BAYLOR UNIVERSITY
SPONSORED PROGRAMS AGREEMENT

THIS AGREEMENT is made by and between the Arkansas-Oklahoma Joint Study Committee, a committee formed pursuant to executive appointment by the Governors of the States of Arkansas and Oklahoma and established by the Second Statement of Joint Principles and Actions under the laws of Arkansas and Oklahoma, (hereinafter referred to as "SPONSOR"), and Baylor University, a Texas nonprofit corporation located at Waco, Texas (hereinafter referred to as "BAYLOR").

WITNESSETH:

WHEREAS, the sponsored Project contemplated by this Agreement is of mutual interest and benefit to BAYLOR and to SPONSOR, will further the instructional and research objectives of BAYLOR in a manner consistent with its status as a nonprofit, tax-exempt, educational institution, and may derive benefits for both SPONSOR and BAYLOR through inventions, improvements, and/or discoveries;

NOW, THEREFORE, in consideration of the premises and mutual covenants herein contained, the parties hereto agree to the following:

ARTICLE 1 - DEFINITIONS

As used herein, the following terms shall have the following meanings:

1.1 "Project" shall mean the description of the project as described in "Appendix A" hereof, under the direction of Dr. Ryan King as the "Project Director."

1.2 "Contract Period" is April 1, 2014 through December 31, 2016.

1.3 "University Intellectual Property" shall mean individually and collectively all inventions, improvements, and/or discoveries, whether patentable or not, copyrights, software, trade secrets, formulae, processes, techniques and other developments and advances with respect to the Project which are developed conceived and/or reduced to practice in the course and scope of performance of this Agreement during the Contract Period by one or more employees or contractors of BAYLOR.

1.4 "University Background Intellectual Property" shall mean individually and collectively all inventions, improvements, and/or discoveries, whether patentable or not, copyrights, software, trade secrets, formulae processes, techniques and other developments and advances with respect to the Project which are developed, conceived and/or reduced to practice by one or more employees or contractors of BAYLOR or licensed to Baylor prior to the

Contract Period, or during the Contract Period but developed independently of the Project.

1.5 Joint Intellectual Property shall mean individually and collectively all inventions improvements and/or discoveries, whether patentable or not, copyrights, software, trade secrets, formulae, processes, techniques and other developments and advances with respect to the Project which are developed, conceived and/or reduced to practice in the course and scope of performance of this Agreement during the Contract Period jointly by one or more employees or contractors of BAYLOR and one or more appointees of SPONSOR.

ARTICLE 2 - SPONSORED PROGRAM WORK

2.1 BAYLOR shall commence the performance of the Project promptly at the start of the Contract Period and shall use reasonable efforts to perform the Project substantially in accordance with the terms and conditions of this Agreement. Anything in this Agreement to the contrary notwithstanding, SPONSOR and BAYLOR may at any time amend the Project by mutual written agreement.

2.2 In the event that the Project Director becomes unable or unwilling to continue the Project, and a mutually acceptable substitute is not available, BAYLOR and/or SPONSOR shall have the option to terminate the Project upon thirty (30) days prior written notice.

ARTICLE 3 - WRITTEN REPORTS

3.1 Written program reports shall be provided by BAYLOR to SPONSOR every quarter, a draft final report shall be provided to the SPONSOR 30 days before a final report is issued and a final report shall be submitted to SPONSOR by BAYLOR within 90 days of the conclusion of the Contract Period or early termination of this Agreement.

ARTICLE 4 - COSTS, BILLINGS, AND OTHER SUPPORT

4.1 It is agreed to and understood by the parties hereto that total costs to SPONSOR hereunder shall not exceed the sum of Six Hundred Thousand Dollars ($600,000). Payment shall be made by SPONSOR to BAYLOR according to the following schedule:

Quarterly invoicing.

4.2 Anything herein to the contrary notwithstanding, in the event of early termination of this Agreement by SPONSOR pursuant to Articles 2 or 9, SPONSOR shall pay all costs for work that has been completed and all non-cancelable obligations incurred by BAYLOR as of the date of termination.

ARTICLE 5 - PUBLICITY

5.1 SPONSOR will not use the name of BAYLOR, nor of any member of BAYLOR's Project staff, in any external publicity, advertising, or news release without the prior written notification to an authorized representative of BAYLOR. BAYLOR will not use the name of SPONSOR, nor any employee of SPONSOR, in any external publicity without the prior written approval of SPONSOR.

Nothing herein shall act to prevent any party to the agreement from complying with the Arkansas Freedom of Information Act found at ARK. CODE ANN §25-19-101 et seq. or the Oklahoma Open Records Act found at Title 51 O.S. Section 24A.1-29.

ARTICLE 6 - PUBLICATIONS

6.1 SPONSOR recognizes that, under BAYLOR's policy, the results of the Project must be publishable and agrees that researchers engaged in the Project shall be permitted to report the methods and results of the Project by means of presentations at symposia and at national or regional professional meetings, by publishing in journals, theses or dissertations, or by other means of their own choosing. However, to permit SPONSOR to determine whether patentable inventions would be disclosed through such publication or presentation, BAYLOR shall furnish SPONSOR copies of any proposed publication or presentation at least sixty (60) days in advance of the submissions of such proposed publication or presentation to a journal, editor, or other third party. SPONSOR shall have thirty (30) days after BAYLOR mails said copies in which to object in writing to such publication or presentation by notice to the Project Director, if it believes in good faith that such publication or presentation will have a negative impact on relevant patent rights or on SPONSOR's trade secrets. BAYLOR will determine if changes are necessary and/or delay such publication or presentation for a period not to exceed sixty (60) days. Any further delay in publication will require subsequent agreement between BAYLOR and SPONSOR.

ARTICLE 7 - INTELLECTUAL PROPERTY

7.1 All rights and title to University Intellectual Property shall be owned by BAYLOR.

7.2 All rights and title to Joint Intellectual Property shall be owned by BAYLOR and SPONSOR jointly.

7.3 Rights to inventions, improvements and/or discoveries relating to the Project, whether or not patentable or copyrightable, made solely by employees of SPONSOR shall not be deemed to be University Intellectual Property and shall belong to SPONSOR. Such inventions, improvements, and/or discoveries shall not be subject to the terms and conditions of this Agreement.

7.4 In the event a determination must be made as to ownership of intellectual property created as part of the Project, BAYLOR, acting reasonably, shall make such determination.

7.5 BAYLOR shall, within its sole discretion, cause patent applications to be filed and prosecuted, both domestically and internationally, in BAYLOR's name with respect to University Intellectual Property. BAYLOR shall notify SPONSOR and provide a copy of the application within thirty (30) days of filing all patent applications for University Intellectual Property developed as part of the Project.

ARTICLE 8 - TERM AND TERMINATION

8.1 This Agreement shall become effective upon commencement of the Contract Period and shall continue in effect for the full duration of the Contract Period, unless sooner terminated in accordance with the provisions of this Article or Article 2. The parties hereto may, however, agree to a short extension of the term of this Agreement to complete the Project work under mutually agreeable terms and conditions which the parties reduce to writing and sign. Either party may terminate this Agreement upon thirty (30) days prior written notice to each other.

