BAYLOR
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Oklahoma Scenic Rivers Joint Phosphorus Study

Scope of Work

Proposed by:

Ryan S. King

Associate Professor, Department of Biology, Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX 76798

www.baylor.edu/aquaticlab

CRASR
Center for Reservoir & Aquatic Systems Research

Framework for Scope of Work

"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."

Second Statement of Joint Principles and Actions, p.2, Mandatory Study Components

Methodological Constraints on Scope of Work

"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."*

Second Statement of Joint Principles and Actions, p.2, Mandatory Study Components

Outline of Scope of Work

- Field gradient study
  - Spatial factors (e.g., number and method of site selection)
  - Temporal component (e.g. sampling frequency and duration)
- Experiments
  - Whole-stream enrichment*
  - Laboratory microcosm experiment
- Results and Reporting
  - Approaches to data analysis
  - Weight-of-evidence derivation of numeric criterion
- Budget

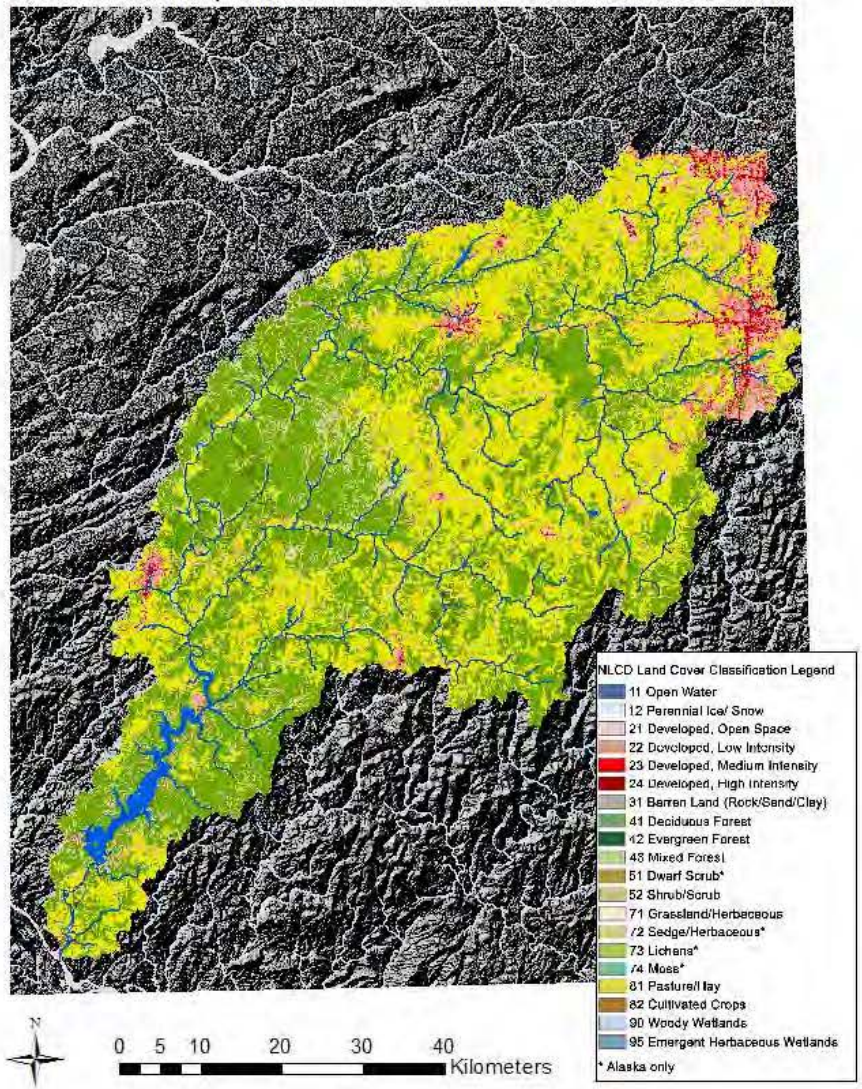
Field Gradient Study

Key elements of a field study

- Spatially extensive & intensive
- High temporal frequency of sampling over 2+ years
- Spans a ***gradient*** of conditions from reference to highly enriched. Probabilistic designs are not efficient and likely to fail.
- Minimizes contagion (clumping) between nutrients and locations in the basin and other confounding factors (sedimentation, geomorphology, light, catchment size)

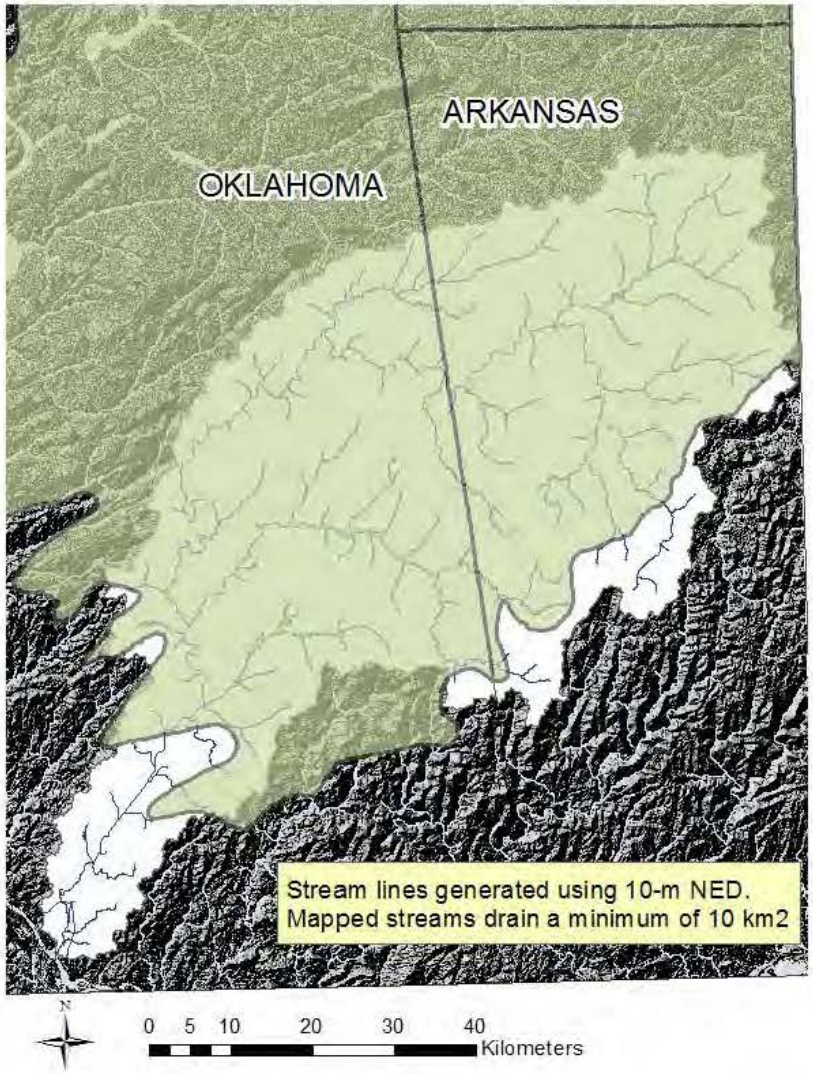
Illinois River Watershed

Catchment Boundary Delineated Using Reconditioned 10-m NED.
Land Use Corresponds to National Land Cover Dataset, 2006.



