best indicator was the combined population of *Utricularia (U. purpurea+ U. fibrosa + U. foliosa)* closely followed by water depth (data not shown). All three *Utricularia* species—a key component of the ecologically significant floating periphyton mat community (*21, 32, 38*)—demonstrated a consistent-pronounced decline with increasing P concentrations (Figure 2C). This may be due to their inability to photosynthesize in waters devoid of $CO_2$, a condition found at higher pH levels (*42*) in P-enriched areas of the Everglades. In fresh waters the total amount of free $CO_2$ available for photosynthesis is variable and highly pH dependent. The p*K* dissociation relationships of $CO_2$, $HCO_3^-$ and $CO_3$ indicate that free $CO_2$ is the dominant form in the water column at a pH of 5 and below, whereas $HCO_3^-$ is dominant from pH 7–9. Above a pH of 9.5, $CO_3$ is the main form of inorganic carbon in the water column (*43*). Supporting the "$CO_2$ limitation" hypothesis is the rise in pH in the Everglades. It averages 7.5 and ranges from a low of 7.2 at night to a high of >10 during the day in the P treatment channels (*29*). Daytime alkalinity values were often found to exceed 300 $\mu$g $L^{-1}$ as $CaCO_3$. Another interaction takes place within the floating periphyton mat, where a significant reduction in *Utricularia* is concurrent with the disappearance of the periphyton community, which primarily occurs at higher P levels (*21*). We have found in periphyton removal experiments that a loss of plant buoyancy occurs when the highly oxygenated periphyton mat disappears and *Utricularia* sinks deeper into the water column (*15, 31*).

Next, we used the Bayesian changepoint method to detect variations in response of the population of *U. purpurea* to TP at different seasons each year (Figure 3A–D). Here each existing data point was assigned a probability of being the changepoint and the TP concentration associated with the highest probability was selected as the TP threshold. For example, *U. purpurea* displayed a variation in TP thresholds over different seasons with geometric mean values of 17.2, (August 1995), 22.3 (March 1996), 12.4 (April 1998), and 16.6 $\mu$g $L^{-1}$ TP (August 1998, near the end of the 6-y experiment) (Figure 3D). In all cases the probability of each selected threshold exceeded 0.97. Importantly, the temporal variation among TP thresholds suggested that seasonal and yearly differences must be integrated into the development of a TP

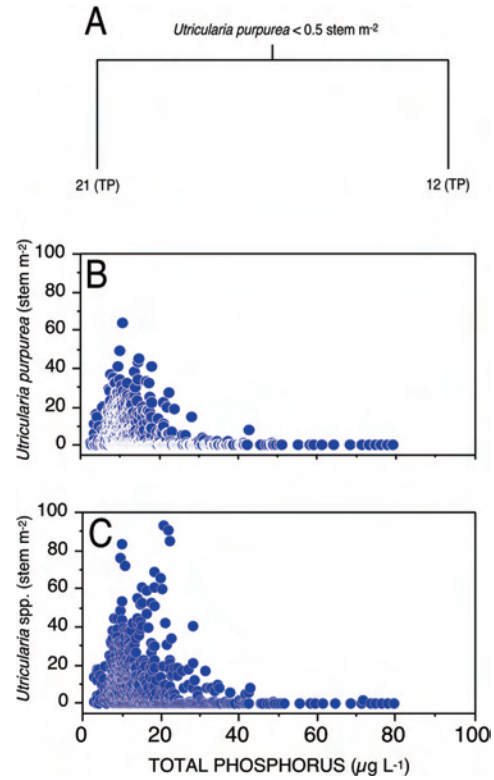


**FIGURE 2. (A). A CART (classification and regression tree) model was used as an inverse ANOVA (*41*) to establish the association between total phosphorus (TP) and candidate biological and abiotic variables (e.g., macrophytes species, water depth, algal species). *Utricularia purpurea* stem counts were selected as the variable that was most closely associated with P concentrations in the water column of the Everglades. (B) The number of all *Utricularia purpurea* stems/$m^2$ in response to mean TP concentrations found in the water column in August. The TP values are the geometric mean ($\mu$g $L^{-1}$ TP) of the water column phosphorus in found during the six months growth period for the plant species. Data for all seasons and years are combined to give a general response pattern for *U. purpurea* during the entire experiment (1992–1998). (C) The number of *Utricularia* spp. stems/$m^2$ in response to mean TP concentration in the water column in August of 1992–1998.**

threshold for each attribute. To accomplish this we calculated an overall mean threshold and credible interval (CI) for *U. purpurea* for the entire experimental period by averaging the geometric mean P thresholds across each time period. The 95% Credible Interval (CI) is the interval of P concentrations that includes 95% of the probability mass of the changepoint distribution. In Bayesian analyses each data point has a probability that it is the threshold but the threshold is assigned to the highest maximum probability data point distribution (*17*). The lower bound ($x_L$) is largest P concentration such that $\sum_{i=1}^{L} p(x_i) \leq 0.025$ and the upper bound ($x_U$) is the smallest P concentration such that $\sum_{i=U}^{n} p(x_i) \leq 0.025$ . Because the distribution is discrete, the selected 95% credible interval ($x_L$, $x_U$) may include more than 95% probability mass. In other words, the CI would give a conservative estimate of the range of upper and lower bounds to the TP threshold. The mean P threshold for *U. purpurea* was 14.8 $\mu$g $L^{-1}$ TP with a 95% CI that ranged from 13.6 to 15.7 $\mu$g $L^{-1}$ TP (Table 1).

To examine the ecological imbalance of the community across trophic levels, we completed a similar hierarchical changepoint analysis over each season and year to identify biological attributes that were consistent metrics of ecological imbalance (*44*). After an initial screening of our database with CART to identify candidate species and other ecological

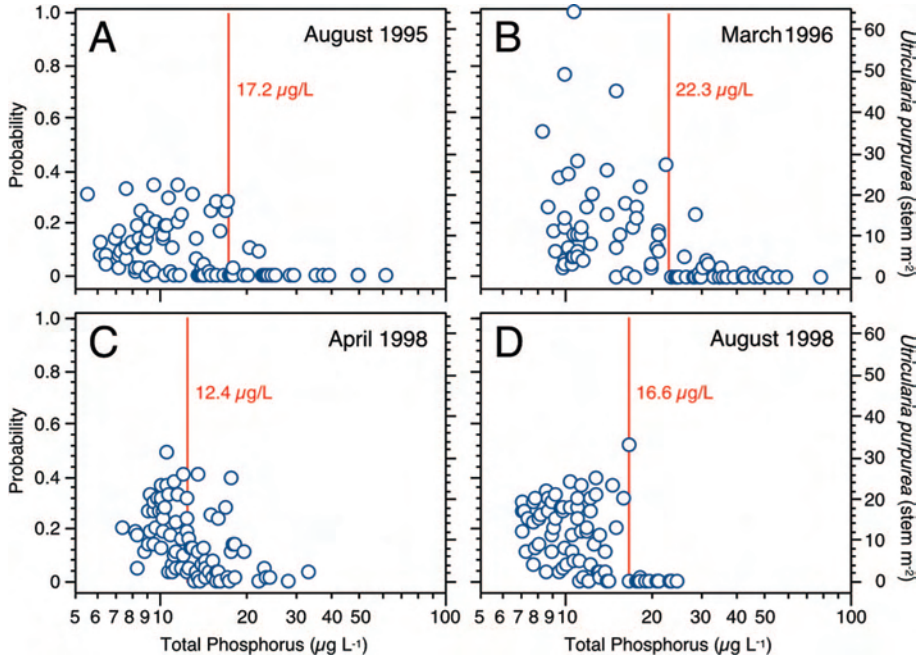


**FIGURE 3. (A to D) A decrease in the stem counts of *Utricularia purpurea* per m$^2$ to increasing water column total phosphorus (TP) concentrations in the dosing channels during the summer and spring of 1995–1998. The selected TP threshold was based on Bayesian analyses and is shown as a "red" vertical line. Values range from a low of 12.4 µg L$^{-1}$ TP in April of 1998 to a high of 22.3 µg L$^{-1}$ TP in March of 1996. The mean and variance of the populations above and below the thresholds have been shown to be significantly different as estimated by a nonparametric deviance analyses (*17*). The probability for all the thresholds exceeds 0.97.**

**TABLE 1. Results from Bayesian Changepoint Analysis on All Trophic Levels from the Phosphorus Dosing Experiment in 1993–1998**

| metric | observed response (*n*)[a] | mean changepoint[b] | maximum probabilities | 95% CI[b] | lower and upper probabilities |
|---|---|---|---|---|---|
| community | | | | | |
| mat cover | decrease (216) | 19.2 | 0.88 | 18.4–20.0 | 0.003–0.000 |
| Bray–Curtis-dissimilarity (macroinvertebrates) | increase (180) | 13.0 | 0.54 | 9.4–15.8 | 0.010–0.011 |
| macroinvertebrates | | | | | |
| % tolerant species | increase (180) | 13.0 | 0.77 | 9.8–19.4 | 0.004–0.004 |
| % sensitive species | decrease (72) | 12.4 | 0.67 | 8.4–26.2 | 0.016–0.012 |
| % predators | decrease (72) | 8.2 | 0.96 | 7.9–8.7 | 0.003–0.664 |
| % microcrustacea | increase (180) | 19.9 | 0.83 | 16.5–20.7 | 0.000–0.000 |
| % oligochaeta | increase (108) | 18.3 | 0.99 | 17.1–18.3 | 0.000–0.000 |
| macrophytes (stem counts) | | | | | |
| total *Utricularia* species | decrease (468) | 15.6 | 0.94 | 15.0–15.9 | 0.002–0.002 |
| *Utricularia purpurea* | decrease (432) | 14.8 | 0.84 | 13.6–15.7 | 0.000–0.005 |
| algae | | | | | |
| % diatom on *Eleocharis cellulosa* | decrease (108) | 14.5 | 0.91 | 12.1–22.2 | 0.003–0.003 |
| % diatom on plexiglas | decrease (72) | 23.5 | 0.68 | 14.0–40.3 | 0.001–0.006 |
| % diatom on floating mats | decrease (144) | 15.3 | 0.75 | 12.0–22.3 | 0.004–0.007 |

[a] (*n* = number of sampling periods times 36 sample locations that displayed a significant TP changepoint)
[b] Changepoints and 95% credible intervals are based on the geometric means of total phosphorus (µg L$^{-1}$ TP) in water.

metrics (over 50 metrics were tested), we selected biological indicator groups that covered multiple trophic levels including "fast" (periphyton), "intermediate" (macroinvertebrate composition, plant density), and "slow" (percent mat cover) process variables from both the natural state and the altered states. Imbalance was calculated as a change in the mean and/or variance of the attribute response variable found above versus below the biological changepoint with the highest maximum probability. The maximum probability is the mode of the changepoint probability distribution: $p(x_r) = \max(p(x_1),...,p(x))$ . It is the P concentration that is most likely to be the threshold, and it gives an indication of how well defined the threshold is within a particular metric (*17*).

