ARKANSAS DEPARTMENT OF ENERGY AND ENVIRONMENTAL, DIVISION OF ENVIRONMENTAL QUALITY

RE: FRL-comment on FRL–11994–01–R6

Exhibit A - *Water Quality and Ecological Assessment of Osage and Spring creeks in the Illinois River Basin*. McGoodwin, Williams and Yates.

Water Quality and Ecological Assessment of Osage and Spring Creeks in the Illinois River Basin, Arkansas

Final Report

McGoodwin, Williams, and Yates

Prepared by:
Center for Agricultural and Rural Sustainability
University of Arkansas Division of Agriculture
Arkansas Cooperative Extension Service

Questions concerning this report should be directed to:
Marty D. Matlock, Ph.D., P.E., C.S.E.
233 Engineering Hall
Fayetteville, AR 72701
mmatlock@uark.edu
479/575-2849

December , 2009

EXECUTIVE SUMMARY

The cities of Springdale and Rogers, Arkansas contracted with McGoodwin, Williams and Yates , the University of Arkansas Center for Agricultural and Rural Sustainability and Arkansas Water Resources Center to conduct a study evaluating water quality and assessing biological conditions in Osage and Spring Creeks in Northwest Arkansas. More specifically, the team collected and analyzed water quality, benthic macroinvertebrate, fish, and periphyton samples from Osage and Spring Creeks in Northwest Arkansas to evaluate the status of attainment of the aquatic life designated use of the streams under Arkansas Pollution Control and Ecology Commission's Arkansas Department of Environmental Quality Regulation 2 (ADEQ Reg. 2). This project was designed to evaluate three tiers of impact: 1) above and below wastewater treatment plants (WWTP) of the Cities of Rogers and Springdale, Arkansas; 2) sites below wastewater treatment plants compared to reference conditions; and 3) gradients across stream reaches from upstream to downstream.

The reaches that were sampled were located in the Illinois River watershed and included five sites on Osage Creek (Reaches 030, 930), three sites on Spring Creek (Reach 931), and two reference sites (Chambers Springs and Little Osage Creek). Sampling began in the Critical Season of 2007 and continued through the Critical Season of 2009. Sites were analyzed for water quality, habitat, and biotic condition using scientifically approved methods, documented through a Quality Assurance Project Plan.

Results of the water quality assessment showed no violations of ADEQ Reg. 2 criteria, with the exception of the site upstream from the Springdale WWTP for dissolved oxygen during Critical Season 1. All other observations across all other sites met the criteria for designated use for water quality during all observation periods. The Tier 1 assessment determined that while upstream and downstream sites differed, discharge of wastewater from the Rogers WWTP to Osage Creek or the Springdale WWTP to Spring Creek resulted in no violation of water quality standards according to the criteria of ADEQ Reg. 2; data suggested that the site below the Springdale WWTP was less impacted than the site above the discharge. The Tier 2 assessment showed overall differences of sites downstream of the WWTPs when compared to the reference sites but no clear indication that nutrients caused these differences. The Tier 3 assessment of the reach continuum from upstream to downstream showed that the impacts of the WWTPs in Osage and Spring Creeks across all metrics were not significant, and any decline in metrics observed was

fully or close to fully recovered by the lower site (OSG5). Water column phosphorus concentration did not cause biotic impairment, and the stream approached reference conditions by the downstream site (OSG5).

In conclusion, based upon the analyses performed during this project water quality in Spring and Osage Creeks met or exceeded designated use criteria for the period measured. Biological data indicated that stream ecosystem processes were not impaired by phosphorus, and biotic communities were not degraded by phosphorus. In fact, by the lower site (OSG5) biotic communities were similar to the reference sites. Phosphorus from the Rogers and Springdale wastewater treatment plants was not shown to cause impairment in water quality or biotic community function.

Table of Contents

List of Figures

List of Tables

List of Appendices

Section 1: Introduction

1.1 Project Background

The headwaters of the Illinois River originate in northwest Arkansas and flow southwest into Oklahoma. The headwaters are influenced by agricultural run-off as well as effluent from the Cities of Fayetteville, Springdale, Rogers, Siloam Springs and Prairie Grove, Arkansas (NPDES permits number AR0020010, AR0022063, AR0020273, AR0043397, AR0022098, respectively). The Cities of Rogers and Springdale, Arkansas (Cities) discharge treated wastewater from publicly owned treatment works (POTWs) into Osage and Spring Creeks, respectively (Figure 1.01).

The Cities contracted with McGoodwin, Williams and Yates (MWY), the University of Arkansas Center for Agricultural and Rural Sustainability, and Arkansas Water Resources Center to collect and analyze water quality, benthic macroinvertebrate, fish, and periphyton samples from Osage and Spring Creeks in Northwest Arkansas to evaluate the status of attainment of the aquatic life designated use of the streams under ADEQ Reg. 2.

1.2 Scope and Objectives

The purpose of this project was to collect water quality and biological data from targeted water bodies in Spring and Osage Creek of the Illinois River watershed in northwest Arkansas in order to assess attainment of the aquatic life use in those stream reaches. This project was designed to evaluate three tiers of impact: 1) above and below wastewater treatment plants (WWTPs) of the Cities of Rogers and Springdale, Arkansas (Cities); 2) sites below WWTPs compared to reference conditions; and 3) gradients across stream reaches from upstream to downstream. The reaches that were sampled in the Illinois River watershed were Osage Creek (reaches 030, 930) and Spring Creek (reach 931) (Figure 1.01). In addition, sampling was performed on two regional reference streams for comparison. Little Osage Creek was selected as a non-point source impacted reference stream and Chambers Springs Creek was selected as a minimally impacted reference stream for this study (Figure 1.01). Samples were collected upstream of the zone of influence and downstream of the mixing zone for Tier 1 analyses. The total number of sampling sites for Tiers 2 and 3 analysis, including those above and below wastewater treatment plants,

was 10 (Figure 1.01, Table 1.01). The data collected, in combination with other existing chemical and biological data, were used to assess the status of each reach with regard to ADEQ Reg. 2 criteria for listing in the ADEQ Section 303(d) list of water quality-impaired waters. All data were collected under a Quality Assurance Project Plan (QAPP) reviewed and approved by the Cities, MWY, ADEQ, and USEPA (Appendix A).

1.3 Existing Information and Data

Water quality studies have been conducted at sites throughout the Illinois River basin over the past 50 years; those reports that are relevant to this investigation are summarized in this section. The Ozark Highlands Ecoregion drains from northwest Arkansas to Missouri (White/Kings River), Kansas (Elk River) Oklahoma (Spavinaw Creek and Illinois River), and east to Arkansas (White River and tributaries to the Black River) (ADEQ, 2002). The Ozark Highlands Ecoregion, also referred to as the Ozark Plateau, is a rapidly urbanizing landscape with agricultural and forest land uses. The headwater of three major river basins (Illinois, Grand, and White) originate in this region. The predominant water quality parameter of investigation has been phosphorus, due in part to the sensitivity of headwater streams to nutrient enrichment. Phosphorus has been identified from point and nonpoint sources, though source allocation has been difficult due to P sorption to sediments, resulting in storage-release cycle that ameliorates the peak discharge concentrations and prolongs the elevated in-stream concentrations after the storm discharge abates (USGS, 1998a). In-stream sediment composition determines P sediment storage capacity (Haggard *et al*., 2001).

Sediment has been another contaminant of concern in this region. Urbanization is a major source of increased sediment to streams (USGS, 1999; Dogwiler, 2003; Chaubey *et al*., 2007). The process of land use change, including transition from forest to pasture and from forest to residential and commercial, results in increased landscape loading of phosphorus (Haggard *et al*., 2007). The impact of this rate of urbanization also affects the way streams respond to nutrient enrichment (USEPA, 2004; Chaubey *et al*., 2007). How and when water quality is sampled in streams determines whether these impacts are observed (Haggard *et al*., 2003).

Municipal WWTPs affect water chemistry at the point of discharge as well as whole-reach nutrient retention. The specific mechanisms of TP retention such as sediment sorption, biological uptake, and biotransformations have been investigated by Ekka *et al*. (2006); Haggard *et al*.

(2005); Haggard *et al*. (2001a); Dorioz *et al*. (1998); House and Denison (1997); and Reddy *et al*. (1996). The influence of effluent discharge on nutrient retention is variable, where nutrients are sometimes retained with a stream reach and under other conditions net release occurs. Nutrients, particularly P, are generally retained and stored within the fluvial channel when effluent concentrations are high; however, these stored nutrients are often released from within the fluvial channel when effluent discharge has lower than average concentrations (Haggard, 2000). Effluent discharged do have a significant impact on water quality chemistry, and this effect is often observed several kilometers downstream in the Ozark Highlands Ecoregion (Haggard *et al*., 2000; Haggard *et al*., 2003; Haggard *et al*., 2004). Sediment from Lake Francis, a small reservoir in the lower reach of the Illinois River, was determined under anaerobic sediment conditions to be as high as 15 mg TP m^{-2} day^{-1}, representing more TP load than all the WWTPs combined (Haggard and Soerens, 2006).

Stream biotic response (particularly algal growth) to increased P and nitrogen (N) is complicated by the number of additional variables besides nutrients. These variables include light, grazing, scouring, and temperature (Ludwig *et al*., 2008; Rodriguez and Matlock, 2008). Periphytic communities in streams dominated by agricultural land use in the Ozark Plateaus are composed of species adapted to higher nitrate, P, and dissolved organic carbon concentrations (USGS, 2002). These communities respond to very low levels of P increase then become saturated very quickly, resulting in a shift often to light limitation (Ludwig *et al*., 2008).

Fish community studies have been conducted in this region of Arkansas as far back as 1963, but more recent studies were conducted in the mid-1980s and 1990s, followed by a 2004 USEPA-funded study. A diverse community of fish species live in Ozark Plateau streams relative to other regions. Approximately 175 species (including protected species) are present in the Ozark Plateaus National Water-Quality Assessment (NAWQA) Program study unit; at least 19 of which are endemic to the Ozark Plateau area. Consequently, widespread and extreme degradation of water quality (chemical or aquatic habitat factors) could affect several species found nowhere else in the world. Many of these 175 species are intolerant of habitat or water chemistry degradation (USGS, 1998b). Land use, watershed size, biotic factors (competition, predator-prey interactions, and periphyton abundance), and riparian habitat characteristics have a significant influence on fish communities within the Illinois River (USEPA, 2004). Changes in land use from forestland to agriculture land over time have resulted in an increased relative abundance of stonerollers and members of the sucker family and a decreased relative abundance of members of the sunfish and

darter families. Most species of darters and some species of sunfish are intolerant of degraded water chemistry and habitat (USGS, 1998b; USEPA, 2004). A common trait of fish communities of Ozark streams in agricultural basins or downstream from WWTPs is increased relative abundance of stonerollers. Increased periphyton production resulting from more nutrients and sunlight provides a more abundant food source for stonerollers and other grazers, such as southern redbelly dace. Often, darters and sunfish compose a smaller percentage of the fish communities of Ozark streams in agricultural basins than in forested basins. USGS (1998b) and USEPA (2004) demonstrated that several other environmental factors (*e.g.* nutrients, organic carbon, suspended sediment, and DO) caused primarily by land-based discharges frequently result in changes in fish communities.

Figure 1.01 Osage Creek basin with sites denoted by circle points and WWTPs denoted by stars. See Table 1.01 below for definition of abbreviations.

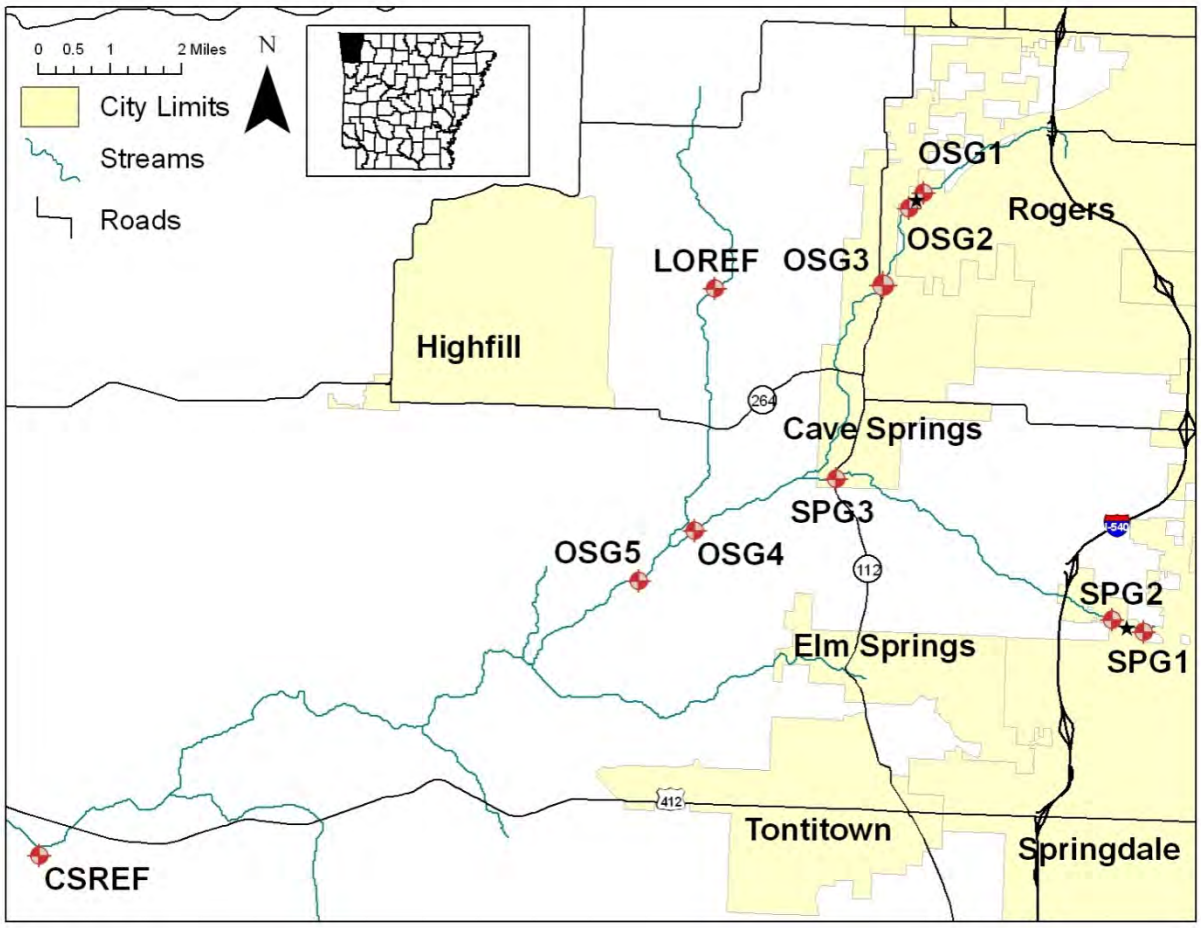


Table 1.01 Descriptions and locations for select sites in the Osage Creek and Illinois River basins.

Location	Abbreviated Identification	Coordinates
Osage Creek, Reach 930, upstream of City of Rogers WWTP	OSG1	Lat: 36°18'8.86"N Lon: 94°12'48.84"W
Osage Creek, Reach 930, downstream of City of Rogers WWTP	OSG2	Lat: 36°17'54.44"N Lon: 94°13'15.22"W
Osage Creek, Reach 930, downstream of City of Rogers WWTP and upstream of Spring Creek confluence	OSG3	Lat: 36°16'56.08"N Lon: 94°13'40.55"W
Spring Creek, Reach 931, upstream of City of Springdale WWTP	SPG1	Lat: 36°12'48.31"N Lon: 94° 9'21.93"W
Spring Creek, Reach 931, downstream of City of Springdale WWTP	SPG2	Lat: 36°12'56.79"N Lon: 94°10'5.38"W
Spring Creek, Reach 931, downstream of City of Springdale WWTP and upstream of Osage Creek confluence	SPG3	Lat: 36°14'38.44"N Lon: 94°14'18.30"W
Osage Creek Reach 030, downstream of Spring Creek confluence and upstream of Little Osage Creek confluence	OSG4	Lat: 36°13'56.40"N Lon: 94°16'21.52"W
Osage Creek Reach 030, downstream of Spring Creek confluence and downstream of Little Osage Creek confluence	OSG5	Lat: 36°13'19.69"N Lon: 94°17'14.11"W
Chambers Creek (Reference Site 1)	CSREF	Lat: 36° 09'53.60"N Lon: 94°26'10.99"W
Little Osage Creek (Reference Site 2)	LOREF	Lat: 36°16'54.20"N Lon: 94°16'8.53"W

Section 2: Methods and Results

2.1. Sample Site Descriptions

Ten sites were sampled for this study (Figure 1.01). Two sites, Chambers Springs and Little Osage (CSREF and LOREF, respectively) were considered reference sites. Little Osage Creek was considered moderately impacted by non-point sources but not point sources. Chambers Springs Creek was considered minimally impacted from human activity although there are several households in the basin, a gravel road travels the length of the stream, portions have been cleared for pasturing cattle, and part is used for pine silviculture in an otherwise oak-hickory forest. Sites upstream of the WWTP outfalls on Osage and Spring Creeks (OSG1 and SPG1, respectively) were selected to evaluate the direct impact, if any, of point sources from the City of Rogers WWTP (OSG1) and the City of Springdale WWTP (SPG1). Two sites were selected immediately downstream of the Cities' WWTP outfalls below the mixing zones (OSG2 and SPG2, respectively). Sites were selected on both Osage and Spring Creeks above the confluence of these two creeks (OSG3 and SPG3), and two more sites were selected on Osage Creek below the confluence with Spring Creek (OSG4 and OSG5). These sites were selected to assess the impact of the WWTP effluent on the individual streams and the basin as a whole based on the three-tiered analysis strategy. Sites were selected to insure safety, accessibility, representativeness, and habitat comparability. Sites varied in watershed size from 8.3 square miles to 130 square miles (Table 2.01). Urban land use varied from 43% to 61% (Table 2.01). Hay meadow/pasture land use varied from 23% to 79% (Table 2.01). Forest land use varied from 61% to 11% (Table 2.01). Each site is described below, and coordinates are presented in Table 1.01.

Site OSG1. Osage Creek 1 (OSG1) was located upstream of the Rogers' WWTP effluent outfall. The site was located on and accessed through the Rogers' WWTP property. This sites' watershed contained high urban land use percent though the immediate area surrounding the site was hay meadow/pasture dominated.

Site OSG2. Osage Creek 2 (OSG2) was located downstream of the Rogers' WWTP effluent outfall below the mixing zone. The site was located on and accessed through the Rogers' WWTP property. This watershed was almost identical to OSG1, as was the area surrounding the site.

Site OSG3. Osage Creek 3 (OSG3) was located upstream of the Highway 112 bridge, downstream of OSG2. The site was accessed across private property with permission from the owner. The watershed was similar to OSG1 and OSG2 with a slight increase in hay meadow/pasture and forested land use. The area immediately surrounding this site was predominantly hay meadow/pasture with a forested riparian zone.

Site OSG4. Osage Creek 4 (OSG4) was located downstream of the confluence of Osage and Spring Creeks. The site was located on City of Springdale property and was accessed across an adjacent land owner's property. The watershed was similar to the other Osage sites with slightly more hay meadow/pasture and forest land percent (Table 2.01). The area immediately surrounding the site was predominantly hay meadow/pasture with a mostly forested, yet disturbed, riparian zone.

Site OSG5. Osage Creek 5 (OSG5) was located downstream of the confluence of Osage and Little Osage Creeks. The site was located on and accessed through Northwest Arkansas Conservation Authority (NACA) property. The watershed contains considerably less urban percent and more hay meadow/pasture percent than other Osage sites (Table 2.01). The area immediately surrounding the site is predominantly hay meadow/pasture with a forested riparian zone.

Site SPG1. Spring Creek 1 (SPG1) was located upstream of the Springdale's WWTP effluent outfall. The site was located upstream of the Silent Grove Road bridge on Spring Creek and was accessed from Pump Station Road. This site had the highest urban percent land use of the study (Table 2.01). The area immediately surrounding the site was urban open space and forested riparian zone. A reservoir with a hydraulic gradient to the creek was adjacent to the south of the creek. There was evidence of seepage of very high redox potential water from the reservoir to the creek. The spring that provided the majority of the flow for the creek originated approximately 1,000 feet upstream of the site.

Site SPG2. Spring Creek 2 (SPG2) was located downstream of the Springdale's WWTP effluent outfall below the mixing zone. The site was located on and accessed through the Springdale's WWTP property. This sites' watershed was almost identical to SPG1 as was the area surrounding the site.

Site SPG3. Site Spring Creek 3 (SPG3) was located upstream of the Highway 112 bridge crossing Spring Creek. The site was located on private property and was accessed from the bridge and across the private property with the landowner's permission. The sites' watershed had substantially less urban percent than the other Spring Creek sites and was mostly replaced with

hay meadow/pasture land use. The area immediately surrounding the site was predominantly hay meadow/pasture with a forested riparian zone.

Site LOREF. Little Osage Creek Reference site (LOREF) was located on upper Little Osage Creek immediately upstream of the Benton County Road 279 bridge and downstream of the Mill Dam Road bridge. This site was located on Osage Mills Baptist Church property and was accessed from that property with the Church's permission. The site's watershed contained the highest percent hay meadow/pasture of any site with a considerable portion (8%) in urban land use but no point source discharge. The area immediately surrounding the site was predominantly hay meadow/pasture with a forested riparian zone. This reference site was selected to represent the typical impacts of urban and hay meadow/pasture non-point source pollution on area streams in the absence of point source contribution.

Site CSREF. Chambers Creek, also referred to as Chambers Springs, Reference Site (CSREF) was located on National Forest Service land in the Lake Wedington unit. Chambers Springs is a small tributary of the Illinois River. The site was located upstream of Benton County Road 196 off of Chambers Springs Road. The sites' watershed was predominantly forest with some hay meadow/pasture. The area immediately surrounding the site was predominantly forest. This site was selected as a least impacted regional reference site, but see previous comments at the beginning of this section for a list of the minor impacts in the basin.

Table 2.01 Watershed areas and dominant land use areas by percent in 2006 for select sites in the Osage Creek and Illinois River Basins (Center for Advanced Spatial Technology, University of Arkansas, 2006).

	OSG1	OSG2	OSG3	OSG4	OSG5	SPG1	SPG2	SPG3	LOREF	CSREF
Watershed Area (Square Miles)	32.1	32.4	35.6	80.6	128.6	12.7	13.2	35.3	35.4	8.3
Percent Urban	43%	43%	40%	34%	24%	60%	60%	36%	8%	0%
Percent Pasture	40%	40%	43%	45%	57%	23%	24%	43%	79%	39%
Percent Forest	13%	13%	14%	17%	17%	13%	14%	17%	12%	61%

2.2 Water Chemistry Methods and Results

2.2.1 Water Chemistry Methods

2.2.1.1 Sample Collection

Water samples were collected during base flow conditions a total of 29 times from the summer of 2007 to the summer of 2009. Grab samples were collected from the vertical centroid of flow (VCF) of the stream and dissolved oxygen (DO), conductivity, and temperature (YSI Model 85, Yellow Springs, OH) and pH (pH Testr 30, Oakton Instruments, Vernon Hills, IL) were measured in the field. Water samples were divided into two unfiltered samples, an unfiltered acidified sample (pH < 2), a filtered unacidified sample (0.45 µm membrane, syringe filtration), and two filtered acidified samples (0.45 µm membrane, syringe filtration, pH < 2). Samples were transported on ice back to the laboratory, stored at 4^{o} C, and subsequently analyzed.

2.2.1.2 Laboratory Methods

The analytical methods for chemical analyses are summarized in Table 2.02 and described in this section. Filtered un-acidified samples were analyzed for Cl^{-} using the automated ferricyanide method (APHA, 2005), nitrite-N (NO_2–N) using the sulfanilamide NED dihydrochloride colorimetric method (APHA, 2005), and (nitrate plus nitrite)-N ((NO_3 + NO_2)–N) using the hydrazine reduction method (APHA, 2005) on a Skalar San Plus Wet Chemistry Autoanalyzer (Skalar, the Netherlands); nitrate–N was obtained mathematically by subtracting NO_2–N from (NO_3 + NO_2)–N. Orthophosphate (OP) and ammonium–nitrogen (NH_4–N) were measured from filtered, acidified samples using the automated ascorbic acid method (APHA, 2005) and the sodium nitroprusside and salicylate method (APHA, 2005). Total phosphorus (TP) was obtained using a persulfate digestion and subsequent automated ascorbic acid method (APHA, 2005). A Skalar San Plus Wet Chemistry Autoanalyzer (Skalar, the Netherlands) was used to determine total nitrogen (TN) in unfiltered acidified samples using an in-line persulfate-ultraviolet oxidation and hydrazine reduction method (Skalar Method, the Netherlands). Total Organic Carbon (TOC) was measured from unfiltered acidified samples using the persulfate-ultraviolet flow injection method (APHA, 2005). Total Suspended Solids (TSS) were obtained using the glass fiber filtration method (APHA, 2005), and turbidity was measured via the nephelometric method (APHA, 2005) on a VWR Scientific 66120-200 Turbidity Meter (VWR International, West

Chester, PA). Chlorophyll-a (Chl-a) was obtained by filtering 1L of stream water through a Pall Type A/E glass fiber filter (Pall Corporation, Ann Arbor, Michigan) which was then shredded in 5 mL of aqueous acetone saturated with $MgCO_3$ and centrifuged. The supernatant was analyzed for Chl–a using the trichromatic method (APHA, 2005).

2.2.1.3 General Quality Assurance and Quality Control Procedures

A field duplicate and a field blank were collected during each sampling event and were analyzed for all project parameters; the field duplicates were compared to collected water samples, and field blanks were evaluated against method reporting limits. All water sample analysis was performed on calibrated instruments using a laboratory control standard to verify method accuracy. Laboratory duplicates were performed on 10% of samples to ensure method precision, and these values were compared against that measured in the water samples. Method accuracy was evaluated by including 10% matrix spikes with each analytical run, and these values were compared against that calculated mathematically. Method blanks were used to reveal any possible analytical process contamination. Laboratory control standards, duplicates, and spikes were considered acceptable within 20% of expected recovery.

Table 2.02 Methods for field and laboratory parameters for water samples collected for the Osage Creek and Spring Creek use attainability assessment.

PARAMETER	UNITS	MATRIX	Method	Reporting Limit (RL)*
Field Parameters				
pH	pH units	water	EPA 150.1	0.1
DO	mg/L	water	EPA 360.1	0.1
Conductivity	uS/cm	water	EPA 120.1	1
Temperature	^{o} C	water	EPA 170.1	NA
Laboratory Parameters				
NH_4-N	mg/L	water	EPA 350.1	0.02
NO_3-N	mg/L	water	EPA 353.2	0.10
NO_2-N	mg/L	water	EPA 354.1	0.01
TN	mg/L	water	Persulfate-Ultraviolet Oxidation and Hydrazine Reduction	0.10
SRP	mg/L	water	EPA 365.1	0.01
TP	mg/L	water	EPA 365.3	0.01
Chl-a	µg/L	water	EPA 446.0	0.1
TOC	mg/L	water	EPA 415.2	0.1
Turbidity	NTU	water	EPA 180.1	0.1
TSS	mg/L	water	EPA 160.2	6.0

*This represents either the method detection limit (MDL) or the practical quantification limit (PQL); however, all concentrations were reported as a value not less than a reporting limit.

2.2.2 Water Chemistry Results

Water quality analyses met the QAPP criteria for quality control (Tables 2.17-2.19); water quality data was within the acceptable quality assurance and quality control ranges defined within the QAPP for water samples across all sites for any of the parameters measured (Tables 2.03 – 2.16). Water quality across all parameters showed significant differences from upstream to downstream sites across all parameters (Figures 2.01), but no violations of ADEQ Reg. 2 criteria were observed since numeric criteria do not exist for nutrients. Water chemistry parameters approached the reference stream conditions by site OSG5 (Figures 2.01 - 2.08), although concentrations were still significantly greater than the reference conditions for phosphorus.

Table 2.03 Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of dissolved reactive phosphorus (e.g., ortho-phosphate), and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	0.021	0.037	0.055	0.035	0.036	0.044	0.032	0.042
LOREF	29	0.021	0.031	0.057	0.031	0.034	0.036	0.028	0.028
OSG1	29	0.018	0.032	0.050	0.032	0.029	0.035	0.031	0.035
OSG2	29	0.029	0.093	0.434	0.114	0.110	0.111	0.077	0.060
OSG3	29	0.030	0.084	0.210	0.110	0.089	0.093	0.073	0.055
SPG1	29	0.042	0.056	0.077	0.060	0.054	0.058	0.056	0.054
SPG2	29	0.070	0.182	0.599	0.133	0.180	0.253	0.167	0.212
SPG3	29	0.092	0.155	0.241	0.170	0.129	0.158	0.145	0.191
OSG4	29	0.077	0.120	0.195	0.143	0.107	0.118	0.112	0.129
OSG5	29	0.061	0.100	0.296	0.121	0.100	0.096	0.086	0.105

Table 2.04 Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of total phosphorus, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	0.029	0.048	0.065	0.045	0.047	0.054	0.041	0.055
LOREF	29	0.029	0.046	0.113	0.047	0.053	0.048	0.040	0.045
OSG1	29	0.030	0.042	0.064	0.040	0.043	0.044	0.040	0.046
OSG2	29	0.040	0.124	0.473	0.143	0.159	0.133	0.104	0.082
OSG3	29	0.044	0.110	0.227	0.131	0.122	0.119	0.093	0.085
SPG1	29	0.051	0.070	0.204	0.073	0.068	0.080	0.063	0.066
SPG2	29	0.131	0.249	0.643	0.180	0.252	0.307	0.257	0.272
SPG3	29	0.112	0.174	0.263	0.189	0.152	0.170	0.164	0.215
OSG4	29	0.090	0.141	0.218	0.160	0.128	0.130	0.130	0.179
OSG5	29	0.074	0.113	0.178	0.139	0.106	0.107	0.100	0.126

Table 2.05. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of (nitrate+nitrite)-nitrogen, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	0.45	1.18	2.71	0.79	1.69	1.72	1.28	0.63
LOREF	29	3.84	5.37	6.88	4.87	5.65	5.62	5.43	5.28
OSG1	29	1.89	3.17	4.26	2.90	3.07	3.37	3.30	3.24
OSG2	29	3.16	4.73	6.69	4.25	4.51	4.59	5.21	5.25
OSG3	29	2.91	4.29	7.32	3.92	3.95	4.05	4.93	4.74
SPG1	29	2.04	2.99	8.32	2.43	3.05	3.27	3.61	2.50
SPG2	29	2.10	3.32	4.56	2.86	3.20	3.72	3.37	3.64
SPG3	29	2.64	3.91	5.40	3.19	4.22	4.18	4.19	3.77
OSG4	29	2.81	3.95	5.47	3.29	4.10	4.03	4.31	4.06
OSG5	29	2.87	4.14	8.14	3.39	4.21	4.22	4.82	4.01

Table 2.06. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of ammonia-nitrogen, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	<0.001	0.010	0.056	0.013	0.013	0.014	0.005	0.012
LOREF	29	0.001	0.013	0.048	0.019	0.011	0.009	0.012	0.019
OSG1	29	0.001	0.010	0.038	0.007	0.013	0.011	0.011	0.009
OSG2	29	0.015	0.032	0.123	0.034	0.039	0.024	0.033	0.031
OSG3	29	0.013	0.026	0.060	0.031	0.029	0.021	0.025	0.022
SPG1	29	0.002	0.013	0.063	0.026	0.012	0.010	0.014	0.008
SPG2	29	0.029	0.059	0.100	0.064	0.059	0.042	0.067	0.067
SPG3	29	0.016	0.029	0.060	0.046	0.026	0.024	0.027	0.027
OSG4	29	0.008	0.025	0.076	0.037	0.022	0.019	0.025	0.031
OSG5	29	0.005	0.020	0.077	0.028	0.019	0.016	0.021	0.016

Table 2.07. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of nitrite-nitrogen, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	<0.001	0.005	0.022	0.008	0.008	0.009	0.001	0.005
LOREF	29	0.005	0.014	0.024	0.016	0.014	0.014	0.011	0.016
OSG1	29	0.003	0.008	0.019	0.010	0.010	0.011	0.006	0.006
OSG2	29	<0.001	0.011	0.039	0.015	0.011	0.013	0.006	0.012
OSG3	29	0.001	0.012	0.024	0.013	0.013	0.015	0.008	0.012
SPG1	29	0.002	0.008	0.019	0.010	0.012	0.011	0.005	0.005
SPG2	29	<0.001	0.010	0.024	0.010	0.013	0.017	0.005	0.013
SPG3	29	<0.001	0.009	0.026	0.013	0.013	0.013	0.003	0.009
OSG4	29	<0.001	0.010	0.021	0.013	0.013	0.015	0.005	0.011
OSG5	29	<0.001	0.011	0.029	0.013	0.013	0.014	0.006	0.012

Table 2.08. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of total nitrogen, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	0.47	1.26	3.11	0.90	1.85	1.62	1.29	0.79
LOREF	29	4.10	5.43	7.37	4.99	6.13	5.06	5.49	5.58
OSG1	29	1.92	3.20	4.74	3.04	3.33	3.02	3.26	3.45
OSG2	29	3.41	4.95	7.23	4.57	5.11	4.21	5.44	5.75
OSG3	29	3.19	4.48	6.45	4.21	4.56	3.74	4.99	5.22
SPG1	29	2.19	2.97	4.31	2.67	3.29	2.97	3.15	2.72
SPG2	29	2.68	4.06	5.53	3.75	4.21	3.88	4.15	4.42
SPG3	29	3.00	4.19	6.00	3.73	4.81	3.90	4.39	4.17
OSG4	29	2.92	4.14	6.01	3.68	4.55	3.68	4.53	4.41
OSG5	29	3.02	4.23	6.21	3.70	4.79	3.85	4.59	4.28

Table 2.09. Overall minimum, geometric mean and maximum concentration (ug L^{-1}) of sestonic chlorophyll-α, and geometric mean concentration (ug L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (ug L^{-1})	Geomean (ug L^{-1})	Maximum (ug L^{-1})	Critical Season 2007 (ug L^{-1})	Primary Season 2007-8 (ug L^{-1})	Critical Season 2008 (ug L^{-1})	Primary Season 2008-9 (ug L^{-1})	Critical Season 2009 (ug L^{-1})
CSREF	29	<0.1	0.1	0.6	0.3	<0.1	0.1	0.3	0.4
LOREF	29	<0.1	0.4	2.8	0.7	<0.1	0.8	0.8	1.2
OSG1	29	0.2	0.7	1.8	0.6	0.9	0.5	0.6	0.8
OSG2	29	0.2	0.8	1.7	0.8	1.0	0.6	0.6	1.1
OSG3	29	0.1	0.8	2.6	0.7	1.1	0.4	0.9	1.3
SPG1	29	0.3	0.6	1.7	0.7	0.6	0.4	0.7	0.9
SPG2	29	<0.1	0.4	3.1	<0.1	0.9	0.4	0.9	1.2
SPG3	29	0.5	0.9	2.3	1.0	0.9	0.8	0.9	1.2
OSG4	29	0.3	1.0	3.9	0.8	1.0	0.8	1.1	2.0
OSG5	29	0.1	0.9	2.6	0.7	1.0	0.7	1.0	1.2

Table 2.10. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of total organic carbon, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	0.25	0.46	0.92	0.49	0.52	0.56	0.37	0.40
LOREF	29	0.26	0.49	1.81	0.59	0.62	0.40	0.44	0.43
OSG1	29	0.15	0.37	1.24	0.39	0.45	0.31	0.34	0.41
OSG2	29	0.92	1.33	2.20	1.51	1.44	1.08	1.30	1.40
OSG3	29	0.72	1.14	1.83	1.23	1.30	0.93	1.17	1.10
SPG1	29	0.24	0.52	1.39	0.62	0.59	0.45	0.40	0.65
SPG2	29	1.76	2.85	4.16	2.63	3.25	2.60	3.15	2.51
SPG3	29	0.76	1.54	2.18	1.77	1.68	1.17	1.62	1.54
OSG4	29	0.74	1.22	2.23	1.50	1.28	0.93	1.29	1.15
OSG5	29	0.66	0.99	1.83	1.24	1.09	0.74	1.02	0.90

Table 2.11. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of total suspended solids, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	<0.1	1.1	3.1	2.4	1.2	1.1	0.5	1.5
LOREF	29	<0.1	4.1	14.7	3.4	4.7	3.7	3.6	6.5
OSG1	29	0.1	1.6	7.0	1.8	2.6	1.8	0.7	2.1
OSG2	29	<0.1	2.0	5.5	2.0	3.3	2.4	1.0	2.2
OSG3	29	0.5	3.8	51.8	3.0	5.1	6.9	1.8	4.6
SPG1	29	<0.1	1.6	5.9	2.7	2.0	1.8	0.9	1.4
SPG2	29	0.2	2.2	15.6	1.8	1.9	2.2	2.3	3.6
SPG3	29	0.5	2.2	14.2	3.4	2.9	1.7	1.6	1.9
OSG4	29	<0.1	3.6	110.9	4.2	3.6	3.2	1.3	19.0
OSG5	29	1.1	3.4	7.2	4.2	4.0	3.6	2.2	3.9

Table 2.12. Overall minimum, geometric mean and maximum pH, and geometric mean during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum	Geomean	Maximum	Critical Season 2007	Primary Season 2007-8	Critical Season 2008	Primary Season 2008-9	Critical Season 2009
CSREF	29	7.1	7.9	8.3	8.0	7.8	7.9	7.9	7.5
LOREF	29	7.6	7.9	8.2	8.0	7.9	7.7	8.0	7.8
OSG1	29	7.5	7.8	8.3	7.7	7.8	7.8	7.9	7.5
OSG2	29	7.6	7.8	8.2	7.8	7.8	7.7	8.0	7.7
OSG3	29	7.6	8.0	8.8	8.0	7.9	7.9	8.2	7.9
SPG1	29	7.5	7.7	8.0	7.7	7.7	7.6	7.8	7.6
SPG2	29	7.5	7.9	8.1	8.0	7.8	7.8	7.9	7.8
SPG3	29	7.8	8.2	8.8	8.4	8.2	8.1	8.4	8.1
OSG4	29	7.8	8.1	8.7	8.2	8.0	8.1	8.3	8.0
OSG5	29	7.8	8.1	8.6	8.2	8.0	8.0	8.2	8.0

Table 2.13. Overall minimum, geometric mean and maximum specific conductance (µS cm^{-1}), and geometric mean (µS cm^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (µS cm^{-1})	Geomean (µS cm^{-1})	Maximum (µS cm^{-1})	Critical Season 2007 (µS cm^{-1})	Primary Season 2007-8 (µS cm^{-1})	Critical Season 2008 (µS cm^{-1})	Primary Season 2008-9 (µS cm^{-1})	Critical Season 2009 (µS cm^{-1})
CSREF	29	101	183	284	217	156	187	177	185
LOREF	29	111	262	378	270	208	285	263	312
OSG1	29	120	275	364	256	236	313	291	288
OSG2	29	172	377	536	430	297	392	370	428
OSG3	29	157	357	520	421	269	373	351	414
SPG1	29	244	321	401	355	279	331	312	340
SPG2	29	452	604	893	707	524	608	573	642
SPG3	29	241	455	800	526	385	456	447	482
OSG4	29	169	393	655	451	294	406	387	485
OSG5	29	260	364	588	441	295	367	335	427

Table 2.14. Overall minimum, geometric mean and maximum water temperature (℃), and geometric mean (℃) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (℃)	Geomean (℃)	Maximum (℃)	Critical Season 2007 (℃)	Primary Season 2007-8 (℃)	Critical Season 2008 (℃)	Primary Season 2008-9 (℃)	Critical Season 2009 (℃)
CSREF	29	2.9	15.2	24.0	18.9	11.2	19.1	11.0	21.9
LOREF	29	7.2	16.1	25.3	19.1	13.1	18.3	13.2	19.6
OSG1	29	8.0	16.6	23.8	18.6	13.7	19.3	14.0	20.4
OSG2	29	9.1	17.6	26.0	20.3	14.5	20.1	14.7	21.7
OSG3	29	6.9	17.4	27.6	20.8	13.8	20.1	14.2	21.9
SPG1	29	10.3	17.6	23.8	19.9	14.9	19.5	15.2	21.4
SPG2	29	12.3	21.1	30.5	24.9	17.8	23.4	17.3	25.9
SPG3	29	6.6	18.1	29.5	22.4	13.8	21.0	14.4	23.9
OSG4	29	4.6	16.9	27.3	20.4	12.7	20.3	13.2	23.1
OSG5	29	3.6	16.1	27.1	19.7	12.0	19.7	12.1	22.3

Table 2.15. Overall minimum, geometric mean and maximum concentration (mg L^{-1}) of dissolved oxygen, and geometric mean concentration (mg L^{-1}) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (mg L^{-1})	Geomean (mg L^{-1})	Maximum (mg L^{-1})	Critical Season 2007 (mg L^{-1})	Primary Season 2007-8 (mg L^{-1})	Critical Season 2008 (mg L^{-1})	Primary Season 2008-9 (mg L^{-1})	Critical Season 2009 (mg L^{-1})
CSREF	29	5.3	8.0	13.7	7.2	9.3	7.3	8.7	7.2
LOREF	29	5.4	9.2	12.5	8.6	10.0	8.8	9.3	9.4
OSG1	29	5.6	8.4	11.7	8.1	9.1	7.7	8.6	8.2
OSG2	29	6.1	8.4	12.0	7.9	9.0	7.7	9.1	8.6
OSG3	29	5.2	8.9	14.5	8.4	9.6	8.2	9.2	9.0
SPG1	29	5.5	8.5	11.0	8.5	9.1	7.8	8.4	8.6
SPG2	29	5.8	8.7	11.7	8.3	9.6	8.3	8.9	8.4
SPG3	29	4.5	9.1	13.6	8.5	10.0	8.4	9.7	9.0
OSG4	29	6.8	9.0	13.8	8.3	9.7	8.3	9.9	8.9
OSG5	29	6.5	8.6	13.3	7.9	9.5	7.9	9.5	8.4

Table 2.16. Overall minimum, geometric mean and maximum turbidity (NTU), and geometric mean (NTU) during critical and primary seasons at select sites in northwest Arkansas, 2007-2009.

Site	n	Minimum (NTU)	Geomean (NTU)	Maximum (NTU)	Critical Season 2007 (NTU)	Primary Season 2007-8 (NTU)	Critical Season 2008 (NTU)	Primary Season 2008-9 (NTU)	Critical Season 2009 (NTU)
CSREF	29	0.2	1.1	2.3	0.7	1.1	1.3	0.9	1.9
LOREF	29	0.5	3.1	13.6	3.1	3.4	2.9	2.6	4.4
OSG1	29	0.6	1.5	6.0	1.4	1.7	1.7	1.2	1.9
OSG2	29	0.8	1.5	4.9	1.3	2.1	1.6	1.4	1.4
OSG3	29	0.9	2.2	27.8	1.5	2.1	3.9	1.6	2.5
SPG1	29	0.6	1.2	4.5	1.1	1.4	1.4	1.1	1.1
SPG2	29	0.3	1.3	6.2	0.6	1.3	1.2	1.8	2.7
SPG3	29	0.6	1.3	2.4	1.4	1.5	1.2	1.0	1.4
OSG4	29	0.3	2.0	32.8	1.4	2.0	2.0	1.1	8.0
OSG5	29	0.8	2.1	6.2	1.6	2.6	2.3	1.7	2.6

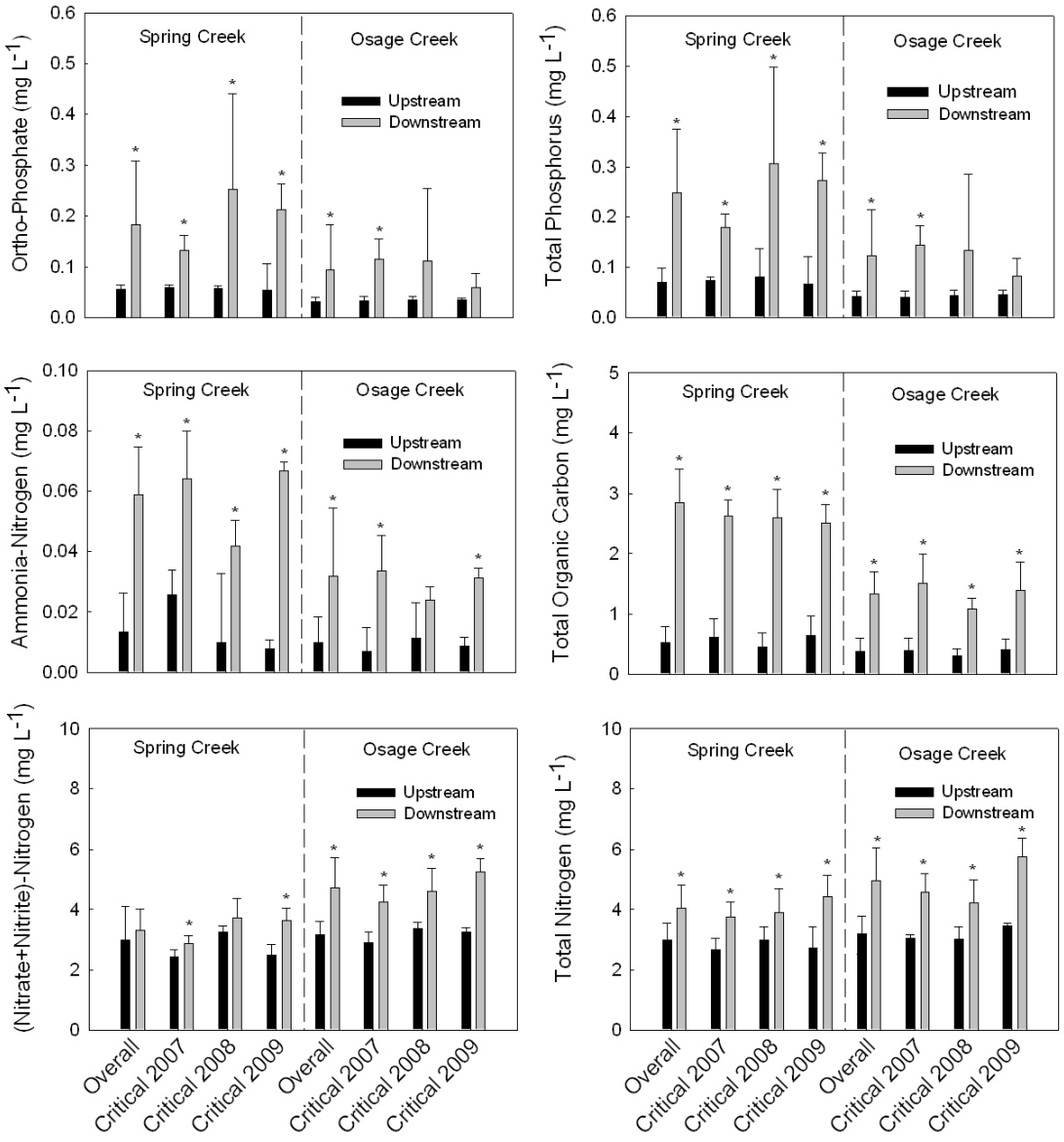


Figure 2.01. Comparisons (mean plus standard deviation) of nutrient concentrations upstream and downstream of the effluent discharges on Osage Creek and Spring Creek; asterisks (*) above the bars and standard deviation denote statistically significant differences (paired T-test, $P<0.05$).