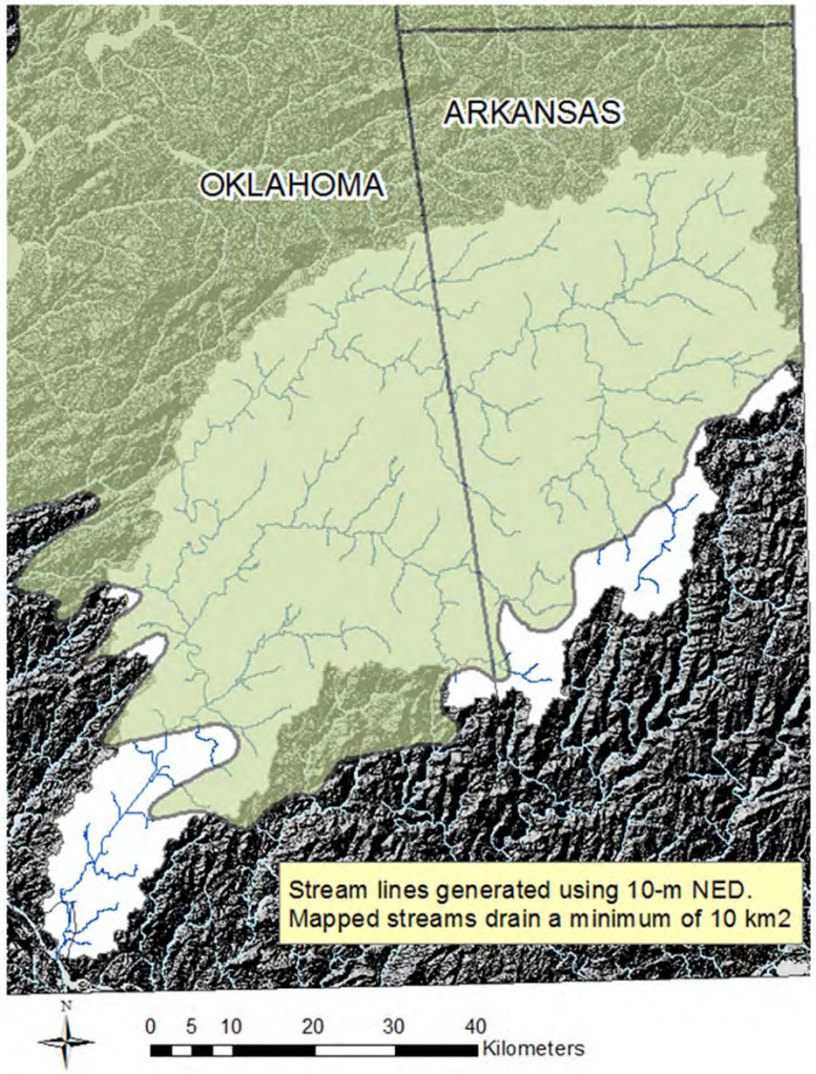
Illinois River Watershed

Shaded Area Corresponds to the
Ozark Highlands Level III Ecoregion



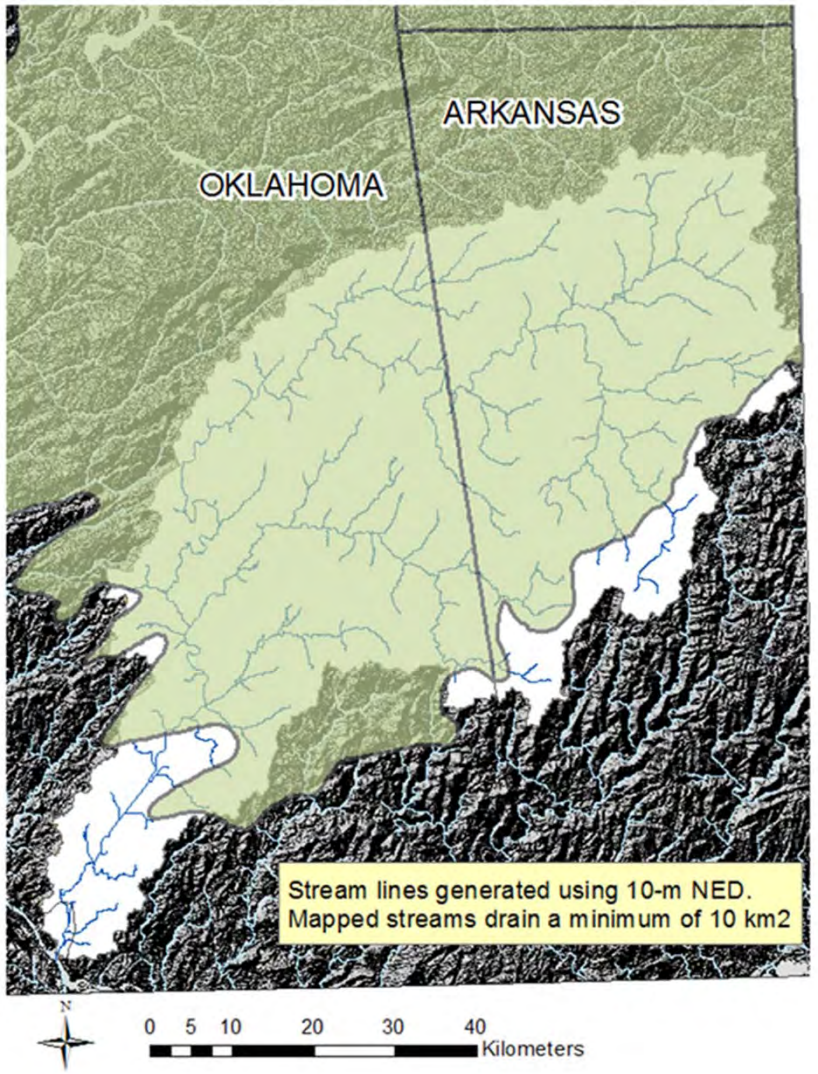
Field study: Spatial

- A minimum of 25 stream reaches will be selected for this study
- Sites will be located in upper Illinois River watershed and neighboring watersheds if necessary
- Sites will be located within and with majority of the catchment covered by the Ozark Highlands Level III Ecoregion (shaded region, right)