8.2 If either party hereto commits any breach of or default in any of the terms or conditions of this Agreement, and then fails to remedy such default or breach within thirty (30) days after receipt of written notice thereof from the other party, the party giving notice may, at its option and in addition to any other remedies which it may have at law or in equity, terminate this Agreement by sending notice of termination in writing to the other party to such effect, and such termination shall be effective as of the date of the receipt of such notice.

8.3 Termination of this Agreement by either party for any reason shall not affect the rights and obligations of the parties accrued prior to the effective date of termination of this Agreement. No termination of this Agreement, however effectuated, shall affect or release the parties hereto from their rights and obligations under Articles 4, 5, 6, 7, 8, 10, 11, and 14.

ARTICLE 9 - INDEPENDENT CONTRACTOR

9.1 In the performance of all services hereunder:

9.1.1 Each party is and shall be deemed to be and shall be an independent contractor of the other. Accordingly, neither party's employees shall be entitled to any benefits applicable to employees of the other.

9.1.2 Neither party is authorized or empowered to act as agent for the other for any purpose and shall not on behalf of the other enter into any contract, warranty, or representation as to any matter. Neither shall be bound by the acts or conduct of the other.

ARTICLE 10 - DISCLAIMER OF WARRANTIES AND LIMITATION ON REMEDIES

10.1 BAYLOR MAKES NO WARRANTIES, EXPRESS OR IMPLIED, AS TO ANY MATTER WHATSOEVER, INCLUDING, WITHOUT LIMITATION, THE CONDITION, ORIGINALITY, OR ACCURACY OF THE RESEARCH OR ANY INVENTIONS, PRODUCTS, OR PROCESSES, WHETHER TANGIBLE OR INTANGIBLE, CONCEIVED, DISCOVERED, OR DEVELOPED UNDER THIS AGREEMENT; OR THE OWNERSHIP, MERCHANTABILITY, OR FITNESS FOR A PARTICULAR PURPOSE OF THE RESEARCH OR ANY SUCH INVENTION, PRODUCT, OR PROCESS. BAYLOR SHALL NOT BE LIABLE FOR ANY DIRECT, CONSEQUENTIAL, OR OTHER DAMAGES SUFFERED BY SPONSOR, RESULTING FROM THE USE OF THE RESEARCH OR ANY SUCH INVENTION, PRODUCT, OR PROCESS.

ARTICLE 11 - GOVERNING LAW

11.1 This Agreement shall be governed and construed in accordance with the laws of the States of Arkansas or Oklahoma. If the claim is against or belongs to the State of Arkansas, Arkansas law shall apply. If the claim is against or belongs to the State of Oklahoma, Oklahoma law shall apply. This Agreement does not constitute a waiver of sovereign immunity by any party.

ARTICLE 12 - ASSIGNMENT

12.1 This Agreement is non-assignable.

ARTICLE 13 - AGREEMENT MODIFICATION

13.1 Any agreement to change the terms of this Agreement in any way shall be valid only if the change is made in writing and approved by mutual agreement of authorized representatives of the parties hereto.

ARTICLE 14 - EXPORT ADMINISTRATION

14.1 It is understood that SPONSOR is subject to United States laws and regulations controlling the export of technical data, computer software, laboratory prototypes and other commodities, and that its obligations hereunder are contingent upon compliance with the Export Administration Act of 1979, 50USC2401-2420 (as implemented by the EAR). Furthermore, it is understood that the transfer of certain technical data and commodities ma require a license from one or more agencies of the United States Government.

ARTICLE 15 - NOTICES

15.1 Notices, invoices, communications, and payments hereunder shall be deemed made if given by certified mail, return receipt requested, and addressed to the party to receive such notice, invoice, or communication at the address given below, or such other address as may hereafter be designated by notice in writing:

If to SPONSOR:

Brian Haggard, Director
Arkansas Water Resources Center
University of Arkansas
Engineering Hall 203
Fayetteville, AR 72701
(479) 575-2879

Derek Smithee
Oklahoma Water Resources Board
3800 North Classen
Oklahoma City, OK 73118
(405) 530-8800

If to BAYLOR:

Office of Sponsored Programs
Baylor University
One Bear Place, #97360
Waco, Texas 76798-7360

cc: Office of General Counsel
Baylor University
One Bear Place, #97034
Waco, Texas 76798-7034

If Technical Matter:

Dr. Ryan King
Baylor University
One Bear Place #97388
Waco, Texas 76798-7388

IN WITNESS WHEREOF, the parties have executed the Agreement in duplicate as of this 9th day of May, 2014.

ATTEST:

By: Marsha J. Duckworth

Marsha J. Duckworth
Assistant Secretary

BAYLOR UNIVERSITY

By: Elizabeth Davis

Elizabeth Davis
Executive Vice President & Provost

SPONSOR: Arkansas-Oklahoma Joint Study Committee

For Arkansas:

Teresa Marks

Teresa Marks
Director
Arkansas Department of Environmental Quality

J. Randy Young, P.E.
Director
Arkansas Natural Resources Commission

For Oklahoma:

Scott Thompson
Executive Director
Oklahoma Department of Environmental Quality

FOR

Mike Thralls
Executive Director
Oklahoma Conservation Commission

J.D. Strong
Executive Director
Oklahoma Water Resources Board

Oklahoma Scenic Rivers Joint Phosphorus Study

Work Plan

4/23/14

Ryan S. King, Ph.D.

Professor, Department of Biology, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX 76798
www.baylor.edu/aquaticlab

BAYLOR
UNIVERSITY

Study Framework

The Baylor work plan is necessarily focused on a few key elements of the Second Statement of Joint Principles and Actions, p.2, Mandatory Study Components:

"to determine the Total Phosphorus threshold response level....at which any statistically significant shift occurs in

1. algal species composition OR
2. algal biomass production

...resulting in undesirable

1. aesthetic OR
2. water quality

...conditions in the Designated Scenic Rivers."

Further, the Baylor study will be:

"completed in accordance with.....

1. U.S. EPA Rapid Bioassessment Protocols
2. EPA Guidance on QA/QC
3. Using Stressor-Response Relationships to Derive Numeric Nutrient Criteria

and shall include....

4. *a sampling population....adequate to determine the frequency and duration of the numeric criterion....and*
5. *limited to streams or rivers within the same EPA ecoregion and comparable to the streams in the designated Scenic River watersheds."*

Here, I present on behalf of the Baylor team a summary of the work to be performed in the context of this study framework. The vast majority of the work will focus on a field gradient study designed to identify levels of total phosphorus that lead to the undesired outcomes described above. A small component of the work plan will leave room for field and/or laboratory experiments that may be performed if sufficient funding and time allows. These experiments may be deemed unnecessary or very important depending upon the preliminary results of the field study in years 1 and 2.

Field Gradient Study

Study Design

Baylor will perform a field study across ≥ 25 stream reaches spanning a steep gradient of total phosphorus (TP) enrichment, from least to highly enriched. Existing TP data from intensively monitored locations will drive the initial screening of sites for inclusion in the gradient study. Oklahoma Water Resources Board (OWRB), Oklahoma Conservation Commission (OCC) and the University of Arkansas (UA) each has been monitoring water chemistry from several locations within the study area for many years. We used data collected by each of these groups to estimate mean levels of total phosphorus (TP) in an effort to help guide site selection and to characterize the distribution of recent TP levels in the region (Tables 1-3, Figure 2).