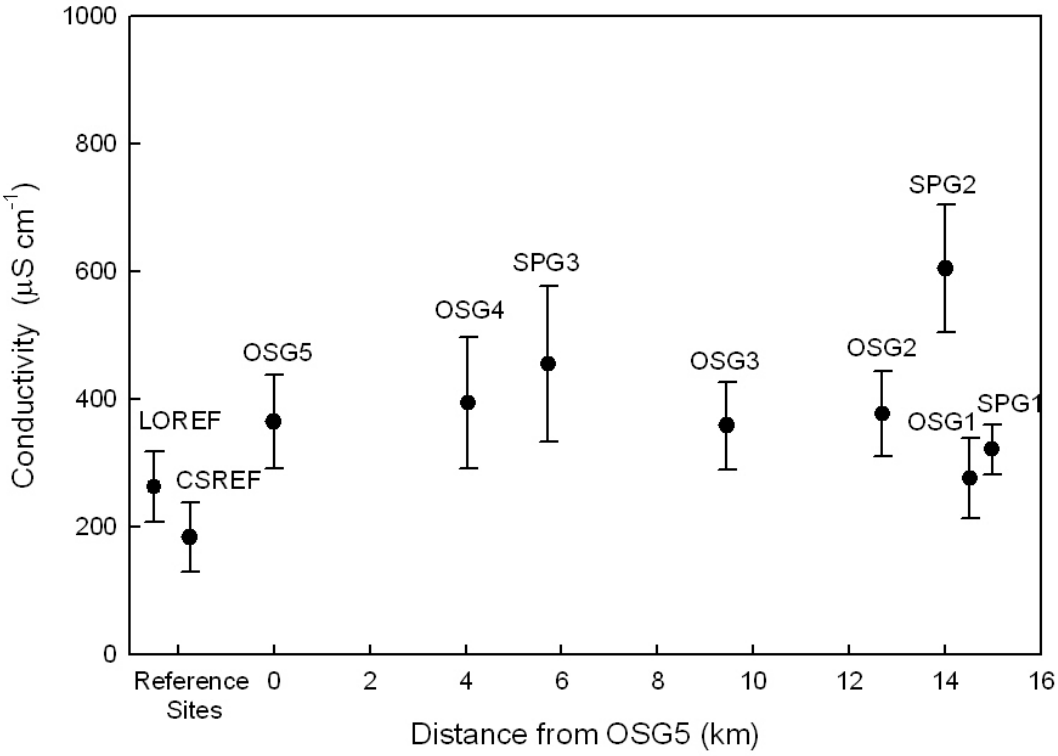


Figure 2.02 Specific Conductance (mean ± standard deviation) across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

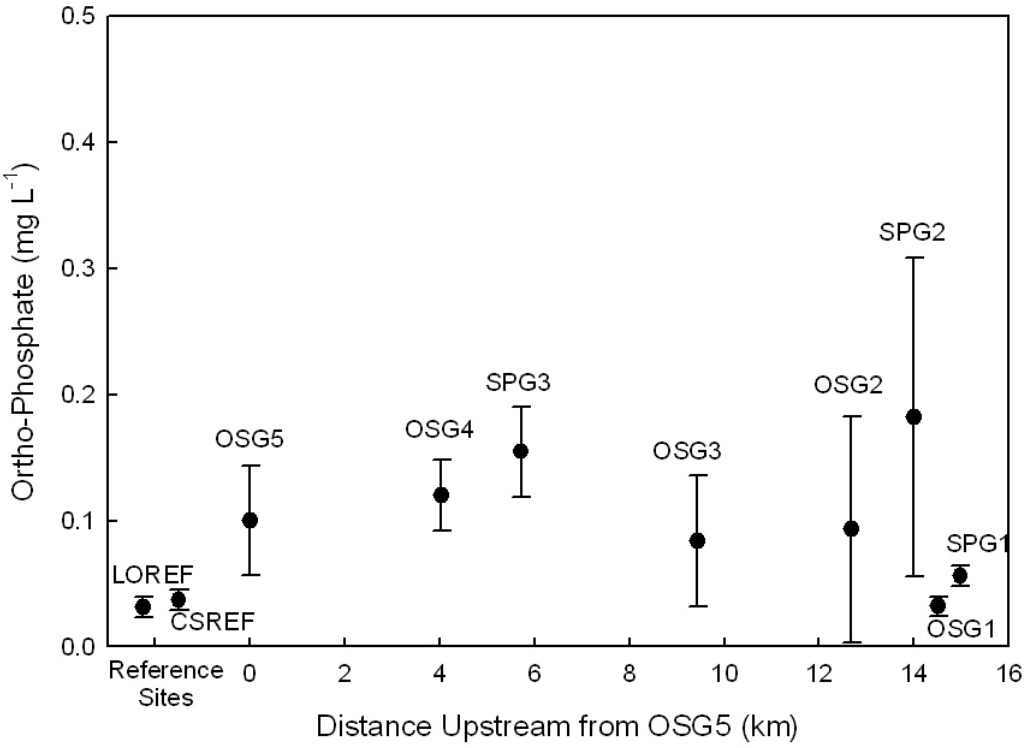


Figure 2.03 Dissolved reactive phosphorus (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

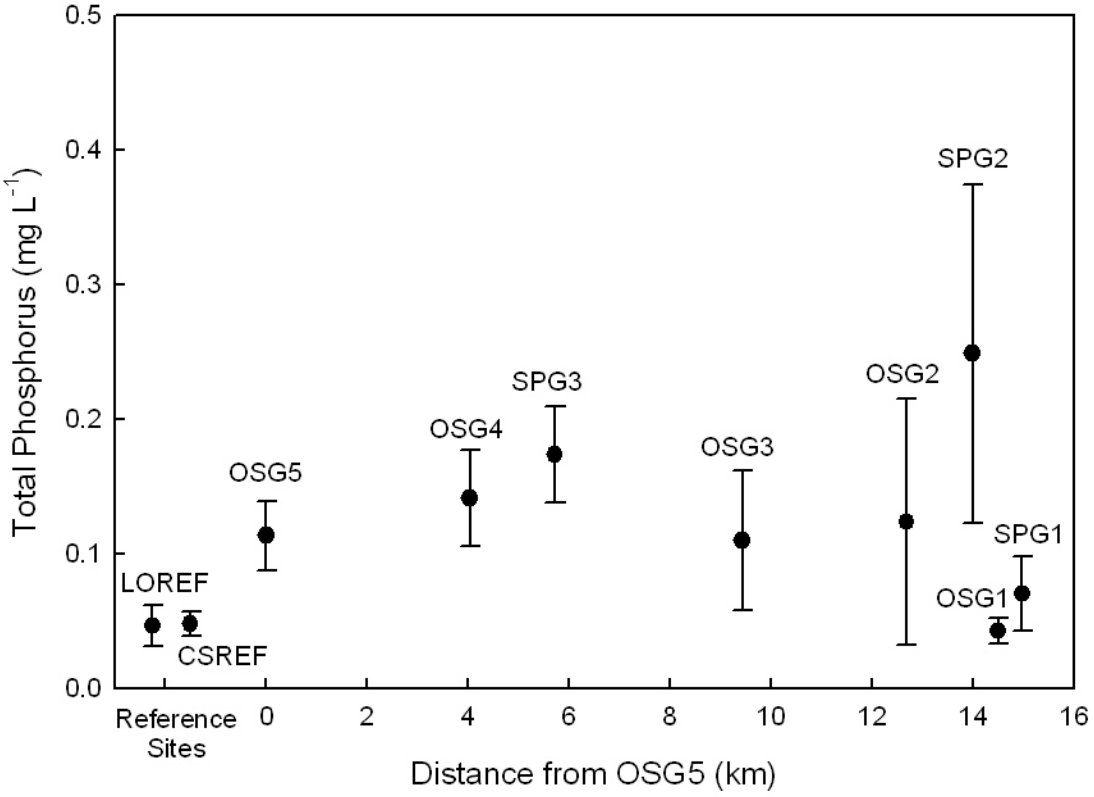


Figure 2.04 Total phosphorus (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

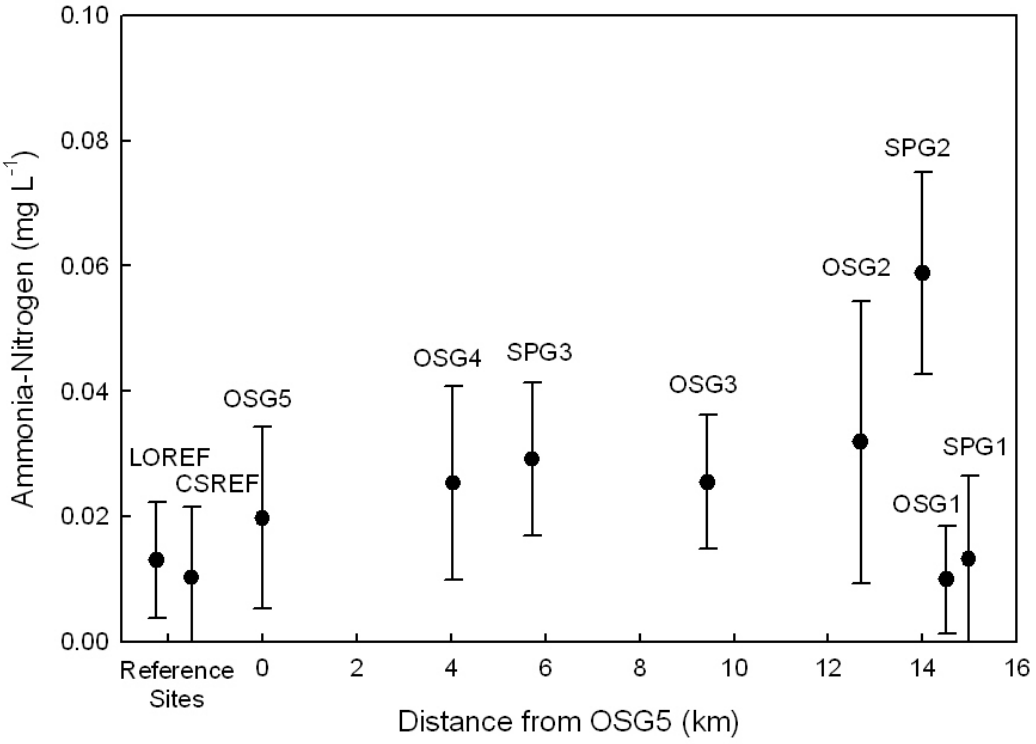


Figure 2.05 Ammonia-nitrogen (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

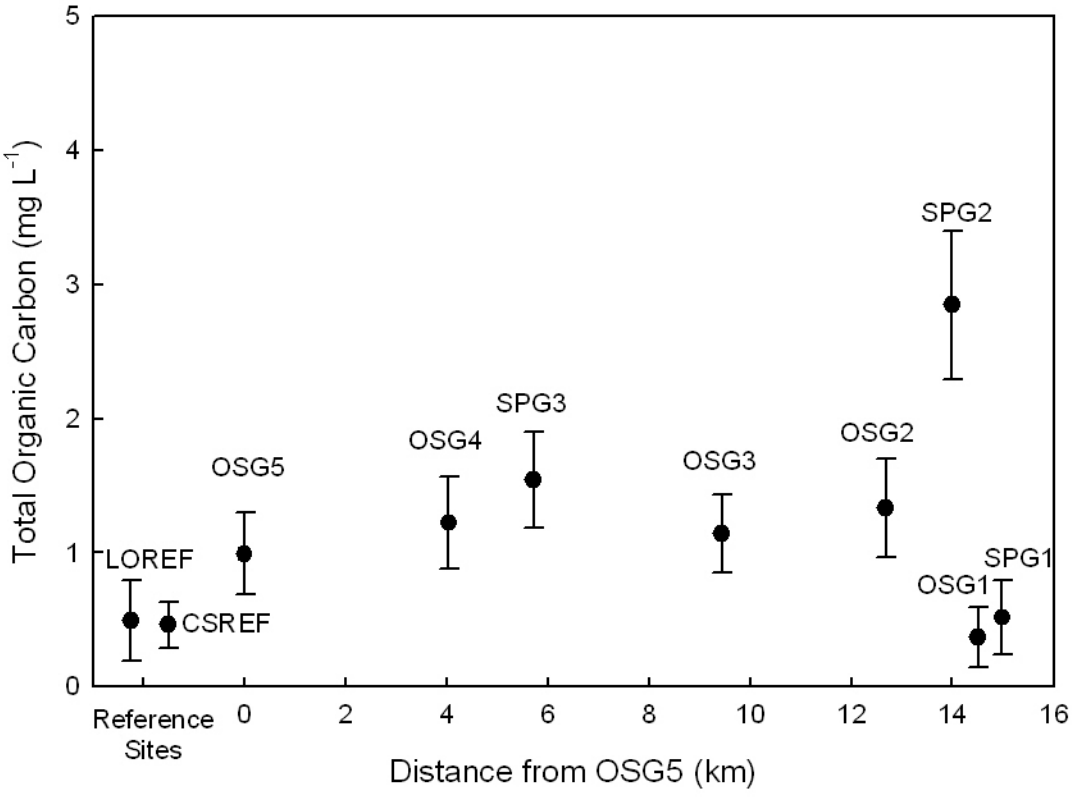


Figure 2.06 Total organic carbon (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

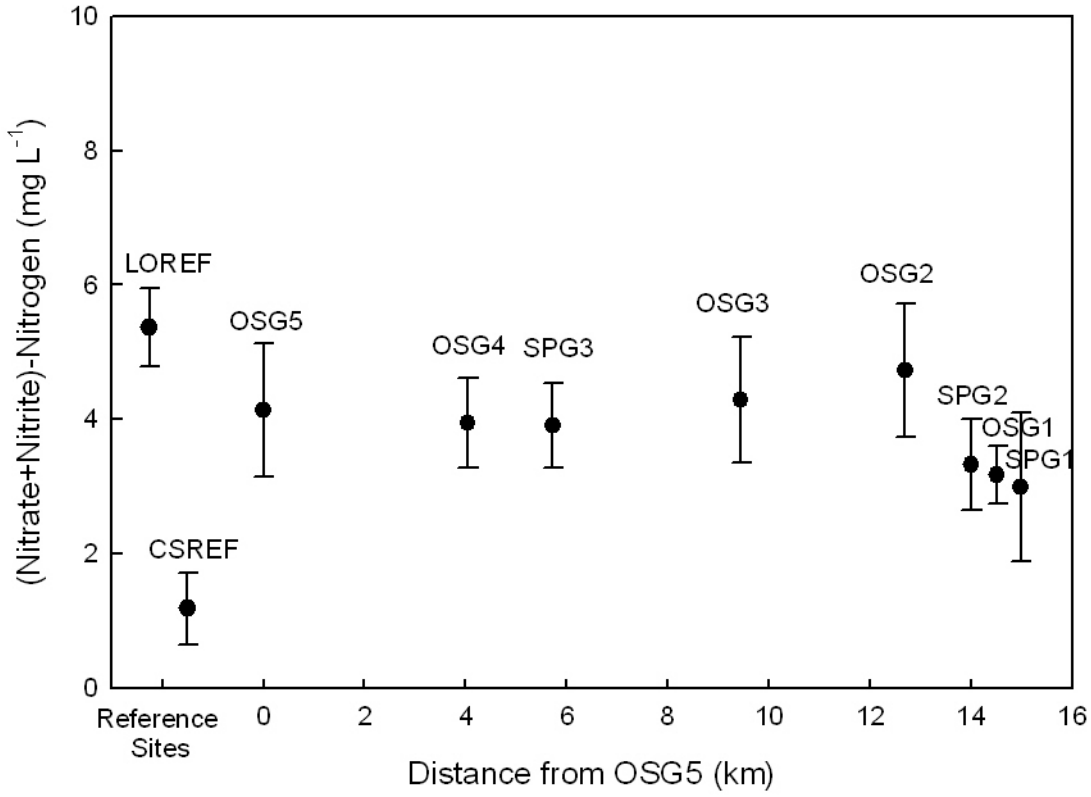


Figure 2.07 Nitrate plus nitrite as nitrogen (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

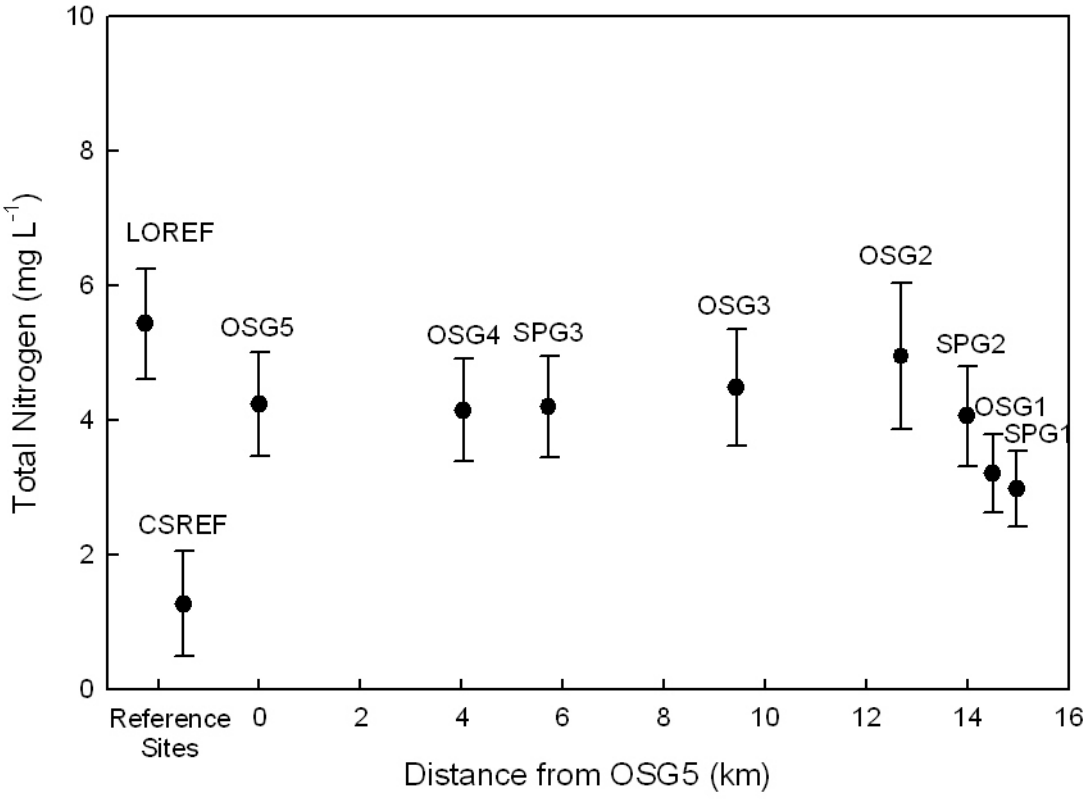


Figure 2.08 Total nitrogen (mean ± standard deviation) concentrations across selected sites within the upper Illinois River Watershed; distance represents approximate river kilometers upstream from the most downstream sampling site on Osage Creek.

Table 2.17 Range, Median and Mean of Percent Recoveries of Field Duplicate Samples Collected by the UA Division of Agriculture Water Quality Research Lab.

Parameter	Range % Recovered	Median % Recovered	Mean % Recovered
pH	99.4-102	100	100
Dissolved Oxygen	98.2-102	100	100
Conductivity	98.3-103	100	100
Temperature	93.9-104	100	99.9
Ammonia-Nitrogen	27.2-217	98.2	107
(Nitrate+Nitrite)-Nitrogen	51.1-116	100	98.7
Nitrite-Nitrogen	10.0-220	102	104
Total Nitrogen	80.2-134	102	105
Ortho-Phosphorus	96.5-103	99.6	99.8
Total Phosphorus	96.9-108	99.9	100
Chlorophyll-α	26.7-168	103	106
Total Organic Carbon	55.7-122	103	101
Turbidity	78.4-147	100	103
Total Suspended Solids	15.8-291	87.5	92.7

Table 2.18 Range, Median and Mean of Percent Recoveries of Laboratory Spikes analyzed by UA Division of Agriculture Water Quality Research Lab.

Parameter	Range % Recovered	Median % Recovered	Mean % Recovered
Ammonia-Nitrogen	84.5-137	101	101
(Nitrate+Nitrite)-Nitrogen	94.6-108	100	100
Nitrite-Nitrogen	85.0-149	100	101
Total Nitrogen	91.6-110	101	101
Ortho-Phosphorus	92.5-110	100	100
Total Phosphorus	90.8-131	101	101
Total Organic Carbon	81.5-111	103	102

Table 2.19. Range, Median and Mean of Percent Recoveries of Laboratory Duplicates analyzed by UA Division of Agriculture Water Quality Research Lab

Parameter	Range % Recovered	Median % Recovered	Mean % Recovered
Ammonia-Nitrogen	84.0-116	100	100
(Nitrate+Nitrite)-Nitrogen	90.3-109	98.6	98.6
Nitrite-Nitrogen	83.9-133	101	101
Total Nitrogen	82.1-112	97.6	97.6
Ortho-Phosphorus	90.0-110	100	100
Total Phosphorus	89.4-115	99.8	99.8
Total Organic Carbon	86.2-116	97.5	97.5

2.3 Diurnal In-Stream Parameter Methods and Results (Data Sondes)

2.3.1 Diurnal In-Stream Methods

An in-situ multi-probe data sonde (YSI 600xlm or YSI 6920 v2, TSI Inc., Yellow Springs, OH) was deployed for two 72-hour periods at each sample site for continuous recording of dissolved oxygen, temperature, pH, and specific conductance during each sampling season under stable base flow conditions. Probes were programmed to record the four field parameters each ten minutes and store the data in the probe's internal memory. Each sonde was deployed in a perforated pvc case for safety and security. The case was anchored to a steel t-post which was driven into the stream substrate. The deployment case was situated in an area which was in constant contact with the main flow of the stream. After retrieval the data were downloaded from the field probes and transferred to the project database. Each sampling event included a standard suite of pre-deployment and post-deployment calibration checks. Data were analyzed for deviations of parameters from ADEQ Reg. 2 standards. Parameter criteria for violation of Reg. 2 are defined below.

Reg. 2.502 Temperature. Heat shall not be added to any waterbody in excess of the amount that will elevate the natural temperature, outside the mixing zone, by more than 5°F (2.8°C) based upon the monthly average of the maximum daily temperatures measured at mid-depth or three feet (whichever is less) in streams, lakes or reservoirs. Maximum allowable temperatures from man-induced causes in the following waters are: Streams - Ozark Highlands 29 °C.

Reg. 2.504 pH. As a result of waste discharges, the pH of water in streams or lakes must not fluctuate in excess of 1.0 unit over a period of 24 hours and pH values shall not be below 6.0 or above 9.0.

Reg. 2.505 Dissolved Oxygen. In streams with watersheds of less than 10 mi^2, it is assumed that insufficient water exists to support a fishery during the critical season. During this time, a D.O. standard of 2 mg/l will apply to prevent nuisance conditions. However, field verification is required in areas suspected of having significant groundwater flows or enduring pools which may support unique aquatic biota. In such waters the critical season standard for the next size category of stream shall apply. All streams with watersheds of less than 10 mi^2 are expected to support a fishery during the primary season when stream flows, including discharges, equal or exceed 1

cubic foot per second (CFS); however, when site verification indicates that a fishery exists at flows below 1 CFS, such fishery will be protected by the primary standard. Also, in these streams with watersheds of less than 10 mi^2, where waste discharges are 1 CFS or more, they are assumed to provide sufficient water to support a perennial fishery and, therefore, must meet the dissolved oxygen standards of the next size category of streams. For purposes of determining effluent discharge limits, the following conditions shall apply:

(A). The primary season dissolved oxygen standard is to be met at a water temperature of 22°C (71.5°F) and at the minimum stream flow for that season. At water temperatures of 10°C (50°F), the dissolved oxygen standard is 6.5 mg/l.

(B). During March, April and May, when background stream flows are 15 CFS or higher, the D.O. standard is 6.5 mg/l in all areas except the Delta Ecoregion, where the primary season D.O. standard will remain at 5 mg/l.

(C). The critical season dissolved oxygen standard is to be met at maximum allowable water temperatures and at Q7-10 flows. However, when water temperatures exceed 22°C (71.6°F), a 1 mg/l diurnal depression will be allowed below the applicable critical standard for no more than 8 hours during any 24-hour period. The following dissolved oxygen standards must be met:

Table 2.20 Minimum dissolved oxygen standards for Ozark Highland Streams (ADEQ Reg. 2).

Waterbodies	**Limit (mg/l)**	
Streams	Primary	Critical
Ozark Highlands		
<10 mi2 watershed	6	2
10 to 100 mi2	6	5
>100 mi2 watershed	6	6

Reg. 2.509 Nutrients. Materials stimulating algal growth shall not be present in concentrations sufficient to cause objectionable algal densities or other nuisance aquatic vegetation or otherwise impair any designated use of the waterbody. Impairment of a waterbody from excess nutrients are dependent on the natural waterbody characteristics such as stream flow, residence time, stream slope, substrate type, canopy, riparian vegetation, primary use of waterbody, season of the year and ecoregion water chemistry. Because nutrient water column concentrations do not always correlate directly with stream impairments, impairments will be assessed by a combination of factors such as water clarity, periphyton or phytoplankton production, dissolved oxygen values,

dissolved oxygen saturation, diurnal dissolved oxygen fluctuations, pH values, aquatic-life community structure and possibly others.

2.3.2 Diurnal In-Stream Results

Diurnal in-stream results indicated one violation of Reg. 2 Numeric Criteria at SPG1 (upstream of the Springdale WWTP) during Critical Season 1, Event 1. (Appendix C). Maximum dissolved oxygen percent saturation measurements (Table 2.21), as well as diurnal dissolved oxygen and pH swings indicated increased primary production at multiple sites, but no violations of Reg. 2 Numeric Criteria were observed other than the one event at SPG1 during Event 1 Critical Season (Appendix C). Additional sampling events at some sites were collected when redeployment was required at other sites due to QA issues with a previous deployment. These deployments were analyzed as *additional events*.

Table 2.21 Diurnal in-stream dissolved oxygen percent saturation maximums from 72 hour data sonde deployments at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. Values greater than 120 are considered elevated.

	Sampling Sites									
Date	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical 1) Event 1	95	95	111	96	103	108	107	112	108	86
Summer 2007 (Critical 1) Event 2	90	100	96	94	98	103	107	120	117	88
Spring 2008 (Primary 1) Event 1	114	104	111	131	113	138	122	108	117	109
Spring 2008 (Primary 1) Event 2	104	100	110	105	108	102	119	131	124	108
Summer 2008 (Critical 2) Event 1	94	90	96	108	104	107	111	120	117	92
Summer 2008 (Critical 2) Event 2	103	99	111	127	117	106	104	113	115	93
Spring 2009 (Primary 2) Event 1	110	99	115	115	109	107	113	122	125	116
Spring 2009 (Primary 2) Event 2	110	104	131	139	129	124	115	127	115	105
Summer 2009 (Critical 3) Event 1	112	121	130	132	139	108	127	131	96	128
Summer 2009 (Critical 3) Event 2	109	108	146	165	151	122	141	121	120	115

2.4 Habitat and Geomorphology Methods and Results

2.4.1 Habitat and Geomorphology Assessment Methods

The ADEQ method for physical habitat assessment of Ozark Highlands, Boston and Ouachita mountain streams was used (modified from Barbour et al., 1999). Both qualitative (visual estimates, RBP Habitat Assessment) and quantitative (in-stream measurements, ADEQ In-stream and Riparian Assessment) approaches were used to develop a habitat profile for each sample reach. During each habitat assessment a measure of reach canopy openness was also conducted along with a measure of stream flow. Geomorphologic assessments were performed once at each site to define the general morphologic characteristics of the reach.

For the qualitative assessment ten broad habitat parameters were rated on a scale of zero to 20. The scores fall into one of four categories, optimal (20-16), sub-optimal (15-11), marginal (10-6), and poor (5-0). Habitat parameters assessed were epifaunal substrate/available cover, sediment deposition, channel flow status, channel alteration, bank stability, vegetative protection, riparian vegetative zone width, frequency of riffles (or bends), velocity/depth regime, and embeddedness. A sample scoring sheet is shown in Appendix D. The scores for the habitat parameters were then added together to give an overall rating score from zero to 200, with 200 being the highest.

For the quantitative assessment five parameters consisting of three to seven variables were measured or estimated. These parameters included: habitat type, habitat quantity, quantity of substrate based on fish use, quantity of in stream cover, and sediment on substrate. Each parameter for substrate type and in stream cover was given a score depending on its abundance. The scores given to the substrate parameters were multiplied by a factor to adjust these scores based on how they relate to fish habitat quality. Habitat type length, depth, and width measurements were measured for each habitat type. A sample scoring sheet is shown in Appendix D. The sediment on substrate parameter was scored according to the degree of embeddedness of substrate. A total score for each habitat type was calculated by summing the scores for the substrate type, in stream cover, and sediment on substrate. The scores from like habitats were averaged for each sampling station. The lengths of each habitat type were also summed. The total habitat type lengths were then divided by 100 and multiplied by the average habitat type score. This results in a single score for each habitat type for the reach for each sampling event.

Canopy openness measures were made at stations at approximately the bottom quarter, middle, and top quarter of each reach. The measurements were made using a convex densiometer. The densiometer was held level at approximately waist height while standing in the middle of the wetted channel. The densiometer face is divided into 24 squares. An estimate was made for each square of percent of canopy openness and a score given for each square from 0 to 4 with 0 denoting no canopy openness (complete vegetative coverage) and 4 denoting complete canopy openness (no vegetative coverage). This was done facing north, south, east, and west at all three stations. These readings were summed for each station, multiplied by 1.04, and subtracted from 100 to get overhead canopy cover. The three readings for the reach were averaged to get the canopy cover estimate for the reach.

Flow measures were taken by spanning the stream with a measuring tape and taking measures at approximately even increments of water depth and velocity. Depth and velocity reading were taken using a Flo-Mate Model 2000 Portable Flowmeter (Marsh-McBirney, Inc.). Flow was calculated using rectangular area estimation around each measured point. Some flow measures for OSG5 were taken from the USGS flow station "Osage Creek near Elm Springs".

Geomorphology assessments were conducted once at each site to characterize channel sinuosity, channel cross sectional area, channel slope, riffle and reach substrate characteristics, and bed-load particle size distribution. In the field the channels were surveyed using a total station (TPS 400 Series, Leica Geosystems). A representative riffle and representative pool cross section was measured at each site. Each cross section was monumented with capped rebar for future survey comparison. A longitudinal profile which included all areas sampled for habitat and biotics was measured at each site and was tied into the cross section monuments for future comparison. Two pebble counts were conducted at each site, a targeted riffle count, and a reach wide count. A bar sample was also collected at each site to assess bed load substrate distribution.

Pebble counts and bars samples were collected following methodology described in *Watershed Assessment of River Stability and Sediment Supply* (Rosgen, 2006) with some modification. Reach-wide and targeted riffle pebble counts were conducted. For the reach-wide count the relative percent of the reach in pool and riffle/run was estimated to 10%. Ten transects of the stream were sampled with the ratio in pools and runs/riffles being determined by the estimated percent, i.e. if 60% of the reach is pool, then 6 transects are in pools and 4 are in riffles/runs. For

the targeted riffle counts 10 transects were conducted in a single representative riffle. For both types of count the same method was used for selecting and measuring the substrate. Ten equally spaced points on the streambed were sampled in each transect. The sample was selected by blindly touching the bottom of the stream and selecting the first object touched. The intermediate, or B, axis was measured and recorded.

Bar samples were collected by selecting an actively depositing gravel bar within the reach. At the bottom 1/3 of bar longitudinally and approximately 1/3 of the distance vertically from the thalweg the largest particle on the surface was found. After removing this particle, to be measured as the D100, approximately 6-8 inches of sediment from an approximately 10 inch in diameter circular area were removed and placed in a 5 gallon bucket and transported to the lab for analysis. In the lab sediment was dried at approximately 100 °C for approximately 24 hours. This was done to get a more accurate depiction of the fine sediment in the sample than wet sieving. D100 particles were measured and weighed after air drying for an extended period (greater than a week). Sieve sizes used were 4", 2.5", 1.25", 5/8", 5/16", No. 5, and No. 10 with the pan catching the remainder. All sieves were 8" diameter brass with steel mesh. The samples were passed through the 4" and 2.5" sieves manually and any particles which could not be passed through were examined for any clinging particles that would be removed if mechanically shaken then set aside for later weighing. The remaining sediment was placed in the remaining sieves in stages as necessary and shaken for 5 minutes. For some sites the No. 5, No. 10, and pan materials were processed a second time due to cohesion of fine clay particles. The materials from each tray were then weighed and the weight recorded. All data for geomorphologic assessment were entered into the computer program RIVERMorph (Version 3.1.0 Rivermorph LLC) for analysis. Longitudinal profiles were analyzed for slope. Cross sections were analyzed for cross sectional areas. Pebble counts and bars samples were analyzed for particle distribution.

2.4.2 Habitat and Geomorphology Results

Results of the qualitative habitat assessment show that while the reference sites have better habitat that most sites were comparable with the exception of SPG1 (Table 2.22), full results can be found in Appendix D. Results of the quantitative habitat assessment were more variable from season to season and among sites, this was mostly due to the transient nature of the woody debris and stage during time of sampling (Tables 2.23-2.27), full results can be found in Appendix D. Canopy cover was notably higher at the reference sites than at most test sites with the lowest

values occurring at OSG4, OSG5, and SPG1 (Table 2.28). Flow varied from season to season at sites but was relatively consistent during biotic events with the increase due to WWTP effluent comprising as much as 50% of the flow at OSG5 (Table 2.29), full flow results for all times flow was measured can be found in Appendix D. Geomorphology results gave the best indication of substrate in each reach, demonstrating the predominance of bedrock at OSG2, SPG2, and SPG3 (Figure 2.09). Overall geomorphology results can be found in Appendix D.

Table 2.22 EPA Rapid Bioassessment Protocol habitat assessment scores at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009..

	Sampling Sites									
Date	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical Season 1)	152	151	161	167	163	141	145	162	175	168
Summer 2008 (Critical Season 2)	117	130	150	136	142	134	140	149	155	170
Summer 2009 (Critical Season 3)	157	143	161	120	146	147	151	157	158	165
Spring 2008 (Primary Season 1)	156	146	158	150	164	135	146	152	156	179
Spring 2009 (Primary Season 2)	152	132	152	140	153	135	130	159	160	163
Averages	147	140	156	143	154	138	142	156	161	169

Table 2.23 ADEQ in-stream and riparian habitat assessment scores summary for select sites in the Osage Creek and Illinois River basins, Summer 2007 (Critical Season 1).

CSREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	42.6	209	89.0
Riffle	29.8	127	37.8
Run	35.6	107	38.1

LOREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	68.7	258	177.2
Riffle	57.5	200	115
Run	0	0	0

SPG1

	Average Habitat Score	Total Length (ft)	IHI
Pool	40.0	271	108.3
Riffle	21.5	84	18.0
Run	18.7	52	9.7

SPG2

	Average Habitat Score	Total Length (ft)	IHI
Pool	42.7	305	130.2
Riffle	27.7	164	45.3
Run	23.8	72	17.1

SPG3

	Average Habitat Score	Total Length (ft)	IHI
Pool	58.4	398	232.2
Riffle	60.3	241	145.3
Run	39.9	134	53.5

OSG1

	Average Habitat Score	Total Length (ft)	IHI
Pool	39.5	258	101.9
Riffle	30.2	201	60.7
Run	28.6	81	23.1

OSG2

	Average Habitat Score	Total Length (ft)	IHI
Pool	44.2	203	89.7
Riffle	35.5	56	19.9
Run	35.6	151	53.8

OSG3

	Average Habitat Score	Total Length (ft)	IHI
Pool	55.0	440	241.8
Riffle	58.5	378	221.1
Run	0	0	0

OSG4

	Average Habitat Score	Total Length (ft)	IHI
Pool	59	105	62.0
Riffle	49.4	243	120.0
Run	52.6	128	67.3

OSG5

	Average Habitat Score	Total Length (ft)	IHI
Pool	NS	NS	NS
Riffle	NS	NS	NS
Run	NS	NS	NS

Table 2.24 ADEQ in-stream and riparian habitat assessment scores summary for select sites in the Osage Creek and Illinois River basins, Summer 2008 (Critical Season 2).

CSREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	52	292	151.8
Riffle	51	135	68.9
Run	0	0	0

LOREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	20.6	255	52.4
Riffle	25.0	197	49.3
Run	0	0	0

SPG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	40.1	191	76.5
Riffle	29.5	180	53.1
Run	0	0	0

SPG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	25.4	150	38.1
Riffle	19	85	16.2
Run	27.9	222	61.9

SPG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	26.8	239	63.9
Run	29.3	419	122.8

OSG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	40.3	440.9	177.7
Riffle	41.3	49.2	20.3
Run	0	0	0

OSG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	32.9	95	31.2
Run	42.7	145	61.9

OSG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	29	210	60.9
Riffle	21.5	51	11.0
Run	25.5	169	43.1

OSG 4

	Average Habitat Score	Total Length (ft)	IHI
Pool	31.9	665	65.7
Riffle	0	0	0
Run	20.4	350	71.5

OSG 5

	Average Habitat Score	Total Length (ft)	IHI
Pool	26.5	159	42.1
Riffle	19.5	315	61.4
Run	0	0	0

Table 2.25 ADEQ in-stream and riparian habitat assessment scores summary for select sites in the Osage Creek and Illinois River basins, Summer 2009 (Critical Season 3).

CSREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	33.3	307	102.2
Riffle	31.8	187	59.5
Run	0	0	0

LOREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	31.9	214	68.3
Riffle	27.4	202	55.3
Run	29	91	26.4

SPG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	34.4	242	83.2
Riffle	22.6	85	19.2
Run	27.5	55	15.1

SPG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	28.3	175	49.4
Run	29.2	356	104.0

SPG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	26.7	117	31.2
Riffle	26.1	146	38.0
Run	33.3	140	46.6

OSG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	31.7	372	118.0
Riffle	24.0	191	45.7
Run	32.8	83	27.2

OSG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	27.6	285	78.7
Riffle	24.6	373	91.6
Run	0	0	0

OSG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	24.9	188	46.8
Riffle	29.7	230	68.3
Run	29.9	193	57.7

OSG 4

	Average Habitat Score	Total Length (ft)	IHI
Pool	35.5	313	111.0
Riffle	19.9	229	45.6
Run	0	0	0

OSG 5

	Average Habitat Score	Total Length (ft)	IHI
Pool	28.6	82	23.5
Riffle	23.2	367	85.0
Run	31.6	215	67.9

Table 2.26 ADEQ in-stream and riparian habitat assessment scores summary for select sites in the Osage Creek and Illinois River basins, Spring 2008 (Primary Season 1).

CSREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	39.4	248	97.7
Riffle	37.6	156	58.7
Run	29.7	23	6.8

LOREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	32.1	252	80.9
Riffle	21.9	90	19.7
Run	37.5	102	38.2

SPG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	27.5	249	68.4
Riffle	28.5	133	37.9
Run	0	0	0

SPG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	25.5	205	52.3
Riffle	17.8	32	5.7
Run	21.0	157	32.9

SPG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	22.1	189	41.8
Run	30.2	318	96.0

OSG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	30.6	235	71.8
Riffle	19.8	129	25.5
Run	31.0	164	50.8

OSG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	24.2	175	42.4
Run	21.9	278	60.9

OSG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	32.7	166	54.3
Riffle	26.7	203	54.1
Run	30.9	135	41.7

OSG 4

	Average Habitat Score	Total Length (ft)	IHI
Pool	36.9	272	100.2
Riffle	31.8	223	70.9
Run	26.2	137	35.9

OSG 5

	Average Habitat Score	Total Length (ft)	IHI
Pool	0	0	0
Riffle	27.6	293	80.9
Run	45.6	155	70.7

Table 2.27 ADEQ in-stream and riparian habitat assessment scores summary for select sites in the Osage Creek and Illinois River basins, Spring 2009 (Primary Season 2).

CSREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	47.3	266	125.7
Riffle	29.2	145	42.3
Run	32.6	61	19.9

LOREF

	Average Habitat Score	Total Length (ft)	IHI
Pool	29.6	241	71.3
Riffle	24.5	199	48.8
Run	26.6	88	23.4

SPG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	28.0	251	70.2
Riffle	20.5	107	21.9
Run	19.5	41	8.0

SPG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	24.2	75	18.2
Riffle	21.1	133	28.1
Run	23.3	183	42.6

SPG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	33.5	150	50.3
Riffle	26.0	138	35.9
Run	34.5	146	50.4

OSG 1

	Average Habitat Score	Total Length (ft)	IHI
Pool	29.2	284	82.8
Riffle	20.5	145	29.7
Run	21.8	150	32.7

OSG 2

	Average Habitat Score	Total Length (ft)	IHI
Pool	23.2	139	32.2
Riffle	16.5	181	29.9
Run	16.5	273	45.0

OSG 3

	Average Habitat Score	Total Length (ft)	IHI
Pool	29.1	192	55.8
Riffle	85.4	144.5	123.3
Run	67.7	217.5	147.3

OSG 4

	Average Habitat Score	Total Length (ft)	IHI
Pool	19.3	184	35.5
Riffle	20.2	336	67.9
Run	32.1	58	18.6

OSG 5

	Average Habitat Score	Total Length (ft)	IHI
Pool	24.3	126	30.6
Riffle	16.9	319	53.8
Run	34.7	246	85.4

Table 2.28 Average reach canopy cover percent for select sites in the Osage Creek and Illinois River basins, critical season 2007 to critical season 2009.

Date	Sampling Sites									
	OSG1	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical Season 1)	40	70	72	18	n/s	28	41	75	63	62
Summer 2008 (Critical Season 2)	35	n/s	68	30	19	22	56	38	77	75
Summer 2009 (Critical Season 3)	46	39	62	9	12	22	31	26	62	69
Spring 2008 (Primary Season 1)	64	78	49	13	10	24	57	47	74	n/s
Spring 2009 (Primary Season 2)	61	37	55	3	17	27	27	33	72	66
Critical Season Averages	40	55	67	19	16	24	43	46	67	69

Table 2.29 Stream flow in cubic feet per second (cfs) for select sites in the Osage Creek and Illinois River basins, critical season 2007 to critical season 2009.

Date	Sampling Sites									
	OSG1	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical Season 1)	23.5	45.9	n/s	73.5	75.0	4.3	21.7	n/s	10.5	1.6
Spring 2008 (Primary Season 1)	44.5	57.5	48.2	146.3	257.0	10.4	37.1	71.8	46.5	14.6
Summer 2008 (Critical Season 2)	14.3	31.9	34.2	66.6	193.4	9.4	34.8	58.6	41.4	7.0
Spring 2009 (Primary Season 2)	45.2	36.5	54.2	102.6	190.0	11.3	34.3	74.6	43.4	6.9
Summer 2009 (Critical Season 3)	18.1	17.1	26.0	61.7	83.4	4.5	27.3	37.6	18.7	1.6

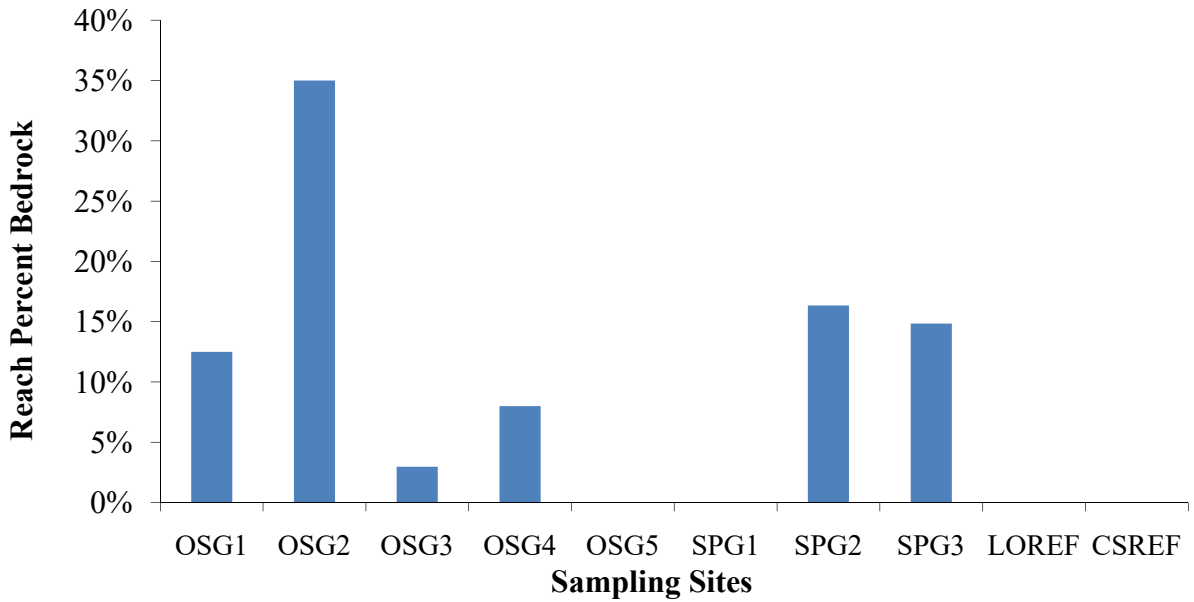


Figure 2.09 Reach percent bedrock for select sites in the Osage Creek and Illinois River basins. Notice that OSG2 and SPG2 have the highest percent bedrock.

2.5 Periphyton Assessment Methods and Results

2.5.1 Periphyton Assessment Methods

The sampling events for periphyton occurred August 2007 through October 2007, and in June 2008, November 2008, March 2009, and September 2009. The field data collections consisted of sampling from natural substrates, as well as two-week deployments of passive diffusion periphytometers (PDPs) at each site.

2.5.1.1 Passive Diffusion Periphytometers (PDPs)

The PDP method was used to measure the response of periphyton to nutrient enrichment. This periphytic response was then used to determine the limiting nutrients (P and/or N) for each stream. The PDPs were constructed of 250 ml polyethylene containers capped with a 0.45 um nylon membrane covered by a 1.5 um glass fiber filter. Each container was filled with treatments of either nitrogen, nitrogen and phosphorus, phosphorus, or a control consisting of reverse osmosis (RO) water. The nutrient treatments consisted of 30 mg/L Na_2HPO_4 and/or 30 mg/L $NaNO_3$. The treatment containers were attached to a flotation device in a random pattern, and covered with aluminum mesh screen to protect the glass fiber filters from grazing (Ludwig, 2007).

The PDPs were then deployed at each site. The flotation devices were oriented parallel to stream flow, with the treatment containers submerged. After a 14-day growth period, the PDPs were retrieved, the treatment arrangements on each flotation device were recorded, and the colonized fiber filters were removed from the treatment containers. The filters were placed in test tubes containing 5 mL of 90 percent acetone solution saturated with magnesium carbonate to preserve the chlorophyll in each sample. The test tubes were numbered according to the container's position on the flotation device in a blind identification system to prevent bias. The samples were then wrapped in aluminum foil, and transported to the laboratory (Ludwig, 2007).

The trichromatic method for spectrophotometric determination of chlorophyll a, b and c was performed on the solution extracted from each glass fiber filter (Method 10200H 2c, APHA 1998). The amount of chlorophyll a per unit exposed filter area was then determined. The Tukey-Kramer multiple comparison test along with a one-way ANOVA test was used to compare

periphytic response of nutrient enrichment from each treatment, and between sites. The significance level α=0.05 was used. Significant differences ($P < 0.05$) between treatments were considered to be indications of nutrient limitation (Ludwig, 2007). In addition, periphyton growth on the control treatments from each site were compared to one another within each season using the one-way ANOVA and Tukey-Kramer tests.

2.5.1.2 Natural Substrate Periphyton Collection

At each site, periphyton grown on natural substrates was collected from a riffle considered to be representative of the sampling reach. Ten rocks were collected at random from across the riffle in a line perpendicular to stream flow. A circle of known area was scribed onto the face of each rock, and the material within the circle was removed and rinsed into sample vials. The vials were then placed on ice and returned to the laboratory for analysis (Barbour et al., 1999, Briggs and Kilroy, 2000).

Five of the samples from each site were analyzed for ash free dry mass composition. The samples were filtered onto 1.5 um glass fiber filters that had been previously ashed at 400°C to remove any organic material. The filtered samples were then placed in a drying oven at 105°C for 24 hours to remove all of the moisture from the filters. The samples were then cooled in a dessicator, weighed, and placed in a muffle furnace at 400°C for four hours. The samples were removed from the furnace, cooled in a dessicator, and weighed. The difference in the dry mass of the samples/filters and their final ashed mass was considered to be the amount of organic material present in the sample (Barbour et al., 1999, Briggs and Kilroy, 2000). The mass of the organic material from each sample per unit of area sampled was then determined, and the amounts were compared between sites using the Tukey-Kramer multiple comparison test along with a one-way ANOVA.