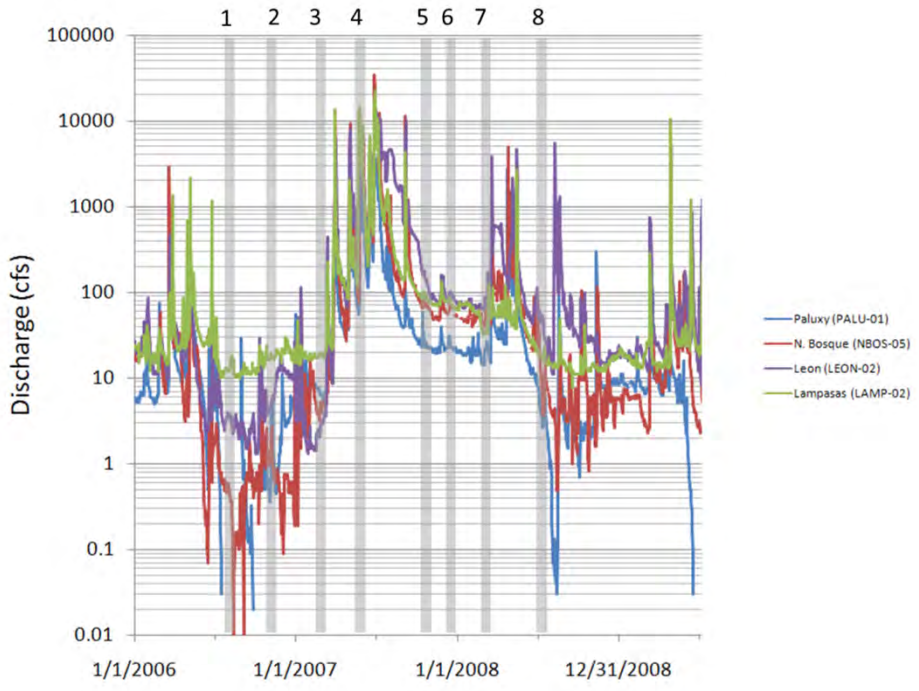
Field study: Spatial, page 2

- Sites will span a gradient from best available (minimum 6-8 "reference" sites) to >0.5 mg/L TP (or higher)
- Catchments: >5-10 km2 and between 2nd to 4th order *at the scale of a 5-10 km2 DA stream map* (blue lines, right)
- Existing data and field reconnaissance with new data to screen sites for final selection (April-July 2014)
- Sites must have riffles with cobble-gravel substrate



Field Study: Temporal

- Mean and max algal biomass is critical critical variable for derivation of this criterion.
- Bimonthly sampling is necessary to do this right



3 year hydrograph from 4 central Texas wadeable streams. Numbers indicate sampling periods from King et al. (2009).

Field Study: Sampling Frequency

Sampling will be scheduled bimonthly but adjusted for field crew safety and consistent field methods.

Proposed sampling will result in minimum of 12 events (at least 25 sites per event) in 2 years.

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				

*expected date of funding; **Jul 2016 desired but dependent upon remaining funds

Field Study: Sampling Methods, Algae

- EPA Rapid Bioassessment Protocols (1999). **Required by *Second Statement of Joint Principles* document.**
- Single habitat (riffle-run)
- 3 riffle-run channel units, 10-50 cm/sec
  - Perpendicular transect, wetted width
  - 5 equidistant points per transect, sample substrate
  - Composite sample of 15 substrate subsamples (also see King et al. 2009, etc).
- Sample immediately placed in dark bottle and on ice for processing in afternoon (filtering onto GFFs for CHLA and AFDM, preservation in M3 for species, remaining left for CNP analysis) and shipment to Baylor

Field Study: Methods, Water

- Water samples collected above 1st riffle upon visit to the site
- Duplicate TP, TN (unfiltered) and NH4-N, NO2NO3-N, and PO4-P (field filtered, 0.45 µm) samples collected in new 50 mL bottles
- Single 1-L seston (CHLA) sample, filtered same afternoon.
- Samples placed immediately on ice and prepared for shipment to Baylor
- Sample collection and analysis follows EPA Guidance on QA/QC and BU Center for Reservoir and Aquatic Systems Research (CRASR) protocols (e.g., Chain-of-Custody, trip blanks, field blanks, filtered blanks, duplicates, etc).
- Other monitoring to follow forms required by EPA RBP (1999).

Field Study, Diel Dissolved Oxygen

- Dissolved oxygen is a key variable that can bridge the "statistically significant shift" and "undesirable water quality" statements in the *Second Statement of Joint Principles* document.
- We propose to estimate minimum DO at least one time across all 25 sites using optical DO sensors and state-of-the-art YSI EXO1 data sondes (Baylor owns 13 brand-new units)
- We will target a low-flow period when streams are most susceptible to senescing algae, high respiration, and low reaeration

Field Study: Methods, Lab, Algae

- CHLA and AFDM determined in accordance with EPA RBP (1999)*, minimum 12 dates
- Species composition on 2 early spring and 2 late summer dates
  - Soft algae (300 cells) and diatoms (600 valves)
  - Dr. Barbara Winsborough, world-class taxonomist (see King et al., 2009a, b, Taylor et al. 2014)
  - Pattern among sites not likely to change markedly if P is driving changes. Not cost effective to analyze all dates.
- Periphyton nutrient content (not mandatory, but very strong indicator)
  - Ratios of % carbon, phosphorus, nitrogen in algal tissue (King and Richardson 2007, King et al. 2009, Taylor, King, et al. 2014)
  - C, N: CHNS autoanalyzer (combustion method)
  - P: colorimetric method