At all levels the composition, diversity or population structure of the attribute were significantly altered above the TP threshold as compared to below the changepoint as shown, for example, in Figure 3 A–D. Importantly, the Bayesian hierarchical model takes into account the natural variation in the attributes and thus provides a robust probability estimate of the TP threshold. We then selected metrics at several trophic levels with the highest ecological value and probability threshold to calculate a mean annual TP threshold concentration for each metric (Table 1). This multimetric approach is based on the premise that while no one particular attribute of a biotic assemblage will always be a reliable indicator of imbalance, a suite of attributes used in combination will greatly increase our ability to detect impairment (*44, 45*). Collectively, our selected attributes represent taxonomic composition, species richness and diversity, tolerance/intolerance, and trophic and community structure (Table 1).

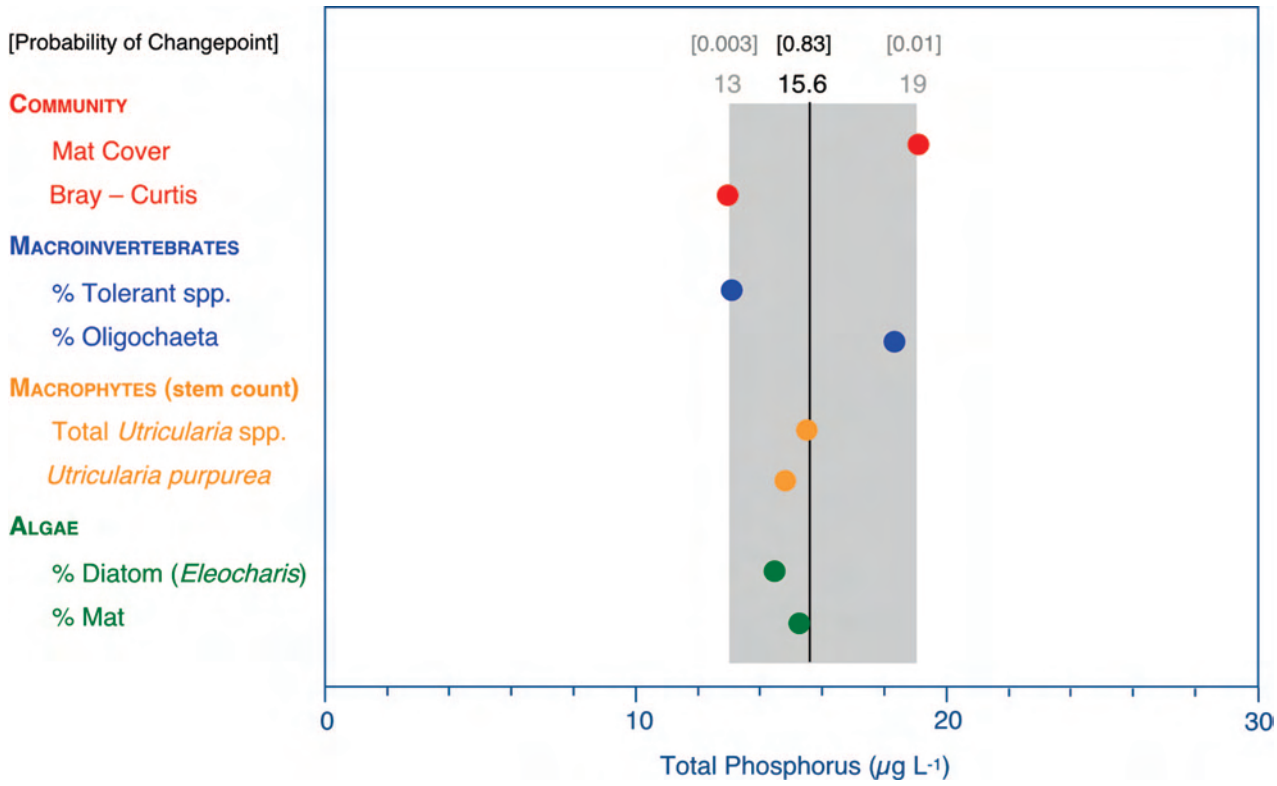


**FIGURE 4. A hierarchical graph of the mean total phosphorus (TP) thresholds along with the corresponding 95% credible interval limits (CI) for each trophic level attribute. The mean TP threshold for all trophic levels combined is 15.6 $\mu$g $L^{-1}$ with a CI of from 13 to 19 $\mu$g $L^{-1}$ TP. The approved EPA TP criterion of 10 $\mu$g $L^{-1}$ TP is below the range of all quantified trophic level thresholds (*27, 28*).**

The observed responses among trophic levels demonstrated a remarkably similar pattern of response (Table 1). Thresholds were almost all above 10 $\mu$g $L^{-1}$ TP and below 20 $\mu$g $L^{-1}$ TP, with macroinvertebrate predators displaying the lowest TP changepoints, whereas diatoms growing on artificial Plexiglas substrates had the highest TP threshold. The most consistent TP threshold with the highest probability was for *Utricularia* species. This metric showed very little variation as noted by the narrow CI (Table 1). The 95% CI intervals were the tightest for the metrics with the highest number of sampling points and varied greatly for the % diatoms on Plexiglas, which had only two sampling dates with changepoints.

To assess uncertainty for each of the selected thresholds, we evaluated the maximum probability and CIs for each metric. Providing an estimate of uncertainty is important because no clear consensus definition for imbalance of natural populations of flora and fauna exists in the ecological literature (*46*). The weakest maximum probabilities were found for Bray–Curtis dissimilarity (BCD, 0.54), % sensitive macroinvertebrate species (0.67) and % diatoms on Plexiglas (0.68) (Table 1). Again, this was probably related to the small number of sampling dates that showed a significant TP threshold for these metrics. Probabilities in excess of 0.90 were found for % Oligochaeta, % predators as well as total *Utricularia* species, and % diatoms on *Eleocharis* stems. Maximum probabilities averaged 0.89, 0.84, 0.78, and 0.71 for the plant macrophytes, macroinvertebrates, algae, and community metrics, respectively. The metrics with both high probabilities and narrow CIs indicate thresholds that accurately reflect a major imbalance in the attributes above the changepoint thresholds for each group as compared to the attribute characteristics below the changepoints (Table 1). The probabilities that the threshold existed at the lower or the higher extreme of the 95% CI were also tested by developing individual probability ratios of the selected changepoint to the lower and higher CIs. In all cases the probability that the changepoint is less than or equal to the lower CI or greater than or equal to the upper 95% CI was <0.01, with most metrics being <0.005 (Table 1). Thus, the probability that the threshold is at the lower or upper 95% CI is close to 0.

To develop a uniformly weighted, integrated threshold, we selected two metrics at each trophic level based on data for at least 100 sampling points, the narrowest CI, and highest maximum probability (Table 1, Figure 4). The overall mean changepoint was 15.6 $\mu$g $L^{-1}$ TP, a value nearly identical to the overall mean of all changepoints shown in Table 1 (Figure 4). The BCD index had the lowest TP threshold (13 $\mu$g $L^{-1}$ TP), lowest maximum probability (0.54), and a wide CI, while mat cover had the highest TP threshold (19.2 $\mu$g $L^{-1}$ TP) with a maximum probability of 0.88 and a narrow CI. The most robust ecological attribute over the entire 6 year period of the experiment was *Utricularia* spp., with a mean changepoint of 15.6 $\mu$g $L^{-1}$, TP a very narrow CI of 15.0–15.9, and a maximum probability of 0.94 (Table 1, Figure 4). The ecological importance of *Utricularia spp.* as a key matrix component of the Everglades periphyton mat community (*1, 21, 31*) coupled with their highly predictable response to P additions ( (*25*), Table 1) suggest they may be highly sensitive indicators of the P threshold for the Everglades, especially *Utricularia purpurea.* These findings also suggest that *Utricularia purpurea* may be both an important keystone and P indicator species for the Everglades.

The 95% CI range for all metrics varied from a low of 13 $\mu$g $L^{-}$ TP to a high of 19 $\mu$g $L^{-1}$ TP. This span of 6 $\mu$g.$L^{-1}$ TP represents a measure of the uncertainty of the estimate, which in part may be due to the natural variation found in the TP thresholds across seasons, water depths, and years (Figure 3A–D). Moreover, 92% of all TP thresholds tested are above 10 $\mu$g $L^{-1}$ TP and below 20 $\mu$g $L^{-1}$ TP. Our dosing study results support the hypothesis that the TP threshold for the Everglades is best represented by a TP zone—not a single number—and that it is above 10 $\mu$g $L^{-1}$ TP, but should not

exceed 15 $\mu$g $L^{-1}$ TP. Importantly, our results at each trophic level show a similar, well-defined TP threshold with a high probability of maintaining a balanced flora and fauna within the Everglades. The dosing research supports our hypothesis that the Everglades has the capacity to assimilate P slightly above background concentrations and that a P threshold protective for all trophic levels in the northern Everglades would best be defined as a threshold zone between 12 and 15 $\mu$g $L^{-1}$ due to seasonal and water level effects.

In the face of these experimental findings, it can be argued that the small spatial scale of our mesocosm study is not representative of the Everglades ecosystem (*15, 30, 38*). To test if our mesocosm results were representative of the TP gradient in the Everglades, we completed TP changepoint analyses (*47*) for macroinvertebrates along a 10 km nutrient (TP, N, Ca, etc.) gradient (*9*) and found that the average for all five metrics (Table 1) in the dosing channels was slightly lower (14.4 $\mu$g $L^{-1}$ TP) than found along the gradient (15.5 $\mu$g $L^{-1}$ TP). In addition our earlier studies (*16*) along this gradient found that no changes in the community structure, productivity or diversity at loadings of 0.4 g $m^2$ $yr^{-1}$, a loading that resulted in <18 $\mu$g $L^{-1}$ TP in the water column. These findings suggest that our mesocosm study results are representative and give a conservative estimate of the existing TP thresholds at the landscape scale.

A current major concern is the fact that the exterior portions of all Everglades areas adjacent to input canals, except the ENP, are not meeting either the proposed EPA criterion of 10 $\mu$g $L^{-1}$ TP or our TP ecological exceedence threshold of 15 $\mu$g $L^{-1}$. Recent TP gradient analyses (*38, 15*) clearly demonstrate elevated TP water column concentrations (>15 $\mu$g $L^{-1}$ TP and sometimes as high as 100 $\mu$g $L^{-1}$ TP) within the first 1 km into the wetlands. Unfortunately, even greatly reduced input TP water concentrations will result in a build up of soil P concentrations (*16*) due to the massive amounts of water being added to the Everglades (i.e., low TP concentrations times large volumes of water equal elevated P loadings). Thus, waters and soils near the input structures will continue to have elevated TP concentrations, although not as high as the northern areas of WCA-2A, which currently have more than 1500 mg $kg^{-1}$ in the top 0–10 cm of soil (*9, 12, 25, 38*). This residual P will result in an upflux of P from the soil sediment to the water column (*25*) and in conjunction with elevated TP input water concentrations will result in TP water concentrations far in excess of the Everglades TP threshold for several km down gradient.