The five remaining samples were filtered onto 1.5 um glass fiber filters and analyzed using the trichromatic method for spectrophotometric determination of chlorophyll a, b, and c (Method 10200H 2c, APHA 1998). Chlorophyll a was expressed in terms of the mass per unit area, and the amounts at each site were compared using the Tukey-Kramer multiple comparison test along with a one-way ANOVA.

2.5.2 Periphyton Assessment Results

2.5.2.1 Passive Diffusion Periphytometers Results

No sampling events at any sites suggested nutrient limitation from the passive diffusion periphytometer nutrient treatments (Appendix E). An example of the results of the one-way ANOVA and Tukey-Kramer comparisons of the nutrient treatments is given in Figure 2.10. Means and Tukey-Kramer groupings are given in Table 2.30. The treatments are given on the y-axis, with the amount of chlorophyll a in mg/cm^2 given on the x-axis. The means diamonds in the one-way ANOVA analysis on the left illustrates the sample mean (central horizontal line) and 95% confidence interval (endpoints in the vertical direction). In addition, the comparison circles on the right can be used to visually compare each group mean by examining the intersection of the circles. If the means are significantly different, the circles do not intersect at all, or intersect such that the outside angle of intersection is smaller than 90°. If the means are not significantly different, the circles intersect such that the outside angle of intersection is greater than 90°. The table of means and Tukey-Kramer groupings also contains this information in that groups of the same letter are statistically the same.

Analysis between sites of the control treatment from the passive diffusion periphytometers showed differences in ambient periphyton growth from reference levels at multiple sites each season (Appendix E). An example of the results of the one-way ANOVA and Tukey-Kramer comparisons of the control treatments is given in Figure 2.11. Means and Tukey-Kramer groupings are given in Table 2.31. The sites are given on the y-axis, and the amount of chlorophyll a in mg/cm^2 is given on the x-axis.

During the PDP sampling events, there were three instances in which the PDPs were lost completely. During the first primary season, the PDP from SPG1 was lost due to high flow, as were the PDPs at OSG1 and OSG4 during the second Critical Season .

2.5.2.2 Natural Substrate Periphyton Collection Results

The statistical comparisons of the amount of organic material per unit area from each site determined by the ash free dry mass analysis showed no statistical differences during Critical Season 1 and Primary Season 2, in Primary Season 1 and Critical Season 2 two sites (a different

one in each season) showed statistically higher amounts, and in Critical Season 3 five sites showed increased mass (Appendix F). An example of the results of the one-way ANOVA and Tukey-Kramer comparisons of the organic material is given in Figure 2.12. Means and Tukey-Kramer groupings are given in Table 2.32. The sites are given on the x-axis, and the amount of organic material per unit area in (g/m^2) is given on the y-axis.

The statistical comparisons of the amount of chlorophyll a per unit area from each site were very similar to the ash-free dry mass results (Appendix F). An example of the results of the one-way ANOVA and Tukey-Kramer comparisons of the organic material is given in Figure 2.13. Means and Tukey-Kramer groupings are given in Table 2.33. The sites are given on the x-axis, and the amount of chlorophyll a per unit area in (mg/cm^2) is given on the y-axis.

During the chlorophyll a analysis of natural substrate periphyton samples, several of the vials broke, and the samples were lost. As a result, only two samples from OSG5 in the second Critical Season , OSG5 in the first primary season, and OSG3 in the second Primary Season were analyzed. When reviewing the results of the means comparisons from these three seasons, it should be noted that $n < 3$ for these sites.

For both PDPs and natural substrate sampling canopy cover was measured with the same method as described in the habitat methods section with the exception that one measurement was taken at each sample area (one at the point of PDP deployment and one at the riffle of natural substrate collection) rather than three across the entire reach since the periphyton are responsive only to immediate light availability (Table 2.34 and 2.35).

Table 2.30 Tukey-Kramer means comparison table with chlorophyll-a (mg/cm^2) means and groupings by nutrient treatment (Level) for passive diffusion periphytometers for OSG5 Critical Season 1.

Level	Group	Mean
P	A	0.0033
NP	A	0.0028
N	A	0.0027
C	A	0.0024

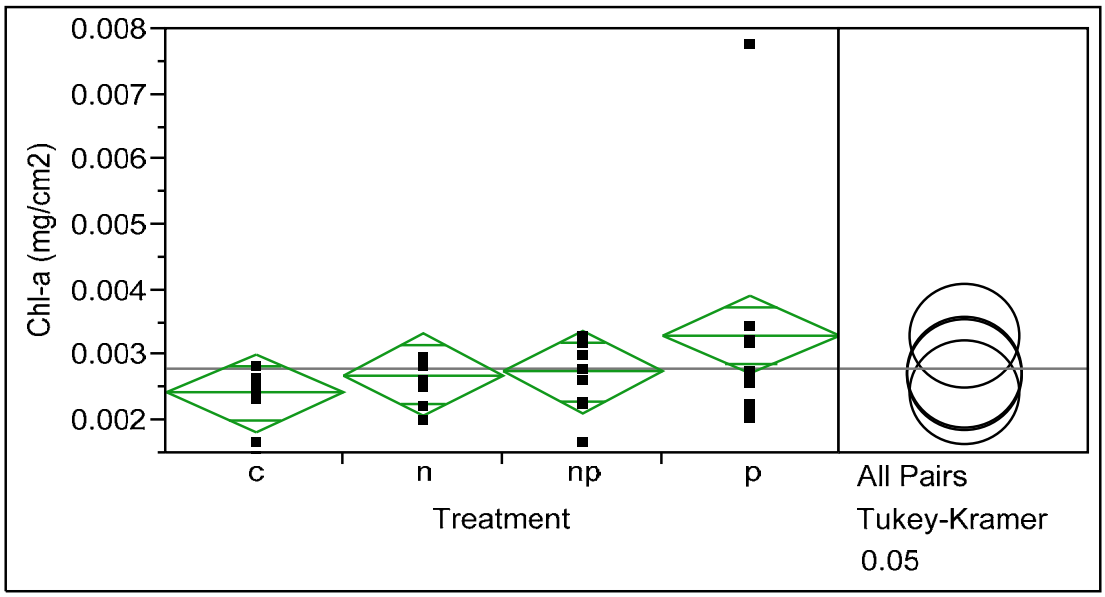


Figure 2.10 Statistical analysis figure for OSG5 Critical Season 1 passive diffusion periphytometer nutrient treatments. The x-axis is nutrient treatment (c – control, n – nitrogen, p – phosphorous, np – nitrogen and phosphorous) and the y-axis is chlorophyll-a concentration in mg/cm^2.

Table 2.31 Tukey-Kramer means comparison table with chlorophyll-a (mg/cm^2) means and groupings by sites (Level) for control treatments from passive diffusion periphytometers for Critical Season 1.

Level	Group	Mean
OSG4	A	0.0121
SPG1	B	0.0046
OSG2	B	0.0037
OSG5	B C	0.0024
SPG2	B C	0.0024
SPG3	B C	0.0019
OSG1	B C	0.0016
CSREF	C	0.0006
OSG3	C	0.0005
LOREF	C	0.0005

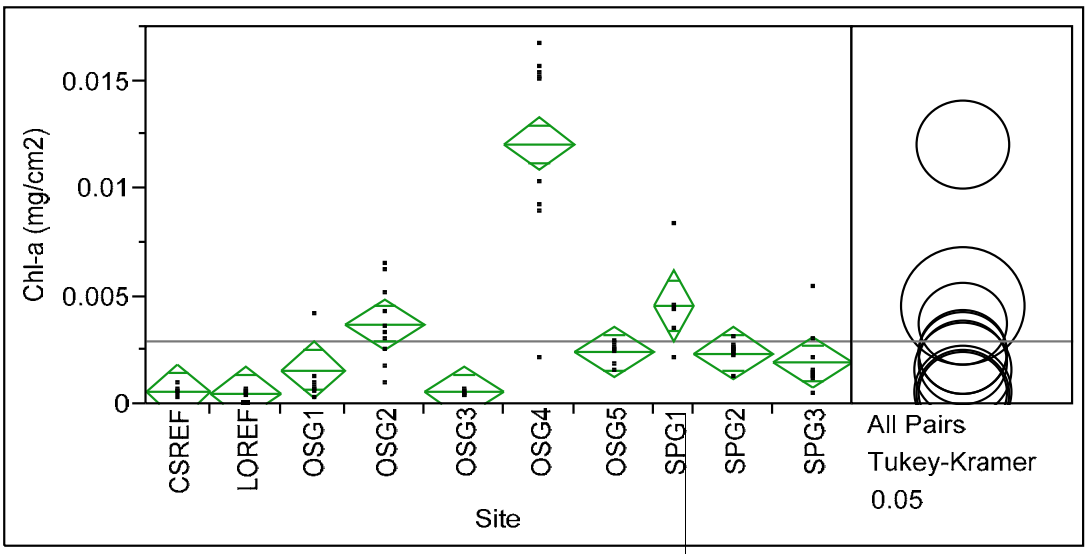


Figure 2.11 Statistical analysis figure for Critical Season 1 passive diffusion periphytometer control treatments. The x-axis is sites and the y-axis is chlorophyll-a concentration in mg/cm^2.

Table 2.32 Tukey-Kramer means comparison table with organic material (g/m^2) means and groupings by sites (Level) for natural substrate periphyton analysis for Critical Season 1.

Level	Group	Mean
OSG2	A	15.653
OSG4	A	8.315
OSG5	A	7.899
CSREF	A	6.148
LOREF	A	6.106
OSG1	A	5.691
SPG1	A	5.415
SPG2	A	4.272
OSG3	A	2.344
SPG3	A	1.741

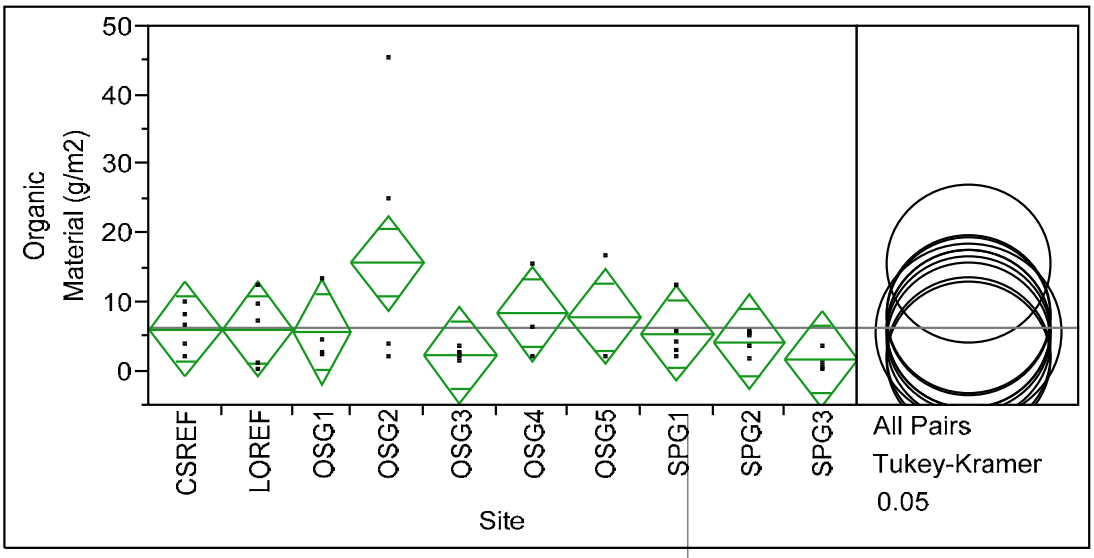


Figure 2.12 Statistical analysis figure for Critical Season 1 ash-free dry mass analysis of natural substrate periphyton samples. The x-axis is sites and the y-axis is organic material mass in g/m^2.

Table 2.33 Tukey-Kramer means comparison table with chlorophyll-a (mg/cm^2) means and groupings by sites (Level) for natural substrate periphyton analysis for Critical Season 1.

Level	Group	Mean
SPG2	A	0.0075
OSG5	A	0.0056
SPG1	A	0.0048
LOREF	A	0.0038
CSREF	A	0.0029
OSG3	A	0.0024
SPG3	A	0.0015
OSG1	A	0.0014
OSG4	A	0.0007
OSG2	A	0.0004

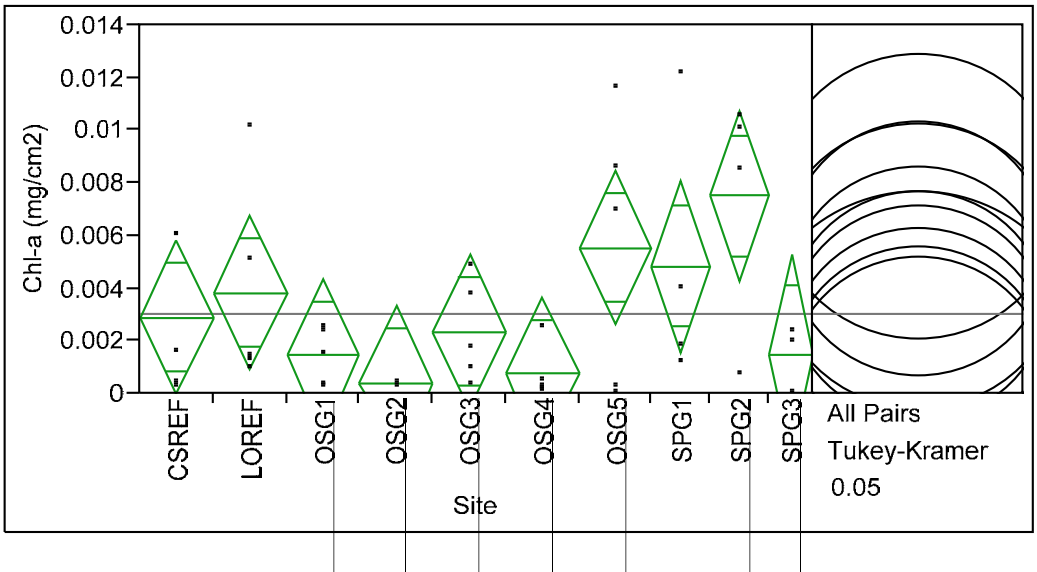


Figure 2.13 Statistical analysis figure for Critical Season 1 chlorophyll-a analysis of natural substrate periphyton samples. The x-axis is sites and the y-axis is chlorophyll-a concentration in mg/cm^2.

Table 2.34 Percent canopy cover results for passive diffusion periphytometer deployments at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009.

Date	Sampling Sites									
	OSG1	OSG2	OSG3	OSG4	OSG5	SPG1	SPG2	SPG3	LOREF	CSREF
Summer 2007 (Critical Season 1)	26	19	42	0	49	44	26	39	61	61
Summer 2008 (Critical Season 2)	n/s	25	20	n/s	11	12	8	18	42	69
Summer 2009 (Critical Season 3)	67	49	42	11	31	29	56	38	63	85
Spring 2008 (Primary Season 1)	46	31	30	23	13	17	0	34	37	72
Spring 2009 (Primary Season 2)	46	19	18	9	14	12	2	1	28	24

Table 2.35 Percent canopy cover results for natural substrate periphyton collections at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009.

Date	Sampling Sites									
	OSG1	OSG2	OSG3	OSG4	OSG5	SPG1	SPG2	SPG3	LOREF	CSREF
Summer 2007 (Critical Season 1)	41	51	38	0	14	38	14	10	53	61
Summer 2008 (Critical Season 2)	22	27	45	29	11	12	0	0	57	61
Summer 2009 (Critical Season 3)	72	23	36	20	8	21	22	6	54	69
Spring 2008 (Primary Season 1)	36	76	41	15	6	32	0	0	72	84
Spring 2009 (Primary Season 2)	1	19	28	9	3	12	0	25	19	29

2.6 Biotic Assessment Methods and Results

2.6.1 Biotic Assessment Methods

We adopted the methods described by the U. S. Environmental Protection Agency (EPA) and ADEQ (Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, USEPA, http://www.epa.gov/owow/monitoring/rbp/download.html). We analyzed fish and macroinvertebrate taxonomic assemblages with attendant habitat assessments at each of two reference and eight test sites (Figure 1.01) during summer of 2007, spring and summer 2008, and spring and summer 2009. Summer samples were planned to occur during the critical season of low flow and high temperatures (>22^0C) each year. However, no conditions representative of a critical season occurred during 2008, so an initially unplanned set of samples was collected in summer 2009 to enable analysis of two critical seasons. After completing analysis of the biological data, it could be seen that the data from September 2008 closely resembled results from the other two years. However, since it did not technically meet the conditions of a "critical season" those data were not included in calculations other than those used for setting scores for invertebrate biometrics.

The study was designed, particularly regarding location of data and sample collection sites, to evaluate water quality impairments, if any, resulting from the Waste Water Treatment Plants (WWTPs) of the cities of Springdale and Rogers on 1) the streams that immediately receive their effluent, and 2) the extended Osage Creek sub-basin of the Illinois River. A critical aspect of this was to obtain sets of samples and accompanying data that were fully comparable to each other among sampling locations. Obviously the samples had to be collected using the same methods, but also during stable weather conditions for the entire week or so required to complete each set.

2.6.1.1 Benthic Macroinvertebrate Methods

2.6.1.1.1 Benthic Macroinvertebrate Field Collections

Benthic macroinvertebrates were collected from two riffles in each of the study sites using a rectangular dip net and a slight modification of the single habitat approach described by USEPA (riffles only). The samples were taken using five locations for kick samples from areas

representing the different water depths and flows from each of the two riffles; collections were biased toward the upstream ends of riffles. The heads of riffles in gravel bed streams with distinct riffle and pool structure have significantly more invertebrates than areas farther downstream (Brown and Brown 1984, Brussock and Brown 1991). The samples were pooled and placed in a tray for picking in the field. The net was examined and invertebrates clinging to it were collected. All visible macroinvertebrates were picked from the samples and placed into 75% ethyl alcohol. Large organic debris and rocks were examined for invertebrates and any found were collected before the organic debris or rocks were discarded. Larger insectivorous invertebrates (crayfish, hellgrammites) were temporarily placed in jars separate from the smaller invertebrates until the larger organisms had succumbed to the alcohol. This was necessary to prevent damage to smaller organisms by the large ones. Samples were appropriately labeled and returned to the lab for identification. Since the collectors and taxonomists were not different persons (Art & Kris Brown) there was no need for chain-of-custody forms to be completed. The biological samples were in the continuous custody of the same persons.

2.6.1.1.2 Benthic Macroinvertebrate Laboratory Methods

Benthic macroinvertebrates were processed in our laboratory following USEPA protocols (see also Barbour et al. 1999). Preserved benthic macroinvertebrates were washed from the respective sample bottles into a 500 um-mesh sieve, rinsed with tap water, and placed into a white tray with 6 cm X 6 cm grids marked on the bottom (total of 12 quadrants). The sample contents were gently mixed and spread in the tray so that they were reasonably homogenous. Numbers were then randomly selected to determine from which four of the 12 grids invertebrates would be picked. All invertebrates were removed from the first four randomly-selected grids and placed in a Petri dish while keeping track of the number picked. If 100 ± 20%, the target number, were picked from the first four grids, sorting was complete. If more than the target number were picked, the contents of the tray (the sample residue) were placed into a sample jar with 75% alcohol and the invertebrates in the Petri dish were returned to the gridded tray. A different set of numbers was randomly selected and corresponding grids were picked using the same method as before. If the number picked from the first four grids exceeded the target number, the whole process was repeated. If the number picked from the four grids was less than the target number, additional random grids were picked until the appropriate number of invertebrates was included. Invertebrates left from the secondary sortings were placed in separate vials and labeled as sorted residue.

Most of the benthic macroinvertebrates were identified to genus using taxonomic keys (e.g., Wiggins 1978, Poulton and Stewart 1991, Smith 2001, Thorp and Covich 2001, Merritt et al. 2008). An *a priori* decision was made to identify the Chironomidae only to family to save time and money required for further taxonomic refinement. Flat worms and leeches, having been preserved using only ethanol in the field, were not relaxed enough to identify past family or order. Instars too young or too badly damaged (missing legs, gills, mouth parts, etc.) were taken to the lowest taxonomic level, generally family, where certainty of identification was not comprised. Organisms were placed in vials with neoprene stoppers containing 75% alcohol and appropriately labeled and stored. Voucher specimens representing each taxon collected were preserved and labeled for subsequent verification and curation in the University of Arkansas Museum.

2.6.1.1.3 Benthic Macroinvertebrate Analysis

The analysis of the macroinvertebrate data is also rather completely prescribed by the USEPA and ADEQ, although ADEQ is still in the process of completing their decisions about analysis and interpretation of benthic macroinvertebrate data from the different ecoregions across the state. We followed their methods as closely as possible including conversing with ADEQ personnel regarding items about which we were unsure. The 11 biometrics we settled upon for the invertebrate Index of Biotic Integrity (IBI) are listed in Table 2.36. With the top score for each biometric assigned as 5, the highest possible total score was 55. It was necessary for us to establish scoring criteria (cut off values) for the biometrics based on our results. We chose to use all of our data from critical and primary seasons from all 10 collecting locations to determine these criteria, and to have them correspond to the 25% and 75% quartiles (Table 2.36). Note that there are only minor differences among the seasonal data (Fig. 2.11), which supports the decision to use all data instead of just those from the critical seasons for determining scoring limits, along with the fact that larger data sets tend to be more normally distributed.

2.6.1.2 Fish Methods

2.6.1.2.1 Fish Field Collections

Fish were collected from a 350 - 1000 foot long reach at each site that was selected to include the diverse habitats representative of each stream, i.e., riffles, pools, and flats (runs, glides). A one

pass, upstream collection was made using a backpack electrofisher with block nets used where needed. The electrofisher output settings were adjusted to optimal performance levels at each site prior to each collection. At least three persons equipped with long-handled dip nets followed the person with the electrofisher to capture stunned fish and transfer them to another person for transport to a site established for holding the fish during identification and counting. The same person (Art Brown) was always responsible for identification of the fish at streamside, and for decisions regarding their release or collection for laboratory examination. Our goal was to release as many fish as quickly as possible to enhance their survival. Fish that were identifiable were released a sufficient distance downstream from the electrofisher to prevent them from being stunned again. Fish not readily identifiable in the field and those needed for voucher specimens were euthanized humanely and preserved in 10% buffered formalin solution, appropriately labeled, and taken to the laboratory for completion of identification and analysis. Fish, as with the macroinvertebrates, were in continuous custody of the same persons (Art and Kris Brown).

Stonerollers (*Campostoma* spp) are often identified only to genus due to the difficulty of identifying them to species and the requirement of microscopy for their specific identification, although it is known that there are two separate species that co-occur in these streams. We chose to separately account for both species of stonerollers. During the first collections (2007) we preserved all stonerollers that were not identifiable at streamside (males in breeding condition can be identified to species in the field), and identified them completely in the laboratory. There were such large numbers at some sites (> 400) that subsequently we began the practice of retaining 40-50 specimens for laboratory identification and applying a ratio of the species to the ones we released in the field. If there were fewer than 50 individuals, we preserved and examined all of them in the laboratory. This enabled us to count and identify each of these species independently of each other (central stoneroller = *C. anomalum,* largescale stoneroller = *C. oligolepis*). We felt that this was necessary because of the importance of these fish. They are very abundant in streams of the south central U. S., tolerant of pollution, primary feeders (grazers), and have a positive response to disturbances (Brown and Matthews 1995, Brown et al. 1998). Stonerollers have a strong impact on the IBI scores because they influence each of the biocriteria. One criterion is percent primary feeders. All but one of the criteria are based on percentages, and at disturbed sites they are very abundant, giving them a large impact. The other criterion is number of species, which is also affected by completely identifying the stonerollers.

2.6.1.2.2 Fish Laboratory Methods

In the laboratory, the preserved fish were washed in tap water to remove as much of the formalin as possible before close examination and manipulation. Fish were examined using a dissecting microscope and taxonomic keys (e. g., Pflieger 1975, Robison & Buchanan 1992). Difficult specimens were sent to Dr. Tom Buchanan at the University of Arkansas at Fort Smith for verification. Representative specimens were placed in museum jars, preserved in 75% ethanol, and appropriately labeled for deposition in the University of Arkansas Museum as voucher specimens. Remaining specimens were disposed of as hazardous waste by the University of Arkansas Office of Environmental Health and Safety.

2.6.1.2.3 Fish Analysis

The fish data were analyzed according to ADEQ methods for the Ozark Highlands Ecoregion as indicated in Table 2.37. This table, as well as tables designating key species and primary feeders were obtained through personal correspondence with ADEQ personnel.

2.6.2 Results of Biological Assessment

2.6.2.1 Benthic Macroinvertebrate Results

The invertebrate IBI scores showing results of individual biometrics (e. g., total taxa) are listed in Tables 2.38-2.42. A summary of the total IBI scores by season and site is in Table 2.43. Figure 2.14 illustrates the pattern of water quality among the sites as indicated by the invertebrate community analyses.

2.6.2.2 Fish Results

Results of the fish community analyses showing each biometric for each season and site are listed in Tables 2.44-2.48. The summary of total IBI scores for the fish community by season at each site is in Table 2.49. The patterns of water quality along Osage and Spring Creeks as indicated by variations in the fish community can be seen in Figure 2.15. The percent primary feeders at each

site for each season was pulled out as an individual figure due to its importance in discerning impairment due to nutrients (Figure 2.16).

Table 2.36 Invertebrate metric scoring ranges established using the 25th and 75th percentile ranking of metric scores from all five collections performed during this study. Note that the % Isopoda metric was changed from "0.0%" indicated by the 25th percentile to "<2 " following our best professional judgment.

A. Invertebrate metric scoring ranges for the Osage and Spring Creek basins of the Illinois River, Arkansas.

Metric	5	3	1
Total Taxa	>17	17 – 12	<12
Number EPT Taxa	>8	8 – 5	<5
%EPT-%Hydropsychidae	>55	55 – 28	<28
% Scrapers	>33	5 – 33	<5
% Clingers	>68	68 – 23	<23
% Diptera	<4	4 – 24	>24
% Chironomidae	<3	3 – 22	>22
% Isopoda	<2	2 – 7	>7
% Tolerant Organisms	<2	2 – 12	>12
HBI	<4.1	4.1 - 5.2	>5.2
% Intolerant Organisms	>24	24 – 6	<6

B. Percentile ranking of metric scores from five collections from summer 2007 through summer 2009 used to establish scoring ranges for each of the biometrics.

Metric	Min	5th	25th	50th	75th	95th	Max
Total Taxa	8	8.45	12	15	17	19.55	23
Number EPT Taxa	2	2.45	5	6	7.75	10.55	14
%EPT- %Hydropsychidae	4.1%	9.3%	28.0%	44.4%	55.3%	67.1%	73.6%
% Scrapers	0.0%	0.0%	4.5%	17.1%	33.1%	48.4%	60.6%
% Clingers	2.8%	5.8%	23.4%	48.7%	67.7%	84.8%	92.1%
% Diptera	0.0%	0.0%	3.9%	10.6%	23.9%	55.9%	66.7%
% Chironomidae	0.0%	0.0%	2.5%	7.2%	21.6%	44.3%	57.5%
% Isopoda	0.0%	0.0%	0.0%	0.4%	6.8%	55.2%	72.5%
% Tolerant Organisms	0.0%	0.0%	1.7%	3.3%	12.1%	53.9%	67.0%
HBI	2.59	3.11	4.11	4.76	5.15	6.40	6.89
% Intolerant Organisms	0.0%	1.9%	5.7%	12.5%	23.8%	52.8%	64.7%

Table 2.37. Fish community biocriteria for Ozark Highland streams established by ADEQ (ADEQ personal communication).

A. Fish metric scoring ranges for the Osage and Spring Creek basins of the Illinois River, Arkansas. If a raw metric score is zero, score as zero, except for the % Primary Feeders metric. Total scores should be interpreted as: 37-45 mostly similar, 25-36 generally similar, 13-24 somewhat similar, and 12-0 not similar to reference streams in the Ozark Highland Ecoregion.

Metric	5	3	1
% Sensitive Individuals	>31	31 - 20	<20
% Cyprinidae (Minnows)	48 – 64	39 – 47 or 65 – 73	<39 or >73
% Ictaluridae (Catfishes)	>2[1]	1 - 2[1]	<1 or >3% bullheads
% Centrarchidae (Sunfishes)	4 - 15[2]	<4 or 15 - 20[2]	>20 or >2% Green sunfish
% Percidae (Darters)	>11	5 – 11	<5
% Primary Feeders	<42	42 – 49	>49
% "Key" Individuals	>23	23 – 16	<16
Diversity	>2.77	2.77 – 2.37	<2.37
# Species	>(watershed area**X**0.034)+16.45	(watershed area**X**0.034)+16.45 to (watershed area**X**0.034)+12.26	<(watershed area**X**0.034)+12.26

[1]no more than 3% bullheads
[2]no more than 2% Green sunfish

B. Watershed areas are used to calculate cut off scores for the # Species metric in Table 2.A above.

	Sampling Sites									
	OSG1	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Watershed Area (square miles)	32.1	32.4	35.6	80.6	128.6	12.7	13.2	35.3	35.4	8.3
(watershed area**X**0.034) + 16.45	18	18	18	19	21	17	17	18	18	17
(watershed area**X**0.034) + 12.26	13	13	13	15	17	13	13	13	13	13

Table 2.38 Invertebrate IBI individual and total metric scores at select sites in the Osage Creek and Illinois River basins for summer 2007 (Critical Season 1). See Table 2.36 for invertebrate metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Total Taxa	5	5	5	3	5	1	3	3	3	5
Number EPT Taxa	3	3	3	3	5	3	1	3	5	5
%EPT- %Hydropsychidae	5	1	1	3	3	1	3	1	3	5
% Scrapers	3	3	5	3	5	1	1	5	3	5
% Clingers	5	3	3	3	5	3	5	3	5	3
% Diptera	3	3	5	3	3	5	3	3	5	5
% Chironomidae	5	3	5	3	3	3	3	3	5	5
% Isopoda	5	1	1	5	5	1	1	1	5	5
% Tolerant Organisms	3	1	1	1	1	1	3	1	3	1
HBI	5	1	1	3	3	1	3	1	3	5
% Intolerant Organisms	5	5	5	1	5	5	5	5	5	5
Invertebrate IBI Total Scores	47	29	35	31	43	25	31	29	45	49

Table 2.39 Invertebrate IBI individual and total metric scores at select sites in the Osage Creek and Illinois River basins for spring 2008 (Primary Season 1). See Table 2.36 for invertebrate metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Total Taxa	3	3	3	1	5	1	1	3	5	5
Number EPT Taxa	3	3	3	3	5	1	1	3	3	5
%EPT- %Hydropsychidae	5	3	3	3	5	1	3	5	3	3
% Scrapers	5	3	3	5	5	1	1	3	5	5
% Clingers	3	3	3	3	3	3	3	3	3	3
% Diptera	3	1	3	3	3	3	3	3	3	5
% Chironomidae	3	1	3	3	3	5	3	3	5	5
% Isopoda	5	1	1	3	5	1	1	5	5	5
% Tolerant Organisms	3	1	3	5	3	1	3	5	3	3
HBI	5	3	3	3	5	1	3	3	5	5
% Intolerant Organisms	5	5	5	5	5	1	5	5	5	5
Invertebrate IBI Total Scores	43	27	33	37	47	19	27	41	45	49

Table 2.40 Invertebrate IBI individual and total metric scores at select sites in the Osage Creek and Illinois River basins for summer 2008 (Critical Season 2). See Table 2.36 for invertebrate metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Total Taxa	3	3	3	3	5	3	1	5	5	5
Number EPT Taxa	3	3	3	3	5	1	1	5	3	5
%EPT- %Hydropsychidae	1	1	1	3	3	1	1	3	3	3
% Scrapers	3	3	1	5	5	1	1	3	3	5
% Clingers	3	3	3	3	5	3	3	3	3	3
% Diptera	3	1	1	3	3	1	1	1	3	5
% Chironomidae	3	1	1	3	3	1	1	3	3	5
% Isopoda	5	5	5	5	5	1	5	5	5	5
% Tolerant Organisms	5	5	3	5	5	3	3	3	5	3
HBI	3	3	1	3	3	1	3	3	3	5
% Intolerant Organisms	5	5	5	5	5	5	5	5	5	5
Invertebrate IBI Total Scores	37	33	27	41	47	21	25	39	41	49

Table 2.41 Invertebrate IBI individual and total metric scores at select sites in the Osage Creek and Illinois River basins for spring 2009 (Primary Season 2). See Table 2.36 for invertebrate metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Total Taxa	3	3	3	1	3	1	1	1	3	5
Number EPT Taxa	3	3	3	3	5	1	1	3	3	5
%EPT- %Hydropsychidae	5	3	3	5	5	3	3	5	5	5
% Scrapers	3	3	3	1	3	1	1	1	3	5
% Clingers	3	3	3	3	3	3	3	3	3	3
% Diptera	3	1	1	1	3	3	1	1	3	3
% Chironomidae	3	1	1	1	3	3	1	1	3	3
% Isopoda	5	1	5	5	5	1	3	5	5	5
% Tolerant Organisms	5	1	3	5	5	1	5	5	3	3
HBI	3	3	3	3	3	1	3	5	5	5
% Intolerant Organisms	5	5	5	5	5	1	1	5	5	5
Invertebrate IBI Total Scores	41	27	33	33	43	19	23	35	41	47

Table 2.42 Invertebrate IBI individual and total metric scores at select sites in the Osage Creek and Illinois River basins for summer 2009 (Critical Season 3). See Table 2.36 for invertebrate metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Total Taxa	3	1	3	3	3	3	3	1	3	5
Number EPT Taxa	3	3	3	5	5	3	3	3	1	5
%EPT- %Hydropsychidae	3	3	3	5	3	1	1	3	3	5
% Scrapers	3	3	3	3	3	1	1	3	3	3
% Clingers	5	5	5	5	5	3	3	5	3	5
% Diptera	3	5	5	5	3	5	1	5	5	5
% Chironomidae	3	3	5	5	3	5	1	5	5	5
% Isopoda	5	5	5	5	5	1	5	5	5	5
% Tolerant Organisms	5	5	5	3	3	1	3	5	5	5
HBI	3	1	3	3	3	1	1	3	5	5
% Intolerant Organisms	5	5	5	5	5	5	5	5	5	5
Invertebrate IBI Total Scores	41	39	45	47	41	29	27	43	43	53

Table 2.43 Invertebrate IBI total scores at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. The maximum possible score for a single sampling event is 55. Summer 2007 and 2009 collections were in critical seasons. During summer 2008 there was no critical season (i.e., low flow, temperature >22 C). Therefore critical season averages are for summer 2007 and summer 2009 only.

Date	Sampling Sites									
	OSG1	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical Season 1)	47	29	35	31	43	25	31	29	45	49
Summer 2008 (Critical Season 2)	37	33	27	41	47	21	25	39	41	49
Summer 2009 (Critical Season 3)	41	39	45	47	41	29	27	43	43	53
Spring 2008 (Primary Season 1)	43	27	33	37	47	19	27	41	45	49
Spring 2009 (Primary Season 2)	41	27	33	33	43	19	23	35	41	47
Critical Season Averages	44	34	40	39	42	27	29	36	44	51

Table 2.44 Fish IBI individual metric and total scores at select sites in the Osage Creek and Illinois River basins for summer 2007 (Critical Season 1). See Table 2.37 for fish metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
% Sensitive Individuals	3	5	3	5	5	1	3	5	5	5
% Cyprinidae	5	5	1	5	1	1	3	5	5	3
% Ictaluridae	0	0	3	5	1	0	0	5	5	5
% Centrarchidae	1	1	1	5	5	3	5	1	3	5
% Percidae	5	5	3	5	3	3	5	3	5	5
% Primary Feeders	5	5	5	5	1	1	1	5	5	5
% Individuals Key Individuals	5	5	5	5	5	1	5	5	5	5
Diversity	5	3	3	1	1	1	1	1	1	3
Total Species	3	3	3	3	1	1	3	5	3	5
Fish IBI Total Scores	32	32	27	39	23	12	26	35	37	41

Table 2.45 Fish IBI individual metric and total scores at select sites in the Osage Creek and Illinois River basins for spring 2008 (Primary Season 1). See Table 2.37 for fish metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
% Sensitive Individuals	1	3	3	5	5	3	1	5	5	5
% Cyprinidae	5	1	1	1	3	1	1	5	1	1
% Ictaluridae	0	0	0	5	5	0	1	5	3	5
% Centrarchidae	1	1	1	5	3	3	5	1	5	5
% Percidae	5	5	5	3	3	5	3	3	5	3
% Primary Feeders	3	5	5	1	5	1	1	5	1	5
% Individuals Key Individuals	5	5	5	5	5	1	1	5	5	5
Diversity	5	1	1	3	1	1	5	1	1	3
Total Species	3	5	5	3	1	1	3	3	3	3
Fish IBI Total Scores	28	26	26	31	31	16	21	33	29	35

Table 2.46 Fish IBI individual metric and total scores at select sites in the Osage Creek and Illinois River basins for summer 2008 (Critical Season 2). See Table 2.37 for fish metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
% Sensitive Individuals	1	1	5	3	5	3	1	5	5	5
% Cyprinidae	3	1	5	1	3	1	1	1	3	1
% Ictaluridae	0	1	0	5	5	0	3	5	5	5
% Centrarchidae	1	1	1	0	3	3	5	5	1	5
% Percidae	5	3	3	3	5	1	1	3	5	3
% Primary Feeders	5	1	5	1	5	1	1	1	5	5
% Individuals Key Individuals	5	1	5	5	5	1	3	5	5	5
Diversity	5	3	3	1	1	1	1	1	1	3
Total Species	3	3	3	1	1	1	5	3	1	5
Fish IBI Total Scores	28	15	30	20	33	12	21	29	31	37

Table 2.47 Fish IBI individual metric and total scores at select sites in the Osage Creek and Illinois River basins for spring 2009 (Primary Season 2). See Table 2.37 for fish metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
% Sensitive Individuals	1	5	3	3	5	5	5	5	5	5
% Cyprinidae	3	3	5	1	1	5	1	5	5	1
% Ictaluridae	0	0	3	5	5	0	1	5	3	5
% Centrarchidae	1	1	1	1	3	0	3	1	1	3
% Percidae	5	5	5	3	5	5	1	5	5	5
% Primary Feeders	5	5	5	1	5	1	1	5	5	5
% Individuals Key Individuals	5	5	5	5	5	1	5	5	5	5
Diversity	3	5	5	5	3	1	5	5	5	5
Total Species	3	1	5	3	1	1	1	3	3	3
Fish IBI Total Scores	26	30	37	27	33	19	23	39	37	37

Table 2.48 Fish IBI individual metric and total scores at select sites in the Osage Creek and Illinois River basins for summer 2009 (Critical Season 3). See Table 2.37 for fish metric cutoff values.

	Sampling Sites									
Metric	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
% Sensitive Individuals	1	3	5	3	5	5	3	3	5	5
% Cyprinidae	5	3	5	5	1	1	1	1	1	5
% Ictaluridae	1	1	5	3	5	0	0	5	3	5
% Centrarchidae	1	1	1	3	5	0	3	3	1	3
% Percidae	5	3	5	5	5	5	1	5	5	5
% Primary Feeders	1	1	5	3	5	1	1	1	5	5
% Individuals Key Individuals	5	3	5	5	5	1	5	5	5	5
Diversity	5	5	5	5	5	1	1	3	5	1
Total Species	5	5	3	3	3	1	5	3	5	3
Fish IBI Total Scores	29	25	39	35	39	15	20	29	35	37

Table 2.49 Summary of fish IBI total scores at select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. Summer 2007 and 2009 collections were in critical seasons. During summer 2008 there was no critical season (i.e., low flow, temperature >22 C). Therefore critical season averages are for summer 2007 and summer 2009 only.

	Sampling Sites									
Date	**OSG1**	**OSG2**	**OSG3**	**OSG4**	**OSG5**	**SPG1**	**SPG2**	**SPG3**	**LOREF**	**CSREF**
Summer 2007 (Critical Season 1)	32	32	27	39	23	12	26	35	37	41
Summer 2008 (Critical Season 2)	28	15	30	20	33	12	21	29	31	37
Summer 2009 (Critical Season 3)	29	25	39	35	39	15	20	29	35	37
Spring 2008 (Primary Season 1)	28	26	26	31	31	16	21	33	29	35
Spring 2009 (Primary Season 2)	26	30	37	27	33	19	23	39	37	37
Critical Season Averages	30.5	28.5	33.0	37.0	31.0	13.5	23.0	32.0	36.0	39.0

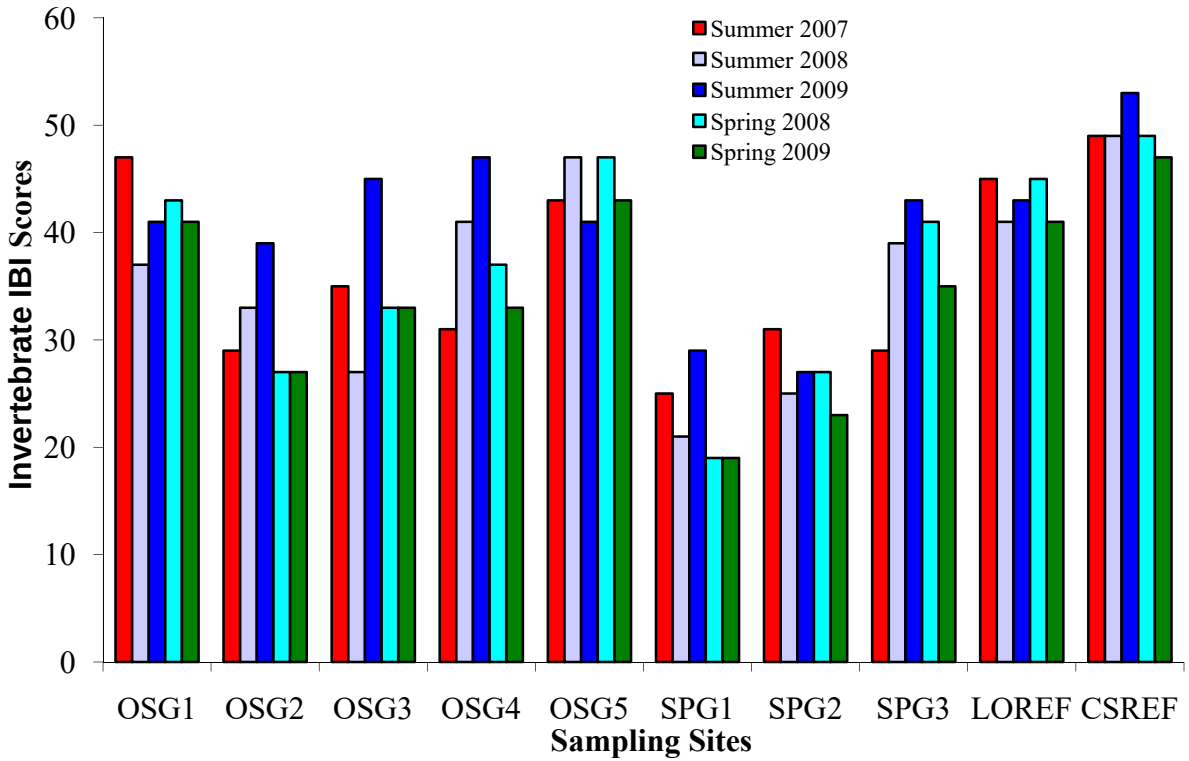


Figure 2.14 Invertebrate Index of Biotic Integrity (IBI) scores for select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. Summer 2007 and 2009 collections were in critical seasons. During summer 2008 there was no critical season (i.e., low flow, temperature >22 C).

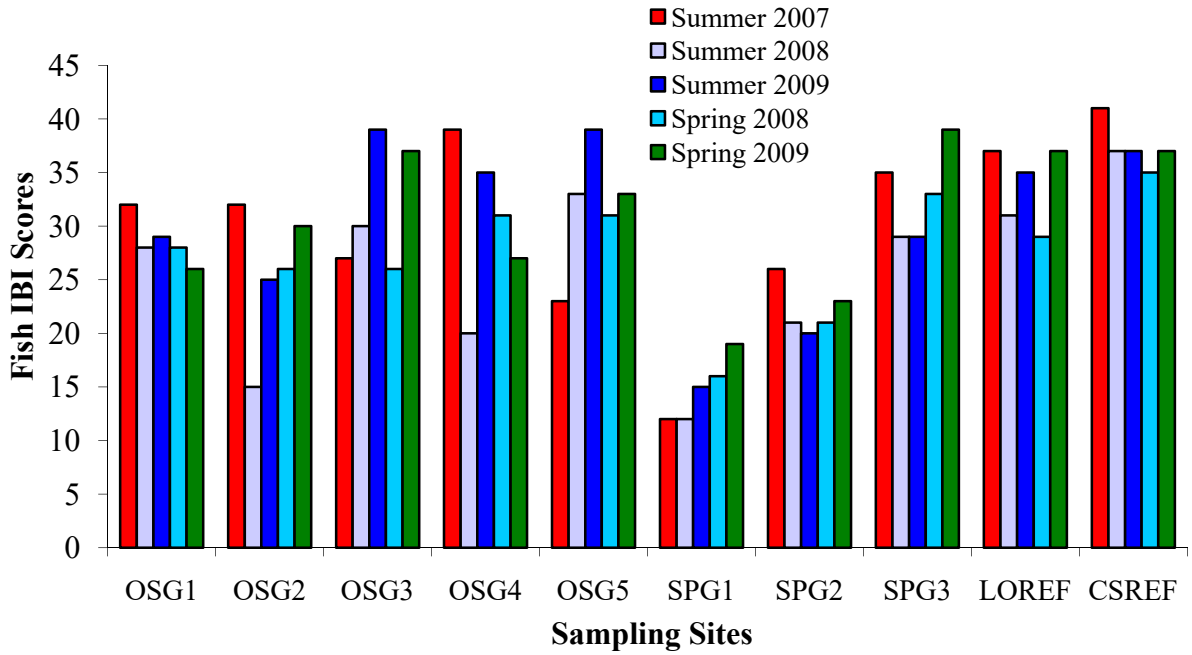


Figure 2.15 Fish Index of Biotic Integrity (IBI) scores for select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. Summer 2007 and 2009 collections were in critical seasons. During summer 2008 there was no critical season (i.e., low flow, temperature >22 C).