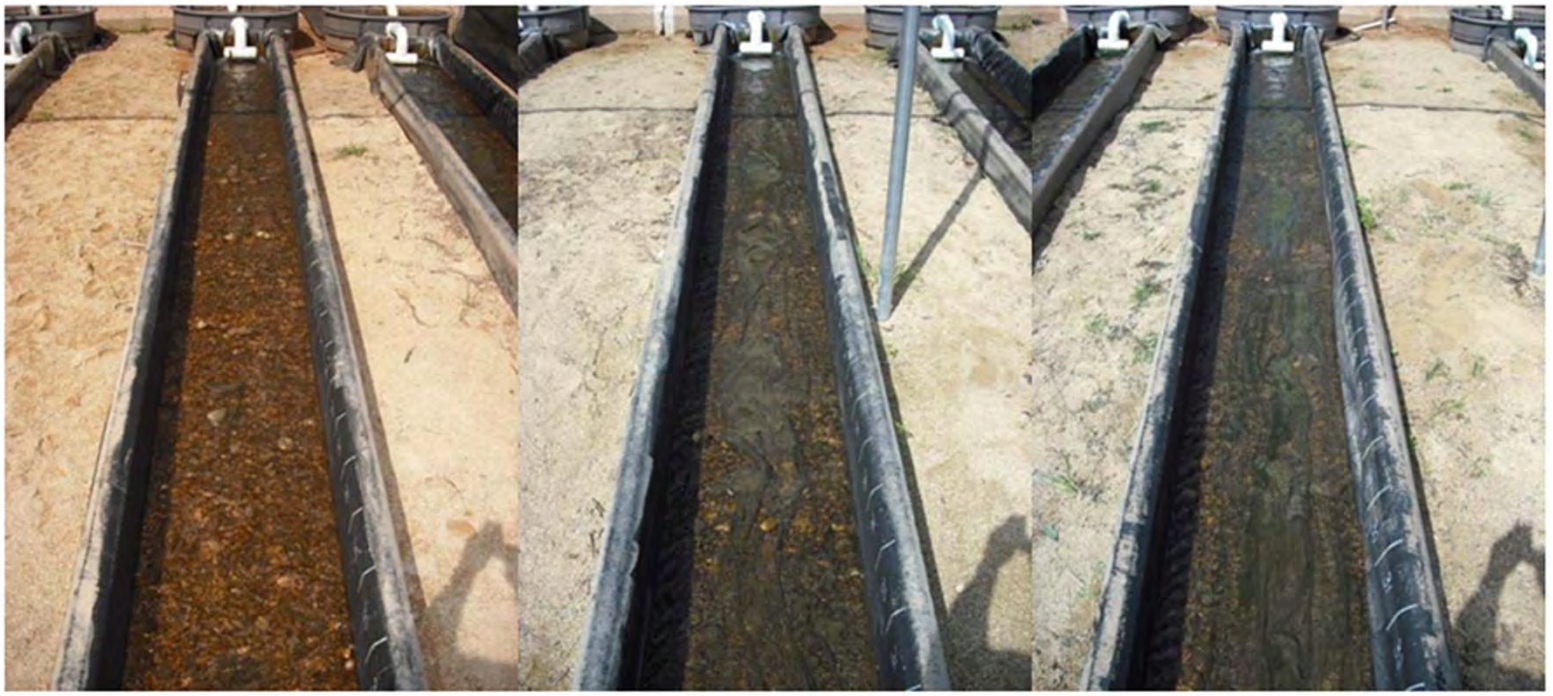
*acetone method is not preferred for CHLA. Hot ethanol is a safer, better extractant, and is more widely used for CHLA analysis (e.g., Biggs and Kilroy 2000)

Field Study: Methods, Lab, Water

- TP, TN, and dissolved nutrient samples run following APHA
- Dr. Jeffrey Back (9 years experience) will conduct nutrient chemistry analysis
- CRASR Lachat Quik-Chem Flow Injection Autoanalyzer
  - TP (MDL 0.001-0.002 mg/L)
  - TN (MDL 0.0025-0.001 mg/L)
  - NO2NO3-N (MDL 0.001-0.002 mg/L)
  - NH4-N (MDL 0.003-0.006 mg/L)
  - PO4-P (MDL 0.0005-0.002 mg/L)

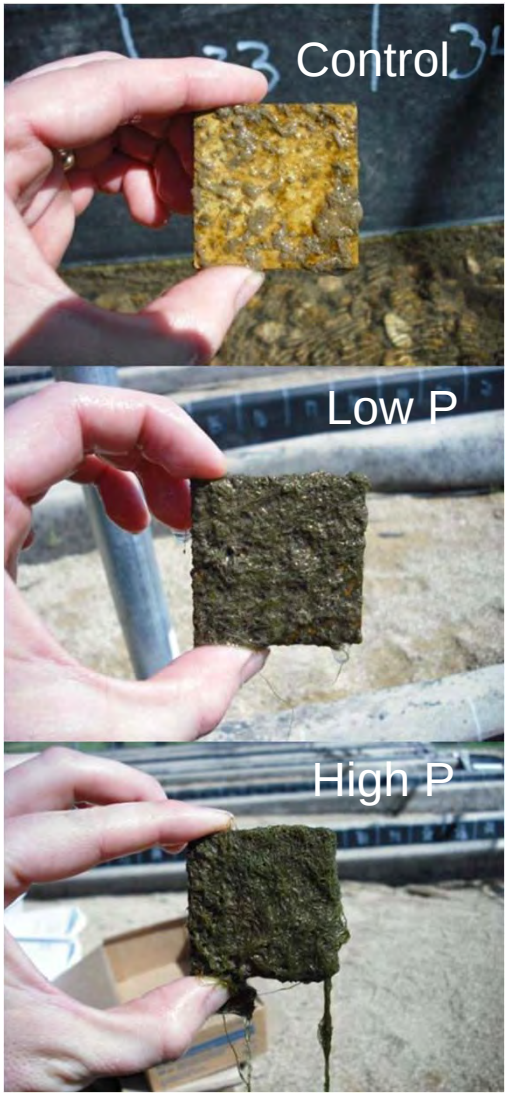
*acetone method is not preferred for CHLA. Hot ethanol is a safer, better extractant, and is more widely used for CHLA analysis (e.g., Biggs and Kilroy 2000)

Experiments



Experiments

- Causation requires multiple lines of evidence
- Field studies or experiments alone are insufficient, but when coupled can demonstrate causation
- Baylor Experimental Aquatic Research (BEAR) stream facility
  - not feasible with this budget
  - good alternatives, however