The weight-of-evidence produced from the dosing and gradient analyses implies that the P threshold protective for all trophic levels would best be defined as a threshold zone between 12 and 15 $\mu$g $L^{-1}$, and a TP concentration >15 $\mu$g $L^{-1}$ is likely to cause degradation of plant and macroinvertebrate assemblage structure and function, a reflection of biological integrity, in the study area. While a TP concentration of 15 $\mu$g $L^{-1}$ TP is a reasonable estimate of a TP concentration that will maintain a balance in the flora and fauna at the northern edge of the Everglades peatland, some species, especially in the interior of the southern Everglades and ENP, may require a value closer to the EPA approved criterion of 10 $\mu$g $L^{-1}$ TP. Our threshold findings result from the development of a numerical estimate of the associated risk for metrics at each trophic level as well as an integrated estimate of risk from the combined hierarchical analysis compared with the Index of Biological Integrity (IBI) estimate (*47*). These methods give very similar results and provide a robust estimate of the TP threshold for the Everglades.

Importantly, our Bayesian approach represents a reliable and innovative way of quantifying ecological thresholds along gradients based on estimates of changes in both mean and population variance coupled with a probability analysis. The quantification of ecological thresholds for multiple-trophic levels rather than relying on single-species responses or arbitrary estimates of imbalance provides for a robust estimate of the TP threshold (*44*) and is applicable to other aquatic ecosystems. However, a Bayesian hierarchical modeling approach needs to be developed in the future to statistically integrate the changepoint analysis method presented here to form an ecosystem level threshold distribution to better represent the interactions among fast and slow responding species. We have explored the potential of combining multiple response variables into a single model to study the interactions between these responses. Preliminary results suggest that a Bayesian multilevel ANOVA method can be used to integrate multiple factors affecting thresholds and the interaction of thresholds at several trophic levels (*48*). This integrated approach should also provide risk-based criteria to assess ecological resilience and predict the threshold for alternative state changes.

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# A new method for detecting and interpreting biodiversity and ecological community thresholds

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## Summary

**1.** Existing methods for identifying ecological community thresholds are designed for univariate indicators or multivariate dimension-reduction of community structure. Most are insensitive to responses of individual taxa with low occurrence frequencies or highly variable abundances, properties of the vast majority of taxa in community data sets. We introduce Threshold Indicator Taxa ANalysis (TITAN) to detect changes in taxa distributions along an environmental gradient over space or time, and assess synchrony among taxa change points as evidence for community thresholds.

**2.** TITAN uses indicator species scores to integrate occurrence, abundance and directionality of taxa responses. It identifies the optimum value of a continuous variable, $x$, that partitions sample units while maximizing taxon-specific scores. Indicator $z$ scores standardize original scores relative to the mean and SD of permuted samples along $x$, thereby emphasizing the relative magnitude of change and increasing the contributions of taxa with low occurrence frequencies but high sensitivity to the gradient. TITAN distinguishes negative ($z-$) and positive ($z+$) taxa responses and tracks cumulative responses of declining [sum($z-$)] and increasing [sum($z+$)] taxa in the community. Bootstrapping is used to estimate indicator reliability and purity as well as uncertainty around the location of individual taxa and community change points.

**3.** Using two simulated data sets, TITAN correctly identified taxon and community thresholds in more than 99% of 500 unique versions of each simulation. In contrast, multivariate change-point analysis did not distinguish directional taxa responses, resulting in much wider confidence intervals that in one instance failed to capture thresholds in 38% of the iterations.

**4.** Retrospective analysis of macroinvertebrate community response to a phosphorus gradient supported previous threshold estimates, although TITAN produced narrower confidence limits and revealed that several taxa declined at lower levels of phosphorus. Re-analysis of macroinvertebrate responses to an urbanization gradient illustrated disparate change points for declining (0·81–3·3% urban land) and increasing (6·8–26·6%) taxa, whereas the published threshold estimate (20–30%) missed the declining-taxa threshold because it could not distinguish their synchronous decline from the gradual increase in ubiquitous taxa.

**5.** *Synthesis and applications*. By deconstructing communities to assess synchrony of taxon-specific change points, TITAN provides a sensitive and precise alternative to existing methods for assessing community thresholds. TITAN has tremendous potential to inform conservation of rare or threatened species, develop species sensitivity models, identify reference conditions and to support development of numerical regulatory criteria.

**Key-words:** biodiversity conservation, change-point analysis, community analysis, constrained classification, ecological thresholds, indicator species, regime shift, species sensitivity distribution

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## Introduction

Ecologists have become increasingly interested in analytical methods for detecting and quantifying ecological thresholds (Brenden, Wang, & Su 2008; Andersen *et al.* 2009; Sonderegger *et al.* 2009). Ecological thresholds may be defined as transition points or zones of relatively rapid change between alternate ecosystem states or ecological condition, often in response to small, continuous changes in one or more causal variables (Toms & Lesperance 2003; Huggett 2005; Groffman *et al.* 2006).

Ecological thresholds may be particularly relevant in the context of anthropogenic environmental gradients because such gradients can represent novel physical and chemical conditions falling outside those experienced by species in evolutionary time. Coevolved communities of interacting species possess unique morphological, behavioural and physiological adaptations, often corresponding to a narrow range of environmental conditions (*sensu* Shelford 1913). Species distributions under otherwise unaltered conditions may simultaneously and abruptly change at a critical level of a novel environmental gradient (May 1977; King & Richardson 2003; Sasaki *et al.* 2008). Detection and description of such ecological *community* thresholds has important implications both for ecological theory and application (Townsend, Uhlmann, & Matthaei 2008; Martin & Kirkman 2009).

Ecological community thresholds may be distinct from ecosystem-level, univariate thresholds described by current models (Groffman *et al.* 2006; Andersen *et al.* 2009; Suding & Hobbs 2009) because community responses are multivariate (i.e., one dimension for each taxon). Such thresholds are theoretically relevant to ecologists because of the evolutionary implications of a synchronous response of species to environmental pressures (Huggett 2005; Okland, Skarpaas, & Kausrud 2009). Potential applications of community thresholds include supporting the development of numerical environmental criteria to prevent loss of biodiversity and ecosystem function (King & Richardson 2003) or identification of biological reference conditions to better characterize community dynamics in the absence of disturbance (Wang, Robertson, & Garrison 2007; Brenden *et al.* 2008; Utz, Hildebrand, & Boward 2009).

Current statistical methods used for identifying thresholds were not developed for multivariate species abundance data (Brenden *et al.* 2008; Andersen *et al.* 2009). The vast majority of taxa in community data sets have low occurrence frequencies (i.e., do not occur in a large proportion of the sample units) and highly variable abundances (McCune & Grace 2002). Consequently, most investigators aggregate community data into univariate responses, selecting *a priori* attributes that presumably represent an important facet of community structure, such as the number of taxa or deriving synthetic variables from multivariate analysis of taxa composition among sites (e.g., dissimilarity metrics, ordination axes; King and Richardson 2003, Walsh *et al.* 2005). While aggregating taxa into one or more response variables may, in some instances, increase community signal in response to anthropogenic gradients, it also likely obscures nonlinear changes in one or more taxa, potentially underestimating or misrepresenting the effect of an anthropogenic gradient on ecological communities. Thus, evaluating ecological community thresholds with existing approaches often involves undesirable generalities, loss of information or assumptions regarding taxon-specific responses.

We introduce a new analytical approach, Threshold Indicator Taxa ANalysis (TITAN), with the goals of (i) exploring and identifying abrupt changes in both the occurrence frequency and relative abundance of individual taxa along an environmental, spatial or temporal gradient; (ii) quantifying uncertainty around locations of abrupt change; and (iii) estimating the relative synchrony and uncertainty of those changes as a nonparametric indicator of a community threshold. We contend that a flexible, taxon-specific approach can yield insight informing and augmenting understanding obtained from existing analytical methods. We illustrate accuracy and diagnostic advantages of TITAN using simulated community data and retrospective analysis of two aquatic macroinvertebrate data sets spanning different types of anthropogenic environmental gradients.

## Materials and methods

### BACKGROUND AND COMPONENT CALCULATIONS

TITAN combines and extends change-point analysis and indicator species analysis. Change-point analysis (nCPA; King & Richardson 2003; Qian, King, & Richardson 2003) is a nonparametric technique that orders and partitions observations along an environmental gradient, identical to a single-split, multivariate regression tree analysis (MRT; De'Ath 2002). MRT replaces the univariate response of typical regression tree (RT; Breiman *et al.* 1984; De'Ath & Fabricius 2000) with a measure of multivariate dissimilarity among sample pairs. In both nCPA and MRT, optimal partitioning is obtained by maximizing a deviance reduction statistic that compares within-group vs. between-group dissimilarity determined by a user-selected ecological distance metric. As optimal partitioning can be sensitive to sample distribution along the environmental gradient, nCPA adds a bootstrap resampling procedure to assess uncertainty associated with the observed change-point value (King & Richardson 2003; King *et al.* 2005).

TITAN replaces the aggregate, community-level, dissimilarity response of nCPA with taxon-specific, indicator value (IndVal) scores from indicator species analysis (Dufrêne & Legendre 1997). Indicator species analysis is a widely accepted method for identifying indicator taxa in noisy biological data, pruning dendrograms from a hierarchical cluster analysis to an optimal number of groups, or evaluating how sampling unit groupings explain species distributions. IndVal scores are a simple and intuitive measure designed specifically to capture strength-of-association between any particular taxon and an external sample grouping (e.g., an *a priori* cluster analysis typology).

Indicator species analysis produces an IndVal score estimating the association of each taxon with each group. Thus, two IndVal scores are computed for a single taxon in a two-group classification. IndVal scores are the product of cross-group relative abundance (proportion of abundance among all sample units belonging to group *i*) and within-group occurrence frequency (proportion of sample units in group *i* with a positive abundance value). IndVal uses occurrence

frequency within each group to weight a taxon's relative abundance by how consistently it is observed in each group. A large abundance within one sample group results in a greater IndVal score only if the taxon also occurs with great regularity in that same group. IndVal scores are superior to simple abundance as a measure of association because integration of occurrence frequency and abundance leads to a measure of association *that is unbiased by group size* (Dufrêne & Legendre 1997). IndVal scores are scaled from 0% to 100% with a value of 100 indicating that a taxon was collected in every sample within a group and not in any other group. Conversely, in a two-group classification, a value of 50 can mean that a taxon occurred in half the samples within only one group, or in equal abundances in all samples of both groups (e.g., Fig. 2). The probability of obtaining an equal or larger IndVal score from random data ($P$) is estimated by comparing the magnitude of each observed IndVal score with those generated when group membership is assigned via ≥250 randomized permutations (Dufrêne & Legendre 1997).