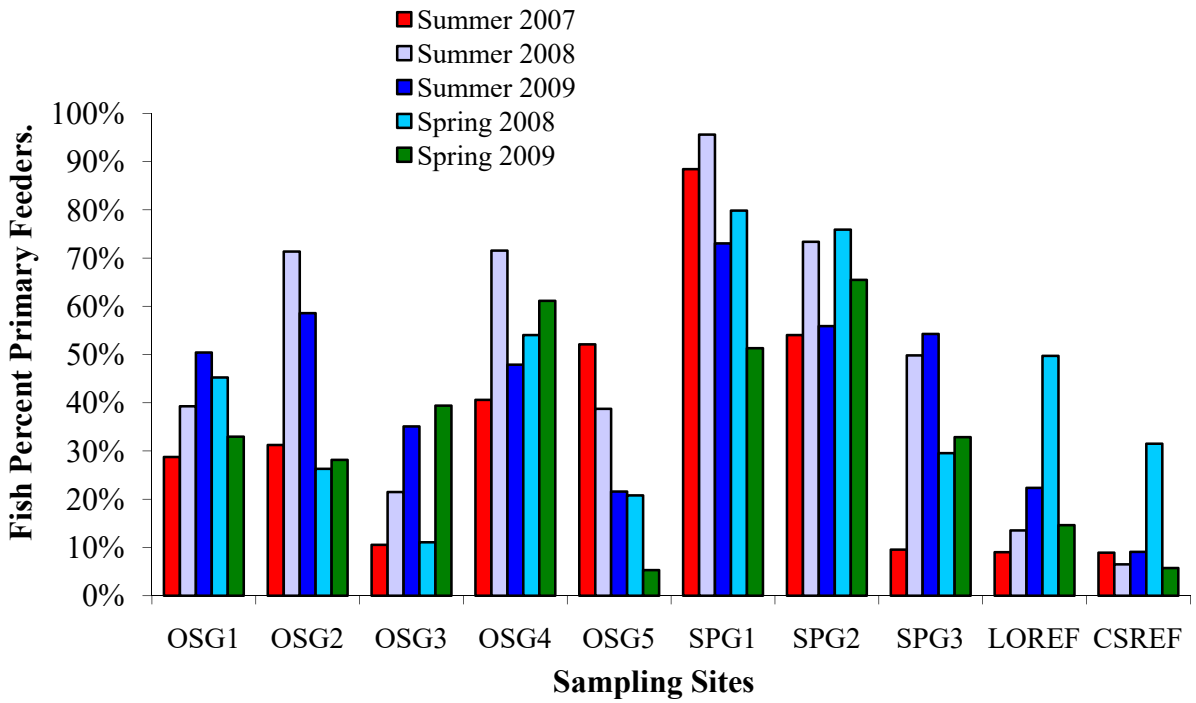


Figure 2.16 Percent primary feeders for select sites in the Osage Creek and Illinois River basins from critical season 2007 through critical season 2009. Summer 2007 and 2009 collections were in critical seasons. During summer 2008 there was no critical season (i.e., low flow, temperature >22 C).

Section 3: Discussion

3.1 Water Chemistry Discussion

3.1.1 Effect of Effluent Discharges – Upstream and Down

Osage Creek

The effluent discharge altered some of the measured physico-chemical properties in Osage Creek, while other parameters showed no statistical differences overall or in any individual season (i.e., primary and critical) (Tables 2.03 – 2.16, Figures 2.01-2.08). The effluent discharge did not significantly alter turbidity, total suspended solids, or sestonic chlorophyll-a concentrations compared to that observed upstream; there were no significant differences overall (all data) or within any critical or primary season (paired T-test, $P>0.05$). Overall, pH and dissolved oxygen concentrations were not significantly different downstream compared to upstream ($P>0.05$), except pH was significantly greater downstream (7.7) compared to upstream (7.5) during critical season 2009 ($P=0.03$) and dissolved oxygen was greater also in primary season 2008-9 (downstream 9.1 mg L^{-1} compared to upstream 8.6 mg L^{-1}, $P<0.01$). Overall nutrient concentrations (including SRP, TP, NH_4-N, NO_2-N, NO_3-N, TN and TOC) were generally greater downstream from the effluent discharge relative to upstream ($P<0.05$). However, there were random times where various nutrient concentrations were not statistically different in individual critical and primary seasons. The effluent discharge also significantly increased water temperature and conductivity relative to upstream ($P<0.05$).

Spring Creek

The effluent discharge at Spring Creek influenced some physico-chemical properties compared to that observed upstream (Tables 2.03 – 2.16, Figure 2.01). However, turbidity, total suspended solids, sestonic chlorophyll-a and nitrate-nitrogen concentrations were not significantly different downstream overall (all data, paired T-test, $P>0.05$); there were a few occurrences where seasonal differences were noted with nitrate-nitrogen, sestonic chlorophyll-a and turbidity, when comparing data upstream and down from the effluent discharge ($P<0.05$). For example, nitrate-nitrogen concentrations were greater downstream from the effluent discharge during the critical seasons ($P<0.05$). Overall, pH, conductivity, water temperature, dissolved oxygen and the other nutrients (including SRP, TP, NH_4-N, NO_2-N, TN and TOC concentrations) were greater

downstream from the effluent discharge compared to upstream at Spring Creek ($P<0.05$). All of the aforementioned physico-chemical properties (except NO_2-N and dissolved oxygen) were generally greater downstream from the effluent discharge in all seasons ($P<0.05$), except pH and TN were not different during primary season 2007-8 ($P>0.05$). Nitrite-nitrogen concentrations were greater downstream compared to upstream in critical seasons 2008 and 2009 ($P\leq0.03$), and dissolved oxygen concentrations were greater downstream during 2008 through primary season 2008-9 ($P\leq0.05$).

Water Quality Standards

The numeric water quality standards that apply to these Ozark Highland streams were compared to the physico-chemical properties measured in the water samples collected upstream and downstream from the effluent discharges in Osage Creek and Spring Creek. The pH of the water samples was slightly basic, ranging from 7.5 to 8.3 across all data collected at these two streams; although pH significantly increased at Spring Creek, the increase was small from 7.7 upstream to only 7.9 downstream. There was a profound increase in conductivity downstream (range: 172-893 µS cm-1), where conductivity upstream (range: 120-401 µS cm-1) was reflective of streams draining catchments with urban and pasture land use. Water temperatures measured in water samples on-site showed a slight but significant increase from upstream to down at Osage Creek (means: 16.6 and 17.6 °C, respectively) while the increase at Spring Creek was greater from upstream to downstream (means: 17.6 and 21.1 °C, respectively), with some maximum values downstream that exceeded the ADEQ Reg. 2 standard of 29.0 °C. The dissolved oxygen concentrations represent single data points during day light hours (typically morning to early afternoon), and the range in concentrations (5.5-12 mg L-1) across all data collected was above the threshold for warm water fisheries (5 mg L-1, Arkansas Regulation 2). The turbidity criterion that applies to these streams is 10 NTU (specific to the Ozark Highlands); there were no values upstream or at the first site downstream that exceeded this criterion in the collected water samples. The effluent discharges did significantly increase nutrient concentrations in both streams, although the biological data needs to be evaluated to ascertain any violations of the narrative nutrient criteria as written in Arkansas Regulation 2.

3.1.2 Longitudinal Patterns in Physico-Chemical Properties

Water quality comparisons across multiple sampling sites are complex, and specific comparisons will be provided within the parameter tables. However, it is more informative to discuss general

longitudinal gradients (upstream to downstream patterns), especially with regard to nutrient concentrations since only narrative nutrient criteria currently exist. Phosphorus (i.e., SRP and TP) concentrations significantly increased downstream (OSG2 and SPG2) of the effluent discharges compared to upstream (OSG1 and SPG1), and then concentrations in upper Osage Creek (OSG3) and Spring Creek (SPG3) decreased from dilution (groundwater and lateral inputs, i.e. tributaries) and possibly in-stream retention. The phosphorus concentrations in lower Osage Creek (OSG4) were increased downstream from its confluence with Spring Creek, but concentrations again decreased in this reach (OSG5). These observations are consistent with previous studies (Haggard et al., 2003a; Haggard, 2005; Ekka et al., 2006) that showed that phosphorus concentrations generally increased upstream in Osage Creek to each effluent discharge. However, phosphorus concentrations are much less in lower Osage Creek and Spring Creek than what was historically observed (see Haggard et al., 2003; Ekka et al., 2006). This change resulted from improved phosphorus management at the Springdale WWTP, and this watershed management change has resulted in decreased phosphorus transport in the Illinois River (B.E. Haggard, unpublished data).

The longitudinal patterns in ammonia-nitrogen and total organic carbon were similar to that observed with phosphorus, where the effluent discharge increased concentrations and then concentrations decreased downstream. The loss in ammonia downstream may be attributed to the incredible nitrification rates often observed in streams (e.g., see Haggard et al., 2005). The longitudinal decrease in total organic carbon was likely from dilution and mineralization of the organic carbon input from the effluent discharge.

The longitudinal gradient in nitrate-nitrogen and total nitrogen was not as consistent moving from upstream to downstream. These concentrations generally increased below the effluent discharge compared to that measured upstream. In Spring Creek, nitrate and total nitrogen increased downstream (from SPG2 to SPG3); this increase may be partially attributed to nitrification of reduced nitrogen in the effluent discharge. However, the concentrations slightly decreased in upper Osage Creek. Further downstream in lower Osage Creek, the concentration of these two constituents increased (from OSG4 to OSG5). The increases in Spring Creek and lower Osage Creek may also be from catchment sources. Several studies have shown that nitrate-nitrogen and total nitrogen concentrations during base flow conditions in streams increase with increases in pasture land use (or decreases in forested areas) within the catchment (e.g., Haggard et al., 2003b,

2007). Thus, the increased concentration likely reflects nitrogen sources from the catchment along the longitudinal profile.

3.1.3 Reference Condition Comparisons

The two selected reference streams, Chamber Springs (CSREF) and Little Osage Creek (LOREF), showed some distinct differences in select physico-chemical properties, while others were not different between the two streams overall (Tables 2.03 – 2.16, Figures 2.01 – 2.08). Total phosphorus concentrations were not significantly different between Chamber Springs (0.048 mg L^{-1}) and Little Osage Creek (0.046 mg L^{-1}), despite substantial differences in catchment land uses. However, dissolved phosphorus was greater at Chamber Springs (0.037 mg L^{-1}) compared to that observed at Little Osage Creek (0.031 mg L^{-1}) overall (all data, paired T-test, $P<0.01$) and particularly during the critical seasons ($P<0.05$). The difference in dissolved concentrations was small between these sites, only 0.006 mg L^{-1}.

Overall, nitrogen concentrations except ammonia-nitrogen were significantly greater at Little Osage Creek compared to Chamber Springs ($P<0.01$); these differences generally persisted across all seasons sampled. While total organic carbon was not different between sites, sestonic chlorophyll-a was greater ($P<0.01$) at Little Osage Creek (0.4 µg L^{-1}) compared to Chamber Springs (0.1 µg L^{-1}). Water temperature and pH were not significantly different between sites overall ($P>0.65$), but conductivity and dissolved oxygen concentration (from the single point samplings) were greater at Little Osage Creek overall ($P<0.01$). Total suspended solids and turbidity were different ($P<0.01$) with Little Osage Creek having three times greater concentrations (4.1 mg L^{-1}) and NTU (3.1), although the values at Little Osage Creek indicated little suspended solids within the water column.

The comparison between sites upstream from the effluent discharges (OSG1 and SPG1) and the reference sites were variable with nutrients, resulting from the variability between the two reference sites. With regard to phosphorus, concentrations were not significantly different between Little Osage Creek and Osage Creek upstream from the effluent discharge (OSG1); however, phosphorus concentrations at all other sites at Osage Creek and Spring Creek were significantly greater than that measured at the two reference sites (all data, paired T-test, $P<0.01$). The phosphorus concentrations at the most downstream site on Osage Creek (OSG5) had concentrations statistically greater than the reference sites.

Ammonia-nitrogen concentrations were not different between the upstream sites (OSG1 and SPG1) and the reference sites (CSREF and LOREF), whereas all other sites had concentrations greater than that observed at the reference streams (all data, paired T-test, $P<0.04$). Nitrite-nitrogen concentrations at Chamber Springs (0.005 mg L^{-1}) were less than that observed at all sites on Osage Creek and Spring Creek, whereas concentrations were not significantly different ($P>0.15$) at Little Osage Creek (0.014 mg L^{-1}) and select sites downstream from the effluent discharges (OSG2, OSG3 and SPG2). Nitrate-nitrogen and total nitrogen concentrations at all sites on Osage Creek and Spring Creek were significantly greater than concentrations observed at Chambers Springs (P,0.01), but less than concentrations at Little Osage Creek ($P<0.03$).

Total organic carbon was generally not different between the upstream sites on Osage Creek (OSG1) and Spring Creek (SPG1) and the two reference streams (CSREF and LOREF), whereas concentrations downstream from the effluent discharges were elevated above that observed in the reference streams. Sestonic chlorophyll-a was least at Chambers Springs compared to all sites on Osage Creek and Spring Creek ($P<0.01$), whereas suspended algae at Little Osage Creek was not different than the other sites. Turbidity and total suspended solids concentration at all sites on Osage and Spring Creek was in between that observed at the two reference streams, with Chambers Springs having the least and Little Osage the greatest. The most downstream site on Osage Creek (OSG5) generally had physico-chemical properties in the collected water samples that were significantly different than the two reference streams (paired T-test, $P<0.05$), but these conditions were approaching those observed at the reference sites (i.e., concentrations generally decreased the further downstream from effluent discharges).

3.2 Diurnal In-Stream Parameter Discussion

Exploration of the diurnal in-stream data began with comparison to ADEQ Reg. 2 standards for potential violations of numeric water quality criteria. The parameters for which there are numeric standards are pH, temperature (°C), and dissolved oxygen (mg/L). Each parameter was compared to the appropriate standard for the season, water temperature, and watershed size.

The criteria for pH is that values must be between 6 and 9 and not vary more than 1 standard unit (SU) over a 24 hour season. These criteria were never observed to be in violation during this investigation; only once was a site at risk of violating the criteria, during Primary Season 1 event 1 at site OSG4, where the pH varied by a maximum of 0.9 SU over a 48 hour season. Multiple

sites showed signs of pH variability greater than that seen at the reference site. This will be discussed in more detail in the section on nutrient narrative criteria.

Temperature criteria are based on a monthly maximum average, which was not addressed in this study, and an instantaneous maximum (29°C). Maximum temperatures recorded in water chemistry samples on-site suggested a potential for exceedance of the standard below the Springdale WWTP. The maximum temperature recorded during the diurnal data sonde deployments occurred at SPG3 (28.9°C). Few other maximums exceeded or approached 28°C. It should be noted that temperature values increased below both WWTP outfalls but that the difference in temperatures from SPG1 to SPG2 was often greater than 4°C. SPG2 was frequently the warmest site during sampling seasons and SPG1 was frequently as cool or cooler than the reference sites. The low temperature at SPG1 is attributed to the fact that the majority of the flow at the site comes from a spring just upstream of the site. The increase in temperature from SPG1 to SPG2 reflects the fact that the WWTP effluent contributes as much as 70% or more of the base-flow of the stream (Appendix C).

Watershed areas can be found in Table 2.01. These areas are important because they set the levels for DO standards. Dissolved oxygen (mg/L) standards appear to have been violated in only one instance, during Critical Season 1 Event 1 at SPG1. Dissolved oxygen was below 5 mg/L for 0.7 hours and the temperature was below 22°C during that time so no 8 hour 1 mg/L deviation tolerance was in effect. The reason for the temperature being below the 22°C during that time is likely its proximity to the spring which contributes the majority of the flow for Spring Creek at SPG1. Other events came close to having criteria violations but did not exceed criteria. During Critical Season 1 sites OSG4, SPG3 and CSREF during event1 and SPG1 during event 2 had periods of DO below 6 mg/L. Since this occurred during the critical season the DO criteria at these sites was 5 mg/L resulting in no violation. During Primary Season 1 event 2 sites OSG3 and OSG4 went below 6.5 mg/L and OSG4 went below 6 mg/L for 2 hours. These do not appear to violate criteria since these occurred in June and water temperatures were above 22°C. During Critical Season 3 sites OSG4, SPG1, SPG3, and LOREF during event 1 and SPG1 and SPG3 during event 2 went below 6 mg/L. These were not violations since the critical season criteria is 5 mg/L for these sites. A minimum value of 4.5 mg/L for DO was measured during water chemistry sampling for site SPG3. Since water temperatures were over 22 °C at the time a measure of how long DO had been depressed below 5 mg/L would be needed to ascertain if this

was a violation of ADEQ Reg. 2 criteria since an 8 hour depression is allowed if temperature exceeds 22 °C. No diurnal data at SPG3 showed a violation of DO criteria.

The narrative criteria for nutrients include analysis of "dissolved oxygen values, dissolved oxygen saturation, diurnal dissolved oxygen fluctuations, pH values..." (Arkansas Reg. 2). The values and daily fluctuations compared to reference site values and daily fluctuations as well as expected values were assessed. Minimum dissolved oxygen values were only in violation of regional standards once, and this above the Springdale outfall on Spring Creek (SPG1). Also values were typically at or near those at the reference sites, so no indication of narrative criteria violation was apparent. Dissolved oxygen saturation was typically high at many of the sites (Table 2.21). Sites upstream of the WWTP outfalls were typically near or below reference conditions, though SPG1 exceeded 120% saturation on three occasions. The sites immediately downstream of the treatment plants were typically higher than above, but still within the range seen at the reference sites with the exception of the last event at SPG2 (141%). Sites farther downstream from the WWTPs (OSG3, SPG3, OSG4, and OSG5) were consistently higher than the reference conditions and the sites farther upstream. Values at these sites routinely exceeded 120% saturation with maximums of 146%, 131%, 165%, and 151% respectively. Diurnal DO fluctuations were varied over sites and seasons. Reference sites (LOREF and CSREF) typically had the lowest swings, but this was not always the case. Some diurnal swings at the reference sites were greater than 3 mg/L. Sites below the treatment plants either had little change from upstream or actually showed a decrease in diurnal swing (SPG2). OSG3 showed increased swings but typically they were similar to OSG2. Sites that showed the greatest swings were SPG1, SPG3, OSG4, and OSG5. At these sites the swings were typically less than 3 mg/L, but with many up to 5 mg/L, and some as high as 6 mg/L. Fluctuations of pH values at the sites pretty much mirrored that of DO. The reference sites often had pH swings of between 0.25 to 0.5. SPG3 and OSG4 had the largest fluctuations. SPG2, OSG3, and OSG5 also exhibited swings that were somewhat elevated from the reference sites.

3.3 Habitat and Geomorphology Discussion

Qualitative habitat scores (EPA RBP Visual Assessment) were relatively comparable with averages for the five sampling events ranging from 138 (SPG1) to 169 (CSREF). Variability in visual habitat scores was mostly due to riparian condition, availability of stable cover, and bank stability. Sites were selected by visual comparison so it is not a surprise that the variability between sites is relatively low.

Quantitative habitat scores (ADEQ Habitat Assessment) varied more by site and season than did the visual score. Designation of areas as riffle, run, or pool varied from year to year depending on stage of flow and shifting substrate. Also many areas of the streams had multiple habitat types in one cross section so that the habitat would be noted in the field notes as partial pool with dominant run habitat, but that value is only entered as run habitat in the calculations.

Two of the most variable habitat parameters from site to site were canopy cover and percent bedrock substrate. Canopy cover variation from site to site was mostly due to width of channel but was also influenced by riparian zone quality and width. The reference sites had averages of close to 70% canopy cover over all five sampling seasons. Of the smaller sites only SPG1 had an average of less than 40% at 24%. Sites OSG4 and OSG5 had much lower canopy cover percents mostly due to natural stream widening, however OSG4 had a disturbed riparian corridor. Overall the test sites had much lower canopy cover than the reference sites. The percent of each reach with bedrock substrate was high at some sites. The reference sites contained no bed rock. Sites OSG1, OSG2, SPG2, and SPG3 all had over 10% of the reach with bedrock substrate. Site OSG2 stood out with 35% bedrock substrate while the other three sites with considerable bedrock had 15% or less.

Change of habitat was a frequent theme in our visits to the sites. Some of the changes were due to flood flows and some were due to direct human influence. Flood flows changed the channel pattern somewhat at all sites. The biggest changes occurred at SPG1, OSG4, and OSG5. At SPG1 the changes were mostly due to flashy flood flows and consisted of a large log jam that was frequently pushed out and replaced with newly fallen trees and brush. The channel changed courses a couple of times during the study but was always in the same general pattern when sampled for biotics. This frequent changing is likely due to hydrologic regime change caused by urban landuse. This site also experienced some direct impact from repairs to a part of the

adjacent lake embankment that was heavily eroded during high flows. Visible impacts to the area immediately upstream were short term and gone after a couple of storm events. At OSG4 the area underwent extreme change of habitat due to transient trees and log jams as well as direct human influence. Areas that were deep scour pool at the beginning of the study were shallow riffles by the end due to root wads and entire trees washing through the reach. Just prior to the Spring 2009 sampling the stream was impacted in the middle of the sampling reach by an adjacent landowner creating a crossing by pushing bank material into the stream and moving material in-stream with a bulldozer. Approximately 200 ft of stream were affected by the immediate physical impacts. Technicians who were checking and deploying equipment and observed the event noted that water turbidity was noticeably increased at OSG5. Site OSG5 suffered from frequent movement of large woody debris though the reach just like OSG4. Prior to the Summer 2009 sampling event as part of the construction of pipelines for the NACA water treatment plant a low water crossing was placed at the upstream end of the sampling reach. This dramatically changed the nature of the upstream portion of the site creating a large scour pool just downstream of the crossing. Increased shallow habitat was created just downstream of the scour pool due to the deposition of the bed-load from the scour area.

3.4 Periphyton Discussion

Multiple methods were used for describing the periphyton communities at each site. Passive diffusion periphytometers (PDPs) were used to explore the possibility of nutrient limitation at sites as well as to explore scour and grazer excluded ambient periphyton growth. Natural substrate was sampled using ash-free dry mass and chlorophyll a methods to describe the standing crop periphyton mass.

In regards to the nutrient limitation no sites had statistically significant results suggesting nutrient limitation. Many sites during multiple seasons had variability in the nutrient treatments that suggested response to the treatments but the means were not statistically different than the controls. This suggests that some factor other than nutrients is limiting periphyton growth in the system. Possibilities include temperature, light, turbidity, or some combination of these factors.

The control treatments from the PDPs were compared between sites for each season to determine if ambient periphyton growth was greater. During Critical Season 1 OSG4, SPG1, OSG2, and OSG5 were significantly higher than the reference sites, with OSG4 being significantly higher

than the other three sites listed above. During Critical Season 2 OSG5 and SPG3 were significantly higher than CSREF though OSG5 was not significantly higher than LOREF. During Critical Season 3 OSG4 and SPG3 were significantly higher than the reference sites. During Primary Season 1 OSG1 and SPG2 were significantly higher than CSREF while only OSG1 was significantly higher than LOREF. During Primary Season 2 SPG3 and OSG4 were significantly higher than the reference sites. Sites OSG4 and SPG3 appear to have the highest ambient periphyton growth from these results.

Natural substrate samples were collected to provide further understanding of periphyton standing crop in the system. Standing crop is affected by many things including nutrients, light, temperature, primary feeder grazing, and scour. The period sampled for this study included many and frequent high flow events. This appeared to have an impact on visible standing crop. Chlorophyll a provides the best assessment of periphyton primary producer standing crop. The chlorophyll a analysis shows very little as far as trends in increased standing crop at any given site. For Critical Season 1 and Primary Season 2 no sites were significantly different than the reference sites. Only SPG3 was significantly higher than the reference sites in Critical Season 2. In Critical Season 3 OSG2, OSG3, OSG4, OSG5 and SPG3 were significantly higher than the reference sites. In Primary Season 1 only SPG2 was significantly higher than the reference sites.

Ash-free dry mass analysis was also conducted on the natural substrate periphyton samples. During Critical Season 1, Primary Season 1, and Primary Season 2 no sites were statistically different than the reference sites. Site SPG3 was significantly higher during Critical Season 2. Sites OSG1, OSG2, OSG4, OSG5, and SPG3 were significantly higher than the reference sites during Critical Season 3. These analyses were from the same collections as the natural substrate chlorophyll a samples and were affected by the same factors in the streams.

Canopy cover is one of the factors that most directly influences periphyton growth on artificial and natural substrate. Measures of canopy cover varied by site and season for both natural and artificial substrate periphyton samples (Tables 2.34 and 2.35). Though it does not appear that all sites with decreased canopy cover always had increased periphyton production on artificial and natural substrate, there does seem to be a correlation in that the sites that did have increased periphyton were from sites with lower canopy cover for that event. It should be noted that this does not necessarily mean that the entire canopy cover for that site is low since periphyton was

sampled from singular locations, but it does indicate that canopy cover is an important factor for periphyton productivity.

3.5 Biotic Discussion

3.5.1 Benthic Macroinvertebrates Discussion

Osage Creek – Comparison of the average critical season invertebrate IBI scores for site OSG1 just above the Rogers WWTP (44) with the average score from downstream at OSG2 (34) indicates a significant decrease in water quality (Table 2.43). The pattern of the water quality indicated by the invertebrate IBI scores can be seen in Figure 2.14. The invertebrate IBI scores substantially rebounded farther downstream in the Osage Creek basin. The upstream site (OSG1) and the farthest downstream site (OSG5) compare favorably with the reference sites (LOREF and CSREF). This pattern of scores indicates that although the effluent from the Rogers WWTP may have caused a decrease in water quality immediately downstream from the plant discharge, water quality recovered farther down Osage Creek and before entering the Illinois River mainstream.

The habitat for the macroinvertebrate species assemblage at the OSG2 site below the Rogers WWTP is not as good as the habitat quality upstream or downstream from that site (Figure 2.10). There were simply no other suitable places for the site, especially because of the golf course downstream. At OSG2 there is a lot of bedrock and little bedload (gravel) to provide interstitial refugia from flow and predators. This confounding factor could be partly responsible for the observed pattern of macroinvertebrates. The invertebrate assemblage showed some recovery at OSG3 where there is much better physical habitat for them (average critical season IBI = 40, Table 2.22).

Spring Creek – The average invertebrate IBI score during critical seasons below the Springdale WWTP (29) although very low, was not quite as low as the average IBI at SPG1 above the plant (27, Table 2.43, Fig. 2.15) indicating that the Springdale WWTP effluent did not lower the water quality of its immediate receiving stream. However, the invertebrate community at SPG1 above the WWTP and SPG2 downstream were both in very poor condition compared to the reference sites' average IBI (47.5, Fig. 2.15). The reason(s) for the poor water quality at SPG1 are not clear, but may be related to the small reservoir near the site. The invertebrate community began to recover from these low values by the SPG3 site (36), and even more by the OSG5 site farther

downstream (42), which compares more favorably with the average critical season IBI scores of the reference sites (47.5). The habitat quality (Figure 2.09) at SPG2 below the Springdale WWTP is low compared to sites upstream and downstream principally in that, like OSG2 below the Rogers WWTP, there is insufficient gravel bedload at the site to provide interstitial refugia for the organisms.

3.5.2 Fish Discussion

Osage Creek – The average IBI scores for the fish assemblages above and below the Rogers WWTP (30.5 and 28.5 respectively) were not very dissimilar (Table 2.49, Fig. 2.16). However, they were lower than the average critical season scores for the reference streams (37.5). The fish IBI scores had increased substantially farther downstream (OSG4 = 37, OSG5 = 31) such that the slight impact seen below the plant did not continue down Osage Creek into the Illinois River.

The habitat at the OSG2 site is a potentially confounding factor for the fish as it is for the invertebrates, as explained earlier in this document. The poor habitat at this site could account for some of the decrease in fish IBI scores. Percent primary feeders, one of the biometrics, was very high at OSG2 (Ave. = 51.4) compared to the other sites, especially in the reference streams (Ave. = 9.6), contributing to the low scores at the site. This is an important metric because excess nutrients can result in excess periphyton, which is the food for "primary feeders" like stonerollers. However, the extensive bedrock at the OSG2 site is excellent substrate for the growth of periphyton. It is doubtful that the habitat, principally the extensive bedrock, accounted for a huge percentage of the average low scores seen there, but it probably was of some significance.

Spring Creek – The patterns of water quality indicated by analyses of the fish community are almost identical to those for the invertebrate community (Figs. 2.15 and 2.16). The fish assemblage at site SPG2 just below the WWTP compared to the fish assemblage just upstream (SPG1) indicated that the water quality below the plant was better, however both are very low compared to those farther downstream and compared to the reference streams. The fish data corroborate the invertebrate data indicating that although the fish assemblage shows low water quality below the plant, the receiving stream is even worse just upstream, so these data do not indicate that the Springdale effluent impairs the quality of the receiving stream. The fish IBI scores for OSG3 indicate that Spring Creek is reaching the level of water quality indicated by the reference stream IBI scores even before the confluence with Osage Creek. The previous

comments regarding the habitat quality at this site for the invertebrates apply in much the same way for the fish. The percentage of primary feeders during critical seasons was high (52 – 88%) at all the Spring Creek sites compared to other sites in the basin, especially the reference sites (9 – 22%).

The total fish IBI scores for this study (Table 2.49) generally fall within the ADEQ designated guidelines for the Ozark Highland streams of "25-36 Generally Similar", meaning that they are generally similar to other streams in this ecoregion regarding the water quality indicated by their fish community total metric scores. One of the two reference streams that we used were at the high end of this range (Little Osage Creek – LOREF) with scores averaging 36 during critical seasons and an overall average of 34 for all five of our collections (Table 2.49). The other reference stream (Chambers Spring – CSREF) scored a little higher and was in the lower end of the highest range for Ozark Highland streams "Mostly Similar" with an average critical season score of 39 and an overall average score of 37. It is becoming very difficult to find high quality reference streams in northwestern Arkansas. Only SPG1 and SPG2 scored in the lower category "13-24 Somewhat Similar", with SPG1 at the lower end of this scale and SPG2 nearer the upper end, suggesting that the fish community was improving immediately below the Springdale WWTP outfall. None of our Osage Creek Basin stream sites scored in the "12-0 Not Similar" category. Farther down Spring Creek at site SPG3 the fish community (and invertebrates, but without a scale for comparison throughout the ecoregion) indicate that the stream had recovered at least to the point of being generally similar to others in the ecoregion. Even farther downstream after confluence with Osage Creek (OSG4 and OSG5) the stream maintained this "generally similar" status. These results for the downstream areas of Spring and Osage Creeks are encouraging because these stream segments are classified as "Ecologically Sensitive Waterbodies" by ADEQ due to their being habitat for the Neosho mucket, a bivalve mollusk that is becoming quite rare and endangered (ADEQ REG. 2).

Section 4: Conclusion and Recommendations

The purpose of this project was to assess attainment of designated aquatic life use in Osage and Spring Creeks in Northwest Arkansas, particularly to evaluate if the cities of Springdale and Rogers, Arkansas WWTP discharges resulted in violations of ADEQ Reg. 2 Criteria. This project was designed to evaluate three tiers of impact: 1) above and below WWTPs of the Cities of Rogers and Springdale, Arkansas; 2) sites below WWTPs compared to reference conditions; and 3) gradients across stream reaches from upstream to downstream.

The results clearly indicated that there are no upstream-downstream impacts from the WWTPs that rise to the level of impairment of water quality (Tier 1). The assessment of Tier 2 Impacts, comparing reference stream conditions to all sites, showed generally higher levels of nutrients at test sites (with the exception of nitrogen when compared to LOREF), lower dissolved oxygen depression and larger diurnal swings, higher standing crops and rates of growth of periphyton, and lower biotic IBI scores. The Tier 3 assessment of the reach continuum from upstream to downstream showed that the impact of the Rogers WWTP in Osage Creek (OSG2) across all metrics was not significant, and any decline in metrics observed was fully or close to fully recovered by the lower site (OSG5). The Springdale WWTP discharge actually appeared to improve water quality in the stream from SPG1, and like Osage Creek, all metrics recovered by OSG5.

Results of the water quality assessment showed no violations of ADEQ Reg. 2 Criteria, with the exception of SPG1 for DO during Critical Season 1. All other observations across all other sites met the criteria for designated use for water quality during all observation periods. The conclusion is that there is no evidence that discharge of wastewater from the Rogers WWTP to Osage Creek or the Springdale WWTP to Spring Creek results in any violation of water quality standards according to the criteria of ADEQ Reg. 2. There appears to be no justification from this data for placing Spring and Osage Creeks on the 303(d) list of impaired waters for impairment by nutrients.

Section 5: References

ADEQ 2002. Integrated Water Quality Monitoring and Assessment Report 2002, Arkansas Department of Environmental Quality.

APHA 2005. Standard methods for the examination of water and wastewater. 21st ed. American Public Health Assoc., Washington, DC.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Brown, A. V. and K. B. Brown. 1984. Distribution of insects within riffles of streams. Freshwater Invertebrate Biology 3: 2-11.

Brown, A. V. and W. J. Matthews. 1995. Streams of the Central United States. In: Cushing, C. E., K. W. Cummins and G. W. Minshall (eds.). River and Stream Ecosystems. Elsevier Press, Amsterdam.

Brown, A. V., M. M. Lyttle, and K. B. Brown. 1998. Impacts of gravel mining on gravel bed streams. Transactions of the American Fisheries Society 127: 981-996.

Brussock, P. P. and A. V. Brown. 1991. Riffle-pool geomorphology disrupts longitudinal patterns of stream benthos. Hydrobiologia 220: 109-117.

Chaubey, I., D. Sahoo, B. Haggard, K. White, and M. Matlock. 2003. Assessment of nutrient retention in an agriculturally dominated stream. Proceedings of the Arkansas Water Resources Center Annual Conference, April 2002. AWRC, University of Arkansas, Fayetteville, AR.

Chaubey, I., D. Sahoo, B. Haggard, M. Matlock, and T. Costello. 2007. Nutrient-Retention, Nutrient-Limitation, and Sediment-Nutrient Interactions in a Pasture Dominated Stream. Transactions of the ASABE. 50(1): 35-44.

Dogwiler, T., 2003. Fluvial Disturbances in Karst Streams: Sediment Transport Processes. Proceedings of the Geological Society of America. Denver, Colorado, Oct. 27-30, 2003.

Dorioz, J. M., E. A. Cassell, A. Orand, and K. G. Eisenman. 1998. Phosphorus storage, transport and export dynamics in the Foron River watershed. Hydrological Processes 12: 285-309.

Ekka, S.A., B.E. Haggard, M.D. Matlock, and I. Chaubey, 2006. Dissolved phosphorus concentrations and sediment-interactions in effluent dominated Ozark streams. Ecological Engineering 26(4):375-391.

Haggard, B.E. 2000. Stream nutrient retention in the Eucha-Spavinaw Basin. Ph.D. Dissertation, Oklahoma State University, Stillwater, Oklahoma.

Haggard, B.E., D.E. Storm, and E.H. Stanley. 2001. Effect of a point source input on stream nutrient retention. Journal of American Water Resources Association 37:1291-1299.

Haggard, B. E, K. L White, I Chaubey, M. D Matlock, P. B DeLaune, and P. A Moore. 2003a, Phosphorus sources in an Ozark catchment, USA: have we forgotten phosphorus from discrete sources?: Pages 54–59 in Proceedings Report, Theme 14, International Water Association Annual Meeting, Dublin, Ireland. International Water Association Publishing, London, UK. (CDrom available from: International Water Alliance, Alliance House, 12 Caxton St., London SW1H 0QS UK.).

Haggard, B.E., Moore, Jr. P.A., Chaubey, I., and Stanley, E.H. 2003b. Nitrogen and phosphorus concentrations and export in an Ozark Plateaus catchment in the United States. Biosystems Engineering 86(1):75-85.

Haggard, B., T. Soerens, W. Green, and R. Richards. 2003c. Using regression methods to estimate stream phosphorus loads at the Illinois River, Arkansas. Applied Engineering in Arkansas 19(2): 187-194.

Haggard, B.E. 2005. Effect of reduced effluent phosphorus concentrations at the Illinois River, Arkansas. Proceedings Report, ASAE Watershed Management to Meet TMDLs and Emerging Water Quality Standards, Albuquerque, New Mexico.

Haggard, B.E., Stanley, E.H., and Storm, D.E. 2005. Nutrient retention in a point source enriched stream. Journal of the North American Benthological Society 24:29-47.

Haggard, B., S. Ekka, M. Matlock, I. Chaubey. 2005. Phosphate equilibrium between stream sediments and water: Potential effect of chemical amendments. Transactions of ASAE, 47(4):1113-1118.

Haggard, B. and T. Soerens. 2006. Sediment phosphorus release at a small impoundment on the Illinois River, Arkansas and Oklahoma, USA. Biological Engineering 28: 280-287.

Haggard, B.E., Smith, D.R. and Brye, K.R. 2007. Variations in stream water and sediment phosphorus among select Ozark catchments. Journal of Environmental Quality 36: 1725-1734.

House, W. A., and F. H. Denison. 1997. Nutrient dynamics in a lowland stream impacted by sewage effluent: Great Ouse, England. The Science of the Total Environment 205: 25-49.

Hynes, H. B. N. 1960. The Biology of Polluted Waters. Liverpool University Press, London. 202 pp.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 66:21-27.

Ludwig, A., M. D. Matlock, B. Haggard, M. E. Matlock, E. Cummings. 2008. Identification and evaluation of nutrient limitation of periphyton growth in headwater streams in the Pawnee Nation, Oklahoma. *Journal of Ecological Engineering.* 32(2008): 178-186.

Merritt, R. W., K. W. Cummins and M. B. Berg (eds.) 2008. An Introduction to the Aquatic Insects of North America (4th edition). Kendall/Hunt Publishing Company, Dubuque, IA. 1158 pp.

Migliaccio, K., B. Haggard, I. Chaubey, and M. Matlock. 2007. Water quality monitoring at the War Eagle Creek Watershed, Beaver Reservoir, Arkansas. Transactions of the ASABE. 50(6):2007-2016.

Pflieger, W. L. 1975. The Fishes of Missouri. Missouri Department of Conservation, publisher, Columbia, MO. 343 pp.

Poulton, B. C. and K. W. Stewart. 1991. The Stoneflies of the Ozark and Ouachita Mountains (Plecoptera). The American Entomological Society, publisher. Philadelphia, PA. 116 pp.

Reddy, K. R., E. Flaig, L. J. Scinto, O. Diaz, and T. A. DeBusk. 1996. Phosphorus assimilation in a stream system of the Lake Okeechobee Basin. Water Resources Bulletin, American Water Resources Association 32(5): 901-915.

Robison, H. W. and T. M. Buchanan. 1992. Fishes of Arkansas. The University of Arkansas Press, Fayetteville, AR. 536 pp.

Rodriguez, A. and M. Matlock, 2008. Measuring Temporal Variability in Algal Nutrient Response in the Lake Waco/Bosque River Watershed. *Journal of Biological Engineering. 2:1 (11Jan2008).*

Roop, R. D. and C. T. Hunsaker. 1985. Biomonitoring for toxics control in NPDES permitting. Journal of the Water Pollution Control Federation 54: 271-277.

Rosgen, D. 2006. Watershed Assessment of River Stability and Sediment Supply (WARSSS). Wildland Hydrology, Fort Collins, CO.

Sen, S., B.E. Haggard, I. Chaubey, K.R. Brye, T.A. Costello, and M.D. Matlock. 2006. Preliminary investigation into sediment phosphorus release at Beaver Reservoir, Northwest Arkansas, 2002-3. Water, Air, & Soil Pollution, 179:67-77.

Smith, D. G. 2001. Pennak's Freshwater Invertebrates of the United States (4th edition). John Wiley & Sons, Inc., New York, NY. 638 pp.

Thorp, J. H. and A. P. Covich (eds.). 2001. Ecology and Classification of North American Freshwater Invertebrates (2nd edition). Academic Press, San Diego, CA. 1056 pp.

USEPA, 2004. Water Quality and Biological Assessment of Selected Segments in the Illinois River Basin and Kings River Basin, Arkansas. http://www.epa.gov/region6/water/ecopro/watershd/monitrng/studies/ill_kings_finrpt.pdf

USGS Report 98-4164, 1998a. United States Geological Survey (USGS), Water-Quality Assessment of the Ozark Plateaus Study Unit, Arkansas, Kansas, Missouri, and Oklahoma – Nutrients, Bacteria, Organic Carbon, and Suspended Sediment in Surface Water, 1993-95.

USGS Report 98-4155, 1998b. USGS Water-Quality Assessment of the Ozark Plateaus Study Unit, Arkansas, Kansas, Missouri, and Oklahoma – Fish Communities in Streams and their relations to Selected Environmental Factors, 1998.

USGS 1999, The Quality of Our Nation's Waters—Nutrients and Pesticides: U.S. Geological Survey Circular 1225, 82 p.

USGS 2002. Relations of Benthic Macroinvertebrates to Concentrations of Trace Elements in Water, Streambed Sediments, and Transplanted Bryophytes and Stream Habitat Conditions in Nonmining and Mining Areas of the Upper Colorado River Basin, Colorado, 1995–98 By Scott V. Mize and Jeffrey R. Deacon. Water-Resources Investigations Report 02–4139. USGS, Denver, CO.

White, K., B. Haggard, M. Matlock, J. Kim. 2005. Periphytic chlorophyll-a response to triclosan exposure: application of a passive diffusion periphytometer. Applied Engineering in Agriculture. 21(2):307-311.

Wiggins, Glenn B. 1978. Larvae of the North American Caddisfly Genera (Trichoptera). University of Toronto Press, Canada. 401 pp.

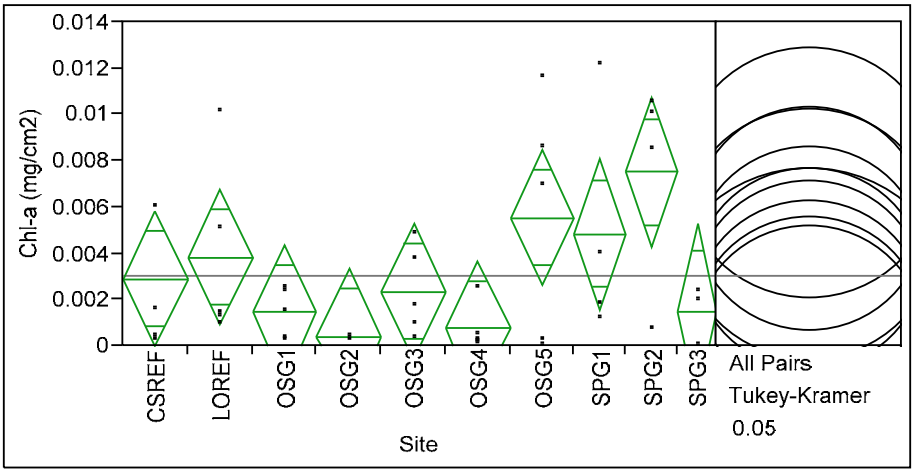
Appendix F

Natural Substrate Periphyton Results

Natural Substrate Chlorophyll-a Analysis

Natural Substrate Chlorophyll-a Comparisons: Critical Season 1

Oneway Analysis of Chl-a (mg/cm2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.362979
Adj Rsquare	0.203724
Root Mean Square Error	0.003203
Mean of Response	0.003045
Observations (or Sum Wgts)	46

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	0.00021050	0.000023	2.2792	0.0386*
Error	36	0.00036942	0.000010		
C. Total	45	0.00057991			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	0.002890	0.00143	-1.6e-5	0.00580
LOREF	5	0.003827	0.00143	0.00092	0.00673
OSG1	5	0.001447	0.00143	-0.0015	0.00435
OSG2	5	0.000415	0.00143	-0.0025	0.00332
OSG3	5	0.002359	0.00143	-0.0005	0.00526
OSG4	5	0.000746	0.00143	-0.0022	0.00365
OSG5	5	0.005550	0.00143	0.0026	0.00846
SPG1	4	0.004837	0.00160	0.0016	0.00808
SPG2	4	0.007511	0.00160	0.0043	0.01076
SPG3	3	0.001500	0.00185	-0.0023	0.00525

Std Error uses a pooled estimate of error variance

Natural Substrate Chlorophyll-a Comparisons: Critical Season 1

Comparison of Chl-a (mg/cm2) By Site

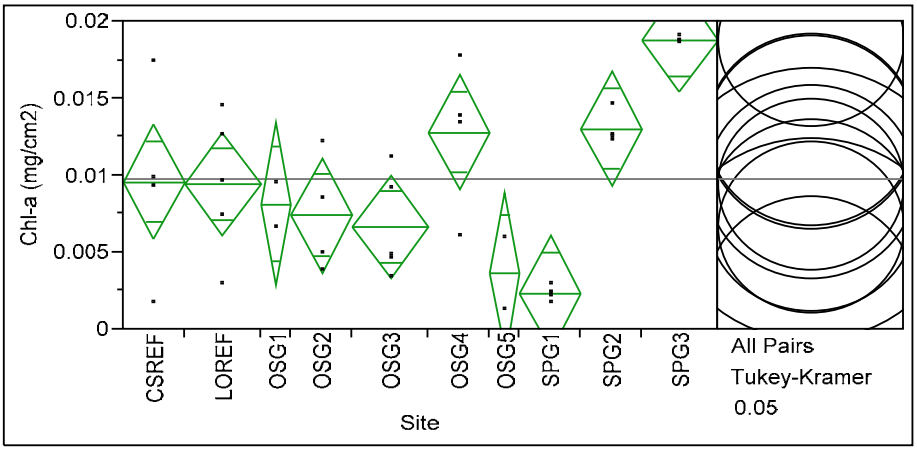
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
SPG2	A	0.00751140
OSG5	A	0.00555003
SPG1	A	0.00483652
LOREF	A	0.00382654
CSREF	A	0.00288963
OSG3	A	0.00235904
SPG3	A	0.00150003
OSG1	A	0.00144663
OSG4	A	0.00074590
OSG2	A	0.00041461

Levels not connected by same letter are significantly different.

Natural Substrate Chlorophyll-a Comparisons: Critical Season 2

Oneway Analysis of Chl-a (mg/cm2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.689344
Adj Rsquare	0.592933
Root Mean Square Error	0.003648
Mean of Response	0.009726
Observations (or Sum Wgts)	39

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	0.00085643	0.000095	7.1501	<.0001*
Error	29	0.00038596	0.000013		
C. Total	38	0.00124239			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	4	0.009595	0.00182	0.0059	0.01333
LOREF	5	0.009468	0.00163	0.0061	0.01280
OSG1	2	0.008124	0.00258	0.0028	0.01340
OSG2	4	0.007422	0.00182	0.0037	0.01115
OSG3	5	0.006675	0.00163	0.0033	0.01001
OSG4	4	0.012825	0.00182	0.0091	0.01656
OSG5	2	0.003660	0.00258	-0.0016	0.00894
SPG1	4	0.002382	0.00182	-0.0013	0.00611
SPG2	4	0.013036	0.00182	0.0093	0.01677
SPG3	5	0.018799	0.00163	0.0155	0.02214

Std Error uses a pooled estimate of error variance

Natural Substrate Chlorophyll-a Comparisons: Critical Season 2

Comparison of Chl-a (mg/cm2) By Site

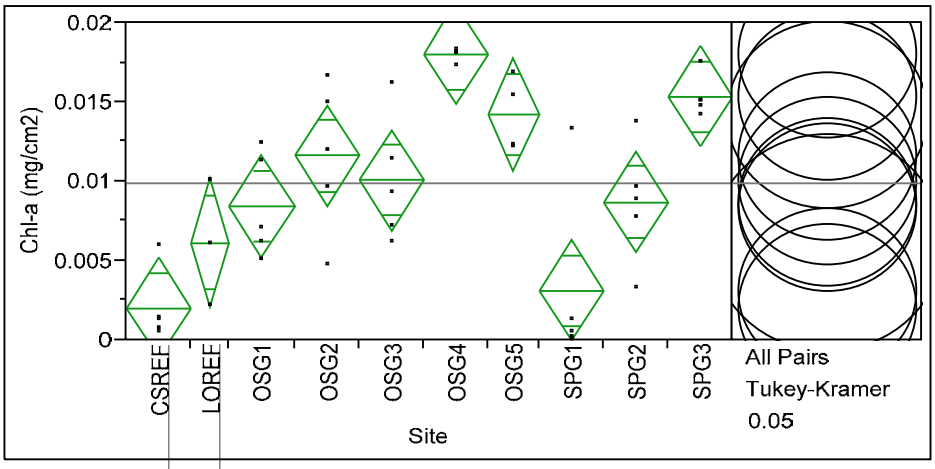
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
SPG3	A			0.01879884
SPG2	A	B		0.01303558
OSG4	A	B		0.01282472
CSREF		B	C	0.00959506
LOREF		B	C	0.00946800
OSG1		B	C	0.00812449
OSG2		B	C	0.00742245
OSG3		B	C	0.00667549
OSG5		B	C	0.00366005
SPG1			C	0.00238171

Levels not connected by same letter are significantly different.