Experiment 1: Whole-stream enrichment

- Year 2 or 3, assuming permission granted
- Reference and treatment reaches
  - Same stream, nearly identical gradient, light, substrate, mean transit time, etc.
  - Monitor each reach for at least 30 days prior to dosing
  - Dose PO4-P as NaH2PO4 continuously for 60 d in treatment reach (12VDC low amp metering pumps)
  - Estimate mean TP:PO4-P in low-enriched streams near threshold zone
  - Supplement with NaNO3 or NH4NO3 to match balance of observed NO3-N, NH4-N, and PO4-P in enriched streams

Experiment 1: Whole-stream enrichment, page 2

- 2-4 ISCO samplers, daily integrated grab samples (Baylor owns 12 ISCOs)
- 4 YSI EXO1 sondes, two station GPP and CR pre and during dosing (propane evasion method)
- FMI VDC metering pumps (very reliable)
- Time series intervention analysis of GPP, CR, and benthic CHLA mg/m2 between reference and treatment reaches (Carpenter et al. 1989)
- Completed 2 whole-stream enrichments in Alaska in 2013 with great success--didn't miss a day of dosing, nailed our target for 10 weeks, both streams

Experiment 2: Laboratory streams

- "Baby BEAR"; Year 2 or 3
- 24 channels
  - 1 x 0.1 x 0.1 m
  - 5-8 L
  - temperature control via recirculating water in tubing submersed in FrigidUnits Living Stream
  - light: up to 1500 umol/m2/s via LED grow lights
  - static renewals via gravity feed and outflow stand pipe in sumps

The updated set-up is 2 levels with suspended LEDs

Experiment 2: Laboratory streams

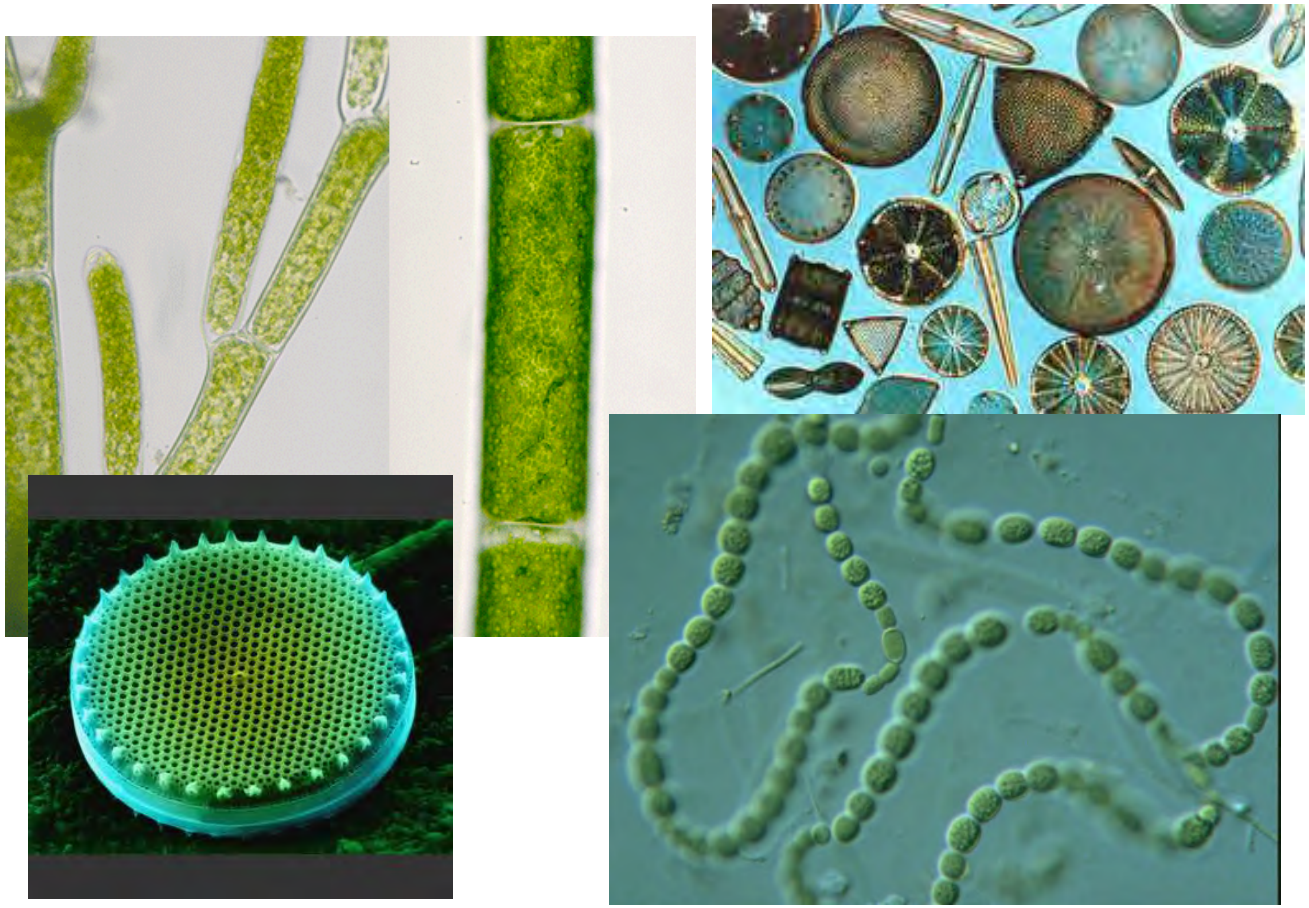
Experimental Design

- gradient of TP using stream water from field sites (6 sites)
- Sites will be chosen based on preliminary field results to target threshold zone
- 12 streams with cobble from reference stream
- 12 streams with cobble from most enriched stream
- 2 reps per field site per cobble type
- daily turnover of stream water
- 4 week study

Measured variables

- All nutrient analytes
- 0, 1, 2, 3, and 4 wk CHLA mg/m2 and AFDM g/m2
- % C, N, and P in periphyton at week 0, 2, and 4
- Dominant soft algal species
- GLM model with mean TP of source water as continuous predictor and cobble type as fixed discrete

Results and Reporting,
Mandatory Study Component #1.
Significant Shifts in Algal Species Composition