## CHANGE-POINT IDENTIFICATION AND UNCERTAINTY FOR A SINGLE TAXON

TITAN uses IndVal scores instead of deviance reduction (as in nCPA or MRT) to identify change points across a continuous environmental gradient ($x$; Fig. 1, Step 1). Midpoints between observed values of $x$ are candidate change points ($x_i$) used to iteratively split observations into two groups, and thus produce two IndVal scores at each split (Fig. 1, Step 2·1). The relative magnitude of IndVal scores for groups on each side of a candidate change point reflects whether a taxon shows greater association with the left (negative response with respect to $x$) or the right (positive response) side of each split (Fig. 1, Step 2·1C, D). The greater the difference in taxon fidelity (association) created by a specific split, the greater the IndVal score for one of the two groups. The greatest IndVal score at each split and the side of the split on which it occurs are retained for comparison with those at other candidate change points (Fig. 2). In practice, we use a minimum group size of five observations (De'Ath & Fabricius 2000), so for any sample of $n$ observations and depending on the number of unique $x$-values observed, TITAN compares up to $2n-20$ IndVal scores for each taxon (i.e., $2n$ for IndVals at each split, less the 10 samples and splits needed to satisfy the minimum group size). Any value of $x$ resulting in an IndVal maximum among candidate splits is identified as the observed change point or the optimal partition for that taxon (Fig. 2).

TITAN estimates uncertainty surrounding taxon-specific responses using the distribution of change-point values across a series of bootstrap replicates of the entire data set (Fig. 1, Step 3; Manly 1997; Toms & Lesperance 2003). The bootstrap procedure is necessary because unlike *a priori* group classification required by indicator species analysis, optimal group partitioning along $x$ is initially unknown in TITAN, and is in fact the objective of the analysis. Whereas the permutation procedure is used to estimate the probability that an equal or larger IndVal could be obtained from random data, the bootstrap procedure estimates uncertainty around change-point locations (optimal partitioning along $x$), as well as consistency in the response direction of each taxon (negative or positive). Variability in change-point location, directionality (positive or negative with respect to $x$) and magnitude (relative to the absence of structure along $x$) constitute the information content of the *indicator response* for each taxon in TITAN.

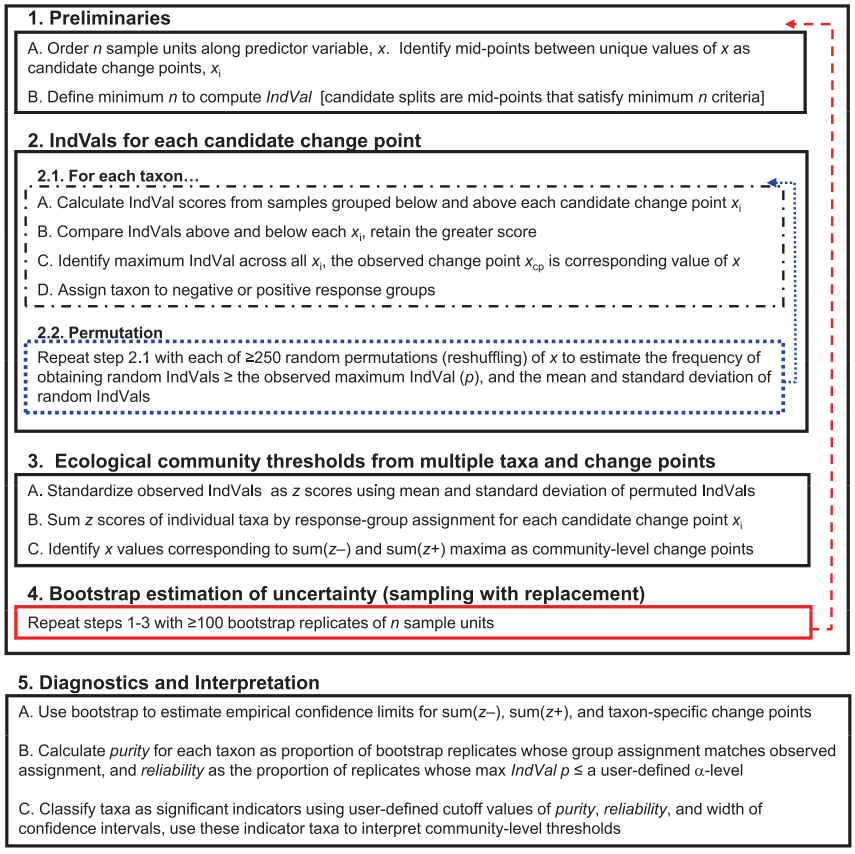


**Fig. 1.** Flow chart of Threshold Indicator Taxa ANalysis (TITAN).

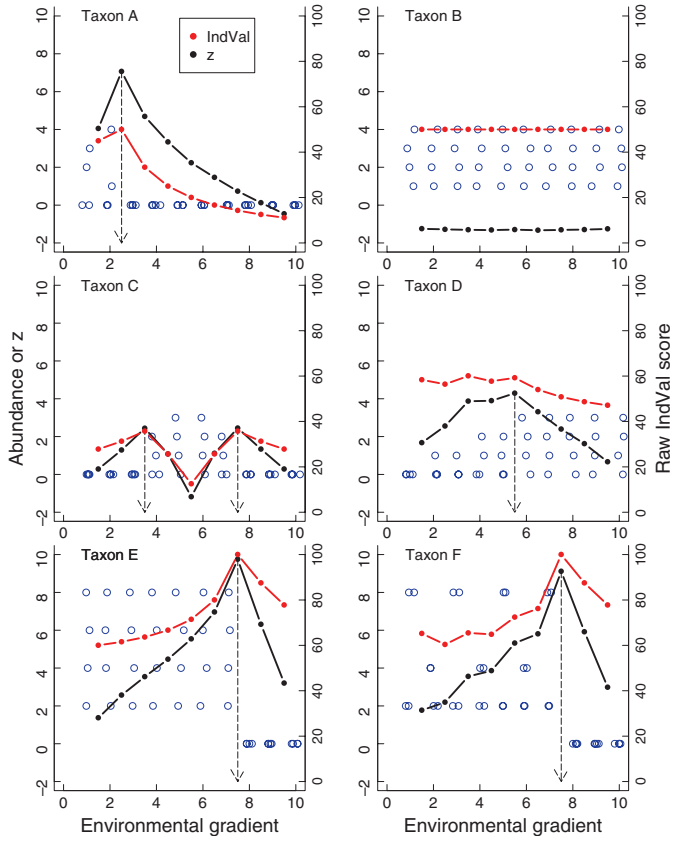


**Fig. 2.** Response of indicator value (IndVal) and *z* scores for six hypothetical taxa abundances along a uniform environmental gradient. Dashed vertical arrows illustrate the maximum *z* score and corresponding environmental change point.

Two important diagnostic indices measuring the quality of the indicator response for any taxon are obtained from bootstrap resampling: purity and reliability. Indicator *purity* is the proportion of change-point response directions (positive or negative) among bootstrap replicates that agree with the observed response. Pure indicators (e.g., purity ≥0·95) are consistently assigned the same response direction, regardless of abundance and frequency distributions generated by resampling the original data.

If bootstrap resampling substantially alters the probability of obtaining an equal or larger IndVal based on 250 random permutations of the data, then that particular taxon is not a reliable indicator. Indicator *reliability* is estimated by the proportion of bootstrap change points whose IndVal scores consistently result in *P*-values below one or more user-determined probability levels (e.g., $P \leq 0{\cdot}05$). Reliable indicators (e.g., ≥0·95 of the bootstrap replicates achieving $P \leq 0{\cdot}05$, or some other user-defined proportion of replicates) are those with repeatable and consistently large IndVal maxima.

For each pure indicator taxon, TITAN uses bootstrap replicates to estimate empirical quantiles of the change-point distribution. Variation in change-point estimates highlights uncertainty in the location of the maximum IndVal with respect to *x*. Sharp, nonlinear responses in taxon abundances are reflected by relatively narrow intervals between upper and lower change-point quantiles (e.g., 5%, 95%), whereas taxa with linear or more gradual responses will have broad quantile intervals spanning most of the range of *x*. If the gradient is long enough to produce a Gaussian-like response, the interval will likely encompass the mode because of reliably strong, but impure bootstrap change points. However, we discourage strict-interpretation of these quantiles as confidence limits because any method of computing confidence limits will be unreliable for taxa with low occurrence frequencies (Manly 1997).

## INDENTIFYING ECOLOGICAL COMMUNITY THRESHOLDS FROM MULTIPLE CHANGE POINTS

Once IndVals for each candidate change point and taxon are classified according to response direction, the aggregate response of all indicator taxa at each candidate change point may be used as evidence for a community-level threshold. As a dendrogram assessment tool, Dufrêne & Legendre (1997) recommended that the number of groups resulting in the largest sum of IndVal scores (scores significant at $P < 0{\cdot}05$ or other criteria) be considered optimal for dendrogram pruning. This approach uses the greatest cumulative IndVal signal to distinguish groups, whether from a single taxon or many taxa, because a primary goal is to facilitate accurate classification of new observations using taxa most characteristic of each group. In contrast, evidence for a community threshold in TITAN requires substantial change across more than the most predominant taxa. As rare taxa are often highly sensitive to environmental alterations and the focus of biodiversity conservation, changes in their distribution are of great interest, although often more difficult to detect. Because the absolute value of IndVal scores is influenced by a taxon's overall abundance, it is less important in TITAN than the magnitude of change *relative to each taxon's abundance distribution* (see below). All taxa are not required to have identical IndVal maxima to produce a community threshold, rather only that large IndVal scores for many taxa occur close together along *x*. Integrating information about taxon-specific changes across groups, we apply the additive indicator

score concept in a novel way, using rescaled indicator responses to partition observations while preserving the information provided by rare taxa.

IndVal scores are rescaled as $z$ scores within TITAN according to degree of departure from expected values by subtracting the mean of randomized permutations from the observed IndVal, and dividing by its permuted SD. Exploratory simulations suggest that permuted IndVal $z$ scores are similar to nonparametric alternatives (i.e., substituting median and interquartile range for mean and standard deviation). We use $z$ scores because the central focus of TITAN is somewhat distinct from the original purpose of IndVal. IndVal was developed to interpret a pre-existing sample typology (Dufrêne & Legendre 1997), whereas TITAN seeks to use IndVal scores to select among candidate groupings. Rather than raw IndVal magnitudes, which would favour the most widely distributed or abundant taxa, standardization facilitates cross-taxa comparison by emphasizing change in IndVals across candidate splits *given a specific pattern of abundance and occurrence* (Fig. 2). Rescaling makes little difference during determination of taxon-specific change points, but it can make a substantial difference during interpretation of their relative information content. Rare or infrequently occurring taxa with smaller IndVal magnitudes can have a very strong $z$ score if their response to environmental change is dramatic. Standardized taxa responses increasing at the change point ($z+$) are distinguished from those decreasing ($z-$) and those showing no response.