Natural Substrate Chlorophyll-a Comparisons: Critical Season 3

Oneway Analysis of Chl-a (mg/cm2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.71979
Adj Rsquare	0.651631
Root Mean Square Error	0.003506
Mean of Response	0.009834
Observations (or Sum Wgts)	47

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	0.00116836	0.000130	10.5604	<.0001*
Error	37	0.00045483	0.000012		
C. Total	46	0.00162319			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	0.002020	0.00157	-0.0012	0.00520
LOREF	3	0.006160	0.00202	0.0021	0.01026
OSG1	5	0.008438	0.00157	0.0053	0.01161
OSG2	5	0.011618	0.00157	0.0084	0.01480
OSG3	5	0.010099	0.00157	0.0069	0.01328
OSG4	5	0.018017	0.00157	0.0148	0.02119
OSG5	4	0.014216	0.00175	0.0107	0.01777
SPG1	5	0.003115	0.00157	-0.0001	0.00629
SPG2	5	0.008701	0.00157	0.0055	0.01188
SPG3	5	0.015362	0.00157	0.0122	0.01854

Std Error uses a pooled estimate of error variance

Natural Substrate Chlorophyll-a Comparisons: Critical Season 3

Comparison of Chl-a (mg/cm2) By Site

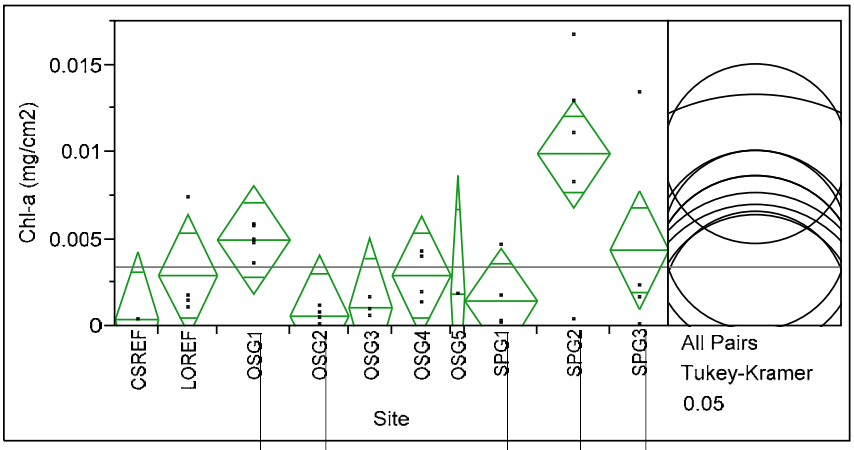
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
OSG4	A					0.01801724
SPG3	A	B				0.01536188
OSG5	A	B	C			0.01421562
OSG2	A	B	C			0.01161829
OSG3		B	C	D		0.01009853
SPG2		B	C	D	E	0.00870103
OSG1		B	C	D	E	0.00843792
LOREF			C	D	E	0.00615970
SPG1				D	E	0.00311544
CSREF					E	0.00201965

Levels not connected by same letter are significantly different.

Natural Substrate Chlorophyll-a Comparisons: Primary Season 1

Oneway Analysis of Chl-a (mg/cm2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.5083
Adj Rsquare	0.350253
Root Mean Square Error	0.003356
Mean of Response	0.003439
Observations (or Sum Wgts)	38

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	0.00032610	0.000036	3.2161	0.0084*
Error	28	0.00031545	0.000011		
C. Total	37	0.00064154			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	3	0.000353	0.00194	-0.0036	0.00432
LOREF	4	0.002936	0.00168	-0.0005	0.00637
OSG1	5	0.004962	0.00150	0.0019	0.00804
OSG2	4	0.000618	0.00168	-0.0028	0.00406
OSG3	3	0.001049	0.00194	-0.0029	0.00502
OSG4	4	0.002902	0.00168	-0.0005	0.00634
OSG5	1	0.001824	0.00336	-0.0051	0.00870
SPG1	5	0.001415	0.00150	-0.0017	0.00449
SPG2	5	0.009893	0.00150	0.0068	0.01297
SPG3	4	0.004367	0.00168	0.00093	0.00780

Std Error uses a pooled estimate of error variance

Natural Substrate Chlorophyll-a Comparisons: Primary Season 1

Comparison of Chl-a (mg/cm2) By Site

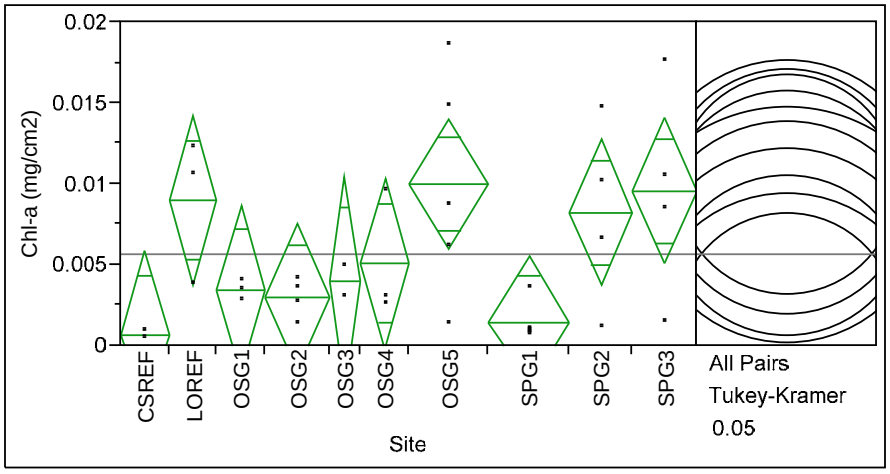
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level			Mean
SPG2	A		0.00989286
OSG1	A	B	0.00496155
SPG3	A	B	0.00436711
LOREF	A	B	0.00293566
OSG4	A	B	0.00290186
OSG5	A	B	0.00182386
SPG1		B	0.00141511
OSG3		B	0.00104884
OSG2		B	0.00061826
CSREF		B	0.00035268

Levels not connected by same letter are significantly different.

Natural Substrate Chlorophyll-a Comparisons: Primary Season 2

Oneway Analysis of Chl-a (mg/cm2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.458428
Adj Rsquare	0.27096
Root Mean Square Error	0.004388
Mean of Response	0.005657
Observations (or Sum Wgts)	36

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	0.00042379	0.000047	2.4454	0.0362*
Error	26	0.00050065	0.000019		
C. Total	35	0.00092444			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	3	0.000688	0.00253	-0.0045	0.00590
LOREF	3	0.008968	0.00253	0.0038	0.01418
OSG1	3	0.003497	0.00253	-0.0017	0.00870
OSG2	4	0.003032	0.00219	-0.0015	0.00754
OSG3	2	0.004037	0.00310	-0.0023	0.01042
OSG4	3	0.005127	0.00253	-0.0001	0.01033
OSG5	5	0.009996	0.00196	0.0060	0.01403
SPG1	5	0.001483	0.00196	-0.0026	0.00552
SPG2	4	0.008235	0.00219	0.0037	0.01274
SPG3	4	0.009573	0.00219	0.0051	0.01408

Std Error uses a pooled estimate of error variance

Natural Substrate Chlorophyll-a Comparisons: Primary Season 2

Comparison of Chl-a (mg/cm2) By Site

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

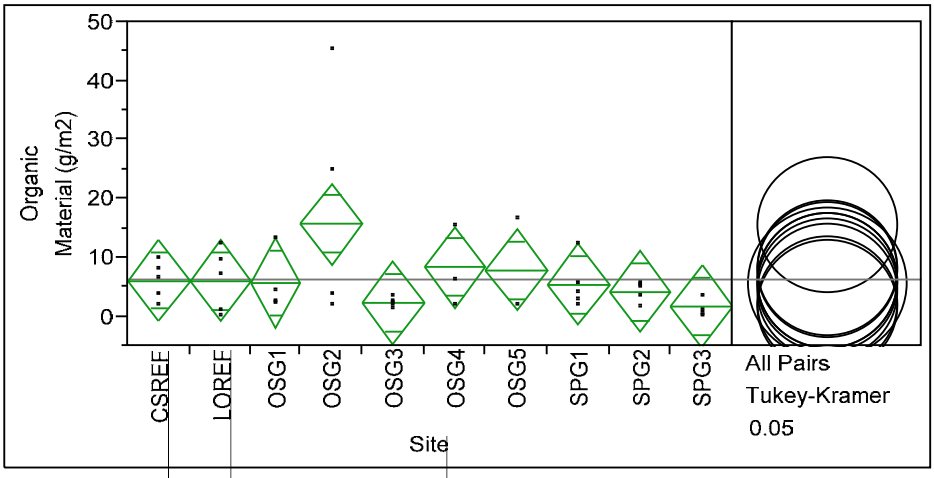
Level		Mean
OSG5	A	0.00999603
SPG3	A	0.00957264
LOREF	A	0.00896799
SPG2	A	0.00823469
OSG4	A	0.00512705
OSG3	A	0.00403698
OSG1	A	0.00349664
OSG2	A	0.00303188
SPG1	A	0.00148267
CSREF	A	0.00068798

Levels not connected by same letter are significantly different.

Ash Free Dry Mass

Ash Free Dry Mass Comparisons: Critical Season 1

Oneway Analysis of Organic Material (g/m2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.230705
Adj Rsquare	0.053176
Root Mean Square Error	7.617625
Mean of Response	6.372031
Observations (or Sum Wgts)	49

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	678.6860	75.4096	1.2995	0.2684
Error	39	2263.1002	58.0282		
C. Total	48	2941.7862			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	6.1479	3.4067	-0.743	13.039
LOREF	5	6.1064	3.4067	-0.784	12.997
OSG1	4	5.6906	3.8088	-2.013	13.395
OSG2	5	15.6531	3.4067	8.762	22.544
OSG3	5	2.3438	3.4067	-4.547	9.235
OSG4	5	8.3150	3.4067	1.424	15.206
OSG5	5	7.8993	3.4067	1.009	14.790
SPG1	5	5.4152	3.4067	-1.476	12.306
SPG2	5	4.2718	3.4067	-2.619	11.163
SPG3	5	1.7410	3.4067	-5.150	8.632

Std Error uses a pooled estimate of error variance

Ash Free Dry Mass Comparisons: Critical Season 1

Comparison of Organic Material (g/m2) By Site

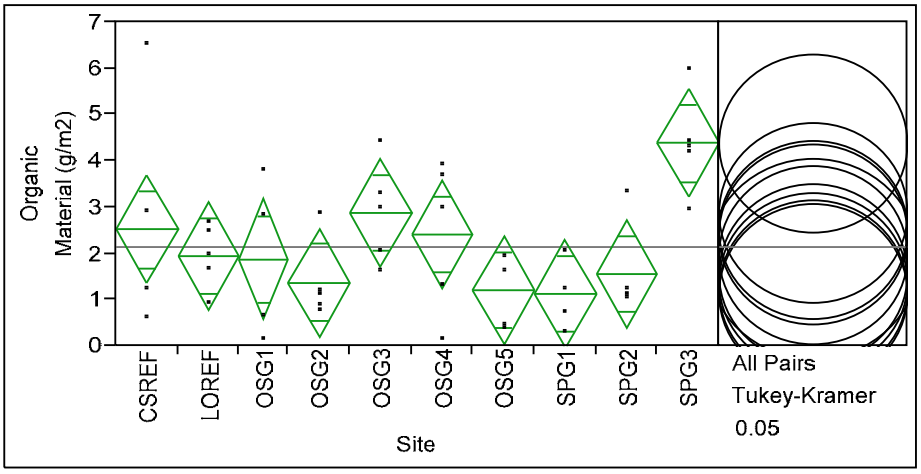
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
OSG2	A	15.653051
OSG4	A	8.315034
OSG5	A	7.899282
CSREF	A	6.147928
LOREF	A	6.106353
OSG1	A	5.690601
SPG1	A	5.415166
SPG2	A	4.271849
OSG3	A	2.343800
SPG3	A	1.740960

Levels not connected by same letter are significantly different.

Ash Free Dry Mass Comparisons: Critical Season 2

Oneway Analysis of Organic Material (g/m2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.400431
Adj Rsquare	0.262068
Root Mean Square Error	1.289514
Mean of Response	2.133556
Observations (or Sum Wgts)	49

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	43.31160	4.81240	2.8941	0.0101*
Error	39	64.85096	1.66285		
C. Total	48	108.16256			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	2.51530	0.57669	1.349	3.6818
LOREF	5	1.95403	0.57669	0.788	3.1205
OSG1	4	1.87088	0.64476	0.567	3.1750
OSG2	5	1.37891	0.57669	0.212	2.5454
OSG3	5	2.87562	0.57669	1.709	4.0421
OSG4	5	2.41829	0.57669	1.252	3.5848
OSG5	5	1.21261	0.57669	0.046	2.3791
SPG1	5	1.12253	0.57669	-0.044	2.2890
SPG2	5	1.55907	0.57669	0.393	2.7255
SPG3	5	4.37579	0.57669	3.209	5.5422

Std Error uses a pooled estimate of error variance

Ash Free Dry Mass Comparisons: Critical Season 2

Comparison of Organic Material (g/m2) By Site

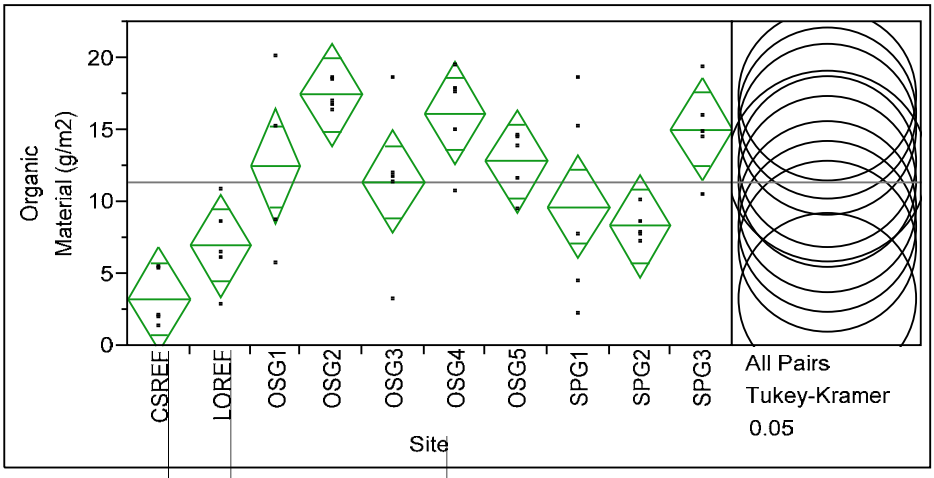
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level			Mean
SPG3	A		4.3757865
OSG3	A	B	2.8756158
CSREF	A	B	2.5152977
OSG4	A	B	2.4182890
LOREF	A	B	1.9540329
OSG1	A	B	1.8708826
SPG2		B	1.5590688
OSG2		B	1.3789098
OSG5		B	1.2126091
SPG1		B	1.1225296

Levels not connected by same letter are significantly different.

Ash Free Dry Mass Comparisons: Critical Season 3

Oneway Analysis of Organic Material (g/m2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.587248
Adj Rsquare	0.491997
Root Mean Square Error	3.952179
Mean of Response	11.34125
Observations (or Sum Wgts)	49

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	866.7026	96.3003	6.1653	<.0001*
Error	39	609.1691	15.6197		
C. Total	48	1475.8717			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	3.2775	1.7675	-0.30	6.853
LOREF	5	6.9777	1.7675	3.40	10.553
OSG1	4	12.4726	1.9761	8.48	16.470
OSG2	5	17.4477	1.7675	13.87	21.023
OSG3	5	11.3985	1.7675	7.82	14.974
OSG4	5	16.1589	1.7675	12.58	19.734
OSG5	5	12.8329	1.7675	9.26	16.408
SPG1	5	9.6870	1.7675	6.11	13.262
SPG2	5	8.3358	1.7675	4.76	11.911
SPG3	5	15.0502	1.7675	11.48	18.625

Std Error uses a pooled estimate of error variance

Ash Free Dry Mass Comparisons: Critical Season 3

Comparison of Organic Material (g/m2) By Site

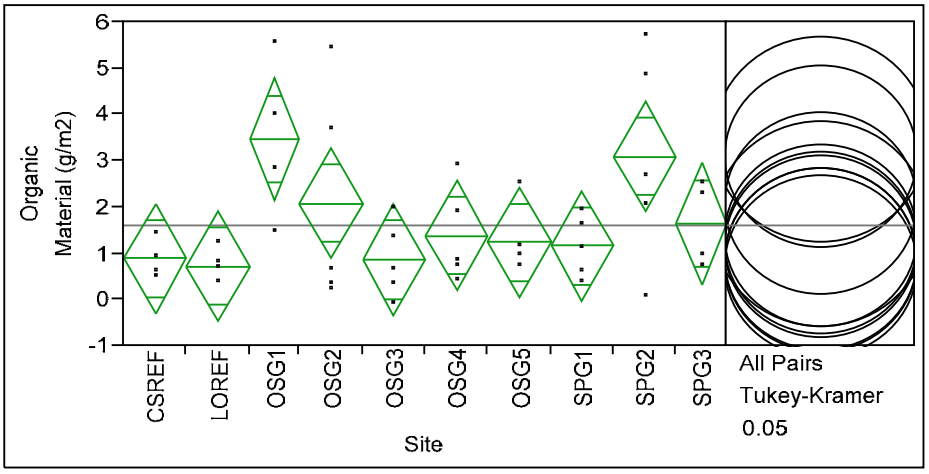
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
OSG2	A				17.447713
OSG4	A	B			16.158882
SPG3	A	B	C		15.050211
OSG5	A	B	C		12.832869
OSG1	A	B	C		12.472551
OSG3	A	B	C	D	11.398525
SPG1	A	B	C	D	9.687014
SPG2		B	C	D	8.335821
LOREF			C	D	6.977699
CSREF				D	3.277509

Levels not connected by same letter are significantly different.

Ash Free Dry Mass Comparisons: Primary Season 1

Oneway Analysis of Organic Material (g/m2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.362437
Adj Rsquare	0.211436
Root Mean Square Error	1.315392
Mean of Response	1.621504
Observations (or Sum Wgts)	48

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	37.37698	4.15300	2.4002	0.0290*
Error	38	65.74978	1.73026		
C. Total	47	103.12676			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	0.89387	0.58826	-0.297	2.0847
LOREF	5	0.72757	0.58826	-0.463	1.9184
OSG1	4	3.48192	0.65770	2.150	4.8134
OSG2	5	2.08915	0.58826	0.898	3.2800
OSG3	5	0.86615	0.58826	-0.325	2.0570
OSG4	5	1.38584	0.58826	0.195	2.5767
OSG5	5	1.24033	0.58826	0.049	2.4312
SPG1	5	1.16410	0.58826	-0.027	2.3550
SPG2	5	3.09735	0.58826	1.906	4.2882
SPG3	4	1.64568	0.65770	0.314	2.9771

Std Error uses a pooled estimate of error variance

Ash Free Dry Mass Comparisons: Primary Season 1

Comparison of Organic Material (g/m2) By Site

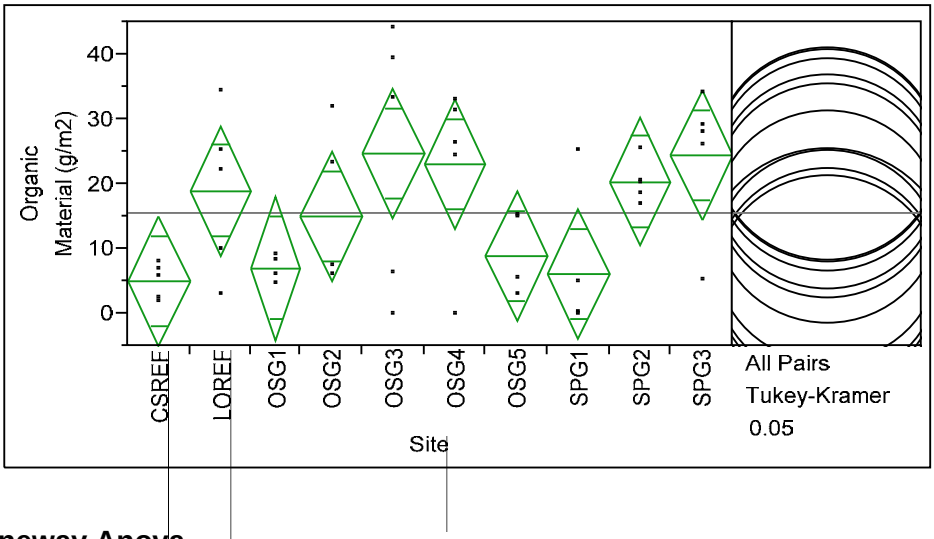
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
OSG1	A	3.4819204
SPG2	A	3.0973501
OSG2	A	2.0891522
SPG3	A	1.6456838
OSG4	A	1.3858390
OSG5	A	1.2403259
SPG1	A	1.1641047
CSREF	A	0.8938661
OSG3	A	0.8661494
LOREF	A	0.7275655

Levels not connected by same letter are significantly different.

Ash Free Dry Mass Comparisons: Primary Season 2

Oneway Analysis of Organic Material (g/m2) By Site



Oneway Anova
Summary of Fit

Rsquare	0.371815
Adj Rsquare	0.226849
Root Mean Square Error	10.98835
Mean of Response	15.53342
Observations (or Sum Wgts)	49

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	9	2787.1976	309.689	2.5648	0.0201*
Error	39	4709.0066	120.744		
C. Total	48	7496.2042			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
CSREF	5	5.0375	4.9141	-4.90	14.977
LOREF	5	18.9583	4.9141	9.02	28.898
OSG1	4	7.0591	5.4942	-4.05	18.172
OSG2	5	14.9948	4.9141	5.05	24.935
OSG3	5	24.6957	4.9141	14.76	34.635
OSG4	5	23.0534	4.9141	13.11	32.993
OSG5	5	8.8486	4.9141	-1.09	18.788
SPG1	5	6.1115	4.9141	-3.83	16.051
SPG2	5	20.3857	4.9141	10.45	30.325
SPG3	5	24.4947	4.9141	14.55	34.434

Std Error uses a pooled estimate of error variance

Ash Free Dry Mass Comparisons: Primary Season 2

Comparison of Organic Material (g/m2) By Site

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
OSG3	A	24.695650
SPG3	A	24.494704
OSG4	A	23.053431
SPG2	A	20.385691
LOREF	A	18.958277
OSG2	A	14.994778
OSG5	A	8.848582
OSG1	A	7.059117
SPG1	A	6.111550
CSREF	A	5.037525

Levels not connected by same letter are significantly different.

ARKANSAS DEPARTMENT OF ENERGY AND ENVIRONMENTAL, DIVISION OF ENVIRONMENTAL QUALITY

RE: FRL-comment on FRL–11994–01–R6

Exhibit B - *King, RS. 2016. Oklahoma-Arkansas Scenic Rivers Joint Phosphorus Study: Final Report*.

Final Report to Governors from the Joint Study Committee and Scientific Professionals

Summary and Recommendations

The committee met between October 2013 and December 2016, selected qualified scientific professionals, developed a scope of work, completed the 2-year joint study, reviewed the results and used a weight of evidence approach to recommend a six-month average total phosphorus level of not to exceed 0.035 milligrams per liter based on water samples collected during critical conditions was necessary to protect the designated [Oklahoma] Scenic Rivers.

Respectfully Approved December 19, 2016

Brian Haggard, PhD
Co-Chair
Arkansas

Derek Smithee
Co-Chair
Oklahoma

Ryan Benefield
Arkansas

Shellie Chard
Oklahoma

Marty Matlock, PhD
Arkansas

Shanon Phillips
Oklahoma

Ryan King, PhD
Baylor University

Technical Summary and Recommendations

Introduction

The six Oklahoma Scenic Rivers, particularly the Illinois River Watershed, have been a focus of conservation and management efforts to improve water quality by Arkansas and Oklahoma. The Illinois River Watershed is a trans-boundary watershed in the Ozark Plateaus with its headwaters in northwest Arkansas, and this watershed includes three of the designated Oklahoma Scenic Rivers – the Illinois River, Flint Creek and Baron Fork. The other Oklahoma Scenic Rivers include Little Lee Creek and Lee Creek in the watershed to the south of the Illinois River Watershed, as well as the Mountain Fork further south in the Ouachita Mountains. However, the focus of the environmental issues, elevated phosphorus (P) concentrations in the streams and rivers, and management have centered on the trans-boundary Illinois River Watershed.

In 2003, the states signed the [first] ***Joint Statement of Principles and Actions*** stating the shared goal of improving water quality in the Illinois River Watershed, resulting in effluent total phosphorus (TP) limits of 1 mg L^{-1} on municipal facilities with a design capacity of greater than 1 million gallons per day (MGD) and Arkansas passing legislation and regulations on poultry litter management. The management changes in the Illinois River Watershed improved water quality, reducing phosphorus concentrations and loads in the Illinois River (Haggard, 2010; Scott et al., 2011). The changes in TP concentrations and loads were subsequent to changes in effluent P inputs from one facility upstream in northwest Arkansas [i.e., Springdale's wastewater treatment plan (WWTP)] (Scott et al., 2011) to which elevated TP concentrations could be traced upstream (see Haggard, 2010).

However, TP concentrations in the Illinois River and select tributaries were still greater than the numeric TP criteria (0.037 mg L^{-1}, OWRB, 2002, OAC 785:45) applicable to Oklahoma's Scenic Rivers seasonally in 2009 and dependent upon flow conditions (Scott et al., 2011). Continuing in a collaborative fashion, the states then adopted a ***Second Statement of Joint Principles and Actions*** (hereafter, ***Second Statement***) in 2013 augmenting the first agreement, providing a three-year extension of commitments. The premise of the Second Statement included the governors' appointment of six individuals to the "**JOINT STUDY COMMITTEE**" who were required to reach agreement on the procurement, execution and conduct of the "**JOINT STUDY**" as defined with the terms of the *Second Statement*. The costs (i.e, $600,000) of the **JOINT STUDY** were paid for by Arkansas parties and funds placed in repository with the Arkansas-Oklahoma Arkansas River Compact Commission. The **JOINT STUDY COMMITTEE** was authorized to formulate the scope of work and select qualified scientific professionals (who do not reside in nor principal business locations within the states) to conduct the **JOINT STUDY**.

The **JOINT STUDY** included mandatory components as defined in the *Second Statement* which guided the formation of the scope of work by the **JOINT STUDY COMMITTEE** and selected contractor, i.e. qualified scientific professionals. The three important mandatory components included:

(1) "The primary purpose of the **JOINT STUDY** is to determine the TP threshold response level, in mg L^{-1}, at which any statistical shift occurs in algal species composition or algal biomass

production resulting in undesirable aesthetic or water quality conditions in the Designated Scenic Rivers."

(2) "The **JOINT STUDY** shall be completed in accordance with U.S. EPA Rapid Bio-assessment Protocols… and follow EPA's most recent guidance 'Using Stressor-response Relationships to Derive Numeric Nutrient Criteria…" (EPA, 2010).

(3) "The **JOINT STUDY** shall include a sampling population that is adequate to determine the frequency and duration component of the numeric criterion."

The **JOINT STUDY COMMITTEE** issued a Request for Qualifications (RFQ), interviewed three professional teams, and then selected Dr. Ryan King's research group at Baylor University to perform the negotiated scope of work specific to the **JOINT STUDY**. All Statement of Qualifications (SOQs), meeting minutes, interim reports and reference materials are available on the web at:

www.ok.gov/conservation/Agency_Divisions/Water_Quality_Division/IR_Joint_Study_Committee.html

The purpose of this report is to provide "an objective analysis of the water quality data" and identifies the relation between TP concentrations and "multiple ecological response levels" targeted at protecting the Oklahoma Scenic Rivers from "undesirable aesthetic and water quality conditions." The **JOINT COMMITTEE** unanimously made "specific recommendations as to what TP levels, and what frequency and duration components of measure, are necessary to protect the aesthetics beneficial use and scenic river (Outstanding Water Resource) designations assigned to the designated [Oklahoma] Scenic Rivers" based on the relation between TP concentrations and "biotic indicators of water quality, including primarily algal taxonomic composition and periphyton biomass." The technical report from the selected scientific professionals is provided as an appendix of to this report, and it provides the expansive details of the sampling, data collected, statistical analysis, and additional supplemental information.

Joint Study Methods and Data Analysis

The sampling sites selected for the **JOINT STUDY** targeted "streams and rivers within the same EPA eco-region and comparable to the streams in the designated Scenic River watershed in terms of stream order and watershed land uses." A total of 35 stream reaches were selected for the **JOINT STUDY**, and the majority of the stream reaches were within five of the six designated Scenic River watersheds, including the Illinois River, Flint Creek, Baron Fork, Little Lee Creek and Lee Creek watersheds. Additionally, stream reaches were also included in adjacent watershed within the same EPA eco-region. The stream reaches were selected based on these criteria: (1) presence of riffles, (2) cobble substrate (10-20 cm), (3) open tree canopy, and (4) fast, turbulent flow. The ultimate goal of the site selection was to have stream reaches or sites with a gradient of TP concentrations sufficient to evaluate thresholds in algal taxa and biomass response with increasing TP concentrations.

Water and biological sampling occurred on an every other month schedule, subject to flow conditions, from June 2014 through April 2016. Water samples were collected at the upstream boundary of each stream reach and then analyzed for TP and other water-quality parameters at the Baylor University Center for Reservoir and Aquatic Ecosystems Research (CRASR) following standard methods and approved quality assurance and quality control protocols. Periphyton was removed from 15 cobbles in the desired size class (10-20 cm) from each stream reach and analyzed for periphyton biomass [mg chlorophyll-a (chl-a) m^{-2}] and algal species composition of diatoms and soft algae. The diatoms and soft algae were enumerated by species and reported as biovolume. Sampling was successfully completed every other month during base flow conditions at all 35 stream reaches or sites over the two-year study, with the exception of two sites where the stream was not flowing in October 2014 and one site observed in backwater conditions (i.e., flooded by Lake Tenkiller) during June 2015 and December 2015. Water and biological samples were collected over a variety of flows across the study (see Appendix, Figure 9), including relatively low conditions and following historic flooding in late December 2015. The **JOINT STUDY COMMITTEE** unanimously defined the '**CRITICAL CONDITIONS**' for the **JOINT STUDY** as the conditions where surface runoff is not the dominant influence of total flow and stream ecosystem processes.

The **JOINT STUDY** used various statistical techniques to analyze for TP thresholds with algal species composition (i.e., biovolume) and periphyton biomass (mg chl-a m^{-2}). The main techniques employed included:

(1) A nonparametric form of change point analysis (nCPA, King and Richardson, 2003) was used to determine threshold in periphyton biomass and select algal species (i.e., Cladophora biovolume). This statistical technique estimates the probability that the variance in the data explained by the model (i.e., threshold) is not better than expected by chance and provides estimates of uncertainty (i.e., confidence intervals) about where the true threshold might be. This technique is recommended for deriving numeric nutrient criteria (see EPA, 2010).

(2) Threshold Indicator Taxa Analysis (TITAN, Baker and King, 2010), which is an analytical approach used to identify thresholds among many algal species simultaneously in in response to a stressor gradient (i.e., increasing TP concentrations; the details of this technique are available in the appendix. TITAN provides TP threshold information on individual species, as well as community-level responses, that is, the groups of algal organisms that are decreasing (Sumz-) in abundance (i.e., biovolume) and increasing (Sumz+) in abundance across the TP concentration gradient. TITAN also provides uncertainty or confidence intervals about the TP threshold.

The final technical report (see Appendix) further outlines additional statistical techniques that were used in the **JOINT STUDY**, providing additional weight of evidence to support the recommendation put forth by the **JOINT STUDY COMMITTEE**. The **JOINT STUDY COMMITTEE** unanimously agreed that the range in TP thresholds from the **JOINT STUDY** were developed based on water and biological samples collected under **CRITICAL CONDITIONS**.

Joint Study Results

Phosphorus Thresholds with Periphyton Biomass and Nuisance Algal Taxa

TP concentrations were relatively consistent within each site over time, although several sites showed seasonal variability associated with dilution of effluent inputs. The concentrations were also depressed below the median TP concentrations over the 2-year **JOINT STUDY** during select samplings when algal biomass and primary production were high. Overall, TP concentrations in individual water samples ranged from less than 0.01 mg L^{-1} to almost 0.20 mg L^{-1} (see Appendix, Figure 10), and 2-year study averages varied from less than 0.01 mg L^{-1} to greater than 0.10 mg L^{-1} (at two sites or stream reaches). The evidence presented in the **JOINT STUDY** showed that a focus on TP as the potential driver of potential nuisance conditions of biomass and algal species composition was supported.

Benthic chl-a varied over time among the study sites or stream reaches, as well as within an individual site when higher productivity often existed. The average benthic chl-a across the 35 sites or stream reaches over the 2-year **JOINT STUDY** varied from ~50 mg m^{-2} to over 600 mg m^{-2}, while the benthic chl-a measured at discrete samplings varied from less than 50 mg m^{-2} across several sites to over 1000 mg m^{-2} (see Appendix, Figures 13-14). The dramatic increases in benthic chl-a observed in two sampling months (i.e., December 2014 and February 2015) coincided with blooms of *Cladophora glomerata* (hereafter, *Cladophora*).

The scientific professionals analyzed the relations between benthic chl-a and TP concentrations over a variety of durations (from 2 to 12 months), producing over 110 TP thresholds for the **JOINT STUDY COMMITTEE** to evaluate. The **JOINT STUDY COMMITTEE** agreed to put more weight on average TP concentrations over a 6 month duration or longer period, providing still almost 70 different TP thresholds with periphyton biomass for consideration. The TP concentrations were a statistical significant shift in periphyton biomass occurred varied from 0.014 to 0.060 mg L^{-1} with instantaneous benthic chl-a and from 0.018 to 0.040 mg L^{-1} for average benthic chl-a over the same duration (see Appendix, Tables 4-5, Figure 16-17). The average benthic chl-a across all sites above the TP threshold (i.e., sites with TP concentrations greater than the change point) was 2 or more times greater than average benthic chl-a at all sites with TP concentrations less than the threshold.

The dominant filamentous algae was *Cladophora*, which is widely known as a nuisance species that increases in abundance [essentially biovolume] with nutrient enrichment (Dodds and Gudder, 1992). *Cladophora* was not present to very low in biovolume at sites or stream reaches with relatively low TP concentrations, but showed a non-linear change in biovolume as TP concentrations increased across the sampling locations. The scientific professionals recommended that the **JOINT STUDY COMMITTEE** focus on mean responses of *Cladopora* biovolume to the increasing TP gradient, because of measurement variability with soft, filamentous algae. The TP thresholds showing an increase in average *Cladophora* biovolume across all sites and at least a six-month duration varied from 0.032 to 0.051 mg L^{-1}, with 16 out of 17 change points evaluated being 0.035 mg L^{-1} or greater (see Appendix, Tables 6, Figure 18).

The **JOINT STUDY COMMITTEE** and scientific professionals also evaluated how the proportion of total biovolume of nuisance algal taxa changed across the increasing TP gradient, where five genera of filamentous green algae that occurred in our data set were classified as nuisance taxa: *Cladophora, Oedogonium, Rhizoclonium, Spirogyra, and Hydrodictyon.* However, *Cladophora* was the dominant species of the total nuisance biovolume (generally greater than 95%); there were a few sites that had blooms of other algal species across the 2-year **JOINT STUDY**. The **JOINT STUDY COMMITTEE** per scientific professional recommendation focused on average proportions of the nuisance algal taxa over durations of six months or longer. The analysis showed that significant TP thresholds were present in 15 out of 17 relations evaluated, where TP thresholds ranged from 0.033 to 0.058 mg L^{-1} with 14 out of 15 TP thresholds being at concentrations of 0.035 mg L^{-1} or greater (see Appendix, Table 7, Figure 20).

Phosphorus Thresholds with TITAN Analysis

The community level analysis of algal species response to an increasing TP gradient provided additional information, and it was considered in the weight of evidence used to make recommendations (see Appendix, Tables 8-9, Figure 21). When looking at community level responses, the **JOINT STUDY COMMITTEE** evaluated the change in average taxa biovolumes over six month durations or longer in TITAN. Various algal species declined in abundance (measured as biovolume) as TP concentrations increased, where mean community level shifts in the natural assemblage of algae occurred at TP concentrations as low as 0.011 mg L^{-1} to as high as 0.049 mg L^{-1}. The algal species that declined (Sumz-) in abundance had a lower range in TP thresholds, where mean cumulative shifts occurred at TP concentrations from 0.011 to 0.025 mg L^{-1}. On the other hand, the algal species that increased (Sumz+) in abundance had TP thresholds that over lapped with the Sumz- scores, ranging from TP concentrations of 0.019 to 0.049 mg L^{-1}. TITAN analysis also shows change points (i.e., TP thresholds) for individual species, and the TP thresholds based on TITAN for *Cladorphora* were within the range reported above specific to nCPA analysis (0.032–0.051 mg L^{-1}) but were on the lower end of this range.

Joint Study Committee Recommendations

The **JOINT STUDY COMMITTEE** met ten times between October 2013 and December 2016, where all meeting were open to the public and information including agendas, minutes, and interim reports were posted on the web site dedicated to this committee; there were eight interim reports prepared and presented by the employed scientific professionals, i.e. Dr. Ryan King, Baylor University. The **JOINT STUDY COMMITTEE** unanimously agreed that the **JOINT STUDY** was performed and provided data to meet the '**CHARGE**' of the **JOINT STUDY COMMITTEE** as defined in the third paragraph of page 3 under 'USE OF STUDY FINDINGS AND RESULTS.' The **CHARGE** was "…to make specific recommendations as to what TP levels, and what frequency and duration components of measure, are necessary to protect the aesthetics beneficial use and scenic river (Outstanding Resource Water) designations assigned to the designated Scenic Rivers." The **CHARGE** goes on to state that the recommendation of the **JOINT STUDY COMMITTEE** will be "…based on overall stream health which shall include evaluating the relationship, if

any' between TP concentrations… and biotic indicators of water quality, including primarily algal taxonomic composition and periphyton biomass."

The **JOINT STUDY COMMITTEE** unanimously agreed on several key, factual elements based on the TP thresholds identified in the **JOINT STUDY** and briefly discussed above, including:

(1) The **JOINT STUDY** showed the change in algal taxonomic composition and periphyton biomass was statistically observed at TP concentrations as low as 0.011 mg L^{-1} and as high as 0.074 mg L^{-1}. [Note: This was based on all thresholds reported in the appendix.]

(2) The **JOINT STUDY** showed that statistical shifts in mean *Cladophora* biovolume and mean nuisance taxa proportion of total biovolume was observed between 0.032 and 0.058 mg TP L^{-1}.

(3) The **JOINT STUDY** showed that the largest mean cumulative shift in the natural assemblage of algal species was observed within the range from 0.011 to 0.049 mg TP L^{-1} where species declined in abundance within the range from 0.011 to 0.025 mg TP L^{-1} and species increased in abundance within the range from 0.019 to 0.049 mg TP L^{-1}.

The **JOINT STUDY COMMITTEE** considered the plethora of scientific evidence and statistical analysis provided by the **JOINT STUDY**, but the focus was on the TP concentration thresholds with regard to nuisance algal species (i.e, Cladophora biovolume and nuisance taxa proportion). The **JOINT STUDY COMMITTEE** and its scientific professionals (Dr. Ryan King) employed to complete the **JOINT STUDY** specifically and unanimously recommend:

> *A six-month average total phosphorus level of not to exceed 0.035 mg L^{-1} based on water samples taken during the CRITICAL CONDITION, as previously defined, was necessary to protect the aesthetics beneficial use and scenic river (Outstanding Resource Water) designations assigned to the designated Scenic Rivers.*

The **JOINT STUDY COMMITTEE** also discussed at length how the recommended TP threshold (0.035 mg L^{-1} under defined conditions) related to the periphyton biomass based on generalized additive modeling (GAM, see Appendix, Tables 10, Figures 23-24), and then how predicted periphyton biomass compared to benthic chl-a thresholds where *Cladorphora* biovolume increased significantly (see Appendix, Figure 22). However, the **JOINT STUDY COMMITTEE** put more weight on the TP thresholds associated with *Cladophora* biovolume and proportion of nuisance algal taxa (relative to total biovolume) in discussion specific to making a recommendation to meet the **CHARGE** of the ***Second Statement***. The **JOINT STUDY** provided "reliable and objective data analysis that will then form the basis for the Parties and EPA to make informed decisions about the scientific merit of any proposed revisions to the TP criterion for the designated Scenic Rivers."

Furthermore, the **JOINT STUDY COMMITTEE** unanimously recommends that the states (Arkansas and Oklahoma) develop a monitoring and assessment program informed by the **JOINT STUDY** and other scientific information to determine attainment of the criteria.

Finally, the **JOINT STUDY COMMITTEE** unanimously recommends that protection of the [Oklahoma] Scenic Rivers needs to extend beyond the phosphorus levels and additionally focus on including but limited to the following:

- Hydrologic alteration
- Riparian zone protection
- Stream bank stabilization
- Fluvial channel habitat
- In-stream mining
- And, other contaminants.

And, the **JOINT STUDY COMMITTEE** unanimously views system wide management as critical to the protection of the [Oklahoma] Scenic Rivers.

References

Dodds, W.K., and D.A. Gudder. 1992. The ecology of *Cladophora*. Journal of Phycology 28:415–427.

EPA. 2010. Using Stressor-response Relationships to Derive Numeric Nutrient Criteria.

Haggard, B.E. 2010. Phosphorus concentrations, loads and sources within the Illinois River drainage area, northwest Arkansas, 1997–2008. Journal of Environmental Quality 39:2113–2120.

Scott, J.T., B.E. Haggard, A.N. Sharpley, and J.J. Romeis. 2011. Change point analysis of phosphorus trends in the Illinois River (Oklahoma) demonstrates the effects of watershed management. Journal of Environmental Quality 40:1249–1256.

Appendix

The next pages contain the final report submitted by Dr. Ryan King to the **JOINT STUDY COMMITTEE** to fulfil his obligations in the completion of the **JOINT STUDY** and the contract with Baylor University.

Oklahoma-Arkansas Scenic Rivers Joint Phosphorus Study

FINAL REPORT

19 December 2016

Principal Investigator:
Ryan S. King
Professor, Department of Biology
Baylor University
Waco, TX 76798
www.baylor.edu/aquaticlab

Joint Study Committee Members:
Brian Haggard; Co-Chair (University of Arkansas)
Marty Matlock (University of Arkansas)
Ryan Benefield (Arkansas Natural Resources Commission)
Derek Smithee; Co-Chair (Oklahoma Water Resources Board)
Shellie Chard (Oklahoma Dept. of Environmental Quality)
Shanon Philips (Oklahoma Conservation Commission)

Study Framework

The Oklahoma-Arkansas Scenic Rivers Joint Phosphorus study was executed in accordance with the *Second Statement of Joint Principles and Actions.* The primary purpose of this study was (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."

Furthermore (p.3-4, *Use of Study Findings and Results*):

"The States of Arkansas and Oklahoma, acting through their respective Parties, agree to be bound by the findings of the Joint Study. Oklahoma, through the Oklahoma Water Resources Board, agrees to promulgate any new Numeric Phosphorus Criterion, subject to applicable Oklahoma statutes, rules and regulations if significantly different than the current 0.037 mg/L standard. "Significantly different" means the new Numeric Phosphorus Criterion exceeds -.010 or +.010 than the current .037 criterion. If the new Numeric Phosphorus Criterion is at or between .027 and .047, then the State of Oklahoma is not required to promulgate the new criterion in its water quality standards. Arkansas agrees to be bound by and to fully comply with the Numeric Phosphorus Criterion at the Arkansas-Oklahoma State line, whether the existing 0.037 mg/L standard is confirmed or a new Numeric Phosphorus Criterion is promulgated. Parties for the States of Arkansas and Oklahoma shall forego any legal or administrative challenges to the Joint Study."

This report summarizes the work performed by Baylor University (the third party contractor) along with Joint Study Committee in the context of this study framework. The results presented herein are based on a field gradient "stressor-response" study designed to identify levels of total phosphorus that lead to the undesired outcomes described above. The study design, site selection, measurement endpoints, field methods, and statistical analyses were vetted and unanimously approved by the 6-member Joint Study Committee. Further, the results presented correspond to specifically requested analyses by members of the Joint Study Committee. This report does not include recommendations or conclusions regarding the numerical criterion. This report serves to guide the Joint Study Committee towards an informed, scientifically grounded recommendation for a numerical phosphorus criterion for the Oklahoma Scenic Rivers based on the results herein.

Study Design

Site selection

Thirty-five stream reaches were selected for the study. These sites were located in watersheds of 5 of the 6 Oklahoma Designated Scenic Rivers (Illinois River, Flint Creek, Barren Fork Creek, Little Lee Creek, and Lee Creek; Table 1, Figure 1). The Joint Study committee elected to exclude the Mountain Fork River for logistical reasons.

Candidate reaches were selected based on the following characteristics: (1) presence of riffle channel unit(s); (2) predominance of medium-to-large cobble substrate (10-20 cm); (3) mostly to fully open tree canopy (full sun), and (4) fast, turbulent flow, which is not always a characteristic of riffles in small streams but is in larger streams and rivers that were the primary focus of this study. The combination of these factors was deemed critical to ensure comparability between smaller streams and rivers in the study region and the Illinois River, the largest river in the study. The mainstem Illinois typically had habitat that met all four of these criteria, thus reaches included in the study from other rivers and streams had to also meet these criteria. For example, had we sampled a subset of streams that had only gravel substrate in their riffles, the results would have been confounded by the fact that gravel is scoured much more easily than cobble because even the slightest changes in flow cause these substrates to roll downstream. Nuisance filamentous algae such as *Cladophora* are much more likely to be collected on larger, more stable substrates, and, when coupled with turbulent flow, are the typical locations where nuisance algal blooms are initiated in the large streams and rivers (Dodds and Gudder 1992). Canopy cover also was important because all of the Illinois River mainstem sites were open canopy and very low light conditions associated with dense tree canopy would have limited algal growth and confounded comparisons to open-canopy sites on the Illinois and other large streams in the study area.

Reaches that met these criteria were prioritized for selection if they (1) had an existing USGS stream gage at or near the site, (2) had been or were being monitored for nutrients by Oklahoma or Arkansas. Additionally, the committee prioritized sites on the Illinois River because of its high levels of recreational use and socioeconomic importance to the region.

Reaches were excluded if obvious gravel extraction activity, construction, or anything unusual at or near the site that could have affected the potential relationship between phosphorus and biological response variables were evident.

If all of these conditions were met, the final, most important criterion for site selection was that the sites spanned a gradient of total phosphorus (TP) representative of the full range of TP conditions in the Scenic Rivers, their tributaries, and adjacent watersheds. Existing TP data from intensively monitored locations by the University of Arkansas, Oklahoma Water Resources Board, and Oklahoma Conservation Commission guided the initial screening of sites for inclusion in the gradient study, along with an extensive sampling of 60 sites in April 2014 to identify additional locations not previously studied by these organizations. Based on these data, 35 stream reaches were chosen. Each site filled a gap in the continuum of total phosphorus

concentrations from the lowest to the highest in the region such that the distribution of TP among sites was roughly log-linear.

Table 1. Site codes, coordinates, and location description of the 35 stream reaches.