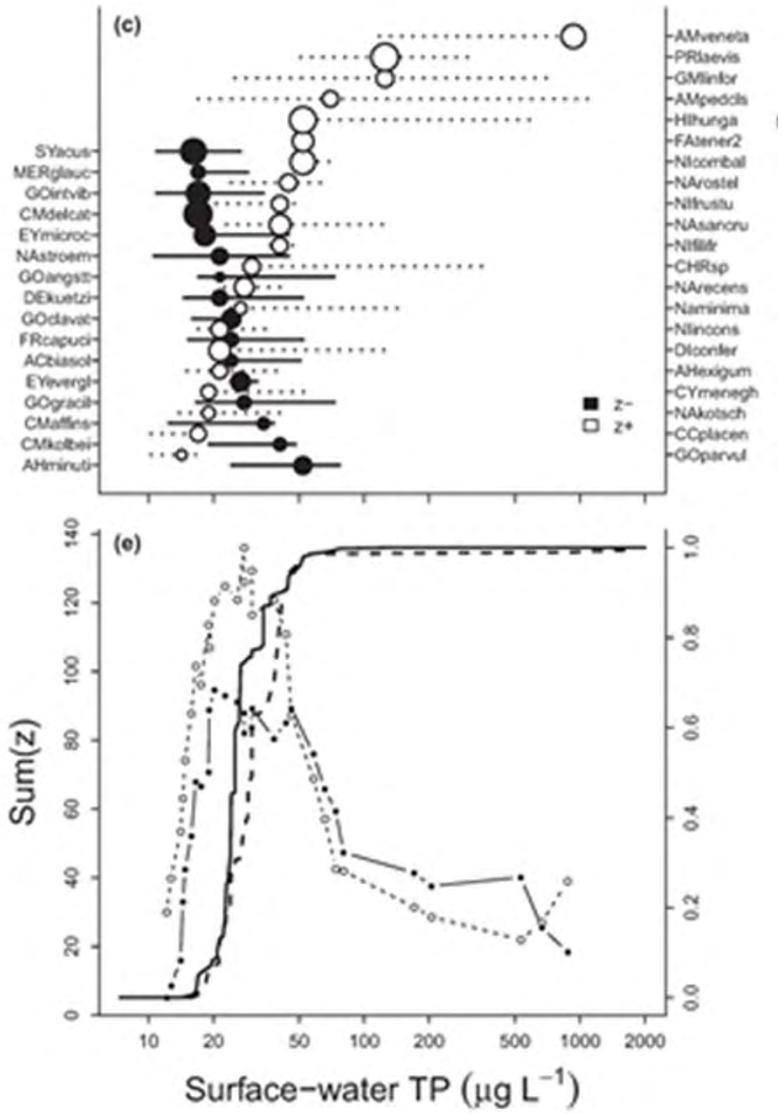
Results and Reporting, Algal Species: Threshold Indicator Taxa Analysis (TITAN)

TITAN will identify synchronous turnover from P-sensitive community to one dominated by eutrophic indicator taxa.

In this Texas example, significant turnover sharply peaked at 0.021 mg/L TP (Sum(z)), a strong indication of a community-level threshold

Consistent with *EPA Stressor-Response Guidance Document*

Mandatory Study Component #1.
Significant Shifts in Algal Species Composition

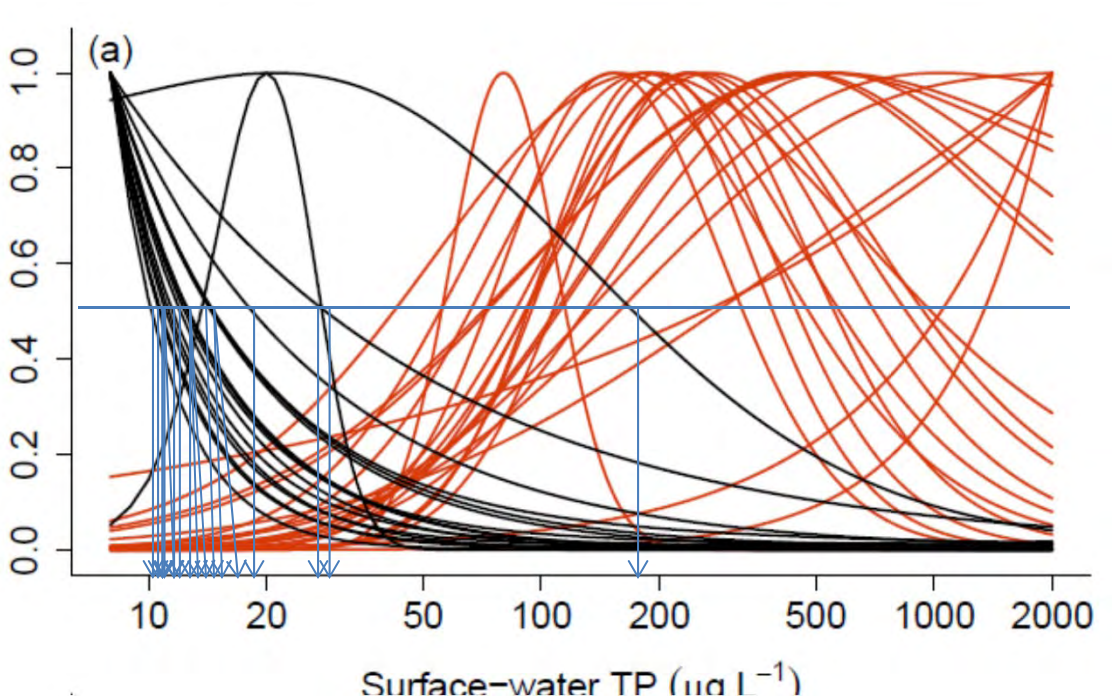


Taylor, King, et al. 2014. *Freshwater Biology*

Results and Reporting, Algal Species: EPA Field-Based Benchmark Method (2011)

- Negative binomial GAM or GLM, individual taxa responses
- "LD50"—50% reduction in population
- TP criterion should protect 95% of the taxa
- TP value where 5%* of taxa decline by 50%
- Fitted (mean) or CIs
- This example: 0.019 mg/L TP