The majority of the intensive monitoring sites have TP levels that exceed the current 0.037 mg/L target adopted by the State of Oklahoma (Figure 2). The gradient study must include a sufficient number of low-level TP reaches to adequately model the relationship between TP and biological response, which should approximate a log-linear distribution from <0.010 to the highest selected site. The current pool of intensive sites does not sufficiently cover the low end of the TP gradient, thus other reaches outside of these sites must be included in the gradient study. These reaches must be within the range of catchment areas, discharge, substrate type, stream velocity, and light (open canopy or nearly so) of the intensive monitoring locations to be selected.

The majority of the selected reaches will be located in the Illinois River watershed, which includes three Oklahoma Scenic Rivers (Flint Creek, Barren Fork Creek, and Illinois River). The minimum number of sites selected from the mainstem of each of these Designated Scenic Rivers will be:

- Flint Creek (minimum 3): at least one site above Sager Creek, below Sager Creek near USGS gage at West Siloam Springs, and between West Siloam Springs and Illinois River confluence
- Barren Fork Creek (minimum 4): at least 1 upper reach in AR near Dutch Mills, between Dutch Mills and Christie, between Christie and Eldon, and near Eldon.
    - Within Barren Fork watershed: at least one site on Evansville Creek
- Illinois River (minimum 6): at least one site above Goose Creek, between Goose Creek and Osage Creek, between Osage Creek and Lake Frances, between Lake Frances and Flint Creek, between Flint Creek and Tahlequah.
    - Within Illinois watershed, excluding Flint Creek and Barren Fork, at least one site on each of the following:
        - Osage Creek
        - Spring Creek (AR)
        - Ballard Creek
        - Caney Creek

However, in order to achieve the full range of TP enrichment among streams in the Scenic Rivers region, additional reaches will be selected from one or more of the following watersheds: Spavinaw Creek, Saline Creek, Spring Creek (OK), Little Lee

Creek, and Lee Creek (Figure 1). Sites will be located within either the Ozark Highlands or Boston Mountains Level III Ecoregions.

The minimum number of sites from the watersheds outside the Illinois River watershed will be:

- Spavinaw, Saline, and Spring (OK): at least 4 sites
- Lee: at least 3 sites on the mainstem and/or large tributaries (Mountain Fork Creek, Cove Creek)
- Little Lee: at least 1 site

If additional sites are included in the study, the priority will be as follows:

- Spring Creek (OK)
- Saline Creek
- Sager Cr., below WWTP
- Beaty Cr
- Little Lee Creek
- Caney Cr.
- Osage Cr below NACA WWTP outflow (lower USGS gage)
- Illinois River
- Barren Fork Cr
- Flint Cr
- Webber Creek
- Sager Cr, above WWTP

Formal site selection will begin following execution of the contract and availability of funding to begin visiting sites and collecting pilot samples. No site will be selected without a personal visit by the Principal Investigator (RSK) and feedback from the committee on the suitability of the site for inclusion in the study.

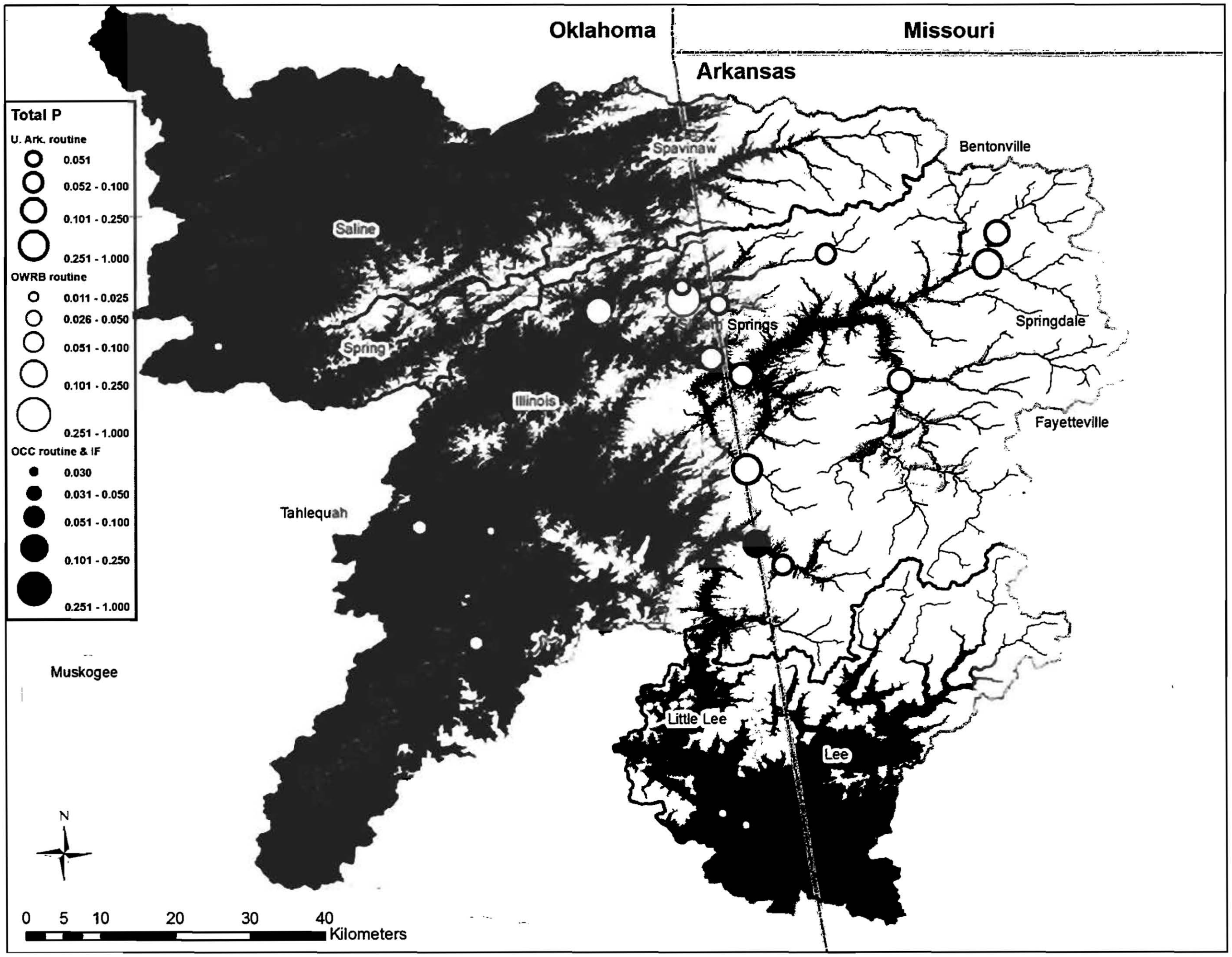

Figure 1. Map of the study area illustrating focal watersheds and spatial distribution of total phosphorus (TP) among streams. Symbols correspond to sampling locations where different organizations have been intensively monitoring TP and other variables. Symbols are sized in proportion to the mean TP concentration for 2011-13 (U. Arkansas) and 2008-2013 (OWRB, OCC).