Name	Latitude	Longitude	Description
BALL1	36.06137	-94.5732	Ballard @ E0660 Rd
BARR1	35.87954	-94.4822	Barren Fk @ SH45 Dutch Mills
BARR2	35.91906	-94.6193	Barren Fk @ SH59 nr Baron
BARR3	35.94727	-94.6935	Barren Fk @ N4670 Rd Christie
BARR4	35.87013	-94.897	Barren Fk @ Welling Br
BEAT1	36.35495	-94.7767	Beaty @ D0458 Rd
CANE1	35.78497	-94.8559	Caney @ Welling Road
COVE1	35.68576	-94.3663	Cove @ Creek Fk Rd
EVAN1	35.87742	-94.5706	Evansville @ D0795 Rd.
FLIN1	36.23973	-94.5007	Flint @ Dawn Hill East Rd nr. Gentry
FLIN2	36.21771	-94.6019	Flint @ D0553 nr West Siloam Springs
FLIN3	36.21454	-94.6655	Flint @ D4680 Rd Hazelnut Hollow
GOOS1	36.05603	-94.2912	Goose @ Little Elm Rd CR19
ILLI1	35.95398	-94.2494	Illinois @ Orr Rd
ILLI2	36.10135	-94.3441	Illinois @ SH16 nr Savoy
ILLI3	36.16864	-94.4355	Illinois @ Chambers Springs Rd
ILLI4	36.1093	-94.5339	Illinois @ SH 59 AR Canoeing
ILLI5	36.14201	-94.6681	Illinois @ N4695 low water xing & River Rd
ILLI6	36.17349	-94.7237	Illinois @ Flint Cr
ILLI7	36.06755	-94.8823	Illinois @ Hanging Rock SH10
ILLI8	35.91667	-94.928	Illinois @ SH62 Tahlequah
LEE1	35.68091	-94.3578	Lee @ Creek Fk Rd
LLEE1	35.57263	-94.5567	Little Lee @ SH101 Nicut
LSAL1	36.28455	-95.0887	Little Saline @ E506 Rd
MTFK1	35.68016	-94.4558	Mountain Fk @ SH59 pulloff S of Davidson
OSAG1	36.26593	-94.2378	Osage @ Healing Springs Rd CR264
OSAG2	36.222	-94.2901	Osage @ Snavely Rd
SAGE1	36.198	-94.5829	Sager @ Beaver Springs Rd.
SALI1	36.28154	-95.0932	Saline @ E6508 Rd USGS site
SPAR1	36.24367	-94.2393	Spring @ SH112 AR
SPAV1	36.38485	-94.481	Spavinaw @ Limeklin Rd CR29
SPAV2	36.32323	-94.6854	Spavinaw @ Colcord Kiethy Rd
SPRG1	36.1429	-94.9091	Spring @ Rocky Ford Rd & N556
SPRG2	36.09092	-95.0147	Spring @ N485 Rd low water xing
SPRG3	36.14833	-95.1548	Spring @ SH82

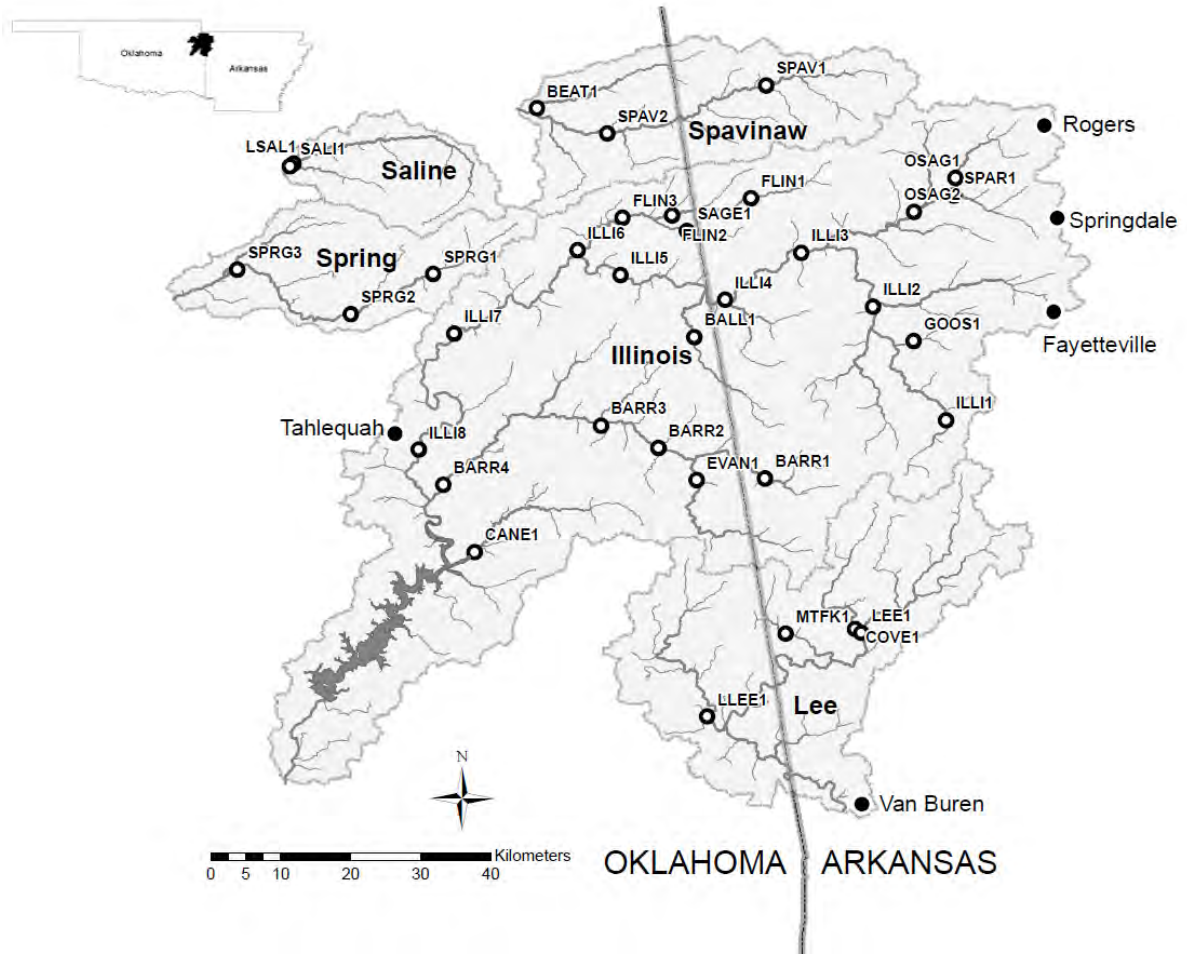


Figure 1. Locations and site codes of the 35 sampling reaches (see Table 1).

Catchment land cover/land use

Land cover and land use in the catchments of the 35 sites varied primarily in the percentage cover of forest, pasture, or developed land (Figure 2, Table 2). Most sites, even those with relatively low levels of total phophorus, had at least 30% cover of pasture land. The exceptions were COVE1, LEE1, LLEE1, and MTFK1, catchments that skirted the edge of the Ozark Highlands and were primarily located in the adjacent Boston Mountains. These sites had steeper uplands that limited extensive ranching and development. However, pasture land in the these catchments was typically located near the stream, where, if a source of phosphorus, may have a greater effect on nutrients than if located farther away (e.g., King et al. 2005). Moreover, these sites had similar levels of total phosphorus as sites with the lowest levels of pasture in the Ozark Highlands ecoregion (0.005-0.01 mg/L TP).

Sites that had relatively high levels of impervious cover associated with urban development were on the low end of urban intensity indices when compared to major metropolitan areas around the world (e.g., Walsh et al. 2005). Only 4 sites exceeded 10% impervious cover, and each of these were included because they had wastewater effluent discharges from sewage treatment plants upstream of our sampling reaches. Although levels of impervious cover exceeding 10% are known to have negative effects on benthic macroinvertebrate diversity (e.g., King et al. 2011), this may be less true in large streams and wadeable rivers such as those in our study, where the effects of imperviousness on storm runoff and peak flows is diminished.

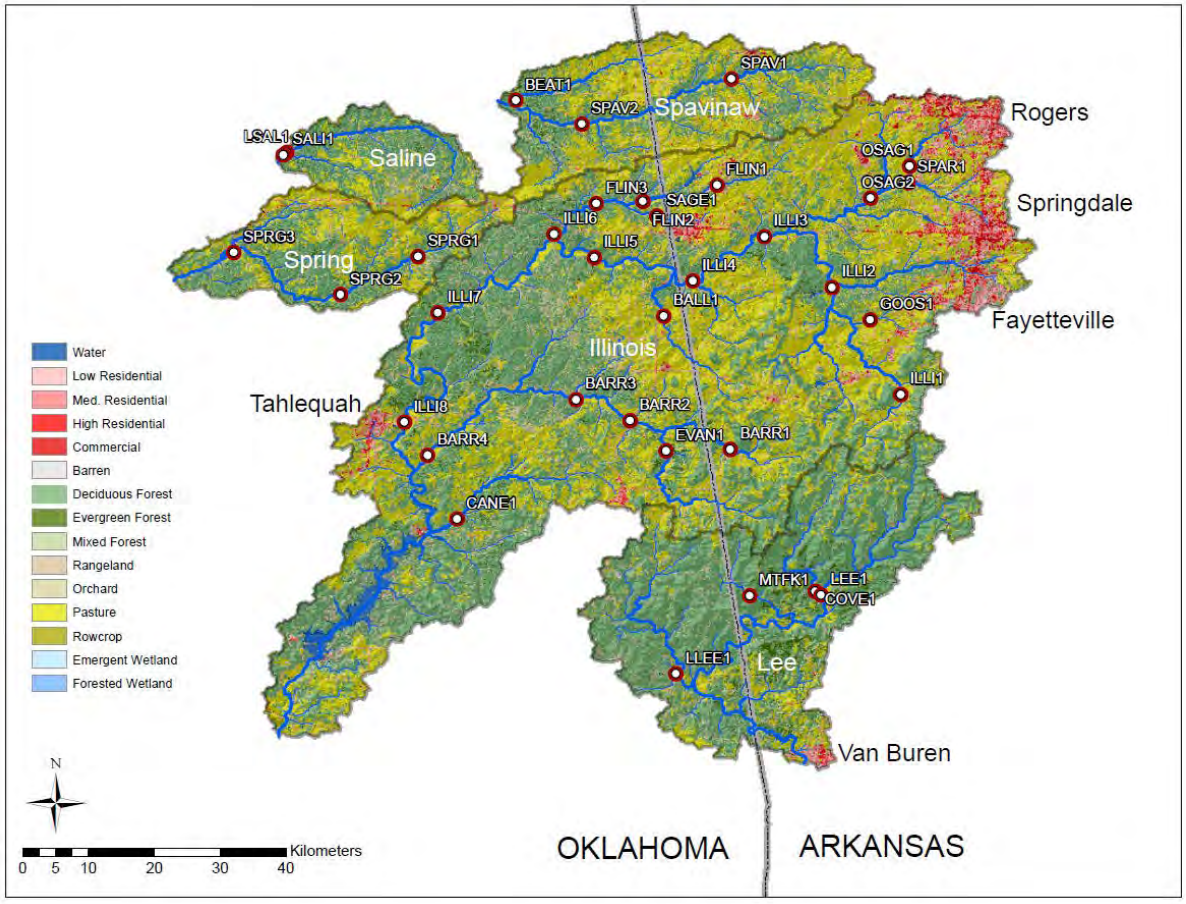


Figure 2. Land use and land cover patterns within the study area.

Table 2. Catchment area and percentages of dominant land cover classes associated with each sampling location. Land cover data was extracted from the most recent version of the National Land Cover Dataset (NLCD 2011).

Site ID	Catchment area (km^2)	% Developed	% Impervious cover	% Forest	% Grassland	% Pasture	% Row crop	% Wetland
BALL1	90.2	7.86	1.31	23.19	0.99	67.73	0.04	0.12
BARR1	105.6	4.22	0.57	45.43	1.82	48.28	0.00	0.14
BARR2	409.5	4.57	0.48	47.63	2.26	44.95	0.09	0.35
BARR3	542.9	4.97	0.56	46.06	2.90	45.37	0.08	0.34
BARR4	879.9	4.73	0.48	49.42	6.18	38.31	0.05	0.33
BEAT1	152.6	5.02	0.70	29.76	2.14	61.72	1.22	0.06
CANE1	232.9	5.93	0.98	43.54	3.37	46.71	0.10	0.09
COVE1	135.3	2.24	0.14	84.33	2.16	11.18	0.00	0.04
EVAN1	164.2	4.29	0.37	52.36	2.69	39.88	0.05	0.59
FLIN1	64.9	9.27	1.83	25.60	2.79	61.50	0.00	0.35
FLIN2	145.9	9.06	1.72	27.56	3.02	58.09	0.20	0.37
FLIN3	245.2	13.18	3.59	27.94	3.62	53.43	0.24	0.36
GOOS1	35.5	23.51	6.96	26.13	0.83	49.21	0.12	0.17
ILLI1	68.9	4.52	0.44	55.61	2.70	36.85	0.06	0.25
ILLI2	420.4	8.33	1.67	34.97	1.43	54.30	0.11	0.44
ILLI3	1239.8	20.84	6.53	27.11	1.16	49.75	0.12	0.42
ILLI4	1473.7	18.38	5.63	28.18	1.18	51.17	0.11	0.44
ILLI5	1716.9	16.85	5.00	29.09	1.25	51.70	0.12	0.48
ILLI6	2092.8	15.73	4.57	30.67	1.99	50.36	0.13	0.49
ILLI7	2294.6	14.64	4.18	34.05	2.84	46.99	0.12	0.55
ILLI8	2465.6	13.91	3.92	36.70	3.01	44.76	0.11	0.66
LEE1	252.2	2.73	0.24	84.62	2.17	9.93	0.01	0.27
LLEE1	264.1	2.79	0.16	77.98	8.53	9.22	0.00	0.19
LSAL1	61.7	3.31	0.33	50.93	8.32	34.89	0.43	0.00
MTFK1	67.1	2.45	0.10	84.70	4.94	7.02	0.00	0.03
OSAG1	100.8	56.47	21.50	7.27	0.37	34.57	0.20	0.20
OSAG2	337.4	36.94	13.02	11.29	0.36	50.38	0.16	0.15
SAGE1	45.9	35.50	12.99	8.99	1.18	53.63	0.03	0.23
SALI1	270.1	4.01	0.40	60.02	7.59	26.34	0.16	0.14
SPAR1	91.7	44.02	16.31	11.69	0.24	42.69	0.01	0.10
SPAV1	173.9	7.34	1.19	38.54	2.30	51.50	0.03	0.07
SPAV2	421.6	6.41	1.09	38.10	2.04	52.91	0.28	0.09
SPRG1	84.0	8.38	1.20	29.87	4.10	56.92	0.00	0.16
SPRG2	194.8	5.79	0.71	39.09	4.01	50.36	0.00	0.25
SPRG3	296.7	4.65	0.51	50.41	3.83	40.38	0.00	0.34

Sampling frequency

Sampling occurred on bimonthly schedule, subject to weather and stream flows. We chose to sample at this frequency for two years to increase the likelihood that we would detect nuisance algal blooms if they occurred (Biggs 2000). This sampling frequency resulted in 12 events (hereafter, Events 1-12), with 35 streams sampled per event, from June 2014 through April 2016 (Table 3), in addition to the total phosphorus (TP) data collected in April 2014 (hereafter, Event 0).

Table 3. Schedule of sampling events. Comprehensive sampling occurred bimonthly starting in June 2014 through April 2016, whereas total phosphorus sampling began in April 2014.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014				Site selection		X		X		X		X
2015		X		X		X		X		X		X
2016		X		X		Analyses, meetings, and final report completion						

Field Methods

Transect delineation

Field methods were patterned after Barbour et al. (1999) and Biggs and Kilroy (2000). Three transects were delineated to span a cross-section of each stream. Transects were delineated upon each site visit and did not necessarily correspond to previous transect locations because of different water levels or flood events that changed channel units between events.

For large streams/rivers (e.g., middle and lower Illinois River, lower Barren Fork Creek, Lee Creek, and several others), we typically identified a single riffle channel unit. The channel unit often was a large riffle that extended to deeper water, whereby three transects began at the wetted margin of the stream out to the point in the stream deemed representative of riffle-glide habitat or before it was too deep or fast to safely sample. The longitudinal distribution of these transects were roughly equidistant from the upper to lower boundaries of the riffle, but were always placed to target medium-large cobble (10-20 cm) habitat.

For streams with riffles that were wadeable from bank to bank and had a series of riffle-pool channel units within a relatively short length of longitudinal reach (<100 m), we selected 3 riffle channel units and placed one transect in each unit. Transects spanned the width of the optimal habitat, which typically was equal to the wetted width of the stream but occasionally was truncated by a pool, a change in substrate, heavy shade, etc, along one margin of the stream. Here, transects extended from one bank out to the margin of the cross-section that had the appropriate depth, velocity, light, and substrate.

Five sampling points were marked along each transect, roughly equidistant but allowing for some variability in location to ensure appropriate depth, velocity, light, and substrate. The first and last points were within 1-2 m from each end transects. Points 2, 3, and 4 were marked at 0.25, 0.5 and 0.75 distances of transects. Points were marked on the stream bottom using flagging tape secured to a large, galvanized metal washer.

Surface water chemistry and phytoplankton collection

Water chemistry and seston samples were collected above the upstream boundary of the reach after the upstream transect was marked. Triplicate TP samples were collected in new 50 mL centrifuge tubes and immediately preserved with sufficient volume of H_2SO_4 to achieve pH < 2. A single grab sample per site was collected for each of the following: TN (unfiltered, preserved with H_2SO_4) and NH_4-N, NO_2+NO_3-N, and PO_4-P (field filtered, 0.45 µm, iced immediately, held at <4 C until frozen that evening.). Separate 1-L sestonic chlorophyll-a and total suspended solid samples were collected in dark bottles and placed on ice immediately. Sample collection followed the Baylor University Center for Reservoir and Aquatic Systems Research (CRASR) approved quality assurance/quality control protocols.

Site characterization

We measured the following physical and chemical variables to characterize the reach on every visit: wetted width (estimated when wading the full width of the stream was not possible), mean depth (m) and velocity (m/s) of riffle channel unit (corresponding to benthic algal sampling transects), canopy cover (0-100%), discharge (ft^3/s, and several conventional water quality variables.

Discharge was estimated using a Marsh-McBirney flowmeter following standard USGS protocols. Discharge generally was not measured at sites that were (a) gaged and had moderate to high flow at the time of sampling, and (b) too large or unsafe wade (mainstem Illinois River). Discharge at gaged sites was estimated during summer low-flow conditions if it can be accomplished safely.

Temperature, specific conductivity, pH and optical dissolved oxygen were measured using YSI EXO1 multiprobes deployed for a minimum of 15 minutes during the site visit. Multiprobes were placed in flowing water above the reach. Readings were recorded manually after sensor readings stabilized. Multiprobes were calibrated prior to each event and post-calibration checked following each event.

Periphyton collection

Cobbles were collected at each of 15 points starting with the most downstream transect. The cobble nearest the transect marker that was 10-20 cm wide was selected regardless of the amount of algae on the top of the substrate, although oil shale fragments were excluded from sampling because they were rare. Rather, calcite or dolomite, the two dominant rock types in these streams, were selected.

Cobbles were removed from the stream by carefully lifting the substrate slowly to the surface. Each substrate was carefully placed in a white sampling basin designated for that transect. This process was repeated until cobbles from each of the 5 points were collected, and repeated again for each of the 2 remaining transects.

Each white basin was partially filled with stream water to keep the periphyton from desiccating and for enhancing the quality of photographs. Each white basin was photographed separately prior to removal of attached periphyton. A small white board with the date, site and transect ID, and event number marked using a dry erase marker was included in each photo to assist with cataloging of photos.

Periphyton was removed from the 15 cobbles before leaving the site. Cobbles were scraped over a clean, deep-sided white pan using a stainless steel wire brush. All attached algae was removed from the upper surface of the cobble. Stream water was used to rinse residue from the cobble into the white pan. After all cobbles were scraped and rinsed, the contents were consolidated into one corner of the pan and poured into a 1 L dark bottle, which was immediately placed on ice to achieve a sample temperature of < 4 degrees C until processing later that day.

Following the removal of periphyton from cobbles, the upper surface of each cobble was wrapped with aluminum foil for estimating the area (cm^2) from which the periphyton was removed. Foil was carefully cut along the margins of the cobble corresponding to the perimeter of the area sampled, removed, and placed in a labeled bag. This process was repeated for all 15 cobbles prior to leaving the site. Foil was cleaned, dried and weighed using analytical balance. Total mass of foil per site was used to estimate area using a simple weight-to-area conversion factor.

Hess (macroinvertebrate) sampling and transect marker characterization

Macroinvertebrate sampling was done primarily to estimate the density and biomass of periphyton grazing taxa, particularly snails in the family Pleuroceridae. Grazing taxa can achieve high densities and exert strong top-down control on algal biomass, hence quantifying their abundance was considered an important ancillary measurement to help explain patterns of benthic algal biomass over time.

Quantitative macroinvertebrate samples were collected using a Hess sampler approximately 0.5 m upstream of each of the 15 transect markers. The Hess sampler was placed upstream to avoid where the periphyton cobble was collected or where anyone had walked or otherwise disrupted the substrate.

Once the Hess sampler was embedded into the substrate, water depth, dominant substrate (gravel or cobble), sedimentation index (qualitative, 1-20, similar to EPA RBP; Barbour et al. 1999), embeddedness of cobbles (0-100%), and stoneroller grazing scars (qualitative, 0-10) within the Hess sampler was recorded prior to disruption of the substrate in the sampler. Next, all gravel and cobble were thoroughly brushed to remove attached periphyton, organic matter, and aquatic macroinvertebrates. Brushing was done inside the sampler where material and organisms were flushed back into the trailing net. Once all surface rocks had been brushed and removed, the remaining substrate was vigorously agitated to a depth of 5 cm for at least 30 seconds to dislodge remaining organisms. Following this step, the Hess sampler was carefully but quickly lifted off of the bottom to help rinse material attached to the net into the dolphin bucket attached to the cod end of the net. Additional rinsing of material from the net into the dolphin bucket was done as necessary. Contents of the dolphin bucket were emptied into a heavy-duty plastic 4-L storage, which was eventually used to composite all 15 Hess samples from one site. Additional storage bags were used if necessary. Before leaving the site, the sample bag(s) was placed on ice for preservation using buffered formal at the temporary field lab later that same day. The final volume-to-volume concentration of formalin after being mixed with the sample material in the bag met or exceeded 5%.

Diel dissolved oxygen and pH

We deployed YSI EXO1 data sondes to measure optical dissolved oxygen (DO) and pH at 15-minute intervals for approximately 48 h at a minimum of 25 sites in summer 2014 and 2015.

The purpose of measuring diel variability in these water quality variables was to determine whether TP was correlated with minimum dissolved oxygen and maximum pH. Both variables are mechanistically related to primary production in streams, but also are strongly influenced by differences in water turbulence (reaeration) among sites, groundwater discharge in the reach, and light conditions during deployment, all of which are very difficult to account for in the large streams and rivers sampled in this study.

Sondes were deployed at a depth of approximately 0.5 m. Sondes were located in shallow glide-pool habitats above riffles in order to reduce the effect of reaeration on DO and pH. Sondes were calibrated immediately prior to deployment, and post-calibration checks were performed following deployment. Sondes that failed post-calibration were excluded from analysis, as were sondes that were affected by factors that biased the results, such as accumulation of drifting debris (which was noted upon retrieval) or an obvious groundwater input immediately adjacent to the deployment site (which was discovered upon reviewing the data).

Frequency and Duration of Stressor and Response Variables

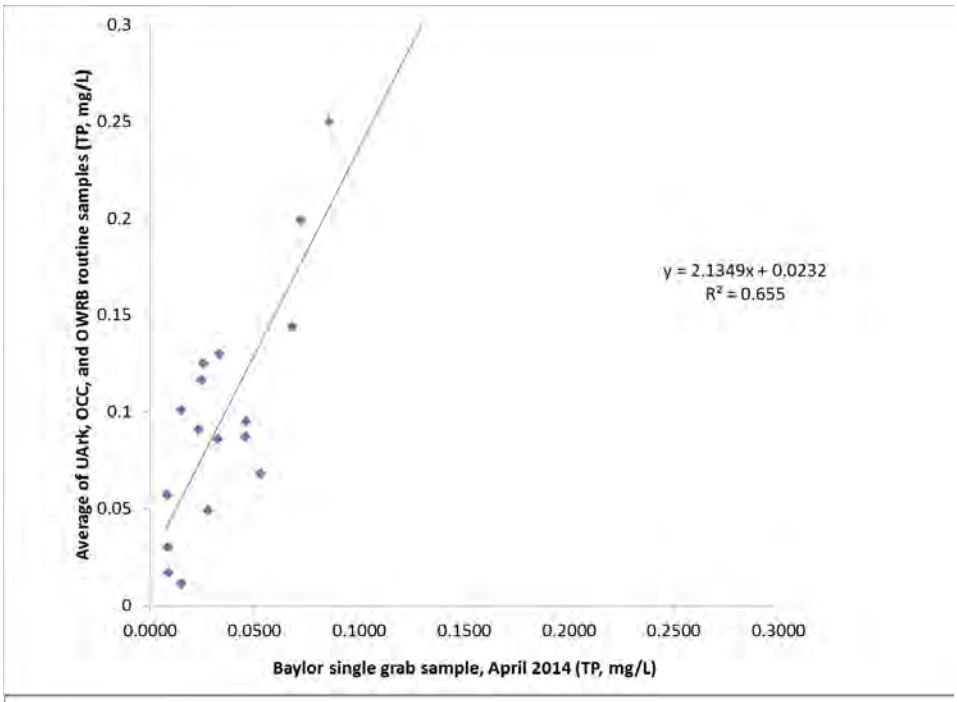
Two critical elements of developing a numerical criterion for total phosphorus for the Designated Scenic Rivers are sampling frequency (how often a TP sample is collected) and duration (over what period of time is the numerical criterion assessed, averaged, and evaluated for exceedance). A third element is frequency of excursion during a defined assessment period to meet the criterion, but this beyond the scope of this report.

Sampling frequency in our study was established during the study design phase prior to collection of any samples. Samples were collected bimonthly during base flow conditions only (or "critical flow" as defined by the Joint Study Committee, which were any flow conditions that were not dominated by surface-water runoff). The decision to sample during base flow conditions was based on several key factors: (1) it was impractical if not impossible under this budget to collect nearly continuous (daily to multiple times per day) samples to estimate phosphorus concentrations representative of all flow conditions from 35 locations over a 2 year period, (2) base flow conditions provide a more representative estimate of phosphorus availability to benthic algae because storm flows usually result in scouring of algae from rocks and very high turbidity which is not conducive for algal growth due to attenuation of light, (3) base flows occur the vast majority of the time, thus they are the typical condition in streams, (4) US EPA recommends and many other states use base flow conditions to establish numerical criteria for streams and rivers, thus there is a precedent for using data collected only during base flow for estimating violations of a numerical criterion, and (5) base flow TP is typically strongly correlated to TP calculated across all flow conditions where such data are available (e.g., Figure 3)

Duration was constrained by the length of the study (2 y) and was assessed by the comparing the strength of the relationships between mean TP calculated across different time intervals to biological response variables, particularly algal biomass. Mean TP (mg/L) was calculated at 2, 4, 6, 8, 10, and 12 month intervals. A 2-month interval included TP samples from 2 events; for example, our first algal sampling event was June 2014, whereas our first phosphorus sampling

event was April 2014 (Event 0). The mean of April and June 2014 TP was the value used when relating 2 month TP to benthic chlorophyll-a collected in June 2014 (see Data Analysis). Similarly, 4 month TP was calculated as the mean of the 2 previous events and the current event (e.g., Events 0, 1, and 2), and so forth. We used arithmetic mean because it was almost perfectly correlated to geometric mean (Figure 4) and is likely a better estimator of cumulative exposure.

Response variables were analyzed as instantaneous measurements (e.g., 4 month mean TP vs. the observed level of benthic chlorophyll-a on a particular event that matched the 4 month TP window) and as mean responses that matched the TP duration (e.g., 4 month mean TP vs. the mean of benthic chlorophyll-a matching the same events used to calculate the 4 month TP; Figure 5). US EPA (2010) recommended calculating mean nutrient and response data if multiple collections were available from the same locations over time because it reduces variability, improves statistical models, and is consistent with the way numerical criteria are assessed (typically over a series of months or a year or more).



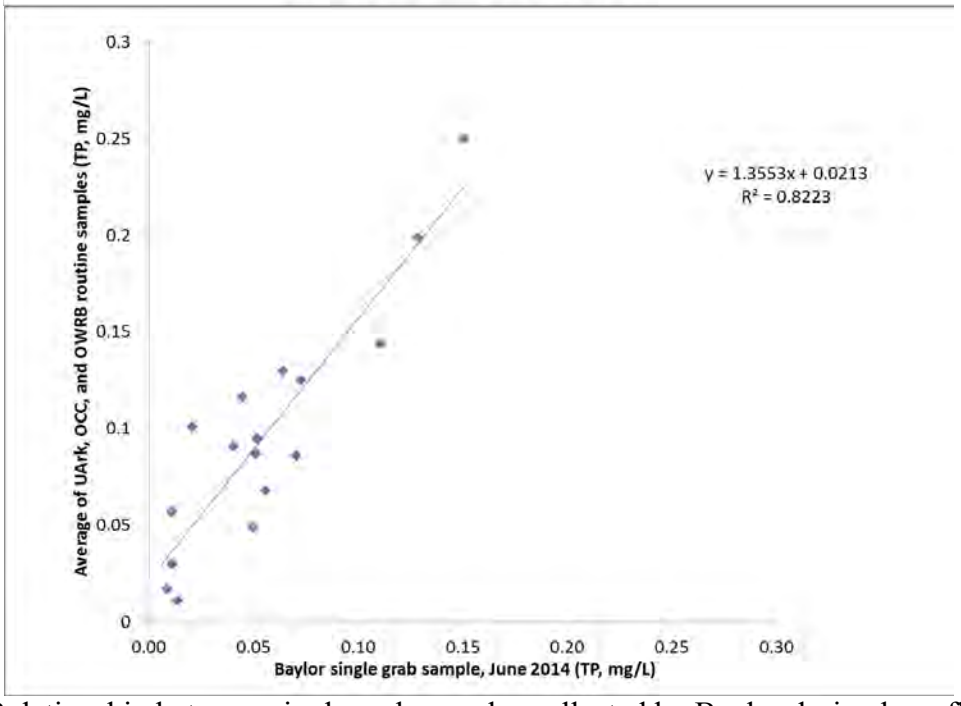


Figure 3. Relationship between single grab samples collected by Baylor during base flow in April (upper panel) and June (lower panel) 2014 to mean TP over 1-2 years prior to the Baylor samples from intensive sites (i.e., samples collected at any flow, including storm flows) monitored by the Oklahoma Conservation Commission (OCC), Oklahoma Water Resources Board (OWRB), and the University of Arkansas (UA).

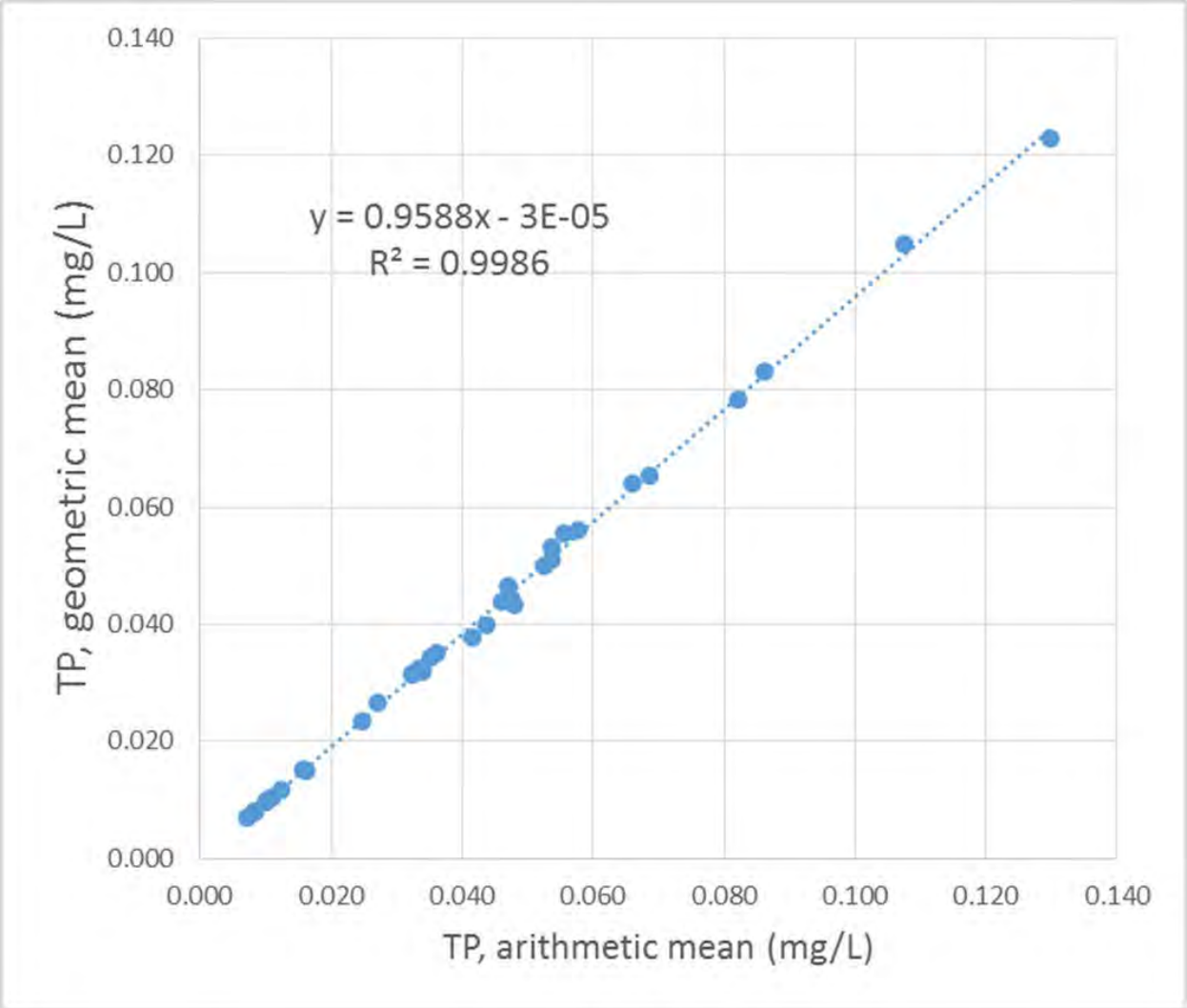


Figure 4. The relationship between geometric and arithmetic mean total phosphorus concentrations from the 35 study sites from April 2014 through April 2016 (n=13).

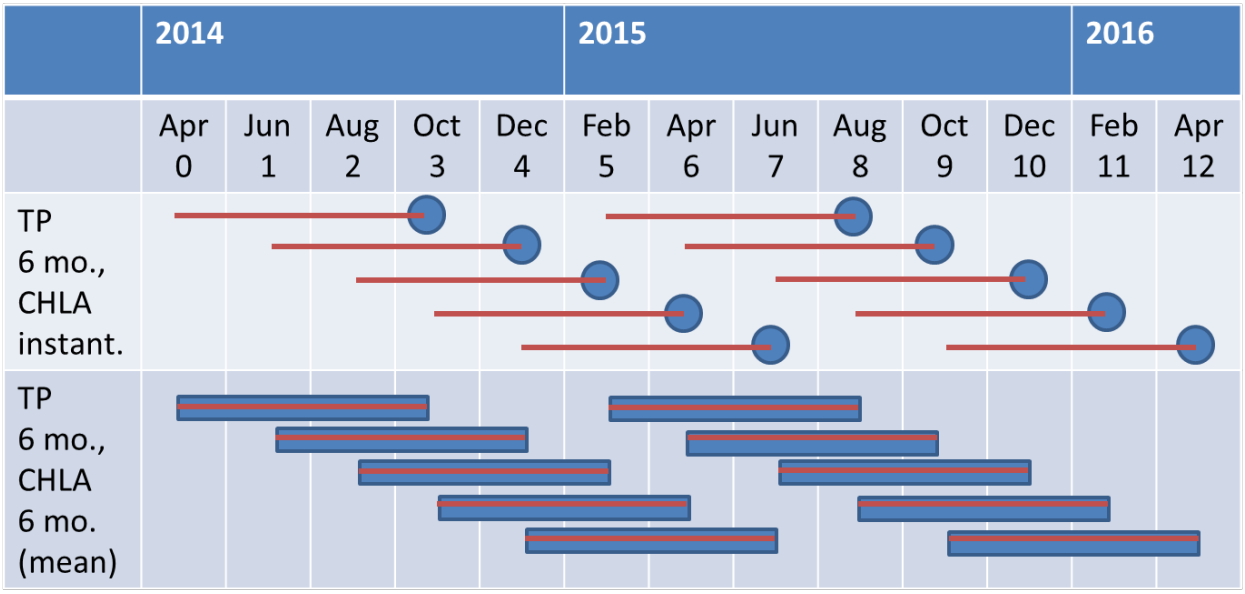


Figure 5. Examples of the two different ways total phosphorus was related to biological response variables. This example is based on a 6 month mean TP. In the top row, each dot represents the "instantaneous" set of values of benthic chlorophyll-a measured on each of the events, and the red line represents the time interval (duration) over which TP was averaged prior to relating to these instantaneous measures of chlorophyll-a. In the bottom row, the blue bars represent the time interval used to calculate the "mean" set of response values of benthic chlorophyll-a, which matches the same set of data used to calculate the mean TP.

Data Analysis

The primary purpose of the Scenic Rivers Joint Phosphorus Study, as stated by the Second Statement of Joint Principals and Actions, page 2, was to identify "*the total phosphorus threshold response level....at which any statistically significant shift occurs in algal species composition or algal biomass production...resulting in undesirable aesthetic or water quality...conditions in the Designated Scenic Rivers."*

A threshold level of TP, defined ecologically, is a where there is a disproportionately large change in an ecological response, such as algal biomass or species composition, with a relatively small incremental increase in concentration of TP (Groffman et al. 2003, Baker and King 2010).

Statistically, a stressor-response threshold can be categorized into two broad, but complementary classes of methods. The first, a change point threshold approach, relates to finding value along a stressor gradient where the response variable, such as algal biomass, changes the most. Here, the goal is to estimate the level of the stressor (the x axis, or predictor variable) where the mean of a response variable increases or decreases disproportionately, such that by splitting the data into two groups defined as above and below that point, the means of those two groups would differ the most when compared to all other possible values of TP in the data set (Figure 6).

The second approach involves identifying the value of the predictor where the mean (or median or other quantile) of the response (the fitted line of a regression, for example) intersects a critical reference value of the response, such as a minimum dissolved oxygen or nuisance levels of benthic chlorophyll-a (Figure 7). This reference value approach is ideal for a policy-based study where an *a priori* management target or standard has been previously established. The first approach is very useful when a management target is not defined or there is an additional goal of identifying where there is the largest change, regardless of a management target (e.g., *"any statistically significant shift occurs…."*, p2, Second Statement of Joint Principles and Actions). However, it should be made clear that the first approach is based on splitting the data at the point of greatest change, but "greatest change" may not correspond to a reference value threshold for a particular endpoint. Here, we describe both approaches and how they were used to satisfy the primary purpose of the Second Statement of Joint Principles and Actions.

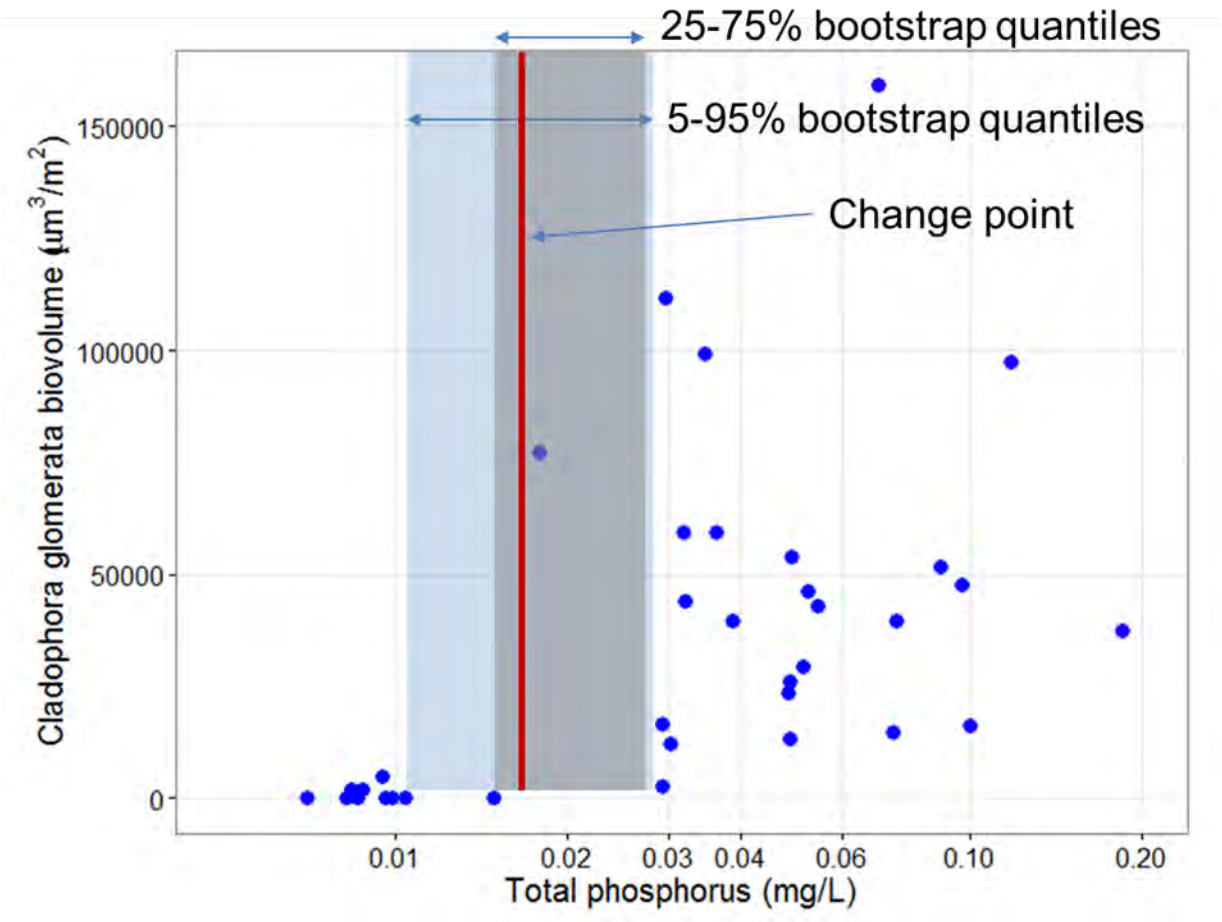


Figure 6. Change point threshold approach based on splitting the data at a TP value that corresponds to the largest change in the response (in this case, biovolume of *Cladophora,* the primary nuisance species in the Designated Scenic Rivers). Here, the data are 2 month TP versus instantaneous *Cladophora* biovolume from June 2014.

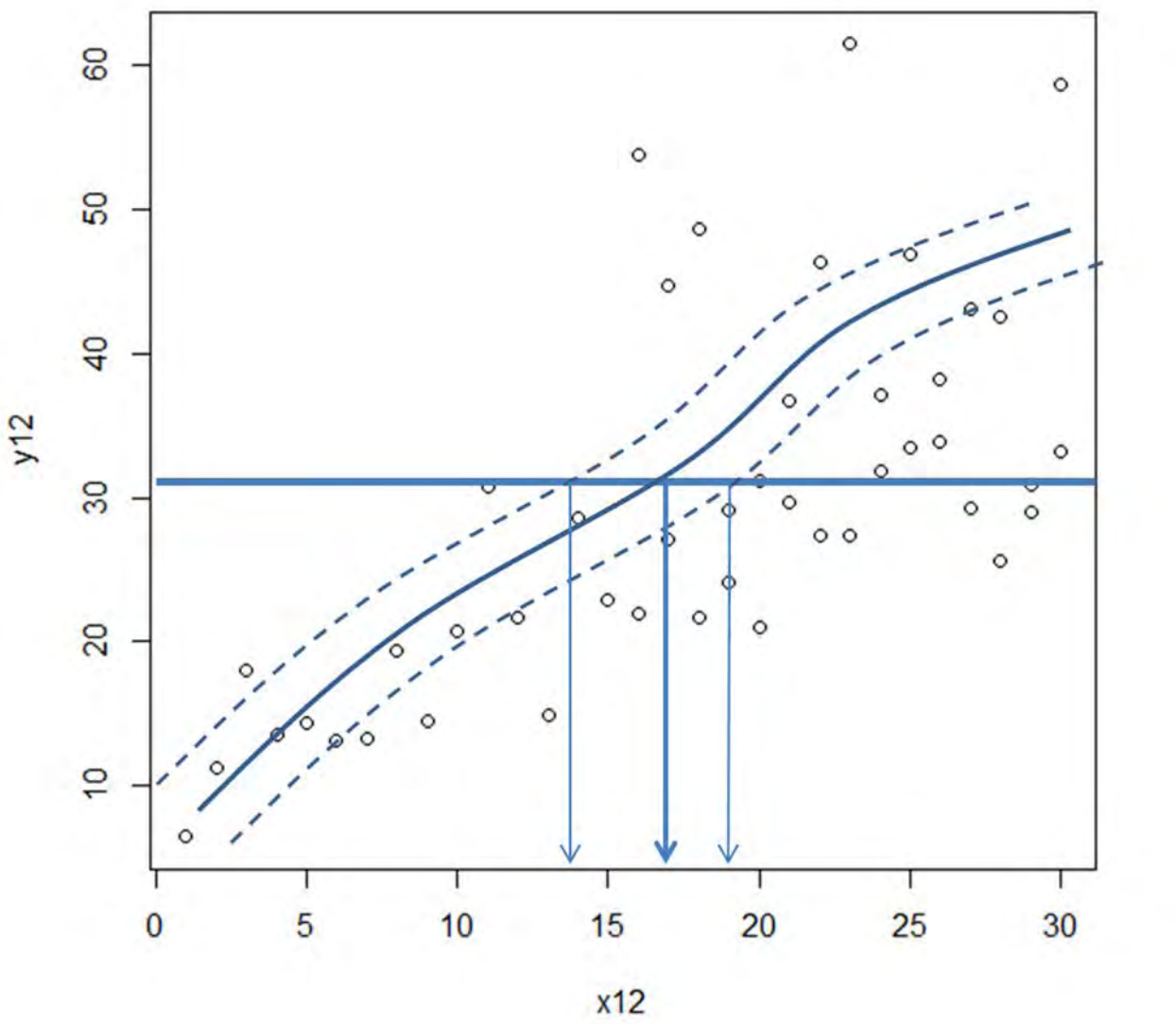


Figure 7. An illustration of the reference value approach. Here, the theoretical reference value is 30, which presumably represents a biological criterion beyond which conditions are considered unacceptable. The fitted line and confidence limits (dotted lines) are used to statistically estimate the level of the predictor (labeled "x12" in this example) that results in an intersection with the y-axis reference value. Here, the mean fitted response intersects the reference value at an x-axis value of approximately 17, whereas the lower and upper confidence limits intersect the reference value at 14 and 19, respectively. Thus, levels of the stressor (x12) that exceed 17, with uncertainty of 14-19, are likely to violate the biological response reference value (y12=30).

Change-point threshold approaches

Nonparametric change-point analysis

There are several methods for estimating statistical change points, but many are not well suited for ecological data (see list of methods in Dodds et al. 2010). A nonparametric form of change point analysis that was employed by King and Richardson (2003) and is included as a recommended technique for deriving numeric nutrient criteria by US EPA (2010) is one of the few techniques that makes few implicit assumptions about the data, particularly ones almost always violated by comparable methods (e.g., piecewise linear regression), despite their widespread use (e.g., Toms and Lesperance 2003). Nonparametric change point analysis, or nCPA as implemented in King and Richardson (2003), is simply a restricted form of regression tree analysis (De'ath et al. 2002) that involves only one predictor and one "branch" in the tree. The branches are defined by the change point. However, there are a few important limitations of using a simple regression tree to identify change points.

First, regression tree analysis identifies one value of the predictor (in this case, TP) that results in the greatest amount of variance explained (more technically, deviance), yet many other values of the predictor may explain very similar amounts of variance. In many stressor-response relationships, there is a zone of disproportionate change (see the gray area in Figure 3) where any one of several values in a relatively narrow range are nearly interchangeable in their ability to explain the variance in the response. To deal with this limitation, the change point approaches employed in this report use a bootstrapping algorithm to estimate quantile intervals (similar to confidence intervals) that provide estimates of uncertainty about where the true change point might be located, if there is one. This is very similar to the use of bootstrapping in Random Forest analysis, a related technique (Breiman 2001).