Evidence for community-level thresholds among negative and positive taxa is assessed separately by tabulating and summing all $z-$ and $z+$ scores for each value of $x$. The value(s) of $x$ resulting in the largest cumulative $z$ scores for negative [sum($z-$)] and positive [sum($z+$)] responses correspond to the maximum aggregate change in the frequency and abundance of their respective taxa. Large values of sum($z$) scores occur when many taxa have strong responses at a similar value of the environmental gradient, whereas weak or variable responses result in lower sum($z$) values without a distinctive maximum. TITAN community-level change points may be assessed by plotting sum($z$) scores vs. $x$, and are easily interpreted via tabular and graphical summaries of change-point distributions of individual taxa.

If sum($z$) maxima involve synchronous change in many taxa, including overlap from bootstrap distributions of taxon-specific change points, then these values of $x$ may be interpreted as evidence for observed community thresholds. Bootstrap replicates used to evaluate taxon-specific change points are also summarized to develop distributions of sum($z$) responses. Variation in the bootstrapped values of $x$ that produce the greatest sum($z-$) or sum($z+$) values is used to estimate uncertainty associated with community change points, and quantiles (e.g., 0·05, 0·95) of these distributions serve as empirical confidence limits (e.g., Qian *et al.* 2003; Toms & Lesperance 2003). Narrow confidence limits represent further evidence for a community threshold, whereas wide confidence bands suggest other response (e.g., linear, modal or random) dynamics are more likely.

## Case studies

### SIMULATED COMMUNITY DATA

We developed two simulations to evaluate how TITAN deals with taxa with distinct distributions, classifies responses, and detects change points. By controlling the statistical properties of the response and predictor variables across 500 unique data sets generated by each simulation, we demonstrate TITAN's efficacy and flexibility.

The first simulation involved a shift in respective taxa at two distinct values of $x$. Eight taxa abundances were simulated along a uniformly distributed environmental gradient (runif in R 2·9·2; $n$ = 100, range = 0–100). Abundances were generated using a negative binomial distribution (rnbinom in R 2·9·2) to simulate noisy, heteroscedastic and sparse site-by-taxa matrices typical of community data (McCune & Grace 2002). Two taxa (Sp1–2) were thus assigned frequency and abundance values that differed below and above a value of 40 along the environmental gradient to simulate samples from populations with a threshold decline (Fig. 3). Likewise, three taxa (Sp6–8) were assigned increasing frequency and abundance values above 60 to assess TITAN's ability to distinguish disparate change points and different response directions. One taxon (Sp3) was assigned an increasing change point at 40 and a decreasing change point at 60 to approximate a unimodal distribution. Finally, two taxa (Sp4–5) differed in frequency and abundance but varied randomly with respect to the environment. The entire simulation was repeated 500 times to generate different data sets, obtain diagnostic statistics, and evaluate the ability of nCPA (Bray-Curtis distance among sample units) and TITAN to correctly identify and assess thresholds in the data.

The second scenario involved similar distributions of the environmental gradient to contrast threshold responses with noisy data and generalized, wedge-shaped distributions typical of complex taxon responses to multiple limiting factors (e.g., Cade & Noon 2003; Brenden *et al.* 2008; Konrad *et al.* 2008). In this scenario, two taxa (Sp1–2) were assigned decreasing frequency and abundance values to simulate samples from populations with a threshold decline at 20 (Fig. 4). Three taxa (Sp6–8) were assigned probabilities of frequency and abundance that increased in proportion to $x$ (i.e., generating wedge-shaped distributions). Sp3 was assigned an increasing change point at 20 and a decreasing change point at 60 to approximate a broad, unimodal distribution, whereas Sp4–5 varied randomly with respect to the environment. This simulation was also repeated 500 times to obtain diagnostic statistics.

### EVERGLADES DATA

These data were taken from a previous study designed to identify a concentration of surface-water total phosphorus (TP) that corresponded to abrupt changes in macroinvertebrate species composition in the Florida Everglades, USA (King & Richardson 2003). Macroinvertebrate densities (no/m$^2$, 164 taxa, species or morphospecies-level taxonomy) were measured from 126 marsh sampling stations along a 10-km TP gradient. Concentrations of TP in the data set ranged from $< 10$ μg/L to $> 100$ μg/L. The authors used several community variables and estimated TP change points using univariate nCPA. The resulting change points ranged from *c.* 10 μg/L to 25 μg/L TP, and authors concluded that TP > 12–15 μg/L likely corresponded to ecologically significant changes in taxonomic composition.

### MARYLAND STREAM DATA

These data were the subject of a previous study on analytical considerations for linking watershed land cover to ecological communities in streams (King *et al.* 2005). In the previous analysis, we used axis scores from non-metric multidimensional scaling (nMDS) of Bray-Curtis dissimilarity in nCPA to identify a level of watershed percent developed land corresponding to an abrupt change in macroinvertebrate community composition in wadeable streams (295 sites, 177 taxa abundances, mostly genus-level identification). Our previous analysis identified a relatively sharp change

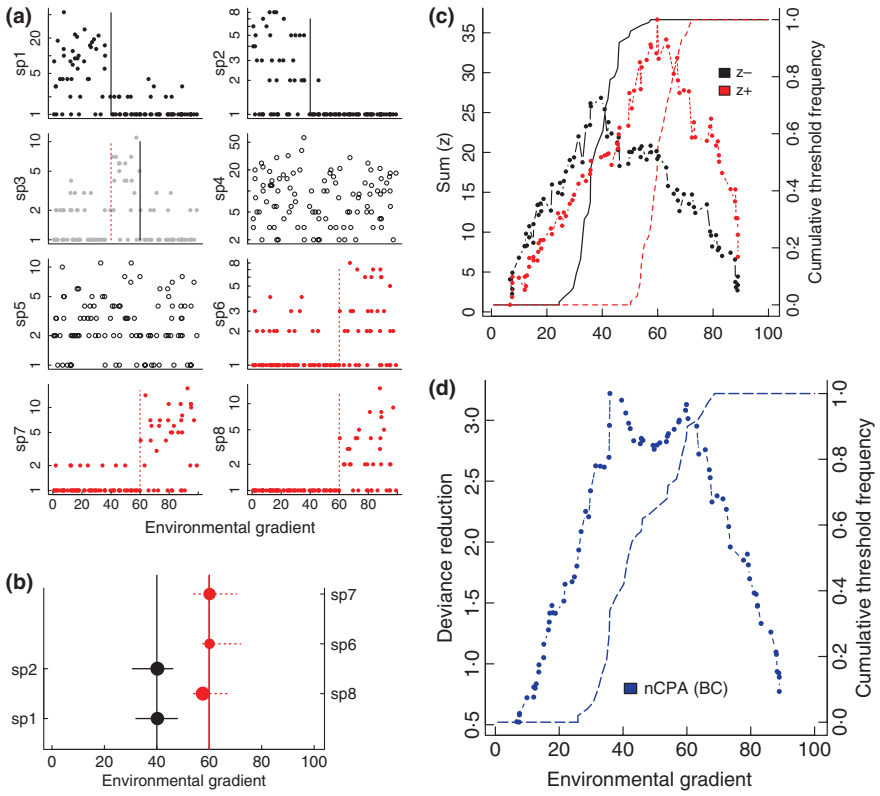


**Fig. 3.** Threshold Indicator Taxa ANalysis (TITAN) and change-point analysis (nCPA, Bray-Curtis distance) of a two-threshold community response to a simulated environmental gradient (Simulation 1). (a) Simulated abundances of eight taxa in response to the environmental gradient (*x*-axis). According to negative binomial probability distributions used in simulating frequency and abundance, Sp1–2 should decline at 40 (black vertical line), Sp3 should increase at 40 (red vertical line) and decrease at 60 (black vertical line), Sp4–5 should not vary predictably with the environment, and Sp6–8 should increase at 60 (red vertical line). (b) Pure (≥0·95) indicator taxa are plotted in increasing order with respect to their observed environmental change point. Black symbols correspond to negative (*z*−) indicator taxa, whereas red corresponds to positive (*z* + ) indicator taxa. Symbols are sized in proportion to *z* scores. Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 500 bootstrap replicates. Vertical lines indicate the simulated true values for negative (black vertical line) and positive (red vertical line) underlying thresholds. (c) TITAN sum(*z*−) and sum(*z* + ) values corresponding to all candidate change points ($x_i$) along the environmental gradient. Black and red vertical lines represent the cumulative frequency distribution of change points ($x_{cp}$, or thresholds) among 500 bootstrap replicates for sum(*z*−) and sum(*z* + ), respectively. (d) Deviance reduction in Bray-Curtis distance values for each candidate change point ($x_i$) along the environmental gradient. The dashed blue line represents the cumulative frequency distribution of change points (thresholds) among 500 bootstrap replicates.

in community composition (inferred from nMDS axis 1 scores) between 20% and 30% watershed developed land (5th–95th bootstrap percentiles).

### DATA ANALYSES

We performed TITAN analysis on all four data sets in R (R Development Core Team 2009, version 2·9·2) using a custom package TITAN (see Appendix S3) written by MEB and RSK. nCPA was performed using a custom function within TITAN based on the db-MRT method of De'Ath (2002) in the package mvpart. We $\log_{10}(x + 1)$ transformed taxa abundances to reduce the influence of highly variable taxa on indicator score calculations in each data set, which was particularly important for taxa with low occurrence frequencies. Taxa with $< 5$ occurrences were deleted (following previous analyses of these data) and we used Bray-Curtis distance as the dissimilarity metric for all nCPA assessments (King *et al.* 2005).

We compared TITAN and nCPA by plotting sum(*z*) and deviance reduction values as a function of increasing values of *x* and identified community change points as the *x* value that resulted in the maximum sum(*z*) or deviance reduction, respectively. We computed cumulative change-point distributions by finding the maximum IndVal (individual taxa), TITAN sum(*z*) and nCPA deviance reduction across 500 bootstrap replicates for the simulated and real data sets. Indicator purity and two levels of reliability (proportion of bootstrap replicates with $P \leq 0{\cdot}05$ and $P \leq 0{\cdot}01$) were also computed from the bootstrap replicates. We used output from the 500 unique iterations of each simulated data set to compute: (i) the frequency at which the 0·05 and 0·95 bootstrap quantile intervals captured the true thresholds, (ii) median change-point values corresponding to IndVal *z*-score maxima along the environmental gradient, and (iii) mean reliability and purity of each taxon.