Table 1. Water chemistry among 10 locations in the upper Illinois River Watershed intensively monitored by the **University of Arkansas.** Data are means of weekly samples collected from 2011 to 2013. Units are mg/L unless otherwise stated. Data provided by Brian Haggard, University of Arkansas.

Site	Lat	Long	Chloride	SpCond	NO2NO3_N	PO4_P	Sulfate	TN	TP	TSS	Turb_NTU
Ballard Creek	35.99611	-94.52944	11.17	339	1.88	0.094	16.08	3.03	0.766	16.6	21.8
Barren Fork	35.88000	-94.48639	8.47	306	1.17	0.034	20.73	1.83	0.080	15.8	13.2
Flint Creek	36.25611	-94.43361	7.75	263	3.43	0.065	5.47	3.75	0.087	9.0	9.7
Flint Creek WSS	36.21200	-94.70600	11.17	295	1.18	0.032	20.42	1.85	0.051	8.1	7.4
Illinois River 59	36.10951	-94.53445	17.49	341	2.09	0.065	19.92	2.66	0.125	35.3	31.9
Illinois River at Watts	36.12994	-94.57151	16.82	337	2.55	0.059	19.40	2.54	0.116	25.6	26.0
Illinois River Savoy	36.10306	-94.34444	17.88	335	3.58	0.048	21.27	3.37	0.130	43.6	41.8
Osage Creek	36.28139	-94.22778	29.35	434	2.99	0.087	35.22	3.92	0.144	42.4	34.1
Sager Creek	36.19500	-94.56361	11.78	300	1.56	0.046	11.88	2.07	0.066	4.1	4.4
Spring Creek (AR)	36.24378	-94.23914	42.74	521	2.92	0.170	61.35	3.65	0.250	48.5	31.9

Table 2. Water chemistry among 8 locations in and around the Illinois River watershed intensively monitored by the **Oklahoma Conservation Commission**. Data are means of integrated flow proportioned (IFP) or routine grab samples collected from 2008 to 2013. Units are mg/L unless otherwise stated. Sample frequency was approximately weekly for nutrients (n~100 for routine samples, n~150 for IFP). Data provided by Shanon Philips, Oklahoma Conservation Commission.

Site	Type	Lat	Long	NH3-N	Chloride	EG Bact	E.coli	Kjeldahl-N	Nitrate-N	PO4-P	TP	Sulfate
Barren Fork: Lower	IFP	35.86286	-94.89910	0.073	4.4			0.608	1.005	0.049	0.119	6.1
Barren Fork: State Line	IFP	35.90620	-94.51824	0.056	6.7			0.693	1.869	0.083	0.185	16.9
Beaty Creek: Upper @ Betty C.	IFP	36.37040	-94.71910	0.060	15.2			0.873	2.924	0.051	0.122	8.7
Caney Creek	IFP	35.79470	-94.84510	0.069	4.5			0.424	0.845	0.066	0.144	7.5
Flint Creek	IFP	36.19610	-94.70780	0.123	19.9			1.320	1.191	0.104	0.251	
Little Saline Creek	IFP	36.27964	-95.07100	0.022	9.2			0.306	0.806	0.015	0.039	5.3
Saline Creek	IFP	36.28200	-95.09292	0.028				0.643	0.618	0.009	0.055	6.5
Spavinaw Creek	IFP	36.34370	-94.77160	0.076	12.6			0.882	2.551	0.022	0.096	10.1
Barren Fork: Lower	Routine Sample	35.86286	-94.89910	0.021	5.8	211	118	0.409	1.222	0.036	0.101	7.7
Barren Fork: State Line	Routine Sample	35.90620	-94.51824	0.044	7.5	297	201	0.359	1.862	0.090	0.153	14.2
Beaty Creek: Upper @ Betty C.	Routine Sample	36.37040	-94.71910	0.031	9.3	393	165	0.501	2.911	0.059	0.095	5.4
Caney Creek	Routine Sample	35.79470	-94.84510	0.024	5.6	236	98	0.218	1.020	0.051	0.086	7.3
Flint Creek	Routine Sample	36.19610	-94.70780	0.043	17.2	282	118	0.583	2.230	0.140	0.199	22.8
Little Saline Creek	Routine Sample	36.27963	-95.07100	0.030	9.7	186	45	0.248	0.763	0.012	0.030	4.9
Saline Creek	Routine Sample	36.28200	-95.09292	0.026	10.1	69	17	0.377	0.775	0.025	0.057	7.2
Spavinaw Creek	Routine Sample	36.34370	-94.77160	0.032	12.1	265	67	0.532	2.864	0.032	0.068	7.9

Table 3. Water chemistry among 9 locations in and around the Illinois River watershed that are intensively monitored by the **Oklahoma Water Resources Board.** Data are means of routine grab samples collected from 2008 to 2013. Units are mg/L unless otherwise stated. Sample collection frequency was monthly (~72 samples per site). Data provided by Monte Porter, OWRB.

Station Description	Long	Lat	CHLA (ug/L)	DO (mg/L)	Q (cfs)	NH4-N	Kjeldahl-N	NO2NO3-N	pH	TP	SpCond (uS/cm)	Turb (NTU)	Temp (C)
ILLINOIS RIVER, US 62, TAHLEQUAH	-94.92380	35.92606	3.81	10.41	819.8	0.079	0.249	1.666	7.76	0.049	286	7.44	17.4
ILLINOIS RIVER, US 59, WATTS	-94.57151	36.12994	3.28	11.18	511.3	0.062	0.289	2.365	7.87	0.056	321	8.52	17.4
CANEY CREEK, OFF SH 100, BARBER	-94.85559	35.78498	0.87	10.05	51.2	0.069	0.135	1.015	7.65	0.029	241	1.68	17.3
BARREN FORK, SH 51, ELDON	-94.83726	35.92173	1.15	9.73	276.6	0.070	0.162	1.475	7.54	0.023	215	3.98	16.2
FLINT CREEK, US 412, FLINT	-94.70680	36.18677	1.02	10.04	85.5	0.058	0.180	2.394	7.62	0.131	305	2.16	16.4
SAGER CREEK, OFF US 412, WEST SILOAM SPRINGS	-94.60538	36.20164	2.06	8.92	16.8	0.215	0.633	4.457	7.58	0.816	445	1.64	17.1
LEE CREEK, SH 101, NEAR SHORT	-94.53153	35.56590	1.92	9.53	395.3	0.066	0.179	0.173	7.54	0.012	105	9.10	17.3
LITTLE LEE CREEK, SH 101, near NICUT	-94.56000	35.58000	0.86	9.71	114.0	0.189	0.160	0.130	7.54	0.017	138	10.69	16.6
SPRING CREEK, OFF US 412, MURPHY (OK)	-95.16500	36.14400	0.42	9.57	77.9		0.114	0.659	7.62	0.011	184	1.96	16.8