Lines are fitted response curves for significant taxa

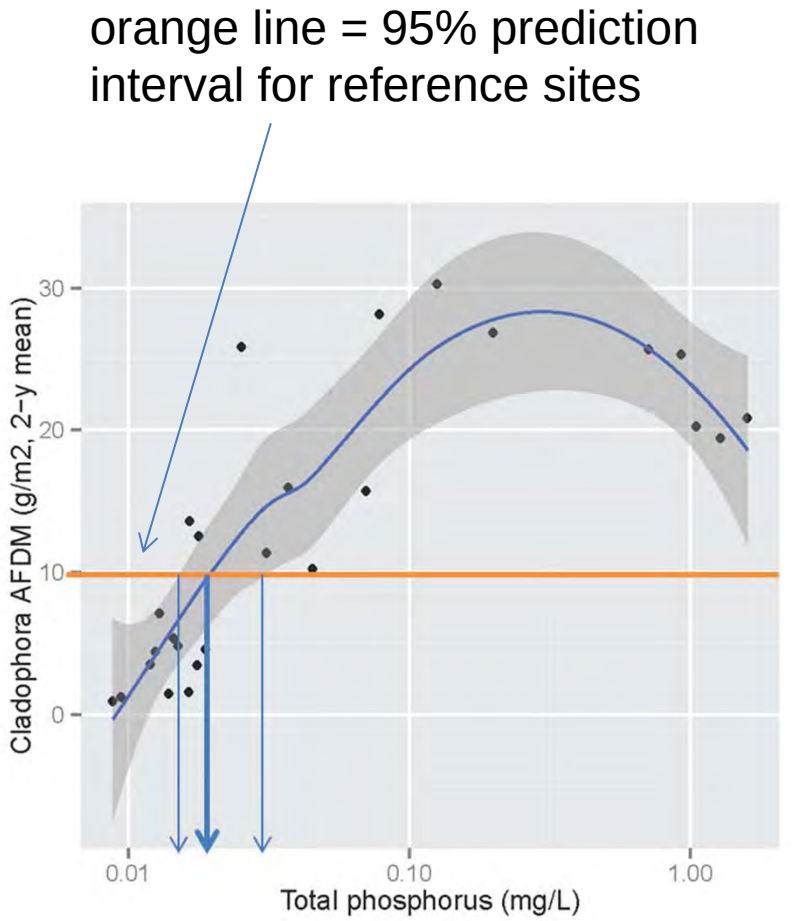


Mandatory Study Component #1.
Significant Shifts in Algal Species Composition

*limited to taxa in analysis data set

Results and Reporting, Algal Species: Algal community metrics

- Will follow US EPA RBP (Barbour et al. 1999) list. Analyze nutrient-relevant metrics in section 6
- GAM or GLM regression (Zuur et al. 2009)
- Fitted responses will be based on average densities over the duration of the study to reduce excessive numbers of tests and enhance signal:noise
- bivariate 95% prediction interval will be computed for reference sites for each metric; the extreme (upper or lower, depending upon metric) will be the basis determining y-axis threshold (figure, right).
- Intersection of fitted response corresponds to TP threshold (more detail in next section)



Mandatory Study Component #1.
Significant Shifts in Algal Species Composition

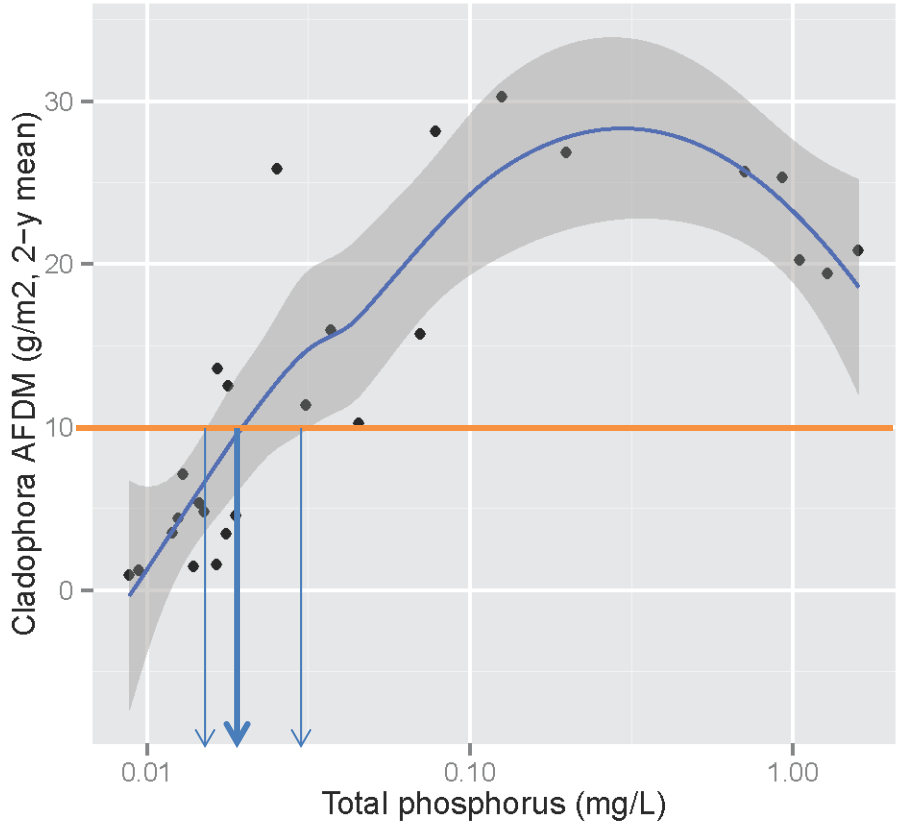
RESULTS AND REPORTING
Mandatory Study Component #2.
Significant Shifts in Benthic Algal Biomass

Results and Reporting: Algal biomass
Fitted response intersection method

Level of TP where benthic algal biomass (mg/m2 chla or g/m2 AFDM) crosses criterion for excessive growth (long-term mean or maximum; USEPA 1999, Biggs 2000).

Use GAM or GLM with appropriate distribution. LOESS used here only for illustration.

Statistical "threshold" model inappropriate for this relationship. We DO NOT want to identify where algae hits the "ceiling" as the TP criterion!