## Results

### SIMULATION 1

TITAN accurately interpreted both negative and positive indicator taxa regardless of their relative position along the environmental gradient, while diagnostic indices helped distinguish the relative information content in taxon-specific

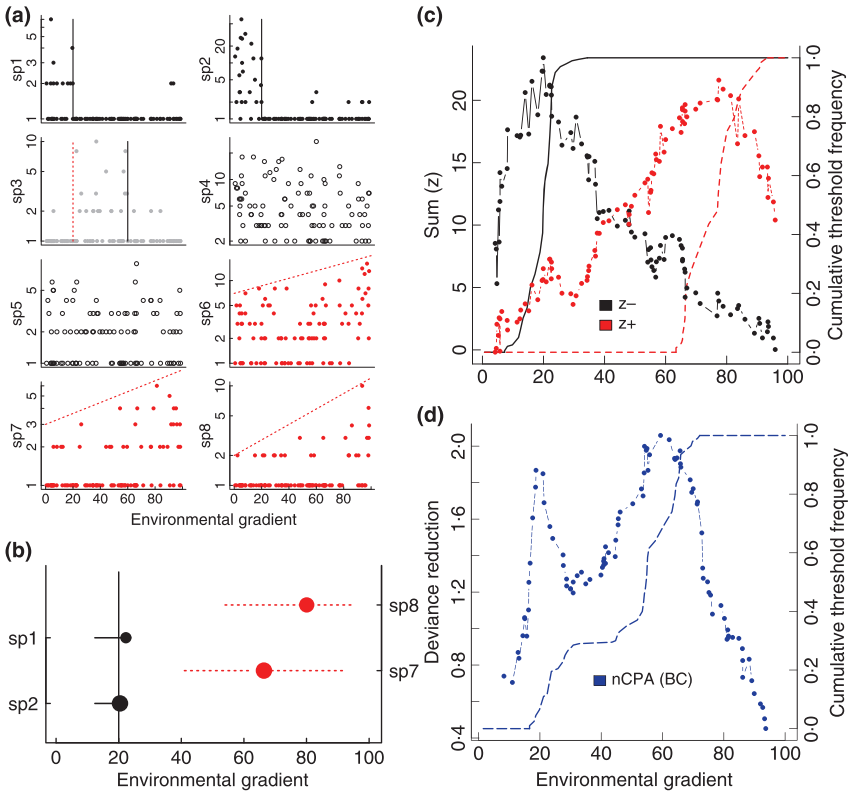


**Fig. 4.** Threshold Indicator Taxa ANalysis (TITAN) and change-point analysis (nCPA, Bray-Curtis distance) of a negative threshold and positive wedge-shaped community response to a simulated environmental gradient (Simulation 2). (a) Sp1–2 should decline at 20 (black vertical line), Sp3 should increase at 20 (red vertical line) and decrease at 60 (black vertical line), Sp4–5 should not vary predictably with the environment, and Sp6–8 should increase with a wedge-shaped distribution (red dotted lines) instead of a threshold response. See Fig. 3 for additional details.

distributions. All 8 taxa produced indicator assignments and observed change points consistent with the simulated parameters, and all had significant ($P \leq 0{\cdot}05$) raw IndVal scores in at least 46% of the 500 data sets. All six taxa simulating a threshold decline or increase had median change-point distributions that overlapped their true threshold value of $x$ (Table 1; see Fig. 3b for example), whereas the distribution for Sp3 (simulated unimodal distribution) included both thresholds. Change-point distributions were much broader for the two random taxa (Sp7–8). All five monotonic taxa were both reliable (mean reliability over 500 data set iterations ≥0·99 for $P \leq 0{\cdot}05$ and $P \leq 0{\cdot}01$) and pure indicators (mean purity over 500 iterations ≥0·99), whereas Sp3 was impure and only moderately reliable (reliability ≥0·69 from $P \leq 0{\cdot}05$ to $P \leq 0{\cdot}01$; mean purity = 0·74). Both taxa (Sp4–5) simulating independence from the environment gradient often produced significant raw IndVal scores at $P \leq 0{\cdot}05$ (64% and 61%, respectively), but were much less reliable at $P \leq 0{\cdot}01$ (38%, 35%) and remained impure indicators (mean purity 0·74, 0·72). The degraded reliability was expected for random taxa, but in the case of Sp3, limited reliability suggests its distribution often resulted in IndVals indistinguishable from random scores.

As an assessment of community-level thresholds, TITAN sum($z-$) peaked at a median of 39·97 (mean = 40·21) across 500 data sets, corresponding closely with the true threshold value of 40 for negative indicator taxa (Table 2; see examples in Fig. 3a and c). The sum($z+$) median peaked at 60·04 (mean = 60·22) close to the simulated true threshold of 60. Cumulative change-point frequency distributions for bootstrap replicates were relatively narrow at the 90% level and straddled the true thresholds for negative and positive taxa in 100% of simulation data sets. nCPA deviance reduction reached its maximum at a median of 59·58 (mean = 53·58; Fig. 3d). The inner 90% of the change-point distribution was broader than either sum($z$) and overlapped the positive threshold in 91% of the iterations, but was slightly above the negative threshold more often, capturing the true value in only 62% of the data sets. This comparison illustrated that despite near-synchronous thresholds among negative and positive responders, TITAN distinguished the two precisely, whereas nCPA produced broader confidence limits and a biased observed change-point estimate.

## SIMULATION 2

All eight taxa produced indicator assignments and observed change points consistent with the simulated parameters, and all had significant ($P \leq 0{\cdot}05$) raw IndVal scores in more than 43% of the 500 data sets. Taxa with higher overall frequencies

**Table 1.** Threshold Indicator Taxa ANalysis (TITAN) individual taxa results from Simulations 1 and 2

| | | | | | Change point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | +/− | IndVal | Freq. | *z* | Obs. | 5% | 95% | Capture | Purity | ≤0·05 | ≤0·01 |
| Simulation 1 | | | | | | | | | | | |
| Sp1 | *z*− | 82·3 | 47·8 | 15·45 | 40·04 | 30·94 | 48·71 | 1·00 | 1·00 | 1·00 | 1·00 |
| Sp2 | *z*− | 54·59 | 29·9 | 10·88 | 39·61 | 25·21 | 48·45 | 0·99 | 1·00 | 1·00 | 1·00 |
| Sp3 | *z*− | 28·81 | 16·9 | 6·04 | 57·5 | 38·21 | 61·71 | 0·66, 0·62* | 0·74 | 0·74 | 0·72 |
| Sp4 | NA | 54·82 | 100 | 1·91 | 48·84 | 8·45 | 92·22 | NA | 0·74 | 0·64 | 0·38 |
| Sp5 | NA | 49·02 | 69·8 | 1·91 | 51·22 | 8·84 | 92·16 | NA | 0·72 | 0·61 | 0·35 |
| Sp6 | *z*+ | 60·61 | 40·3 | 10·47 | 60·33 | 51·16 | 73·03 | 1·00 | 1·00 | 1·00 | 1·00 |
| Sp7 | *z*+ | 82·19 | 48·3 | 15·5 | 59·94 | 51 | 68·61 | 1·00 | 1·00 | 1·00 | 1·00 |
| Sp8 | *z*+ | 59·59 | 40 | 10·37 | 60·33 | 50·9 | 72·78 | 1·00 | 1·00 | 1·00 | 1·00 |
| Simulation 2 | | | | | | | | | | | |
| Sp1 | *z*− | 50·41 | 11 | 12·49 | 19·63 | 9·51 | 27·13 | 0·99 | 1·00 | 1·00 | 0·99 |
| Sp2 | *z*− | 83·61 | 26 | 15·51 | 20·08 | 13·04 | 28·5 | 1·00 | 1·00 | 1·00 | 1·00 |
| Sp3 | *z*− | 52·42 | 32·6 | 9·63 | 60·18 | 49·49 | 67·63 | 0·99, 0·14* | 0·96 | 0·96 | 0·96 |
| Sp4 | NA | 55·28 | 100 | 1·91 | 49·2 | 8·74 | 92·16 | NA | 0·73 | 0·63 | 0·38 |
| Sp5 | NA | 45·04 | 59·6 | 1·91 | 53·22 | 8·96 | 92·1 | NA | 0·73 | 0·62 | 0·37 |
| Sp6 | *z*+ | 49·94 | 51·8 | 4·36 | 71·75 | 30 | 90·37 | NA | 0·93 | 0·90 | 0·77 |
| Sp7 | *z*+ | 51·2 | 28·8 | 8·69 | 70·5 | 52·12 | 87·14 | NA | 1·00 | 1·00 | 0·98 |
| Sp8 | *z*+ | 45·42 | 24·3 | 8·12 | 71·68 | 51·79 | 88·72 | NA | 1·00 | 0·99 | 0·97 |

TITAN observed change points (obs.) and bootstrap confidence intervals (median among 500 simulation iterations) correspond to the value of *x* resulting in the largest indicator value (IndVal) *z* scores for each taxon (see Figs 3a and 4a). Capture rates show the proportion of 500 simulation iterations in which the 90% bootstrap confidence interval included the true threshold. Purity is the mean proportion of correct response direction (*z*− or *z*+) assignments, reliability is the mean proportion of *p*-values ≤0·05 or ≤0·01 among 500 simulation iterations, and Freq. is the number of non-zero observations.
*Values correspond to increasing and decreasing change points, respectively for unimodal taxa.
NA, not applicable to random or wedge-shaped responses.

tended to have higher raw IndVals, but not necessarily higher *z* scores. Rather, *z* scores corresponded more closely to the relative difference between the left and right side of the threshold in the assigned probabilities of the negative binomial distribution function used to simulate abundances (Table 1; see Fig. 4b for example). Both taxa simulating threshold declines had median change-point distributions that overlapped their true change-point value of *x*, whereas the distribution for Sp3 included only the declining change point. All five monotonic taxa and unimodal Sp3 were both reliable and pure indicators (reliability ≥0·96 from $P \leq 0{\cdot}05$ to $P \leq 0{\cdot}01$; mean purity over 500 data sets ≥0·92) with the exception of Sp6 (wedge-shaped increaser; inconsistent reliability; mean purity = 0·93). Both random taxa (Sp4–5) produced significant raw IndVal scores at $P \leq 0{\cdot}05$ in 63% and 62% of the iterations, respectively, but were much less reliable at $P \leq 0{\cdot}01$ (38%, 37%) and were impure indicators (mean purity 0·73, 0·73).