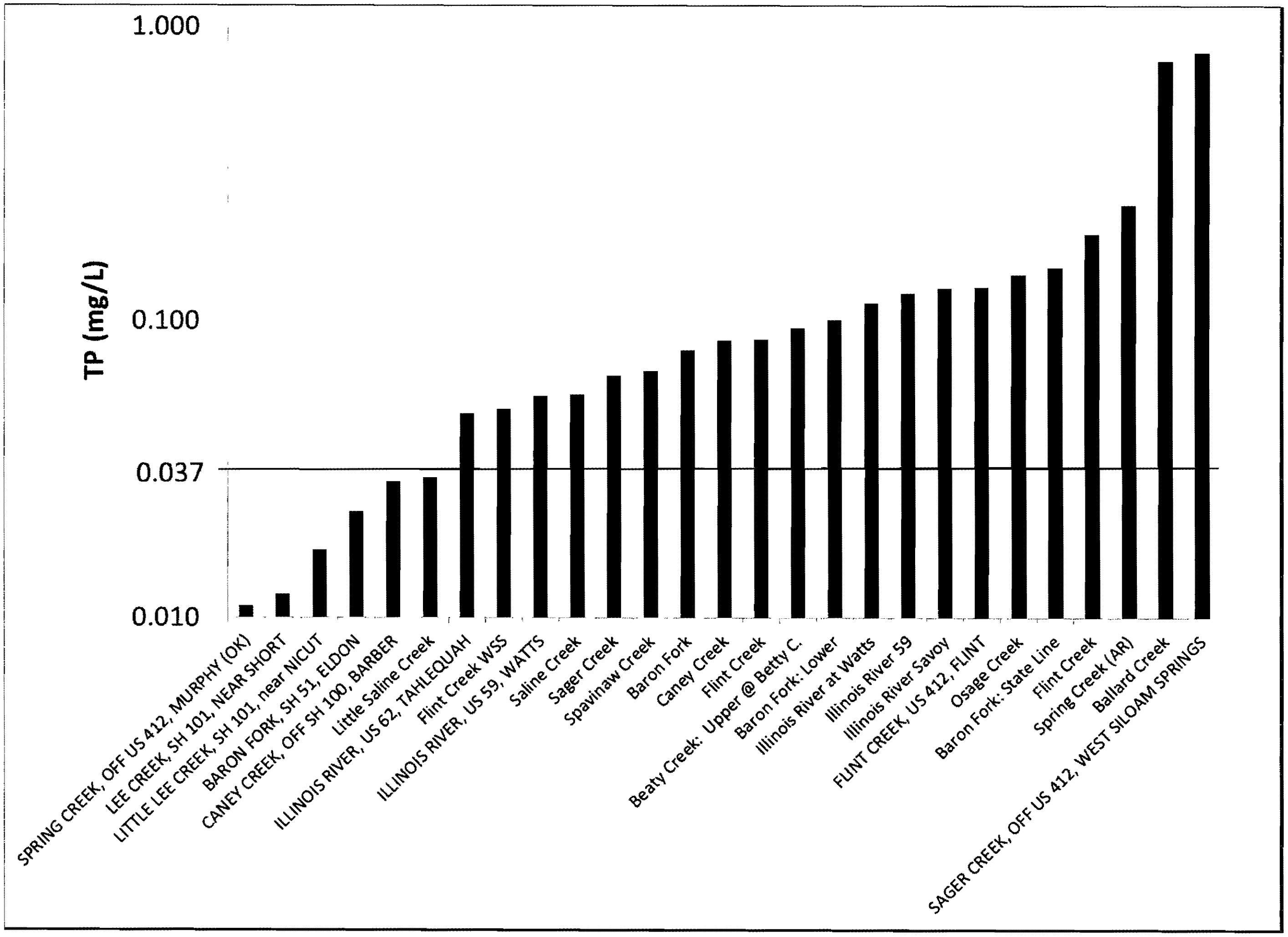

Figure 2. Distribution of mean TP concentrations among intensively monitored locations (U. Ark, OCC, OWRB).

Field Methods

Sampling frequency

Sampling will be bimonthly. This sampling frequency will result in 12 events of at least 25 sites per event if the data collection extends through May 2016 (Table 4). Sampling will begin no later than July 2014, barring extreme weather conditions that prevent sampling during that time. Timing of events may be adjusted over the course of the study to avoid sampling during high flows.

Table 4. Schedule of events, including site screening and selection, sampling frequency (X), final analyses, and report writing. *Contract finalized. **Depends upon remaining funds.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014				* Site screening, selection			X		X		X	
2015	X		X		X		X		X		X	
2016	X		X		X		X**	Final analyses & report writing				

Sampling will be conducted in teams of a minimum of 2 scientists, typically 3 or more. The goal will be to sample all of the selected streams in a few days per bimonthly event. However, we anticipate some sampling events may be interrupted by heavy rain and high flows. We will pause sampling for a sufficient number of days to ensure safe working conditions before moving forward to the remaining sites. If runoff was sufficiently heavy that the streambed may have been scoured, we will revisit sites sampled before the high flows to assess whether all sites must be sampled again. If stream flow remains very high and unsafe for wading for an extended period of time (e.g. several weeks), a bimonthly sampling event may be cancelled or replaced with an additional event at a later time.

Sampling: surface water chemistry and phytoplankton

Water and phytoplankton samples will be collected at the upstream boundary of the reach upon first reaching a site. Duplicate TP, TN (unfiltered) and NH_4-N, NO_2+NO_3-N, and PO_4-P (field filtered, 0.45 µm) samples will be collected in new 50 mL centrifuge tubes TP and TN samples will be preserved with sufficient volume of H_2SO_4 to achieve pH < 2. A single 1-L seston (CHLA) sample will also be collected in a dark bottle and placed on ice. A turbidity sample also will be collected. All samples will be placed on ice in a cooler immediately following collection. Sample collection follows EPA Guidance on QA/QC and the BU Center for Reservoir and Aquatic Systems Research (CRASR) protocols (e.g., Chain-of-Custody, trip blanks, field blanks, filtered blanks, duplicates, etc).

Site characterization

We will measure an efficient set of physical and chemical variables to characterize each site. The goal of the gradient study is to sample a large number of locations frequently; thus we must avoid performing intensive habitat assessments that add little information relative to the high cost (i.e., reducing the number of sites due to prohibitive amount of time required to complete each assessment).

We will measure the following variables at each site visit, if water conditions allow: wetted width, mean depth and velocity of riffle-run channel unit (corresponding to benthic algal sampling transects), mean particle size (based on the 15 benthic algal substrates, see next), substrate embeddedness (%, RBP), qualitative sediment score (RBP), and canopy cover (should be close to zero at all selected sites).

Discharge will be estimated using a Marsh-McBirney flowmeter following standard USGS protocols. However, discharge will not be measured at sites that are (a) gaged and have moderate to high flow, and (b) too large or unsafe wade (mainstem Illinois River). Discharge at gaged sites will be estimated during summer low-flow conditions if it can be accomplished safely. Gages will be spot checked at least once, but, again, only if it is safe to wade.