Second, most simple regression tree analyses do not include an estimate of statistical significance, and those that do often assume a normal distribution, which is inappropriate. The nCPA method employed in this report uses a randomization test to estimate the probability that the variance explained by the model is not better than expected by chance, with a minimum of 1000 randomizations.

Third, the version of nCPA used in this study employs several different probability distributions for calculating deviance reduction (Gaussian, binomial, Poisson) depending upon the type of response data. For example, the proportion of biovolume as nuisance algae species is a binomial response variable and thus a binomial form of nCPA was employed for that analysis.

Change point analysis has its own share of limitations, however. First, the analysis can yield biased change point estimates if the predictor data is strongly skewed (i.e., many high values and very few low, or vice-versa). However, this is a problem for all statistical methods and is a particular problem in observational stressor-response studies that are not carefully designed to sample a stressor gradient in a relatively uniform manner (King and Baker 2014). Second, the method will find a change point even if the response to the predictor is a linear relationship because there is significant change associated with a linear relationship. However, the bootstrapping method largely alleviates this concern because the quantile intervals will span most of the range of x, indicating that the point of greatest change is highly uncertain and could

be almost anywhere along the gradient. Thus, using the bootstrap results in conjunction with common sense (i.e., visualizing the data using scatterplots prior to conducting the analysis, e.g. Zuur et al. 2010) allows for strong inferences to be made.

In accordance with recommendations by the SRJSC, change-point analysis was used to estimate TP change-points for the following variables: algal biomass (benthic chlorophyll-a), *Cladophora* biovolume, and the proportion of nuisance algal taxa, the three primary variables of interest for assessing the relationship between TP and nuisance levels of algal biomass.

Threshold Indicator Taxa Analysis (TITAN)

TITAN (Baker and King 2010) is an analytical approach for identifying and distinguishing threshold-type responses among many species simultaneously in response to a stressor gradient (e.g., algal species composition). King and Baker (2014) provide explicit detail on its use, misuse, and limitations for natural resource management. Briefly TITAN works by integrating a relatively simple and elegant measure of association in taxon abundance with a nonparametric technique for detecting change. Indicator species analysis (Dufrene and Legendre 1997) uses abundance-weighted occurrence frequency to describe association between a particular taxon and groups of samples defined by their order along an environmental gradient. To facilitate comparison across taxa, TITAN compares each taxon's maximum IndVal score to those expected if the same sampled abundances were randomly distributed across the environmental gradient. A good indicator species is one that occurs frequently at one end of a gradient, so that changes in its abundance are easy to detect, but that is not the only kind of response worth noting. IndVal scores will always be small for rare, variable, or sensitive taxa, even though they can nonetheless represent important changes within a community. By comparison to the average IndVal scores derived by random permutation, TITAN standardizes measures of change for any given taxon to units of standard deviation (z scores; Baker and King 2010). Standardization emphasizes observed changes for each taxon relative to their own patterns of variability in abundance and occurrence.

To better understand uncertainty surrounding the observed change points, TITAN employs a bootstrap resampling technique in the same way the previously described nCPA method does. Information provided by the bootstrap is critical for interpreting results in TITAN. In addition to estimation of change-point quantiles, TITAN evaluates consistency in the response direction as purity, and the frequency of a strong response magnitude as reliability (Baker and King 2010). Combined with a minimum occurrence frequency, these diagnostic indices are used as filters to help distinguish the signal produced by indicator taxa responses from stochastic noise along the gradient. This filtering is part of what distinguishes TITAN from many other multivariate techniques based on weighted averaging or dissimilarity.

Once indicator taxa have been identified, TITAN provides information that can be used to identify a potential community-level threshold. A plot of filtered indicator taxa showing change-point quantiles from bootstrap replicates provides evidence regarding the existence of synchronous changes in the community structure (Figure 8, Texas stream example). Because the magnitude of all responses is standardized across taxa as z scores, their sum reflects the magnitude of community change at any point along the gradient. Distinct peaks in the sum(z)

curve (maxima) plotted across the environmental gradient are another indication of coincident change in community structure. When bootstrap replicates used to compare the location of the sum(z) maxima across many sample replicates show a narrow band, this constitutes evidence for a threshold response (Baker and King 2010; King et al. 2011).

TITAN was used to estimate taxa-specific change points and community-level thresholds in algal species abundance (biovolume/cm^2) in response to TP.

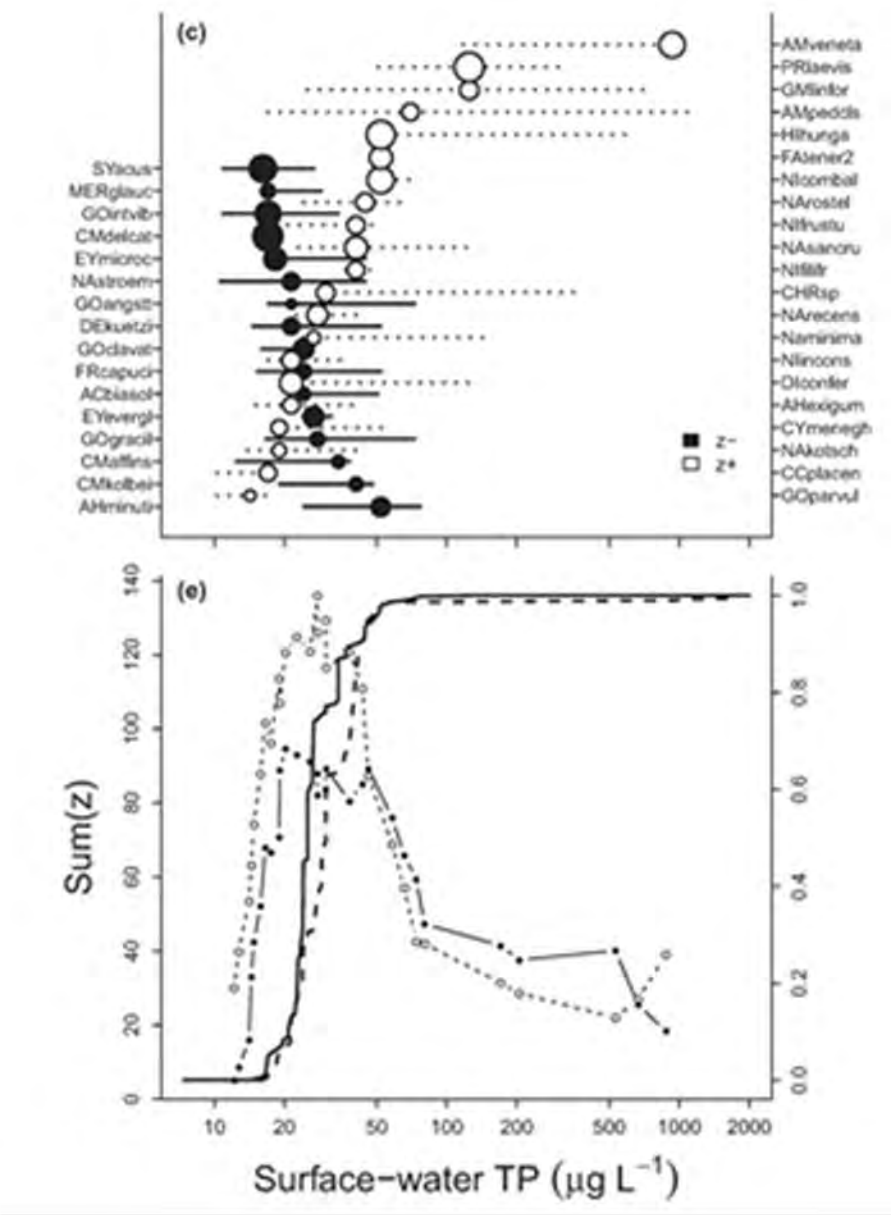


Figure 8. Example of output from Threshold Indicator Taxa Analysis (TITAN). In this example from a study conducted in wadeable streams in central Texas (Taylor et al. 2014), species with negative responses to total phosphorus are shown as filled symbols, whereas species that increased in response to TP are shown as open circles (upper panel). The location of the symbols corresponds to the level of TP resulting the greatest change in the frequency and abundance of each taxon (the change point) and the horizontal lines span the lower to upper quantile intervals (uncertainty). The lower panel illustrates the sum of the responses of the pure and reliable threshold indicator taxa. Sum(z-) (negative responding taxa) sharply peaks at 0.021 mg/L TP with lower and upper quantile limits of 0.016-0.052 mg/L. Sum(z+) (positive responding taxa) sharply peaked at 0.028 (0.018-0.048) mg/L TP. Both results are indicative of a significant shift in species composition between ~0.02-0.05 mg/L TP.

Reference value threshold approach

Neither Oklahoma nor Arkansas has numerical standards for benthic algal biomass or species composition. Scientific literature and a few states (e.g., Montana, Suplee et al. 2009) have either recommended or adopted ~150-200 mg/m^2 benthic chlorophyll-a as a management threshold, such that levels above this value represent nuisance levels of algal biomass. Thus, values of benthic chlorophyll-a at or above 150-200 mg/m^2 could be used as a reference values in this study for use in analyses that are set up to ask "at what level of TP does benthic chlorophyll-a exceed x mg/m^2?". However, differences between large streams and rivers in this study and those from typically much smaller streams in other regions of the world where these numbers have been adopted must be considered prior to using these reference values. Further, differences in taxonomic structure of periphyton in pristine streams of this region relative to other regions where those numbers have been adopted could result in lower or higher natural levels of benthic chlorophyll-a.

For these reasons, we examined values of benthic chlorophyll-a at sites at the low end of the TP gradient to assess the natural range of conditions that might be expected at reference sites in the Ozark Highlands and Boston Mountains ecoregions. Second, we fit an empirical relationships between benthic chlorophyll-a and biovolume of the dominant nuisance algal species in these streams, *Cladophora glomerata*, to refine estimates of nuisance levels of benthic algal biomass that were calibrated to these waterbodies (see Results for greater details).

Based on these assessments, we identified 150, 200, 250, and 300 mg/m^2 benthic chlorophyll-a as reference values representing potential nuisance levels of algae for the Designated Scenic Rivers. We assessed these reference levels using two methods.

First, we related mean benthic chlorophyll-a to year 1, year 2, and years 1 and 2 combined mean TP using a generalized additive modeling approach (GAM; Zuur 2009). A GAM model was the most appropriate for these response data because of nonlinearity that did not match a functional relationship (e.g., power, log, exponential). We used a Gamma probability distribution with an identity link function because the variance in the response was highly correlated to the predictor. Further, we weighted each mean by the inverse of its standard deviation (1/sd) so that points with higher variance associated with their means (more uncertainty) received less weight in the model.

Second, we analyzed the frequency of exceedance of each of those values as response variables to year 1, year 2, and years 1 and 2 combined mean TP using generalized linear models (GLM; Zuur 2009). We calculated the number of times each site exceeded 150, 200, 250, and 300 mg/m^2 benthic chlorophyll-a and fit a model based on a binomial (logistic) probability distribution to the data. The proportion of the total number of events per site in which benthic chlorophyll-a exceeded each of these values (4 separate response variables) was used as a response to mean TP. The total number of events, which was 12 for all but 3 sites that were either not flowing (ILLI1 and EVAN1, October 2014) or flooded (CANE1, June and December 2015) during our sampling event, was used as the weight for the binomial model (Zuur et al. 2009). The resulting models generated fitted responses of the proportion of times in which

benthic chlorophyll-a exceeded each of those 4 critical values for all levels of mean TP in the study.

Results

Temporal patterns in stream discharge, nutrients, and algal biomass

Sampling was successfully completed every two months during critical flow conditions at all of the 35 sites over the 2 year study, with the exception of two sites in October 2014 (ILLI1, EVAN1; streams were not flowing) and another site during June 2015 and December 2015 (CANE1; site was flooded by backwater from Lake Tenkiller).

Hydrographs (Figure 9) illustrate that 2014 through early 2015 was largely devoid of major storm flows associated with large precipitation events. This was not a particularly dry period, either, as precipitation was normal and base flows remained near the historical median for gaged sites. By April 2015, a much wetter weather pattern associated with El Niño conditions developed for the rest of the year, resulting in frequent storm flow conditions and culminating in an historic flood in late December 2015. The period following the historic flood was relatively dry and allowed the streams to return to high critical flow conditions by early February and relatively normal stream levels through March and April 2016.

Total phosphorus concentrations were relatively consistent within each stream over time with the exception of SAGE1, which was wastewater effluent dominated, and several other sites during periods of high primary production associated with blooms of *Cladophora glomerata*. In the latter instances, uptake by benthic algae reduced TP to levels 0.01-0.04 mg/L below the median TP value at these sites over the 2-y study (Figure 10). The patterns of benthic chlorophyll-a in this figure (symbols sized in proportion to chlorophyll-a values) also corroborate a very consistent pattern of sharp declines in TP with high levels of benthic chlorophyll-a.

Although not necessarily a focus of this study, it is important to acknowledge that nitrogen is also critical to primary production in streams, and has been suggested as possibly a stronger correlate of benthic chlorophyll-a in Ozark Highland streams in Arkansas. Because sources of phosphorus are almost always sources of nitrogen, too (e.g., wastewater discharges), it is logical that nitrogen should correlate well with benthic chlorophyll if phosphorus is also a good correlate. The problem with using simple correlations to ascribe causation is demonstrated, in part, in Figure 10 because it shows that during periods of high primary production, phosphorus is rapidly removed from the water column such that the relationship between TP and benthic chlorophyll-a at the particular point in time was weak, and probably weaker than the relationship to total nitrogen if nitrogen is not removed at the same rate as phosphorus, and particularly if it does not change relative to typical concentrations at that site.

To illustrate this point further, we plotted TP as the difference (deviation) from the median value measured at each site during the 2 year study (Figure 11). Large, negative deviations were almost always associated with disproportionately high levels of benthic chlorophyll and increasingly high N:P ratios, typically > 100 (Figure 11). Thus, it was the antecedent TP conditions that led to blooms, and when blooms were present, TP was being taken up more rapidly than it was desorbing from sediment or being supplied by wastewater (Figure 12). Conversely, TN showed no temporal pattern that related to benthic chlorophyll-a (Figure 12). Thus, this study's focus on P as the primary driver of potential nuisance conditions of algal biomass is well supported.

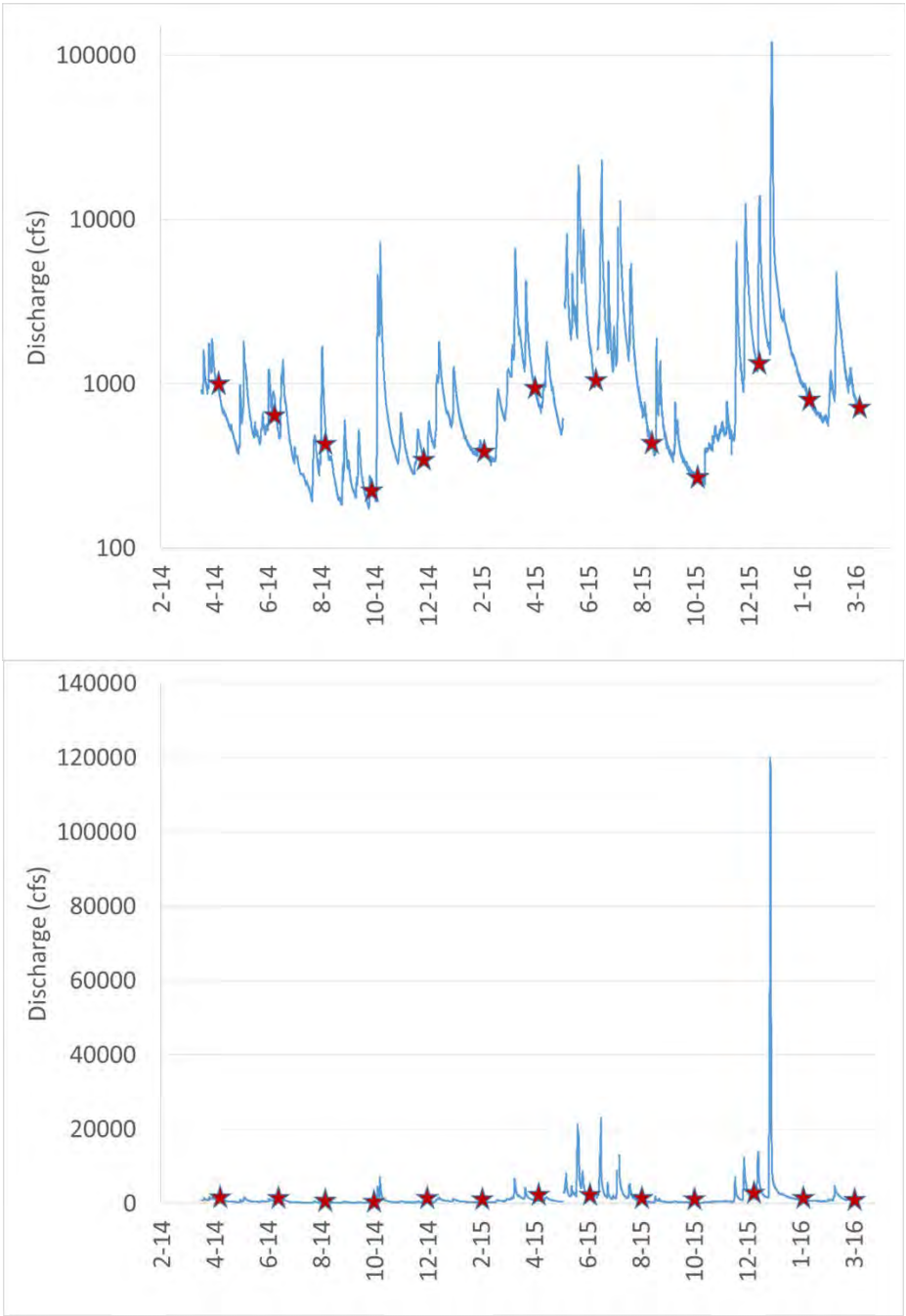


Figure 9. Daily mean discharge at USGS gage 07196500, Illinois River at Tahlequah, from April 2014-2016. Location of the stars indicates the approximate timing of sampling. Discharge is log-scaled in the upper panel, whereas an untransformed scale is used in the lower panel. The huge peak in the lower panel corresponds to the historic flood event in late December 2015.

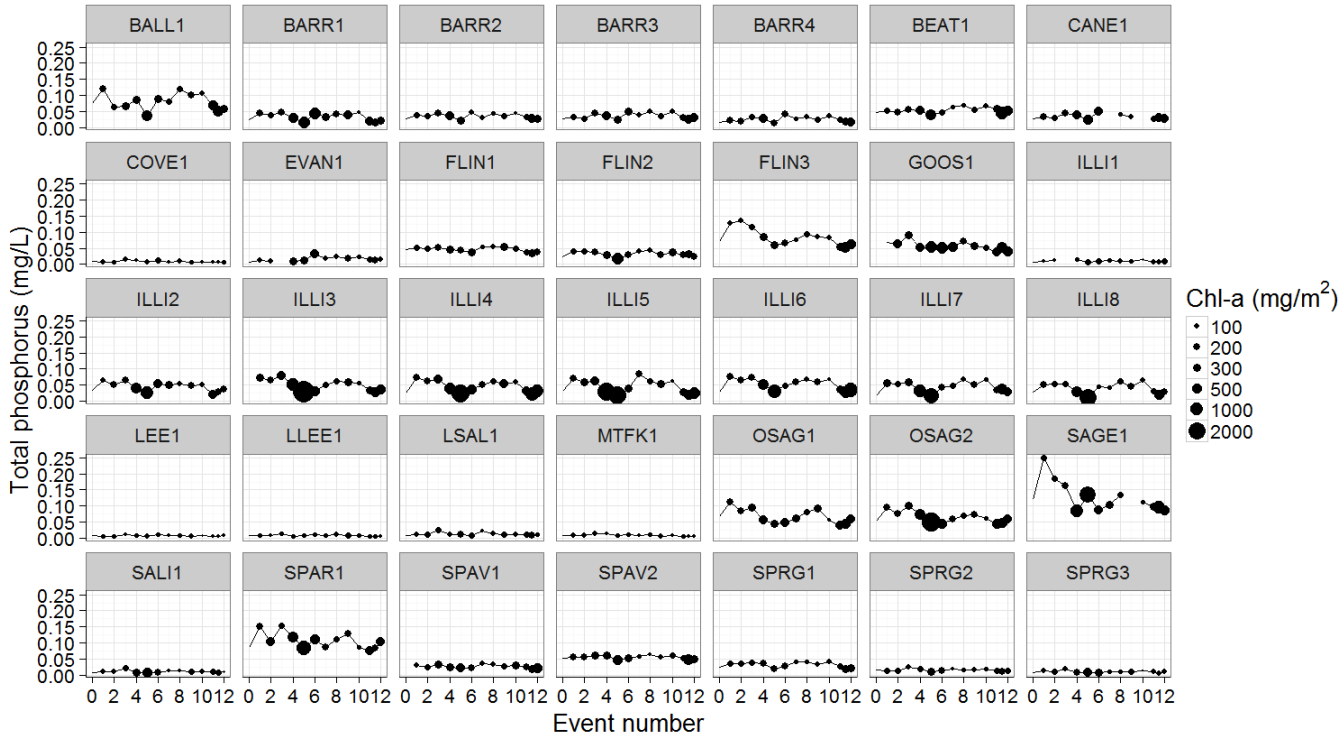


Figure 10. Temporal patterns of total phosphorus among the 35 study sites. Symbols are sized in relative proportion to benthic chlorophyll-a measured at the time of sampling. Note that, with the exception of SAGE1, which was effluent dominated, and to some degree, SPAR1 (also with a large proportion of base flow as wastewater effluent), most of the variability in TP over time within a site was related to whether there were high levels of benthic chlorophyll on the stream bottom at the time of sampling. In these cases, TP values declined sharply, very likely due to biological uptake. Sites with relatively low levels of TP and benthic chlorophyll-a throughout the study tended to have relatively consistent TP concentrations.

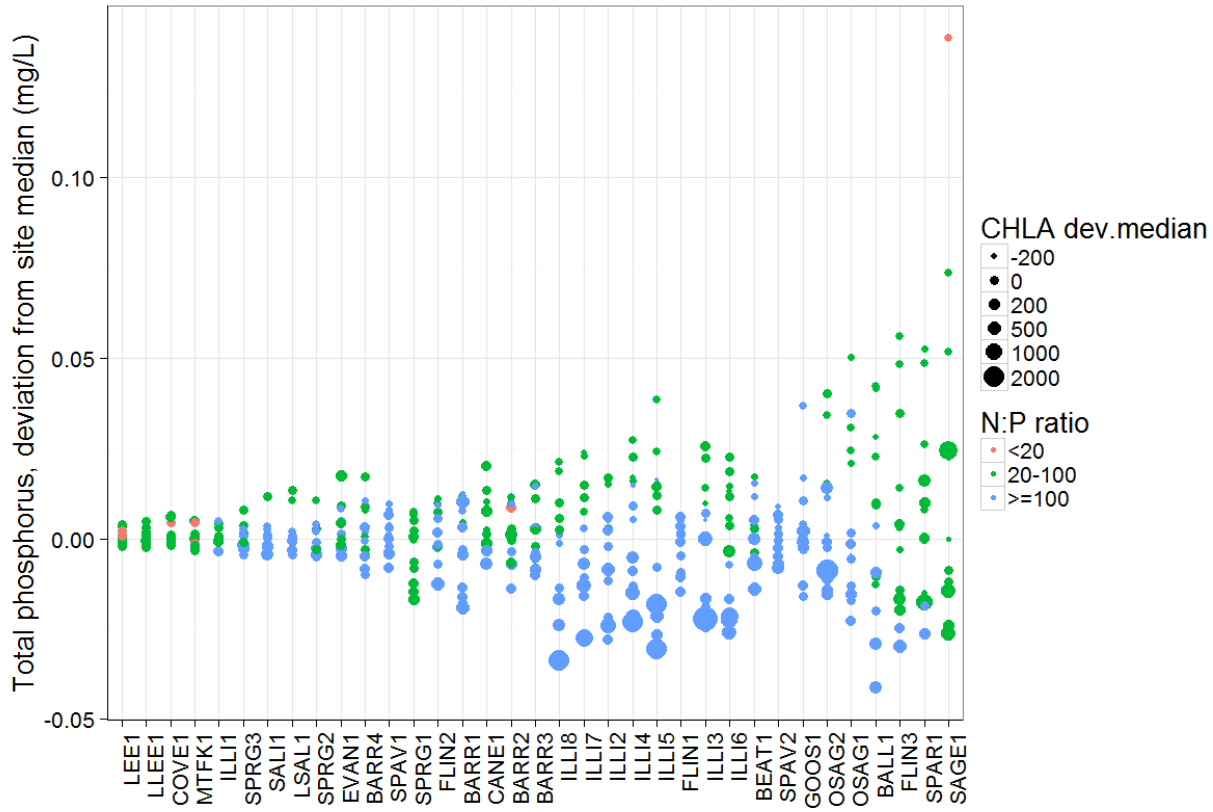


Figure 11. Dot plot of total phosphorus by sites (n=12 events), expressed as the deviation from the median 2-year concentration in mg/L. The 35 study sites are listed in rank order of their median 2-y TP concentrations. Each TP value is sized by the deviation from the site median for benthic chlorophyll-a; large values represent large, positive deviations from the typical level of chlorophyll at that site over the 2-year study. The colors represent the total nitrogen to total phosphorus ratio (N:P ratio) based on the measured TN and TP on that sampling event. N:P ratios <20 can be associated with N limiting conditions, whereas values above 20 increasingly demonstrate P limitation, or, at least, that there was a surplus of nitrogen relative to phosphorus. Note that in almost every case where benthic chlorophyll-a was much higher than the median (large dots), the total phosphorus value was lower, sometimes much lower, than the median. Further, under these conditions, the N:P ratio was >20 (green) and typically >100 (blue), but never <20 (orange). This implies that phosphorus, not nitrogen, was the driver of primary production among the study streams, although the high concentrations of nitrogen in these systems ensured that blooms were not restricted by N.

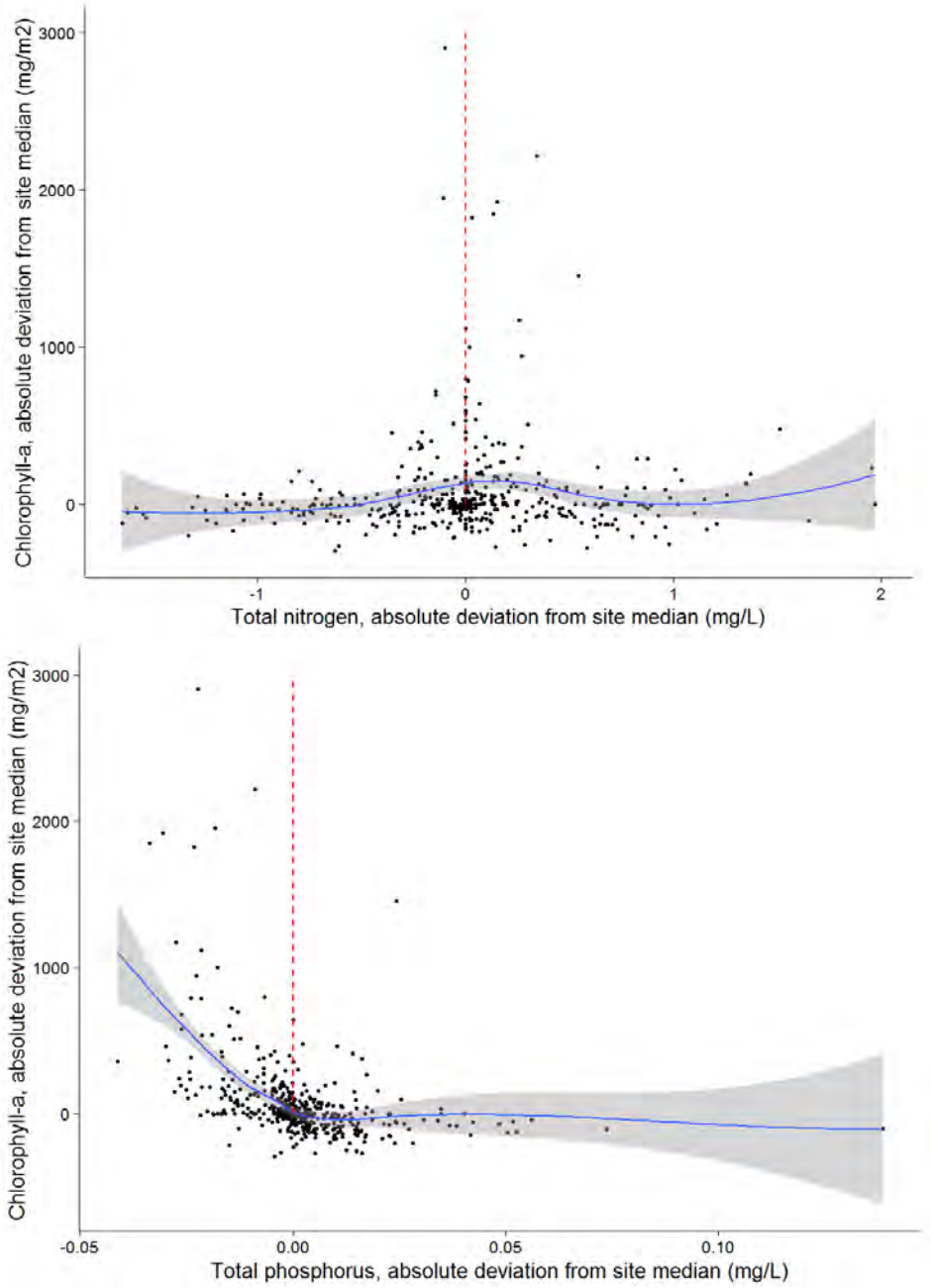


Figure 12. Plots of benthic chlorophyll-a in response to TN (upper) and TP (lower) deviations from site medians. The upper panel shows that the largest chlorophyll-a values were associated with mostly normal TN concentrations, with no relationship to benthic chlorophyll-a. The lower panel shows that almost all of the high chlorophyll-a levels corresponded to sharp reductions in TP. The fitted relationship shows that as TP levels were increasingly reduced, chlorophyll was at its highest. TP levels that are far above the median appear to be related to below normal levels of benthic chlorophyll-a.

Relationships between total phosphorus and algal biomass

Benthic chlorophyll-a varied markedly over time among the study sites (Figure 13). Levels of chlorophyll-a increased only slightly between June and October 2014, but increased dramatically during the months of December 2014 and February 2015 when a bloom of *Cladophora glomerata* was ongoing.

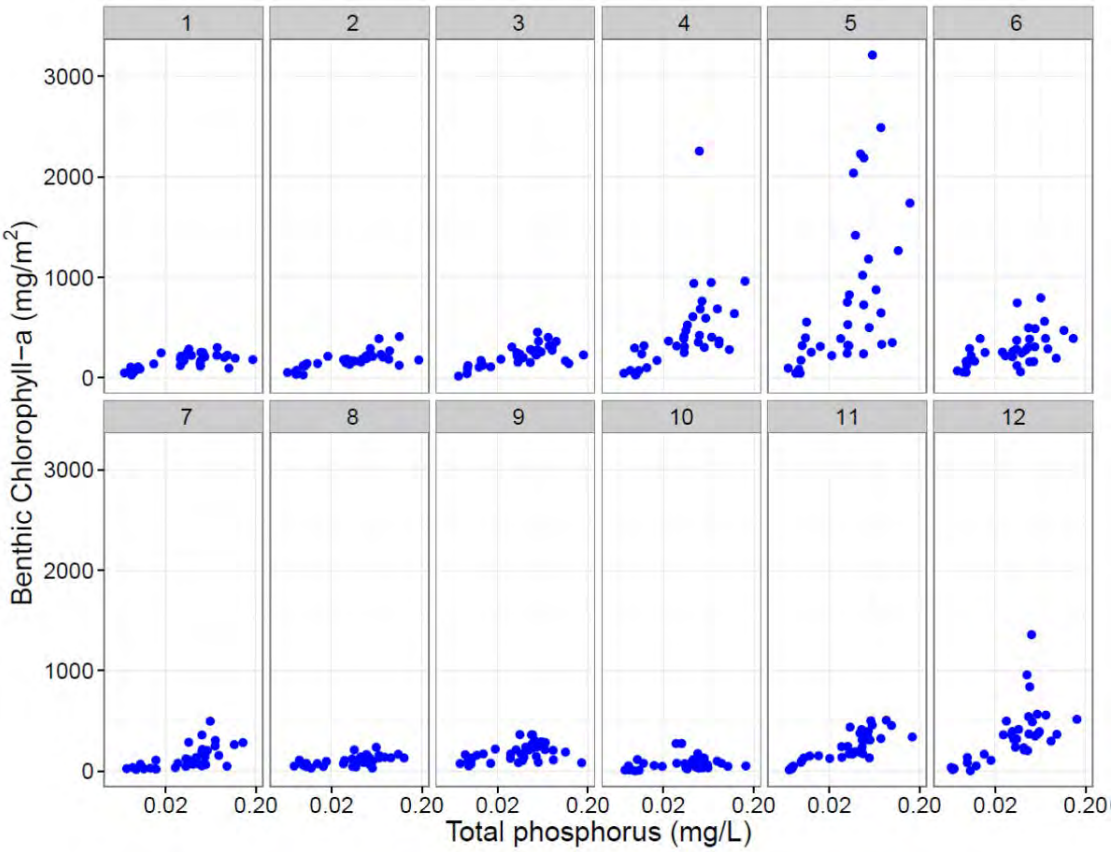


Figure 13. Relationship between benthic chlorophyll-a and 6-month mean TP across each event. Event 1 (June 2014) and 2 (August 2014) are based on 2 and 4 month mean TP values, respectively, because 6 month data was not available.

Benthic chlorophyll-a was reduced markedly by April 2015 following moderate storm flows that scoured much of the *Cladophora* off the stream bottom (Figure 13, 14). Reduction in benthic algal biomass continued through the summer and fall of 2015 (events 7-10). During this period, many large precipitation events resulted in very high stream flows and heavy scouring of algae, but often disproportionately among sites. Between event 10 (early December 2015) and 11 (early February 2016), the historic flood occurred that resulted in a complete scouring of substrate to the extent that channel morphology at most sites did not resemble previous conditions.

Despite the complete scouring following the historic flood, algal biomass recovered very quickly in by early February 2016, with some sites supporting levels up to 500 mg/m^2. However, filamentous green algae was not abundant during this event, and it appeared to be mostly dominated by diatoms and cyanobacteria. Further, due to a complete elimination of grazing macroinvertebrates, particularly pleurocerid snails, and the dormancy of the dominant vertebrate grazers (stonerollers, *Campostoma anomalum* and *Campostoma oligolepsis*; Taylor et al. 2012), the relationship between 6-month TP and algal biomass very closely resembled a theoretical growth-response curve, with a steep increase at low levels of TP and a gradual reduction in the slope (Figure 14, panel 11). By April 2016, *Cladophora glomerata* had become well established and contributed to even higher levels of algal biomass, with one site exceeding 1000 mg/m^2 (Figures 13 and 14, panel 12)

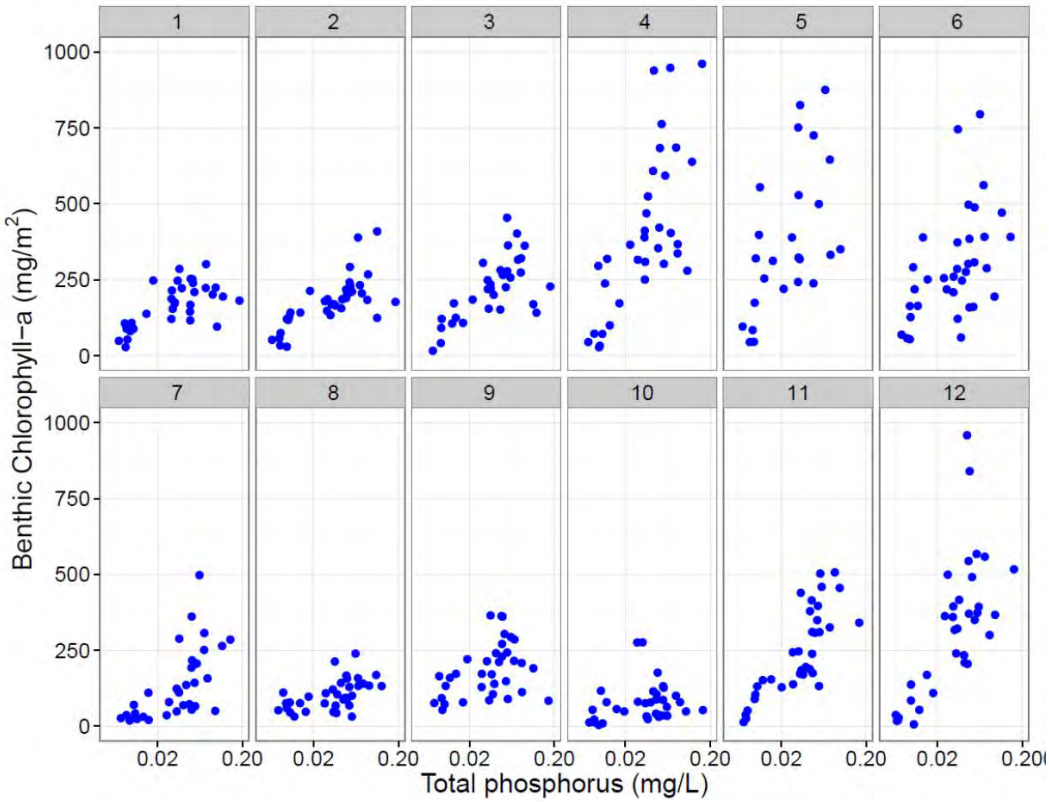


Figure 14. Relationship between benthic chlorophyll-a and 6-month mean TP across each event. This figure is identical to figure 13 except that the y-axis was truncated at 1000 mg/m^2 so that the relationship between TP and benthic chlorophyll-a during periods outside the massive *Cladophora* blooms could be better visualized.

Change point analysis: TP vs. benthic chlorophyll-a

The series of plots in the section *Temporal patterns in stream discharge, nutrients, and algal biomass* revealed the problem of relating nutrients to primary production or algal biomass. Despite the overall consistent levels of TP within a site over time, periods of high primary production depleted TP and caused the relationship between instantaneous measures of TP and algal biomass to break down. Thus, TP change points were estimated using means calculated at durations of 2, 4, 6, 8, 10, and 12 months. TP at these different durations were related to both instantaneous and mean chlorophyll-a (Figures 16 and 17, Tables 4 and 5).

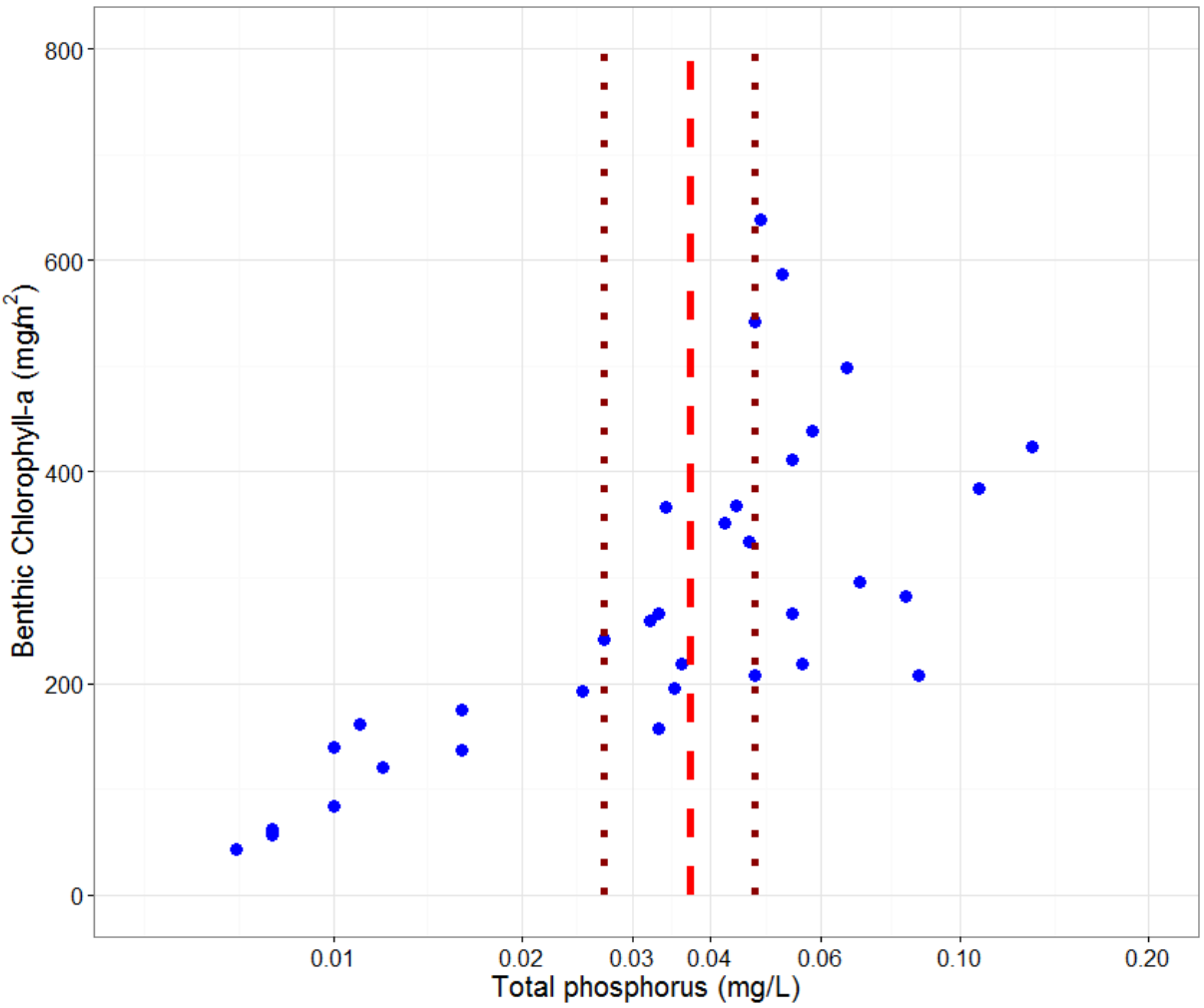


Figure 16. Two-year mean TP (April 2014-2016) vs. 2 year mean benthic chlorophyll-a. The dashed red line corresponds to 0.037 mg/L TP, whereas the dotted lines correspond to 0.027 and 0.047 mg/L TP, respectively.

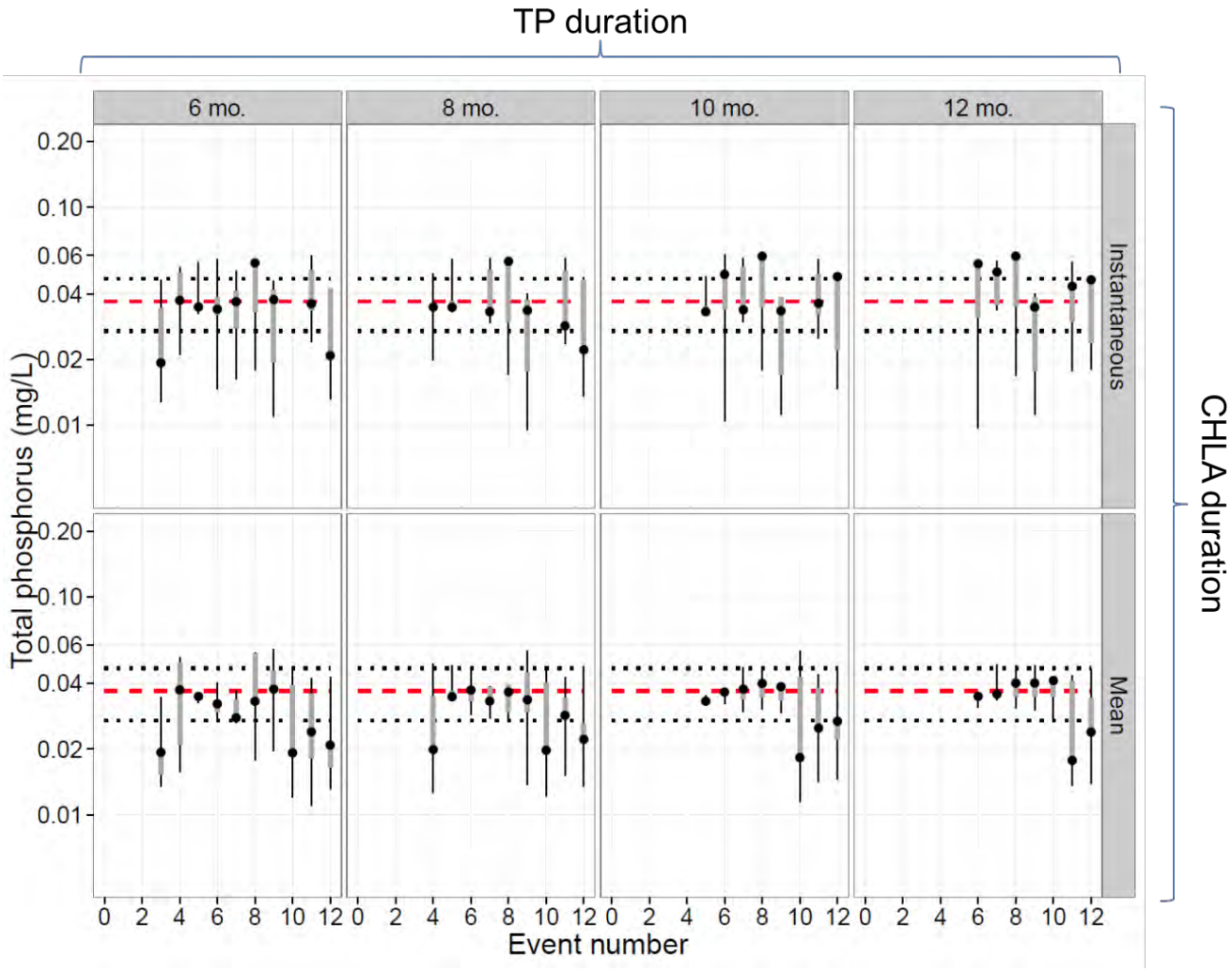


Figure 17. Total phosphorus change points in relation to benthic chlorophyll a. The columns represent 6, 8, 10, and 12 month TP durations, whereas the rows separate instantaneous and mean chlorophyll-a. Points correspond to the observed change point, gray bars span the 25-75% bootstrap quantiles, and black bars span the 5-95% bootstrap quantiles. The dashed red line is 0.037 mg/L TP, whereas the upper and lower dotted lines correspond to 0.027 and 0.047 mg/L. Results for 2 and 4 month TP are not shown, but are included in tables 4 and 5.

Table 4. Change points for 2, 4, 6, 8, 10, and 12 month mean total phosphorus in relation to instantaneous benthic chlorophyll-a.