**Table 2.** Threshold indicator taxa analysis (TITAN) community-level results from Simulations 1 and 2

| Method | Obs. | 0·05 | 0·95 | Capture (−) | Capture (+) |
|---|---|---|---|---|---|
| Simulation 1 | | | | | |
| TITAN sum(*z* −) | 39·97 | 30·76 | 49·84 | 1·00 | ≤0·01 |
| TITAN sum(*z* +) | 60·04 | 51·41 | 68·39 | ≤0·01 | 1·00 |
| nCPA (Bray-Curtis) | 59·58 | 38·10 | 64·82 | 0·62 | 0·91 |
| Simulation 2 | | | | | |
| TITAN sum(*z* −) | 20·17 | 12·53 | 28·22 | 1·00 | NA |
| TITAN sum(*z* +) | 70·21 | 54·27 | 85·08 | ≤0·01 | NA |
| nCPA (Bray-Curtis) | 20·03 | 15·35 | 61·29 | 0·93 | NA |

TITAN observed change points (Obs.) and bootstrap 5th and 95th quantiles of change points (median among 500 simulation iterations) correspond to the value of the *x* resulting in the largest sum of indicator value (IndVal) *z* scores among all negative (*z*−) and positive (*z*+) taxa, respectively (see Figs 3c and 4c), whereas nCPA thresholds correspond to the maximum deviance reduction among sample units (Bray-Curtis distance; Figs 3d and 4d). Capture rates show the proportion of 500 simulation iterations in which the 90% bootstrap confidence interval included the true threshold.
NA, not applicable.

During assessment of community-level response, TITAN sum(*z*−) peaked at a median of 20·17 (mean = 20·02) across 500 iterations, corresponding closely with the true threshold value of 20 for negative indicator taxa (Table 2; see examples in Fig. 4a and c). Cumulative change-point frequency distributions for bootstrap replicates were quite narrow at the 90% level (Fig. 4c), yet straddled the true threshold in 100% of simulation data sets. The sum(*z*+) median peaked at 70·21 (mean = 69·78) and appropriately exhibited a broader cumulative frequency distribution of change points in accordance with the gradual, wedge-shaped increasing response for Sp6–8 (Fig. 4b and d). The nCPA deviance reduction reached its maximum at a median of 20·03 (mean = 25·34), close to

the negative threshold decline, and increased again between 55 and 75. The inner 90% of the change-point distribution was broader than either sum($z$), stair-stepped, and overlapped the negative threshold in 93% of simulation data sets. This comparison illustrated that when negative and positive responders have asynchronous change points, the aggregate dissimilarity metric used by nCPA can be biased by different change-point locations.

### EVERGLADES DATA

The majority of the negative ($z-$) indicator taxa declined sharply between 8 and 20 µg/L TP, resulting in a sum($z-$) change point of 14·5 µg/L (Fig. 5a and b; Table 3; Appendix S1). Positive ($z+$) indicators increased sharply between 20 and 40 µg/L resulting in a distinct sum($z+$) peak at 30·3 µg/L. Most individual taxa change points overlapped considerably in the 15–40 µg/L range, providing evidence in support of an ecological community threshold.

The observed peak in nCPA deviance reduction occurred at 18·6 µg/L, with a secondary peak at ~35 µg/L (Fig. 5d). The cumulative distribution of nCPA change points spanned the range of both sum($z-$) and sum($z+$) distributions. TITAN sum($z-$) and the lower end of the nCPA change-point distribution supported TP community changes reported in King & Richardson (2003), although sum($z-$) results implied a much sharper, synchronous change with TP. This comparison demonstrates the increased precision obtained through taxon-specific analysis in TITAN.

### MARYLAND STREAM DATA

TITAN identified >20 taxa with synchronous declines in response to developed land between 0·5% and 2·5%, resulting in a distinct peak in sum($z-$) at 1·89% (Fig. 6a and b; Table 3; Appendix S2). The cumulative distribution of sum($z-$) change points among bootstrap replicates was quite narrow (Fig. 6b; Table 3). A few additional taxa fell out in an approximately linear sequence from 5% to 20% developed land. The strong synchrony of change in many taxa at low levels of development was consistent with an ecological community threshold.

Relatively few taxa exhibited positive associations with increasing amounts of watershed development (Fig. 6a and b; Appendix S2). Those that did were widely distributed along the developed-land gradient, spanning most of the range of values and approximating a linear distribution of observed taxon change points with increasing urbanization (Fig. 6a). The asynchronous

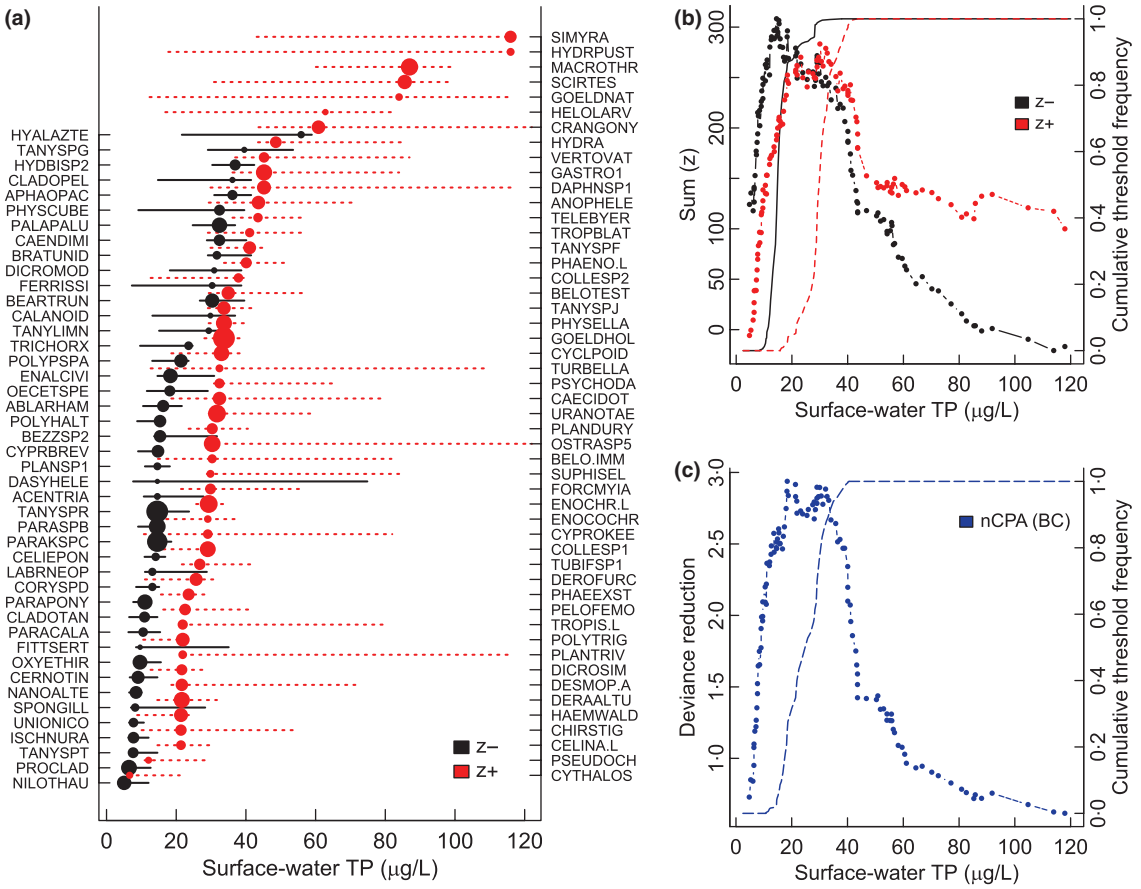


**Fig. 5.** Threshold Indicator Taxa ANalysis (TITAN) and change-point analysis (nCPA, Bray-Curtis distance) of macroinvertebrate community response to a surface-water total phosphorus gradient in the Everglades ($n$ = 126). For clarity, only taxa with purity = 1·0 are shown in (a). Taxa codes in (a) are explained in Appendix S1. See Fig. 3 for additional details.

**Table 3.** TITAN community-level thresholds estimated from macroinvertebrate taxa responses to total phosphorus (TP) in the Everglades and percent developed land in Maryland watersheds

| | Everglades (TP, µg/L) | | | | Maryland (developed land) | | | |
|---|---|---|---|---|---|---|---|---|
| Method | Obs. | 5% | 50% | 95% | Obs. | 5% | 50% | 95% |
| TITAN sum($z-$) | 14·5 | 11 | 14·7 | 21·6 | 1·89 | 0·81 | 1·96 | 3·38 |
| TITAN sum($z+$) | 30·3 | 21·3 | 29·8 | 39·5 | 16·3 | 6·15 | 13·3 | 26·2 |
| nCPA (Bray-Curtis) | 18·6 | 16·8 | 25·7 | 36·1 | 23·7 | 10·5 | 21 | 30·6 |

TITAN observed change points (Obs.) correspond to the value of the $x$ resulting in the largest sum of indicator value (IndVal) $z$ scores among all negative ($z-$) and positive ($z+$) taxa, respectively, whereas nCPA thresholds correspond to the maximum deviance reduction among sites (Bray-Curtis distance). Quantiles (5%, 50%, 95%) correspond to change points from 500 bootstrap replicates.