Temperature, specific conductivity, pH and dissolved oxygen will be measured using a YSI multiprobe, although because measurements will be taken at different times of the day among sites, only specific conductivity will be of much value.

Photographs of the site and of benthic algae substrates (prior to scraping) will be taken at each visit unless conditions do not permit photography (low light, rain).

Benthic algae

We will follow the sampling framework outlined in EPA Rapid Bioassessment Protocols (1999). This protocol is required by the *Second Statement of Joint Principles* document.

We will target riffle-run habitat in erosional zones of the stream reach with particle sizes >= small cobble. Most, if not all of the streams in this study are partially to mostly open canopied, mid-order systems with relatively long riffle-run segments that are representative of others in the reach. We will select a single large riffle-run segment from most streams and continue to sample the same riffle-run segment throughout the study to maintain consistency. In the case of smaller streams that have a series of short riffles, we will select 3 riffle-run channel units for sampling, if applicable.

Benthic algae will be collected from areas within riffle-run channel units. Locations within the reach for selecting benthic algal substrates will be identified using a perpendicular transect that (a) spans the wetted width of the stream or (b) spans the wetted width of habitat that can be safely sampled by wading, if flows are too fast or the channel is too deep on one side of the stream. Option (b) may be employed routinely in the mainstem Illinois River where flows frequently exceed 1000 cfs.

A minimum of five equidistant points will be selected along each transect within the sampling zone. Substrates will be lifted quickly from the water with a small net trailing behind to catch filaments that may come loose during collection. Substrates will be placed in a large pan with site water. A minimum of 15 substrates will be collected (three transects, minimum 5 substrates per transect; King et al. 2009).

Benthic algae will be removed from substrates on-site using standard procedures (EPA 1999, Biggs and Kilroy 2000). The algae-water slurry will be immediately transferred to a dark bottle and placed on ice for processing later the same day.

Surface area of rocks will be estimated using measurements of each dimension (Biggs and Kilroy 2000) or by the aluminum foil mass-to-area conversion method (e.g., King et al. 2009 and references therein).

Macroinvertebrates

Obligate grazers of attached algae (e.g., gastropods, psephenid beetles, some limnephilid caddisflies) will confound the relationship between algal biomass and total phosphorus. A quantitative sampling method focused on large-bodied grazing taxa will be performed at each site visit over the course of the study. The method(s) used will be determined following site visits and final site selection to identify the optimal scale and number of samples required to characterize macroinvertebrate grazing pressure. Samples will be preserved in buffered formalin (5% v/v) stained with rose bengal.

Vertebrates

Vertebrate grazers, particularly central stonerollers (*Campostoma anomalum*), will confound the relationship between algal biomass and total phosphorus. Quantitative fish sampling is not feasible given the relatively large number of sites, temporal frequency of sampling, and budget; however, we will attempt to develop a field protocol for quantifying grazing scars left behind by central stonerollers. Grazing scars are usually obvious if recent. We will consider photographing a random sample of stones for quantification of grazing-scar density. We will also document the presence of large schools of stonerollers, which are typically easily seen if actively grazing in the reach.

Diel dissolved oxygen and pH

Diel variation in dissolved oxygen and pH are key variables that can bridge the "statistically significant shift" and "undesirable water quality" statements in the *Second Statement of Joint Principles* document. We will estimate daily minimum, maximum, and range of dissolved oxygen and pH at least one time across all sites using pH and optical DO sensors associated with YSI EXO1 data sondes. We will target summer low-flow period when streams are most susceptible to senescing algae, high respiration, and low reaeration. Sondes will be deployed for a minimum of 72 hours. We will deploy sondes in several streams simultaneously, retrieve, and then redeploy in several other streams. This process will be repeated until sondes have been deployed for 72 hours in all of the study streams.

Laboratory Methods

Surface water chemistry

TP, TN, and dissolved nutrient samples will be run following standard protocols employed by the Center for Reservoir and Aquatic Systems Research (CRASR). Dr. Jeffrey Back will conduct nutrient chemistry analysis using a Lachat Quik-Chem Flow Injection Autoanalyzer. Method detection limits for each analyte are typically as follows:

- TP (MDL 0.001-0.002 mg/L)
- TN (MDL 0.0025-0.001 mg/L)
- $NO_2 + NO_3$-N (MDL 0.001-0.002 mg/L)
- NH_4-N (MDL 0.003-0.006 mg/L)
- PO_4-P (MDL 0.0005-0.002 mg/L)

Turbidity will be measured in the laboratory in nephelometric units.

Benthic algae and phytoplankton

Phytoplankton samples will be filtered onto a 0.8 µm glass fiber filter. Filters will be wrapped in aluminum foil and immediately frozen for CHLA analysis following Biggs and Kilroy (2000).

Benthic algae species composition will be processed in accordance with EPA RBP (1999) and Biggs and Kilroy (2000). Samples will be transferred from the dark bottle to a 1-L plastic beaker on stir plate. Samples will be lightly homogenized to break up large filaments using a hand blender. After recording sample volume, 10 mL aliquots will be drawn from the sample (with a stir bar maintaining a homogeneous mixture) until 100 mL has been transferred to a bottle for soft-algae taxonomy. The process will be repeated for a second 100 mL sample for diatom taxonomy. Both bottles will be immediately preserved with buffered formalin to a final concentration of 4% v/v. Soft algae and diatoms will be preserved in this manner on every samples event (n=12), but cost will limit species identification to 2 dates per year (4 events total). We will target spring and late summer events, roughly 6 months apart, for taxonomic identifications.

Benthic algal biomass will be also processed in accordance with EPA RBP (1999) and Biggs and Kilroy (2000). After species aliquots are removed, the remaining slurry will be further homogenized until the slurry has no obvious large filaments or particles. Maintaining a high rate of stirring, at least 3 aliquots of 2-5 mL will be drawn from the sample with a volumetric pipette and filtered onto a 0.8 um glass fiber filter (GFF). The filter will be immediately wrapped in aluminum foil, labeled, and frozen for spectrophotometric analysis of CHLA following Biggs and Kilroy (2000). A second sample will be filtered onto a pre-ashed, pre-weighed GFF for estimation of dry mass and ash-free dry mass (AFDM) following EPA RBP (1999).

Soft algae (minimum 300 natural units) will be identified by Dr. Stephen Porter (Dr. Porter is a retired algal taxonomist from USGS and has published numerous reports related to benthic algae as part of the NAWQA program, including samples from the Ozark Highlands). In addition to counts of natural units, Dr. Porter will estimate biovolume of all non-diatom taxa (diatoms are not identified in these samples, only counted as "centric" or "pennate", thus biovolume is not applicable).

Dr. Barbara Winsborough will identify diatom samples (see King et al., 2009a, b, Taylor et al. 2014). Dr. Winsborough will identify a minimum of 500 valves per sample.

The remaining slurry will be transferred to one or more 50 mL centrifuge tubes, evaporated and dried in a drying oven at 60C, and pulverized to a fine powder using a BioSpec bead beater. Once pulverized, benthic algae will be analyzed for nutrient content to estimate ratios of P and nitrogen (N) to carbon (C), which have been shown to be excellent integrative indicators of nutrient status among streams (King et al. 2009, Taylor et al. 2014).