Mandatory Study Component #2.
Algal biomass resulting in undesirably aesthetic

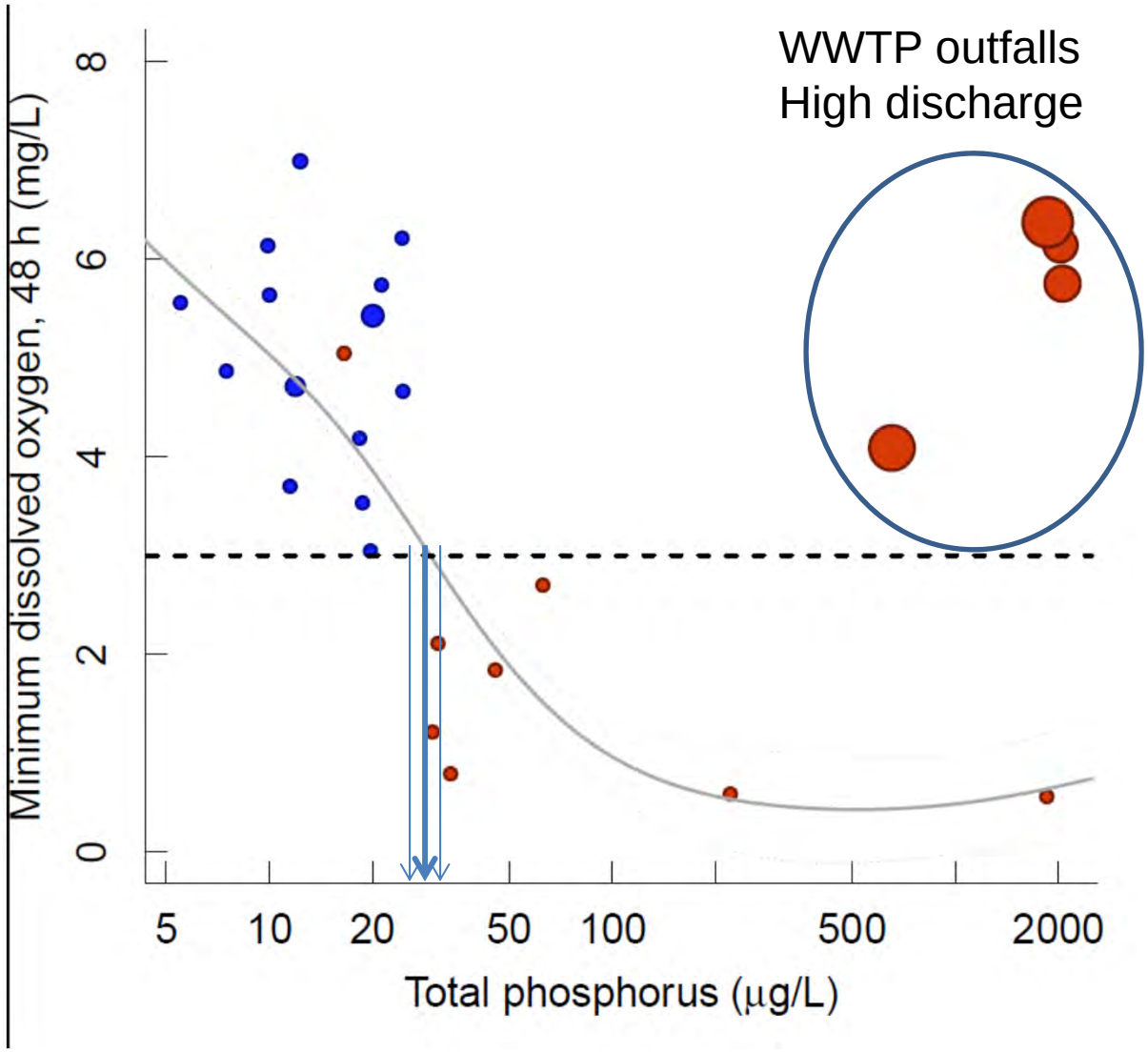
Data from King et al. (2009)

Results and Reporting: Algal biomass
Minimum DO as indicator of undesirable water quality

Fitted response intersection method.

Here, a GAM model with mean velocity and TP revealed that DO crashed in streams with low flow and elevated TP.

The partial fitted response (controlling for velocity) intersected the Texas DO criterion of 3 mg/L at 0.025 mg/L TP.



Data from King et al. 2009

Red=low carbon:chlorophyll ratio

Weight-of-evidence derivation of numeric criterion

- Final report will comprehensively address mandatory components and experimental results.
- Second Statement of Joint Principles document:
  - "*the committee and the scientific professionals employed....will be asked to make specific recommendations*."
  - This clearly indicates my team's findings will be discussed in collaboration with the committee to make a final recommendation
  - Weight-of-evidence approach will be strongly advocated by the Baylor team, including USEPA causal assessment and confounding factors analysis (Cormier et al 2013, Suter and Cormier 2013).

Personnel budget breakdown: All about data collection

Personnel	Sponsor funding, 2014	Sponsor funding, 2015	Sponsor funding, 2016	Breakdown of effort
Director (King)	None	1 mo. summer	1 mo. summer	25% data collection, 45% study design/data analysis, 30% report writing, presentations. Actual time 3-4 months per year. (BU cost).
Dr. Jeffrey Back	None	None	None	Fully funded by Baylor. Will facilitate field collections, oversee QA/QC, and run all water and periphyton chemistry samples in CRASR
Technician #1	Jun-Dec	Jan-Dec	Jan-Sep	MS level, field leader. Trained by King and Back. 100% data collection
Technician #2	Jun-Dec	Jan-Dec	Jan-July	BS level with experience. Trained by King and Back. 100% data collection
Grad student #1	Jun-Dec	Jan-Dec	Jan-Aug	PhD student in year 2 or 3; 100% data collection
Grad student #2	Jun-Dec	Jan-Dec	Jan-Aug	MS student with experience, 1st year. 100% data collection

Multiyear Budget

Category	Year 1 Apr-Dec 2014	Year 2 Jan-Dec 2015	Year 3 Jan-Dec 2016	Total
Personnel (salary+fringe)	73,545	152,580	111,052	**$337,177**
Consumable supplies	13,000	22,150	9,525	**$45,500**
Shipping	2,100	3,150	2,100	**$7,350**
Equipment	0	0	0	**-0-**
Algae species identifications (Dr. Barbara Winsborough)	7,500	15,000	7,500	**$30,000**
Water and periphyton CNP chemistry*	5,700	7,950	5,700	**$13,350**
Travel: lodging, food, incidentals	8,940	17,880	8,940	**$35,760**
Travel: Vehicle use and mileage**	0	0	0	**-0-**
Indirect costs (26.5% instead of BU std 38.5%)***	29,358	57,958	38,377	**$125,693**
Total	**$140,143**	**$276,669**	**$183,162**	**$600,000**

*CRASR will run at least 50% of samples in-kind (no charge)
**included in IDC
***Baylor will match IDC rate of any proposal that beats 26.5%

References
(a very truncated list of literature reviewed)

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