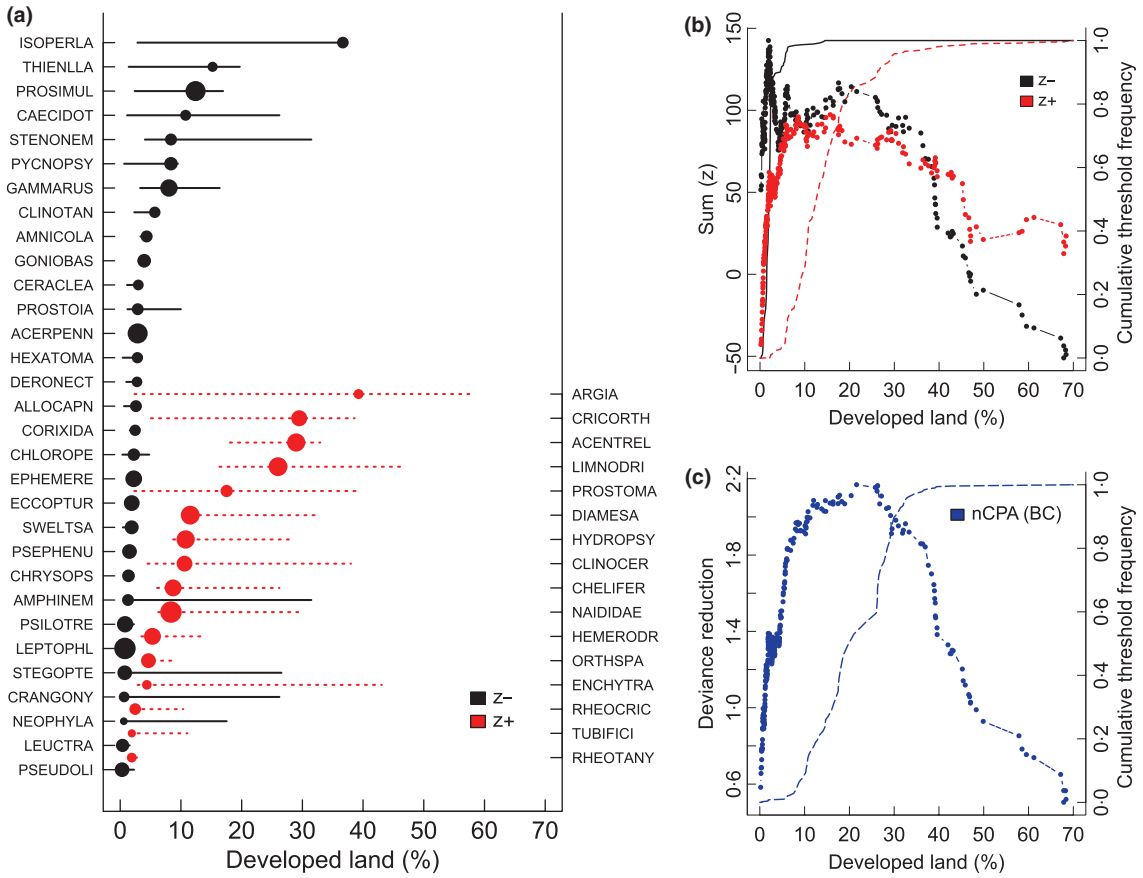


**Fig. 6.** Threshold Indicator Taxa ANalysis (TITAN) and change-point analysis (nCPA, Bray-Curtis distance) of macroinvertebrate community response to a watershed developed land gradient in Maryland ($n$ = 295). Taxa codes in (a) are explained in Appendix S2. See Fig. 3 for additional details.

distribution of positive taxa change points meant that the corresponding maximum of their sum($z+$) showed a relatively weak (poorly defined) peak at 16·3% developed land (Fig. 5b). Further, tolerant taxa exhibited relatively wide bootstrap frequency distributions representing substantial uncertainty about the existence of a threshold because of gradual increases in frequency and abundance. The lower distribution of most tolerant taxon change points only marginally overlapped with those from the majority of declining, sensitive taxa.

nCPA deviance reduction trends closely resembled TITAN sum($z+$) (Fig. 6b and c). nCPA peaked at 23·7% developed land and yielded bootstrap frequency distributions similar to sum($z+$), although nCPA distributions were skewed slightly higher. nCPA bootstrap frequency distributions did not overlap with TITAN sum($z-$) bootstrap distributions. The nCPA and TITAN sum($z+$) change points corresponded closely to the threshold range of 20–30% developed land reported in King *et al.* (2005), but these estimates were far higher than the TITAN sum($z-$) threshold. This comparison demonstrates how the subtle bias exhibited by nCPA analysis of aggregate metrics in Simulation 2 can be exacerbated to distort estimates of community change.

## Discussion

Estimation of taxon-specific change points is arguably the key output from TITAN because this information is precisely what many aggregate community metrics obscure. Response to any novel environment likely differs when considering different biophysical gradients or taxon-specific life history, so discernment of negative and positive response patterns makes sense from an evolutionary perspective (Huggett 2005). There is little reason to expect that all taxa will respond to environmental perturbation at the same level or in the same manner, and responses may vary considerably between infrequent and more widespread taxa. Further, taxa positively associated with anthropogenic environmental gradients do not represent well-organized communities, but rather are a comprised of historically native taxa that either directly (resource subsidy) or indirectly (e.g., realized niche expansion, reduced competition or predation) benefit from it, or an invasive taxon that was not historically present but is able to cross ecosystem boundaries because of a variety of factors related to the novel environment. Therefore, unbiased measures of association along environmental gradients are critical for distinguishing response type, magnitude, and interpreting their relevance.

TITAN deconstructs community-level dissimilarity to assess synchrony of taxon-specific change points. Output can be used alone in novel biodiversity conservation applications, analogous to a species sensitivity distribution used in ecological risk assessment (Newman, Ownby, *et al.* 2000). As in other binary partitioning techniques, TITAN will find taxon-specific or sum($z$) maxima in most data sets, so interpretation of change-point values makes little sense without measures of uncertainty and information content. While this approach will not prove the existence of ecological community thresholds, graphical and tabular display of change-point distributions provides simple and intuitive supporting evidence for interpreting biotic responses, as do diagnostic measures of rescaled IndVals, uncertainty, reliability and purity.

Our simulations demonstrate TITAN's precision, efficacy and flexibility. In the first simulation, the negative ($z-$) and positive ($z+$) indicator taxa thresholds were indistinguishable by nCPA, yet they remained distinct for TITAN. In the second simulation, we illustrated how, through wide confidence bands and reduced sum($z$) scores, TITAN communicates uncertainty when underlying abundance distributions do not show a clear threshold response. In both cases, IndVal scores emphasized sharp changes in frequency and abundance, but many ($>40\%$) randomly generated distributions were nonetheless deemed to contain significant change points following permutation. This pattern illustrated how frequent or abundant taxa with only modest differences in IndVals between groups are often statistically significant ($P \leq 0{\cdot}05$) in large data sets despite dubious ecological significance. However, such patterns are readily distinguished from more meaningful responses through the diagnostic use of reliability and purity. We note that those taxa deemed significant by permutation do not always achieve reliability or purity $\geq 0{\cdot}95$, but taxa with reliability or purity $\geq 0{\cdot}95$ are by definition significant at $P \leq 0{\cdot}05$, and usually much lower.

The Everglades data set represented a synchronous, balanced shift in taxonomic structure during the transition from an oligotrophic community to one more typically associated with southeastern USA wetlands where nutrient limitation is less severe (King & Richardson 2003; Qian, Pan, & King 2004). In contrast to the two closely related thresholds in Simulation 1, this example illustrates how, where a relatively synchronous shift from negative to positive indicator taxa exists, nCPA and TITAN produce similar results. However, the sharp decline of several negative indicator taxa prior to any observed increase in positive indicator taxa with increasing TP resulted in a lower sum($z-$) threshold and narrower quantile intervals than nCPA and sum($z+$). Thus, through TITAN, individual taxon or sum($z$) graphics and quantitative indices may be used in a diagnostic fashion to better understand the general nature of community response.

In the Maryland data, we illustrate new insight gained by distinguishing between taxa subsets with distinct responses to an environmental gradient. Here, most observed taxa are native and evolved without the hydrological and chemical alterations linked to urbanization (e.g., Walsh *et al.* 2005), thus watershed development likely represents a novel selective pressure. Taxa sensitive to the novel environment (or a strong covariate) responded synchronously at strikingly and remarkably low levels of development and exhibit a classic pattern consistent with decline or even potential extirpation. King *et al.* (2005) missed this pattern because we relied upon a whole-community metric similar to the nCPA results in this article. The multivariate nCPA approach did detect a change, but it did not distinguish which taxa were changing and how substantial this change was for each taxon. In this example, so-called 'tolerant' taxa occurred all along the gradient and simply became generally more prevalent in streams draining more heavily developed watersheds. In contrast with negative-responding taxa, positive or tolerant responses did not support characterization as a threshold because of wide confidence bands and gradual cumulative change-point probabilities, much like Sp6–8 in Simulation 2. Because loss of sensitive taxa or other environmental changes may not benefit all community remnants in the same way, the causes for increases in tolerant taxa along the watershed development gradient are likely taxon-specific and there is no reason to expect a synchronous tolerant community response.

In both simulations and the Maryland data set, nCPA was necessarily less sensitive to disparate change points. However, there was clearly a tendency in each data set for nCPA to average away the acute response of some taxa with that of widely distributed taxa. In the Everglades, where negative and positive threshold taxa were relatively synchronous, nCPA fell between the negative ($z-$) and positive ($z+$) responses. In both Simulation 1 and Maryland, nCPA cumulative probabilities tracked the positive ($z+$) response more closely and were

more likely to capture a greater number of positive threshold values. In Simulation 2, the mean nCPA value shifted to the right of its median, whereas the mean and median sum($z-$) were nearly identical. Our contrast between nCPA and TITAN should not be construed as criticism, but rather an illustration of how TITAN can be used to help interpret results from explanatory or predictive models developed using nCPA or MRT, and how under conditions where taxa turnover is synchronous on both sides of a change point, nCPA and TITAN will yield comparable results.

Sample size, minimum split size and taxon frequency are important considerations in TITAN. Small numbers of sample units will necessarily result in uncertain change-point estimates, but an appropriate minimum sample size will be data set specific. At least three and preferably five sample units should be used as the minimum split size to compute $z$ scores. Similarly, we tentatively recommend that taxa with $<3$–5 occurrences be excluded because these taxa are too infrequent to estimate interpretable $z$ scores. In general, the recommendations of De'Ath & Fabricius (2000) for RT, De'Ath (2002) for MRT and Dufrêne & Legendre (1997) for IndVal will also apply to TITAN for these considerations.

As with any classification model, long gradients with high beta-diversity or multiple change points on the same gradient (e.g., multiple interacting limiting factors, Gaussian or otherwise modal distributions) will require recursive partitioning of sample units to isolate thresholds. In Simulation 1, TITAN was able to distinguish unimodal Sp3 as reliably impure, but in Simulation 2, the positive response was close enough to the edge of the gradient that resampling only emphasized the negative threshold. TITAN is identical in form to tree-based modelling, thus could be used in a similar way to identify covarying secondary and tertiary limiting factors corresponding to sharp community changes. Our focus here was on single-predictor thresholds, so evaluation of additional variables fell beyond the scope of this paper. However, multiple predictors may be useful in identifying a hierarchical set of environmental limiting factors of species distributions, or to assess variable importance.

Emerging analytical techniques such as Random Forests (Breiman 2001) can be powerful methods for exploring community responses along multiple environmental gradients, and TITAN would fit naturally within a similar framework. At each level of the tree, sum($z$) could be contrasted among several covarying predictors and the variable that consistently had the highest sum($z$) among bootstrap replicates could be selected as the best predictor, as done in current tree-based predictive models (e.g., De'Ath 2007). We also recommend examination of the continuous trend in sum($z$) along with individual taxa change points, where secondary peaks may reveal additional transition points in community structure. TITAN and extensions of this method should prove useful for detecting taxon-specific and community-level thresholds and for addressing a variety of basic and applied ecological questions.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Taxon-specific results from Threshold Indicator Taxa Analysis (TITAN) of macroinvertebrate community composition in response to surface-water total phosphorus (TP, ug/L) in the Everglades.

**Appendix S2.** Taxon-specific results from Threshold Indicator Taxa Analysis (TITAN) of macroinvertebrate community composition in response to watershed developed land cover (%) in Maryland.

**Appendix S3.** Program TITAN for R 2·9·2 for performing all analyses described in this paper.

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