Benthic algal carbon and nitrogen content will be estimated using a CHNS autoanalyzer, which combusts the sample at a very high temperature and measures C and N as gases. C and N samples will be exposed to concentrated HCl after they are weighed to remove carbonates that can affect estimates of organic C.

Phosphorus content will be estimated using a colorimetric method (Taylor et al. 2014) using the Lachat Quik-Chem FIA.

Prior to drying and analysis of benthic algae P, N and C samples, we will examine the need to use colloidal silica to separate organic matter from inorganic sediment and other debris. We will complete the analysis with the colloidal separation and without the colloidal separation for all samples from the first sampling event. Ideally, the method will prove to be unnecessary because it is very time intensive. Colloidal separation yielded a nearly statistically identical relationship between surface-water TP and periphyton nutrient to carbon ratios when compared to the bulk periphyton (no separation) in a previous study (King et al. 2009).

Macroinvertebrates

All large-bodied grazing individuals will be removed from samples unless densities warrant subsampling. Identifications will be to operational taxonomic units, typically genus. Biovolume will be estimated based on size-frequency classes and published length-mass equations or proximate shapes, or direct measurement as ash-free dry mass. Identifications will be performed by trained taxonomists in the King lab. Voucher specimens will be verified.

Results and Reporting

The following is a list of variables that will be analyzed in response to TP.

- Benthic algal biomass as chlorophyll-a (CHLA)
- Benthic algal biomass as ash-free dry mass (AFDM)
- Benthic algal biomass as carbon (C)
- Benthic algal phosphorus to C (P:C) and nitrogen to C (N:C) ratios (supplemental indicators)
- Biovolume of nuisance algal species (individually and collectively)
- Cell densities of nuisance algal species (individually and collectively)
- Cell densities of all species (assemblage level analysis)
- Sestonic algal biomass as CHLA
- Minimum and daily range of dissolved oxygen (DO)
- Maximum and daily range of pH
- Grazing macroinvertebrate densities and biomass

<u>Algal assemblage analysis</u>
Diatom and soft algae taxonomic data will be combined into single data matrices (sites= rows, species=columns) and expressed as cell densities for assemblage-level analysis. Analyses will be conducted by season (n=4 collections) and by average densities within sites over the study duration. The latter approach will yield a more meaningful assessment of the shifts in composition because it will correspond to a scale that is relevant for management.

We will further stratify analyses using data sets that include all species and others that only include soft algae taxa, the latter being focused primarily on taxa that may be considered nuisance algae.

We will use at least two analysis methods to identify significant shifts in species composition: Threshold Indicator Taxa Analysis (TITAN; Baker and King 2010, 2013) and the EPA Field-Based Benchmark method (EPA 2011)

Threshold Indicator Taxa ANalysis (TITAN)

TITAN is a method designed to identify synchronous changes in the frequency and abundance of individual taxa along an environmental gradient (e.g., TP--see Figure 3). 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 will perform TITAN on cell densities of algal species occurring in >= 3 sites. TITAN will be run with the TITAN 2.0 package (Baker & King, 2010, 2013) in R.3.0.1.

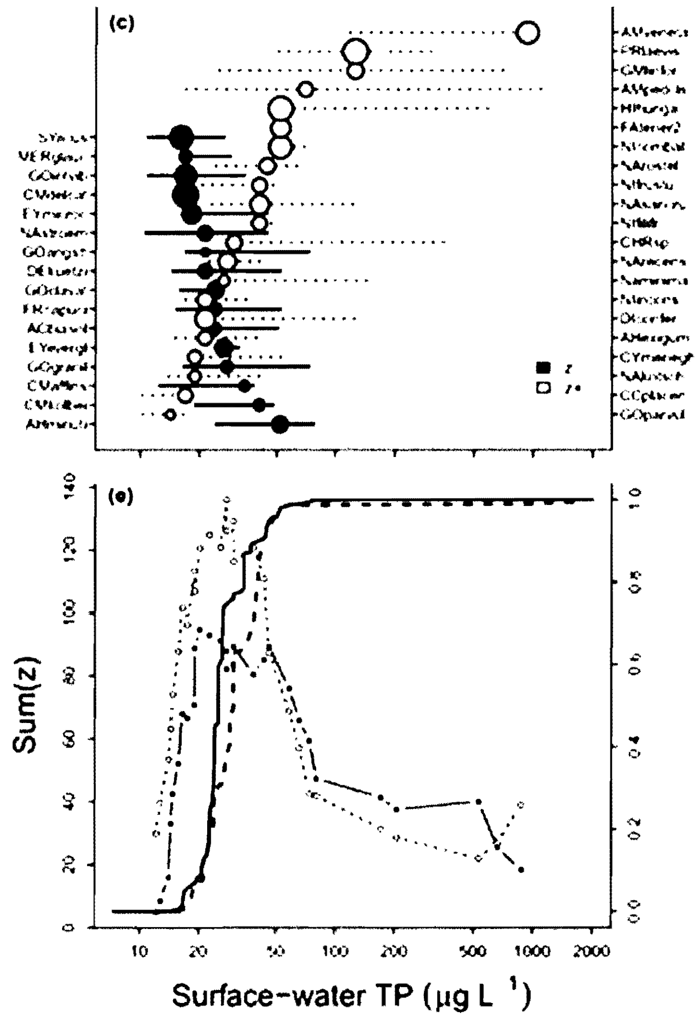

Figure 3. Results from TITAN on algal cell densities from 38 streams in central Texas. Filled symbols represent locations where taxa sharply declined, whereas open symbols are taxa that increased with TP. Synchronous shifts sharply peaked at 0.021 mg/L TP (Sum(z)), a strong indication of a community-level threshold (Taylor et al. 2014). This approach is consistent with methods used in the *EPA Stressor-Response Guidance Document.*

EPA Field-Based Benchmark Method

The EPA Field Based method is an extension of the LD50 species sensitivity distribution approach used by EPA for setting water quality standards for most contaminants. Here, abundances of individual taxa that occur in reference sites are regressed against a contaminant or other variable (in the case of EPA 2011, electrical conductivity associated with alkaline mine drainage). The value of the predictor where the natural abundance or occurrence frequency is reduced below a critical value (e.g., 50% of reference) is identified as a taxon's threshold, beyond which unacceptable declines in abundance or even extirpation are expected. The cumulative distribution of all taxa that decline can then be used to identify the level at which at least 5% of all taxa have declined beyond their critical value. This value is the assemblage-level threshold because EPA demands that a criterion protect 95% of the species (or taxa).

I will use this approach by fitting negative binomial generalized additive models to the mean cell density of each taxon for the duration of the study. Taxa must occur in at least 5 sites to analyze in this manner.

Figure 4 illustrates how this approach was used to identify a critical level of TP in Texas streams. The value at which >5% of the total taxa in the data set had declined 50% or more in abundance was 0.019 mg/L TP (Figure 2).

In addition to identifying the TP level associated with 50% declines in species, we will also identify the level where soft algae, particularly nuisance species, increase in biovolume by 50% over reference (low TP) levels.

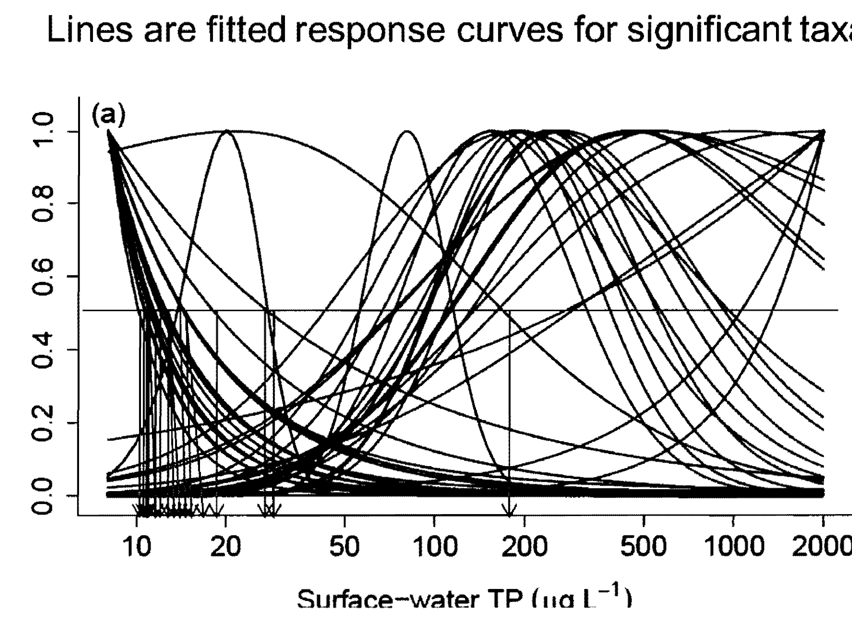

Figure 4. Negative binomial GAM response curves for algal species in response to total P across 38 central Texas streams (data from Taylor et al. 2014).

Algal Biomass, Nutrient Ratios, and Dissolved Oxygen, pH, and Grazer Abundance Analyses

Benthic and sestonic algal biomass, diel DO and pH, nutrient ratios (supplemental indicators of enrichment) and grazer abundance will be analyzed using a few different but complementary analytical techniques.

Generalized additive models (GAM) or generalized linear models (GLM) will be used to identify the level of TP where these variables intersect a level deemed unacceptable based on the levels found at reference sites or literature values (e.g., USEPA 1999, Biggs 2000) (Figure 4).

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 will fit responses of these variables to surface water nutrients using GAMs with the mgcv package in R 3.0.1 (Wood, 2006). Cross-validation will used to determine the optimal amount of smoothing, and AICc will be employed to determine whether covariates (e.g., grazer density or biomass) are warranted.

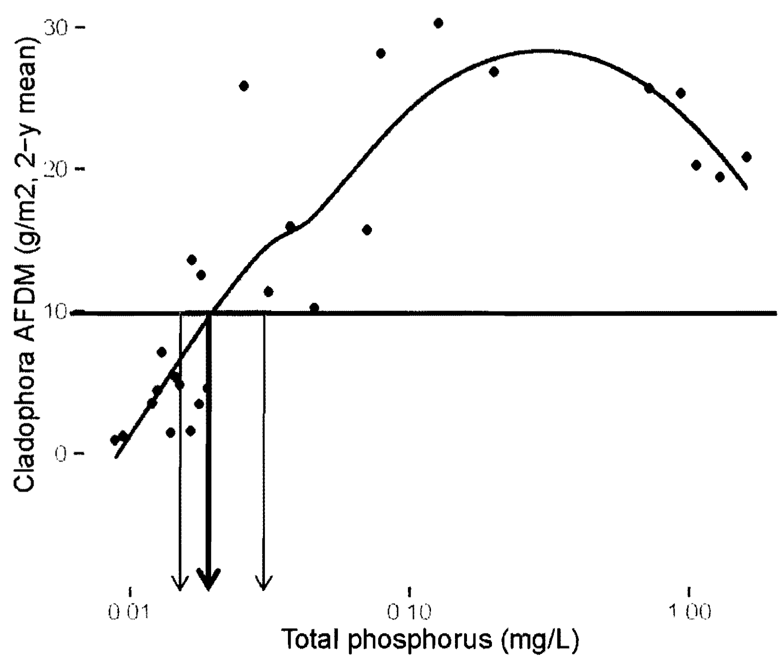

Figure 5. Fitted response of mean values of *Cladophora* biomass (AFDM, g/m^2) over a two year period across 26 central Texas streams (data from King et al. 2009). The fitted response intersects the management threshold of 10 g/m^2 at ~0.030 mg/L TP, with lower CI at 0.018 mg/L TP. *Note that the orange line value of 10 g/m^2 is only used here as an example.*

Nonparametric change point analysis (nCPA; King and Richardson 2003) will be used to identify the level of TP that corresponds to the largest change in the response variable. Bootstrapping will be used to estimate confidence limits around the TP change point.

Quantile regression (Koenker 2005) will be used to fit a linear or nonlinear regression line to the upper or lower boundary of the response to TP. Confidence limits around the regression line will be used to estimate uncertainty.

Other statistical methods may be used if deemed sufficiently unique from those listed or if new methods become available that are superior.

Synthesis and Recommendation of a TP criterion

The final report will comprehensively describe the site selection process, site and catchment characteristics, sampling methods, laboratory procedures, data summaries, analyses and results.

The remainder of the report will be focused on the derivation of a TP criterion for the Oklahoma Scenic Rivers based on the weight of evidence (Cormier et al. 2013, Suter and Cormier 2013) revealed by the analyses in this study as well as previous research (e.g., Stevenson et al. 2012) in the study area. Additional analyses may be employed in this section using existing data if relevant, although I reserve the right to discuss with the committee and make a final decision as to whether a data set can be defensibly analyzed in a manner consistent with the *Stressor-Response Guidance Document*. If not, it will be excluded from the final report.

The committee will be involved throughout the study, but will be particularly involved at this stage of the project. The committee will be aware of preliminary findings via meetings at least twice per year. By the time the final results are presented to the committee, each member will be well informed about the results up to that point of the study and should be prepared to engage in discussions about the final results as it relates to a numerical criterion. Input from all committee members will be considered before specific recommendations are made.

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Addendum—Experiments

Experiments are invaluable for determining causes in field studies. I proposed two experiments to the committee, but these studies were viewed as secondary to the field gradient stressor-response study.

Thus, the Baylor team is not committing to performing any experiments as part of this study. However, we would like to revisit the possibility of performing experiments if time and funding allows, and particularly if specific recommendations about a TP criterion would clearly benefit from them, given preliminary results through year 2.

I proposed two types of experiments: 1) whole-stream enrichment with a paired upstream reference reach, and 2) laboratory microcosm gradient studied designed to mimic the field gradient using algal biomass as a response variable.

Both studies have potential to alleviate or completely remove ambiguity regarding the effect of phosphorus on algal species composition or biomass in the Oklahoma Scenic Rivers, particularly if designed to target concentrations that appear to result in significant shifts and undesirable aesthetics.