				TP change points (mg/L)			Chlorophyll-a (mg/m2)		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	Chl-a Duration	Observed	Median (boot)	p-value	Mean (low)	Mean (high)	5% (boot)	25% (boot)	75% (boot)	95% (boot)
1	14-Jun	2 mo.	Instantaneous	0.016	0.016	0.001	81.9	200.8	0.010	0.013	0.016	0.029
2	14-Aug	2 mo.	Instantaneous	0.017	0.017	0.002	89.4	210.7	0.011	0.012	0.051	0.061
3	14-Oct	2 mo.	Instantaneous	0.022	0.022	0.001	97.1	260.7	0.016	0.017	0.027	0.055
4	14-Dec	2 mo.	Instantaneous	0.038	0.038	0.027	186.3	634.7	0.024	0.035	0.041	0.043
5	15-Feb	2 mo.	Instantaneous			0.071						
6	15-Apr	2 mo.	Instantaneous	0.029	0.029	0.023	194.4	383.9	0.017	0.028	0.029	0.044
7	15-Jun	2 mo.	Instantaneous	0.037	0.037	0.005	49.3	192.2	0.031	0.037	0.048	0.048
8	15-Aug	2 mo.	Instantaneous	0.061	0.055	0.031	87.7	143.0	0.019	0.031	0.061	0.069
9	15-Oct	2 mo.	Instantaneous	0.040	0.039	0.010	131.3	229.2	0.012	0.037	0.040	0.047
10	15-Dec	2 mo.	Instantaneous			0.275						
11	16-Feb	2 mo.	Instantaneous	0.031	0.031	0.001	111.7	318.8	0.018	0.027	0.042	0.044
12	16-Apr	2 mo.	Instantaneous	0.017	0.017	0.006	66.2	461.9	0.012	0.014	0.017	0.031
2	14-Aug	4 mo.	Instantaneous	0.016	0.016	0.001	89.4	210.7	0.010	0.011	0.043	0.051
3	14-Oct	4 mo.	Instantaneous	0.020	0.020	0.001	97.1	260.7	0.015	0.020	0.027	0.054
4	14-Dec	4 mo.	Instantaneous	0.037	0.037	0.020	213.1	659.4	0.022	0.037	0.038	0.049
5	15-Feb	4 mo.	Instantaneous	0.027	0.034	0.034	240.7	1105.2	0.025	0.027	0.035	0.065
6	15-Apr	4 mo.	Instantaneous	0.030	0.030	0.017	194.4	383.9	0.012	0.028	0.035	0.047
7	15-Jun	4 mo.	Instantaneous	0.030	0.033	0.008	49.3	192.2	0.028	0.030	0.041	0.053
8	15-Aug	4 mo.	Instantaneous	0.059	0.048	0.017	89.8	149.5	0.020	0.038	0.059	0.061
9	15-Oct	4 mo.	Instantaneous	0.037	0.037	0.019	128.7	221.6	0.012	0.029	0.040	0.046
10	15-Dec	4 mo.	Instantaneous			0.129						
11	16-Feb	4 mo.	Instantaneous	0.034	0.034	0.001	124.8	329.7	0.023	0.031	0.048	0.058
12	16-Apr	4 mo.	Instantaneous	0.021	0.021	0.008	66.2	464.7	0.013	0.016	0.038	0.042
3	14-Oct	6 mo.	Instantaneous	0.019	0.019	0.001	97.1	260.7	0.013	0.019	0.034	0.046
4	14-Dec	6 mo.	Instantaneous	0.037	0.037	0.010	213.1	659.4	0.021	0.037	0.050	0.053
5	15-Feb	6 mo.	Instantaneous	0.035	0.035	0.003	326.4	1319.5	0.033	0.033	0.035	0.056
6	15-Apr	6 mo.	Instantaneous	0.034	0.034	0.022	185.5	371.7	0.015	0.032	0.038	0.058
7	15-Jun	6 mo.	Instantaneous	0.037	0.034	0.010	74.1	208.4	0.016	0.028	0.041	0.051
8	15-Aug	6 mo.	Instantaneous	0.056	0.043	0.011	89.9	157.7	0.018	0.033	0.056	0.057
9	15-Oct	6 mo.	Instantaneous	0.038	0.038	0.025	130.3	220.5	0.011	0.020	0.042	0.046
10	15-Dec	6 mo.	Instantaneous			0.124						
11	16-Feb	6 mo.	Instantaneous	0.036	0.036	0.001	124.8	329.7	0.024	0.034	0.052	0.060
12	16-Apr	6 mo.	Instantaneous	0.021	0.021	0.003	66.2	461.9	0.013	0.021	0.043	0.043
4	14-Dec	8 mo.	Instantaneous	0.035	0.035	0.005	213.1	659.4	0.020	0.034	0.036	0.049
5	15-Feb	8 mo.	Instantaneous	0.035	0.035	0.007	297.1	1292.0	0.033	0.035	0.037	0.058
6	15-Apr	8 mo.	Instantaneous	0.014	0.036	0.055	142.7	336.1	0.010	0.014	0.048	0.059
7	15-Jun	8 mo.	Instantaneous	0.033	0.038	0.005	49.3	192.2	0.029	0.033	0.052	0.058
8	15-Aug	8 mo.	Instantaneous	0.056	0.055	0.012	89.9	157.7	0.017	0.030	0.056	0.056
9	15-Oct	8 mo.	Instantaneous	0.034	0.034	0.029	130.3	220.5	0.010	0.018	0.038	0.040
10	15-Dec	8 mo.	Instantaneous			0.106						
11	16-Feb	8 mo.	Instantaneous	0.029	0.043	0.001	93.1	310.1	0.024	0.029	0.051	0.058
12	16-Apr	8 mo.	Instantaneous	0.022	0.022	0.002	66.2	461.9	0.014	0.022	0.046	0.047
5	15-Feb	10 mo.	Instantaneous	0.033	0.033	0.004	297.1	1292.0	0.032	0.032	0.035	0.048
6	15-Apr	10 mo.	Instantaneous	0.049	0.041	0.025	229.9	410.6	0.010	0.034	0.049	0.061
7	15-Jun	10 mo.	Instantaneous	0.034	0.044	0.009	49.3	192.2	0.030	0.034	0.053	0.058
8	15-Aug	10 mo.	Instantaneous	0.060	0.045	0.011	89.9	157.7	0.018	0.035	0.060	0.060
9	15-Oct	10 mo.	Instantaneous	0.033	0.033	0.027	130.3	220.5	0.011	0.017	0.039	0.039
10	15-Dec	10 mo.	Instantaneous			0.116						
11	16-Feb	10 mo.	Instantaneous	0.036	0.040	0.001	120.5	322.8	0.025	0.032	0.049	0.058
12	16-Apr	10 mo.	Instantaneous	0.048	0.047	0.003	200.9	570.6	0.015	0.022	0.048	0.049
6	15-Apr	12 mo.	Instantaneous	0.055	0.035	0.042	245.9	447.2	0.010	0.031	0.054	0.055
7	15-Jun	12 mo.	Instantaneous	0.050	0.050	0.003	78.5	233.9	0.034	0.036	0.050	0.057
8	15-Aug	12 mo.	Instantaneous	0.060	0.048	0.014	89.9	157.7	0.017	0.035	0.060	0.060
9	15-Oct	12 mo.	Instantaneous	0.035	0.035	0.025	130.3	220.5	0.011	0.018	0.039	0.040
10	15-Dec	12 mo.	Instantaneous			0.215						
11	16-Feb	12 mo.	Instantaneous	0.043	0.041	0.001	150.7	350.1	0.018	0.030	0.043	0.056
12	16-Apr	12 mo.	Instantaneous	0.046	0.045	0.001	200.9	570.6	0.018	0.024	0.046	0.046

Table 5. Change points for 2, 4, 6, 8, 10, and 12 month mean total phosphorus in relation to mean benthic chlorophyll-a.

				TP change points (mg/L)			Chlorophyll-a (mg/m2)		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	Chl-a Duration	Observed (mg/L)	Median (boot)	p-value	Mean (low)	Mean (high)	5% (boot)	25% (boot)	75% (boot)	95% (boot)
2	14-Aug	2 mo.	Mean	0.017	0.017	0.001	85.7	209.5	0.011	0.013	0.017	0.057
3	14-Oct	2 mo.	Mean	0.022	0.022	0.001	91.0	235.7	0.017	0.017	0.022	0.055
4	14-Dec	2 mo.	Mean	0.038	0.038	0.012	164.9	451.9	0.019	0.035	0.041	0.043
5	15-Feb	2 mo.	Mean	0.016	0.016	0.047	182.5	811.9	0.010	0.016	0.021	0.032
6	15-Apr	2 mo.	Mean	0.026	0.026	0.017	235.8	736.2	0.022	0.024	0.027	0.027
7	15-Jun	2 mo.	Mean	0.037	0.037	0.017	121.9	278.8	0.015	0.035	0.040	0.048
8	15-Aug	2 mo.	Mean	0.053	0.053	0.017	76.6	165.2	0.025	0.036	0.061	0.061
9	15-Oct	2 mo.	Mean	0.040	0.040	0.004	101.2	180.0	0.019	0.039	0.040	0.047
10	15-Dec	2 mo.	Mean	0.019	0.019	0.009	76.1	150.1	0.007	0.015	0.033	0.042
11	16-Feb	2 mo.	Mean	0.023	0.023	0.001	65.1	199.2	0.011	0.018	0.023	0.031
12	16-Apr	2 mo.	Mean	0.017	0.017	0.001	77.4	382.5	0.012	0.017	0.019	0.031
2	14-Aug	4 mo.	Mean	0.016	0.016	0.001	85.7	209.5	0.010	0.012	0.016	0.051
3	14-Oct	4 mo.	Mean	0.020	0.020	0.001	87.3	226.1	0.015	0.016	0.020	0.038
4	14-Dec	4 mo.	Mean	0.037	0.037	0.004	152.9	379.5	0.016	0.022	0.038	0.049
5	15-Feb	4 mo.	Mean	0.029	0.034	0.017	203.0	667.5	0.020	0.027	0.035	0.035
6	15-Apr	4 mo.	Mean	0.027	0.027	0.010	218.3	696.2	0.020	0.026	0.028	0.028
7	15-Jun	4 mo.	Mean	0.030	0.030	0.006	173.7	574.8	0.027	0.029	0.030	0.037
8	15-Aug	4 mo.	Mean	0.038	0.038	0.011	105.7	224.5	0.015	0.035	0.045	0.059
9	15-Oct	4 mo.	Mean	0.037	0.037	0.003	82.7	177.9	0.019	0.037	0.046	0.060
10	15-Dec	4 mo.	Mean	0.019	0.019	0.003	72.1	138.7	0.011	0.019	0.042	0.048
11	16-Feb	4 mo.	Mean	0.023	0.023	0.001	84.2	202.0	0.011	0.018	0.031	0.039
12	16-Apr	4 mo.	Mean	0.021	0.021	0.001	65.4	288.1	0.013	0.016	0.021	0.038
3	14-Oct	6 mo.	Mean	0.019	0.019	0.001	87.3	226.1	0.013	0.015	0.019	0.034
4	14-Dec	6 mo.	Mean	0.037	0.037	0.001	160.4	385.1	0.016	0.021	0.050	0.053
5	15-Feb	6 mo.	Mean	0.035	0.035	0.003	245.4	759.2	0.033	0.033	0.035	0.035
6	15-Apr	6 mo.	Mean	0.032	0.032	0.006	219.4	718.2	0.027	0.029	0.032	0.040
7	15-Jun	6 mo.	Mean	0.028	0.031	0.004	157.2	548.5	0.026	0.028	0.034	0.037
8	15-Aug	6 mo.	Mean	0.033	0.037	0.008	105.7	224.5	0.018	0.033	0.055	0.056
9	15-Oct	6 mo.	Mean	0.038	0.038	0.002	84.3	176.9	0.020	0.038	0.046	0.058
10	15-Dec	6 mo.	Mean	0.019	0.030	0.004	72.1	138.7	0.012	0.019	0.039	0.047
11	16-Feb	6 mo.	Mean	0.024	0.024	0.001	84.2	202.0	0.011	0.018	0.036	0.042
12	16-Apr	6 mo.	Mean	0.021	0.021	0.001	65.4	288.2	0.013	0.016	0.021	0.043
4	14-Dec	8 mo.	Mean	0.020	0.020	0.001	101.7	315.4	0.013	0.020	0.035	0.049
5	15-Feb	8 mo.	Mean	0.035	0.035	0.002	201.5	615.8	0.033	0.034	0.037	0.048
6	15-Apr	8 mo.	Mean	0.037	0.036	0.002	243.6	657.2	0.029	0.033	0.037	0.046
7	15-Jun	8 mo.	Mean	0.033	0.033	0.002	176.8	589.6	0.028	0.033	0.038	0.039
8	15-Aug	8 mo.	Mean	0.037	0.037	0.002	172.0	473.4	0.028	0.030	0.040	0.040
9	15-Oct	8 mo.	Mean	0.034	0.034	0.010	111.8	223.1	0.014	0.030	0.045	0.056
10	15-Dec	8 mo.	Mean	0.020	0.033	0.003	62.1	145.5	0.012	0.020	0.040	0.047
11	16-Feb	8 mo.	Mean	0.029	0.029	0.001	82.3	183.8	0.015	0.029	0.034	0.043
12	16-Apr	8 mo.	Mean	0.022	0.022	0.001	79.7	267.8	0.014	0.022	0.026	0.046
5	15-Feb	10 mo.	Mean	0.033	0.033	0.001	185.1	537.6	0.032	0.032	0.033	0.035
6	15-Apr	10 mo.	Mean	0.037	0.037	0.001	203.0	567.2	0.032	0.036	0.037	0.037
7	15-Jun	10 mo.	Mean	0.038	0.037	0.001	211.7	564.9	0.030	0.034	0.038	0.047
8	15-Aug	10 mo.	Mean	0.040	0.039	0.003	194.7	534.5	0.031	0.035	0.040	0.048
9	15-Oct	10 mo.	Mean	0.039	0.038	0.001	173.8	444.1	0.029	0.033	0.039	0.039
10	15-Dec	10 mo.	Mean	0.018	0.030	0.005	80.7	185.8	0.011	0.018	0.043	0.056
11	16-Feb	10 mo.	Mean	0.025	0.032	0.001	72.0	178.5	0.014	0.025	0.038	0.044
12	16-Apr	10 mo.	Mean	0.027	0.027	0.001	84.5	240.8	0.015	0.022	0.027	0.048
6	15-Apr	12 mo.	Mean	0.035	0.035	0.001	189.1	510.7	0.031	0.035	0.035	0.035
7	15-Jun	12 mo.	Mean	0.036	0.036	0.001	170.9	497.6	0.034	0.036	0.036	0.049
8	15-Aug	12 mo.	Mean	0.040	0.040	0.003	188.8	492.0	0.031	0.035	0.040	0.048
9	15-Oct	12 mo.	Mean	0.040	0.039	0.002	185.9	483.2	0.030	0.035	0.040	0.048
10	15-Dec	12 mo.	Mean	0.041	0.038	0.002	158.9	383.4	0.028	0.035	0.041	0.041
11	16-Feb	12 mo.	Mean	0.018	0.030	0.001	81.3	204.3	0.014	0.018	0.041	0.043
12	16-Apr	12 mo.	Mean	0.024	0.024	0.001	71.0	226.7	0.014	0.024	0.034	0.046

Change point analysis: TP vs. Cladophora glomerata biovolume

Cladophora glomerata was the dominant filamentous green alga identified in the study. *Cladophora* is widely known as a nuisance species that proliferates with nutrient overenrichment (Dodds and Gudder 1992). Benthic algal biomass values that exceeded 200-300 mg/m^2 were typically associated with high levels of *Cladophora* biovolume.

Cladophora biovolume was very low to completely absent at relatively low levels of TP, but a clear, nonlinear change in its frequency and abundance occurred at moderate to high levels of TP (Figures 18 and 19, Table 6).

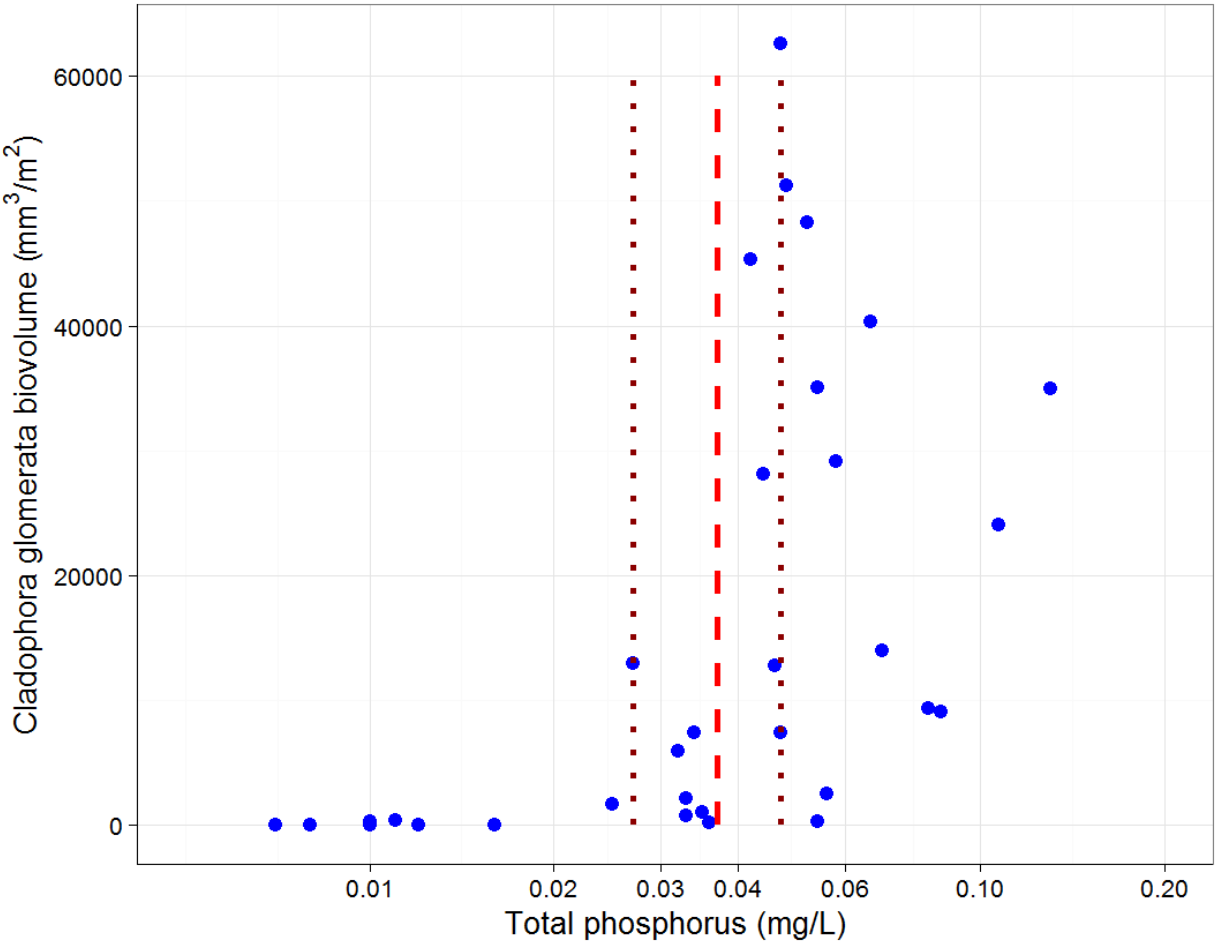


Figure 18. Two-year mean TP (April 2014-2016) vs. mean *Cladophora glomerata* biovolume from events 1, 3, 5, 6, 9, and 12. The dashed red line corresponds to 0.037 mg/L TP, whereas the dotted lines correspond to 0.027 and 0.047 mg/L TP, respectively.

Table 6. Change points for 6, 8, 10, and 12 month mean total phosphorus in relation to instantaneous and mean *Cladophora glomerata* biovolume. *Cladophora* biovolume was measured only on events 1, 3, 5, 6, 9, and 12.

				Change points (mg/L)			Biovolume (mm3/m2)		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	*Cladophora* Duration	Observed	Median (boot)	p-value	Mean (low)	Mean (high)	5% (boot)	25% (boot)	75% (boot)	95% (boot)
3	14-Oct	6 mo.	Instantaneous	0.048	0.048	0.001	406	9028	0.033	0.033	0.035	0.037
5	15-Feb	6 mo.	Instantaneous	0.037	0.035	0.004	3277	109728	0.033	0.034	0.037	0.041
6	15-Apr	6 mo.	Instantaneous			0.065						
9	15-Oct	6 mo.	Instantaneous	0.038	0.039	0.048	47	1031	0.035	0.037	0.052	0.066
12	16-Apr	6 mo.	Instantaneous	0.025	0.025	0.005	0	22428	0.018	0.024	0.042	0.043
5	15-Feb	8 mo.	Instantaneous	0.035	0.033	0.003	3277	109728	0.032	0.033	0.035	0.039
6	15-Apr	8 mo.	Instantaneous			0.058						
9	15-Oct	8 mo.	Instantaneous	0.034	0.034	0.035	47	1031	0.031	0.033	0.042	0.094
12	16-Apr	8 mo.	Instantaneous	0.026	0.026	0.032	0	22428	0.019	0.026	0.046	0.048
5	15-Feb	10 mo.	Instantaneous	0.035	0.035	0.007	3277	109728	0.033	0.033	0.035	0.048
6	15-Apr	10 mo.	Instantaneous									
9	15-Oct	10 mo.	Instantaneous	0.033	0.033	0.033	47	1031	0.030	0.033	0.039	0.085
12	16-Apr	10 mo.	Instantaneous	0.027	0.027	0.014	0	22428	0.019	0.026	0.048	0.049
6	15-Apr	12 mo.	Instantaneous			0.074						
9	15-Oct	12 mo.	Instantaneous	0.035	0.035	0.029	47	1031	0.032	0.034	0.047	0.083
12	16-Apr	12 mo.	Instantaneous	0.024	0.024	0.033	0	22428	0.015	0.021	0.046	0.048
3	14-Oct	6 mo.	Mean	0.048	0.036	0.007	1515	6848	0.016	0.033	0.050	0.055
5	15-Feb	6 mo.	Mean	0.035	0.034	0.002	1771	58932	0.033	0.034	0.035	0.040
6	15-Apr	6 mo.	Mean	0.032	0.031	0.014	1235	49174	0.027	0.031	0.032	0.038
9	15-Oct	6 mo.	Mean	0.051	0.051	0.017	492	6327	0.049	0.050	0.052	0.058
12	16-Apr	6 mo.	Mean	0.043	0.039	0.042	2229	15714	0.018	0.024	0.043	0.046
5	15-Oct	8 mo.	Mean	0.040	0.039	0.004	1334	40199	0.035	0.039	0.040	0.042
6	16-Apr	8 mo.	Mean	0.046	0.046	0.033	2229	15714	0.019	0.026	0.046	0.049
9	15-Feb	8 mo.	Mean	0.037	0.035	0.003	1980	40855	0.033	0.034	0.037	0.041
12	15-Apr	8 mo.	Mean	0.037	0.037	0.001	1343	42562	0.036	0.037	0.037	0.039
5	15-Feb	10 mo.	Mean	0.035	0.033	0.001	1980	40855	0.032	0.033	0.035	0.039
6	15-Apr	10 mo.	Mean	0.038	0.037	0.001	1605	33097	0.036	0.036	0.038	0.048
9	15-Oct	10 mo.	Mean	0.039	0.038	0.001	1334	40199	0.036	0.038	0.039	0.041
12	16-Apr	10 mo.	Mean	0.048	0.047	0.015	2248	16650	0.019	0.027	0.048	0.049
6	15-Apr	12 mo.	Mean	0.037	0.035	0.001	1605	33097	0.034	0.035	0.036	0.037
9	15-Oct	12 mo.	Mean	0.039	0.038	0.001	1069	32183	0.036	0.038	0.039	0.041
12	16-Apr	12 mo.	Mean	0.046	0.046	0.025	1907	14665	0.021	0.040	0.046	0.049
12	16-Apr	24 mo.	Mean	0.039	0.039	0.002	1832	26752	0.035	0.035	0.039	0.047

Figure 19. Photograph of *Cladophora glomerata* covering the stream bottom of the Illinois River at Tahlequah (ILLI8), February 2015.

Change point analysis: TP vs. nuisance taxa proportion of total biovolume

Five genera of filamentous green algae that occurred in our data set were classified as nuisance taxa: *Cladophora, Oedogonium, Rhizoclonium, Spirogyra, and Hydrodictyon.* Although *Cladophora* represented most of the total nuisance biovolume (>95%), there were a few sites that had blooms of other taxa during the 2 year study. The committee recommended that the analysis be conducted on the proportion of the total biovolume as nuisance taxa as a complementary but different way of examining the data (Figure 20, Table 7). Because diatoms were identified on only 4 events compared to 6 events for soft algae, proportions were calculated based on the total soft-algae biovolume. A binomial form of change point analysis was used for these data, which is appropriate for proportion data (Zuur et al. 2009).

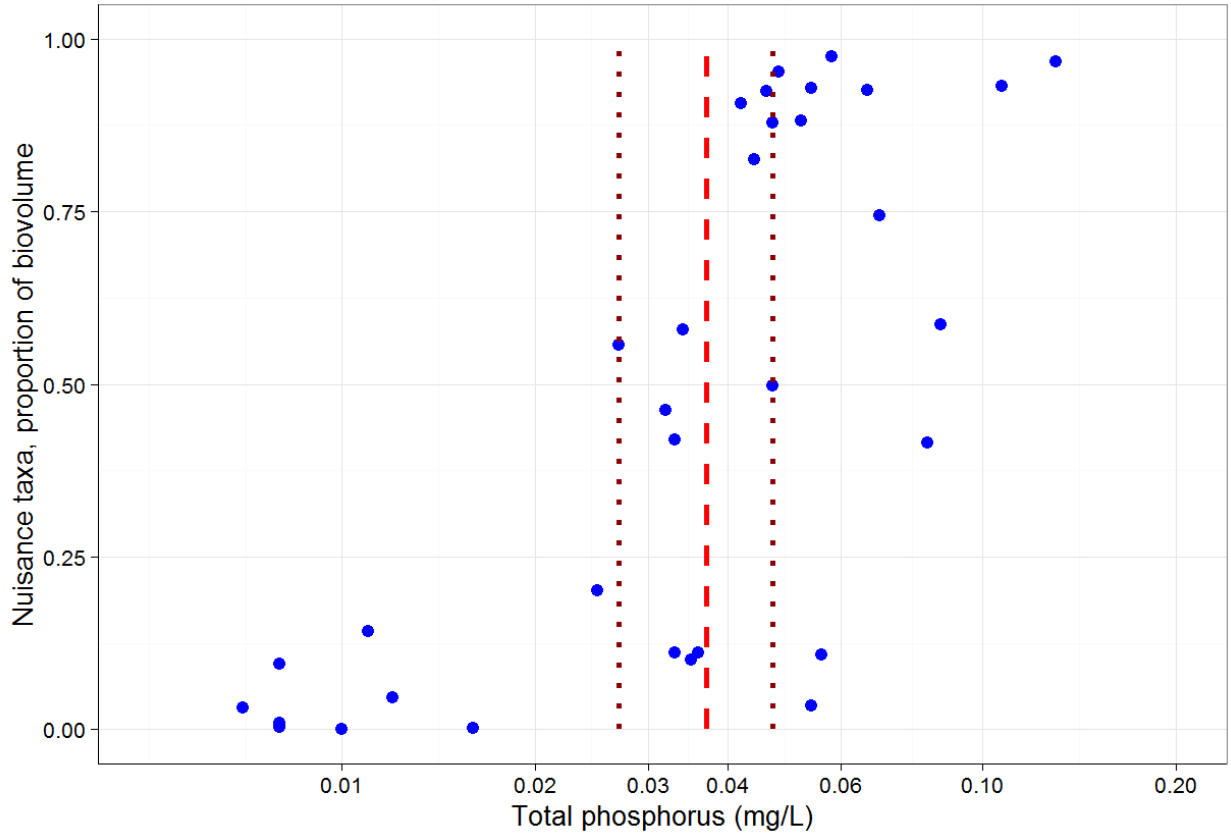


Figure 20. Two-year mean TP (April 2014-2016) vs. mean nuisance taxa proportion from events 1, 3, 5, 6, 9, and 12. The dashed red line corresponds to 0.037 mg/L TP, whereas the dotted lines correspond to 0.027 and 0.047 mg/L TP, respectively.

Table 7. Change points for 6, 8, 10, and 12 month mean total phosphorus in relation to instantaneous and mean nuisance taxa proportion of total biovolume. Soft algal species composition was measured only on events 1, 3, 5, 6, 9, and 12.

				TP change points (mg/L)			Nuisance proportion		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	Nuisance Duration	Observed	Median (boot)	p-value	Mean (low)	Mean (high)	5% (boot)	25% (boot)	75% (boot)	95% (boot)
	14-Oct	6 mo.	Instantaneous	0.074	0.073	0.001	0.238	0.781	0.047	0.052	0.077	0.085
	15-Feb	6 mo.	Instantaneous	0.035	0.035	0.005	0.153	0.856	0.033	0.034	0.052	0.056
	15-Apr	6 mo.	Instantaneous			ns						
	15-Oct	6 mo.	Instantaneous	0.051	0.051	0.036	0.112	0.330	0.045	0.051	0.052	0.097
	16-Apr	6 mo.	Instantaneous	0.043	0.042	0.002	0.148	0.878	0.035	0.038	0.043	0.058
	15-Feb	8 mo.	Instantaneous	0.035	0.036	0.002	0.106	0.861	0.033	0.034	0.056	0.058
	15-Apr	8 mo.	Instantaneous			ns						
	15-Oct	8 mo.	Instantaneous			ns						
	16-Apr	8 mo.	Instantaneous	0.046	0.046	0.005	0.148	0.878	0.038	0.042	0.047	0.060
	15-Feb	10 mo.	Instantaneous	0.033	0.033	0.004	0.106	0.861	0.032	0.033	0.048	0.058
	15-Apr	10 mo.	Instantaneous			ns						
	15-Oct	10 mo.	Instantaneous			ns						
	16-Apr	10 mo.	Instantaneous	0.047	0.047	0.004	0.148	0.878	0.036	0.043	0.048	0.060
	15-Apr	12 mo.	Instantaneous			ns						
	15-Oct	12 mo.	Instantaneous			ns						
	16-Apr	12 mo.	Instantaneous	0.046	0.046	0.003	0.148	0.878	0.037	0.043	0.046	0.058
	14-Oct	6 mo.	Mean	0.052	0.057	0.049	0.116	0.464	0.036	0.051	0.074	0.113
	15-Feb	6 mo.	Mean	0.035	0.035	0.004	0.154	0.840	0.033	0.034	0.048	0.056
	15-Apr	6 mo.	Mean	0.040	0.040	0.040	0.273	0.800	0.028	0.037	0.055	0.064
	15-Oct	6 mo.	Mean	0.058	0.057	0.096	0.263	0.579	0.036	0.050	0.059	0.102
	16-Apr	6 mo.	Mean	0.043	0.042	0.015	0.223	0.777	0.031	0.038	0.044	0.058
	15-Feb	8 mo.	Mean	0.035	0.049	0.011	0.102	0.701	0.033	0.035	0.056	0.105
	15-Apr	8 mo.	Mean	0.037	0.037	0.003	0.160	0.820	0.036	0.037	0.048	0.059
	15-Oct	8 mo.	Mean			ns						
	16-Apr	8 mo.	Mean	0.046	0.046	0.010	0.223	0.777	0.037	0.040	0.047	0.061
	15-Feb	10 mo.	Mean	0.033	0.035	0.009	0.102	0.701	0.032	0.033	0.054	0.100
	15-Apr	10 mo.	Mean	0.037	0.038	0.002	0.120	0.821	0.036	0.036	0.048	0.055
	15-Oct	10 mo.	Mean			ns						
	16-Apr	10 mo.	Mean	0.048	0.048	0.005	0.238	0.794	0.036	0.046	0.048	0.060
	15-Apr	12 mo.	Mean	0.035	0.036	0.009	0.107	0.703	0.035	0.035	0.055	0.095
	15-Oct	12 mo.	Mean	0.039	0.039	0.003	0.170	0.803	0.036	0.038	0.040	0.056
	16-Apr	12 mo.	Mean	0.046	0.046	0.020	0.274	0.754	0.037	0.042	0.047	0.059
	16-Apr	24 mo.	Mean	0.039	0.039	0.005	0.179	0.734	0.035	0.036	0.040	0.061

TITAN: TP vs. algal community composition

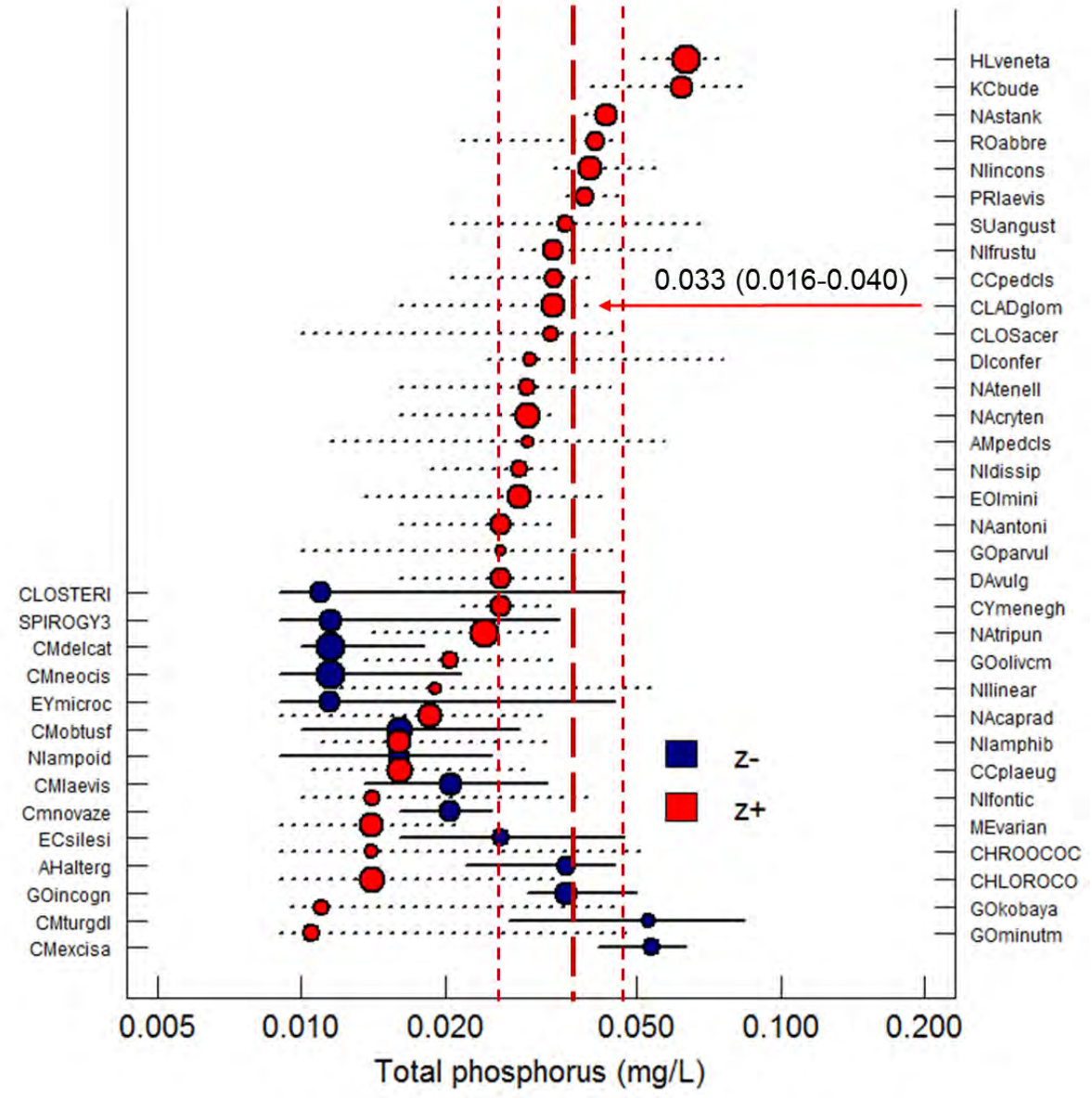


Figure 21. Results of TITAN using two-year mean TP (April 2014-2016) as the predictor vs. mean biovolume of all taxa that occurred at least 3 times from events 1, 3, 5, 6, 9, and 12. Shown are pure and reliable threshold indicator taxa. Negative responding taxa are listed on the left y-axis and marked by dark blue points, whereas positive responding taxa are on the right y-axis and marked by red points. Points are located at change point. Error bars represent the 5-95% bootstrap quantile intervals. The community-level threshold for negative-responding taxa (sumz-) was 0.021 (0.010-0.025) mg/L, whereas the positive-responding community threshold was also 0.021 mg/L, but had higher bootstrap quantile intervals (0.016-0.033). The vertical, red dashed line corresponds to 0.037 mg/L TP, whereas the vertical dotted lines correspond to 0.027 and 0.047 mg/L TP, respectively.

Table 8. TITAN community-level negative (declining taxa only) change points for 6, 8, 10, and 12 month mean total phosphorus in relation to instantaneous and mean taxa biovolumes.

					TP change points (mg/L)		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	Taxa Duration	Response direction	Observed	Median(boot)	5% (boot)	10% (boot)	90% (boot)	95% (boot)
1	14-Jun	6 mo.	Instantaneous	sumz- (negative)	0.019	0.019	0.016	0.017	0.025	0.025
3	14-Oct	6 mo.	Instantaneous	sumz- (negative)	0.013	0.013	0.010	0.011	0.035	0.037
5	15-Feb	6 mo.	Instantaneous	sumz- (negative)	0.025	0.025	0.013	0.013	0.029	0.033
6	15-Apr	6 mo.	Instantaneous	sumz- (negative)	0.017	0.016	0.012	0.012	0.021	0.022
9	15-Oct	6 mo.	Instantaneous	sumz- (negative)	0.026	0.026	0.016	0.021	0.029	0.030
12	16-Apr	6 mo.	Instantaneous	sumz- (negative)	0.010	0.013	0.010	0.010	0.023	0.027
5	15-Feb	8 mo.	Instantaneous	sumz- (negative)	0.033	0.033	0.013	0.013	0.035	0.037
6	15-Apr	8 mo.	Instantaneous	sumz- (negative)	0.012	0.014	0.011	0.011	0.020	0.021
9	15-Oct	8 mo.	Instantaneous	sumz- (negative)	0.024	0.024	0.014	0.015	0.027	0.029
12	16-Apr	8 mo.	Instantaneous	sumz- (negative)	0.011	0.013	0.011	0.011	0.029	0.037
5	15-Feb	10 mo.	Instantaneous	sumz- (negative)	0.024	0.024	0.012	0.012	0.032	0.033
6	15-Apr	10 mo.	Instantaneous	sumz- (negative)	0.012	0.013	0.010	0.011	0.020	0.021
9	16-Apr	10 mo.	Instantaneous	sumz- (negative)	0.023	0.023	0.014	0.015	0.027	0.029
12	15-Oct	10 mo.	Instantaneous	sumz- (negative)	0.011	0.012	0.009	0.010	0.033	0.034
6	15-Apr	12 mo.	Instantaneous	sumz- (negative)	0.011	0.012	0.011	0.011	0.020	0.021
9	15-Oct	12 mo.	Instantaneous	sumz- (negative)	0.023	0.023	0.015	0.017	0.028	0.030
12	16-Apr	12 mo.	Instantaneous	sumz- (negative)	0.012	0.012	0.009	0.010	0.030	0.035
3	14-Oct	6 mo.	Mean	sumz- (negative)	0.019	0.019	0.010	0.011	0.025	0.025
5	15-Feb	6 mo.	Mean	sumz- (negative)	0.025	0.028	0.013	0.021	0.037	0.037
6	15-Apr	6 mo.	Mean	sumz- (negative)	0.020	0.020	0.010	0.013	0.024	0.025
9	15-Oct	6 mo.	Mean	sumz- (negative)	0.020	0.020	0.014	0.016	0.027	0.029
12	16-Apr	6 mo.	Mean	sumz- (negative)	0.021	0.021	0.010	0.011	0.024	0.027
5	15-Feb	8 mo.	Mean	sumz- (negative)	0.025	0.028	0.013	0.021	0.037	0.039
6	15-Apr	8 mo.	Mean	sumz- (negative)	0.016	0.016	0.011	0.012	0.021	0.023
9	15-Oct	8 mo.	Mean	sumz- (negative)	0.024	0.021	0.012	0.013	0.024	0.027
12	16-Apr	8 mo.	Mean	sumz- (negative)	0.022	0.022	0.011	0.013	0.029	0.029
5	15-Feb	10 mo.	Mean	sumz- (negative)	0.019	0.019	0.011	0.016	0.024	0.025
6	15-Apr	10 mo.	Mean	sumz- (negative)	0.014	0.015	0.010	0.012	0.026	0.034
9	15-Oct	10 mo.	Mean	sumz- (negative)	0.023	0.021	0.010	0.011	0.023	0.026
12	16-Apr	10 mo.	Mean	sumz- (negative)	0.011	0.022	0.011	0.011	0.025	0.027
6	15-Apr	12 mo.	Mean	sumz- (negative)	0.020	0.019	0.012	0.012	0.021	0.023
9	15-Oct	12 mo.	Mean	sumz- (negative)	0.023	0.018	0.013	0.013	0.023	0.025
12	16-Apr	12 mo.	Mean	sumz- (negative)	0.018	0.018	0.011	0.012	0.024	0.024
12	16-Apr	24 mo.	Mean	sumz- (negative)	0.021	0.021	0.010	0.011	0.024	0.025

Table 9. TITAN community-level positive (increasing taxa only) change points for 6, 8, 10, and 12 month mean total phosphorus in relation to instantaneous and mean taxa biovolumes.

					TP change points (mg/L)		Bootstrap quantiles (mg/L)			
Event	Date	TP Duration	Taxa Duration	Response direction	Observed	Median(boot)	5% (boot)	10% (boot)	90% (boot)	95% (boot)
1	14-Jun	6 mo.	Instantaneous	sumz+ (positive)	0.035	0.034	0.018	0.019	0.056	0.056
3	14-Oct	6 mo.	Instantaneous	sumz+ (positive)	0.056	0.056	0.042	0.051	0.064	0.070
5	15-Feb	6 mo.	Instantaneous	sumz+ (positive)	0.020	0.025	0.019	0.020	0.035	0.037
6	15-Apr	6 mo.	Instantaneous	sumz+ (positive)	0.038	0.034	0.022	0.027	0.042	0.042
9	15-Oct	6 mo.	Instantaneous	sumz+ (positive)	0.050	0.038	0.020	0.021	0.050	0.050
12	16-Apr	6 mo.	Instantaneous	sumz+ (positive)	0.025	0.021	0.018	0.020	0.043	0.043
5	15-Feb	8 mo.	Instantaneous	sumz+ (positive)	0.025	0.025	0.019	0.019	0.037	0.039
6	15-Apr	8 mo.	Instantaneous	sumz+ (positive)	0.039	0.038	0.027	0.029	0.043	0.048
9	15-Oct	8 mo.	Instantaneous	sumz+ (positive)	0.034	0.034	0.016	0.018	0.043	0.044
12	16-Apr	8 mo.	Instantaneous	sumz+ (positive)	0.022	0.026	0.019	0.021	0.046	0.047
5	15-Feb	10 mo.	Instantaneous	sumz+ (positive)	0.024	0.024	0.019	0.019	0.035	0.036
6	15-Apr	10 mo.	Instantaneous	sumz+ (positive)	0.037	0.037	0.031	0.032	0.049	0.049
9	16-Apr	10 mo.	Instantaneous	sumz+ (positive)	0.039	0.038	0.017	0.021	0.042	0.042
12	15-Oct	10 mo.	Instantaneous	sumz+ (positive)	0.022	0.027	0.019	0.021	0.047	0.048
6	15-Apr	12 mo.	Instantaneous	sumz+ (positive)	0.035	0.035	0.029	0.031	0.041	0.043
9	15-Oct	12 mo.	Instantaneous	sumz+ (positive)	0.040	0.035	0.015	0.018	0.048	0.048
12	16-Apr	12 mo.	Instantaneous	sumz+ (positive)	0.024	0.024	0.020	0.021	0.046	0.046
3	14-Oct	6 mo.	Mean	sumz+ (positive)	0.019	0.021	0.017	0.018	0.035	0.040
5	15-Feb	6 mo.	Mean	sumz+ (positive)	0.037	0.037	0.023	0.025	0.049	0.051
6	15-Apr	6 mo.	Mean	sumz+ (positive)	0.029	0.035	0.023	0.025	0.043	0.048
9	15-Oct	6 mo.	Mean	sumz+ (positive)	0.049	0.047	0.032	0.033	0.050	0.050
12	16-Apr	6 mo.	Mean	sumz+ (positive)	0.016	0.021	0.014	0.016	0.031	0.035
5	15-Feb	8 mo.	Mean	sumz+ (positive)	0.033	0.035	0.021	0.024	0.049	0.051
6	15-Apr	8 mo.	Mean	sumz+ (positive)	0.039	0.037	0.020	0.026	0.043	0.046
9	15-Oct	8 mo.	Mean	sumz+ (positive)	0.030	0.030	0.021	0.024	0.042	0.043
12	16-Apr	8 mo.	Mean	sumz+ (positive)	0.022	0.024	0.015	0.017	0.035	0.041
5	15-Feb	10 mo.	Mean	sumz+ (positive)	0.019	0.021	0.016	0.017	0.032	0.033
6	15-Apr	10 mo.	Mean	sumz+ (positive)	0.037	0.037	0.024	0.026	0.041	0.049
9	15-Oct	10 mo.	Mean	sumz+ (positive)	0.029	0.029	0.022	0.023	0.040	0.041
12	16-Apr	10 mo.	Mean	sumz+ (positive)	0.027	0.023	0.016	0.017	0.036	0.041
6	15-Apr	12 mo.	Mean	sumz+ (positive)	0.025	0.025	0.019	0.020	0.035	0.035
9	15-Oct	12 mo.	Mean	sumz+ (positive)	0.028	0.028	0.018	0.018	0.040	0.042
12	16-Apr	12 mo.	Mean	sumz+ (positive)	0.024	0.024	0.016	0.018	0.042	0.042
12	16-Apr	24 mo.	Mean	sumz+ (positive)	0.021	0.024	0.016	0.019	0.029	0.033

Reference value threshold approach

We related biovolume of *Cladophora glomerata*, which was measured during events 1, 3, 5, 6, 9, and 12, to benthic chlorophyll-a, which was measured during all 12 events, to evaluate whether there was a level of benthic chlorophyll that corresponded to a nonlinear increase in *Cladophora*. The rationale was that (1) we did not have biovolume of *Cladophora* for all events, because this requires manual microscopic estimation by an expert taxonomist, a tedious and expensive process beyond the budget of this study, (2) "nuisance" levels defined by the literature are subjective and context dependent, and (3) some of our sites with low phosphorus consistently yielded benthic chlorophyll-a levels that approached or exceeded literature values for "nuisance" conditions (>150-200 mg/m^2), yet virtually none of this algal biomass was *Cladophora* or other nuisance species of filamentous green algae, and (4) our sampling protocol required large substrates (10-20 cm) for chlorophyll-a estimation, whereas most other protocols do not specify substrate size and thus are more likely to include smaller substrates that are much more prone to tumbling and scouring and thus would bias chlorophyll-a estimates downward, especially at sites dominated by small gravel.

Graphical visualization of the relationship between mean *Cladophora* biovolume and benthic chlorophyll-a suggested that segmented regression would be the most appropriate method for estimating the level of algal biomass that corresponded to a shift to the dominant nuisance species in the Designated Scenic Rivers. This particular method is generally not appropriate for most types of ecological data because it requires that the relationship between two variables can be represented by two or more linear segments that conform to parametric assumptions of normality and homoscedasticity. However, in this instance, these two variables were dependent on each other and exhibited a relationship that was ideal for segmented regression. Lack of independence was not an issue here because we were not testing a hypothesis that required this assumption.

Results of these analyses (Figure 22) indicated that 290 and 183 mg/m2 benthic chlorophyll-a were levels corresponding to a shift from essentially no *Cladophora* to a linear increase in *Cladophora* biovolume during years 1 and 2, respectively. Year 1 was not dry, but lacked significant scouring events, particularly during fall 2014 when the *Cladophora* bloom began to take hold. Year 2 was wet and had many significant scouring events including the historic flood in December 2015.

Based on these results, after rounding up/down to account for statistical uncertainty (see confidence limits, Figure 22), we agreed that 150-200 mg/m^2 likely represented the lower end of potential nuisance levels of algal biomass in the Designated Scenic Rivers during a wet year, whereas levels above 300 mg/m^2 should be considered nuisance levels under most conditions, acknowledging that a few sites with the lowest levels of TP in the region achieved benthic chlorophyll-a >300 mg/m^2 in February 2015, an event marking the end of several months of relatively stable flow.

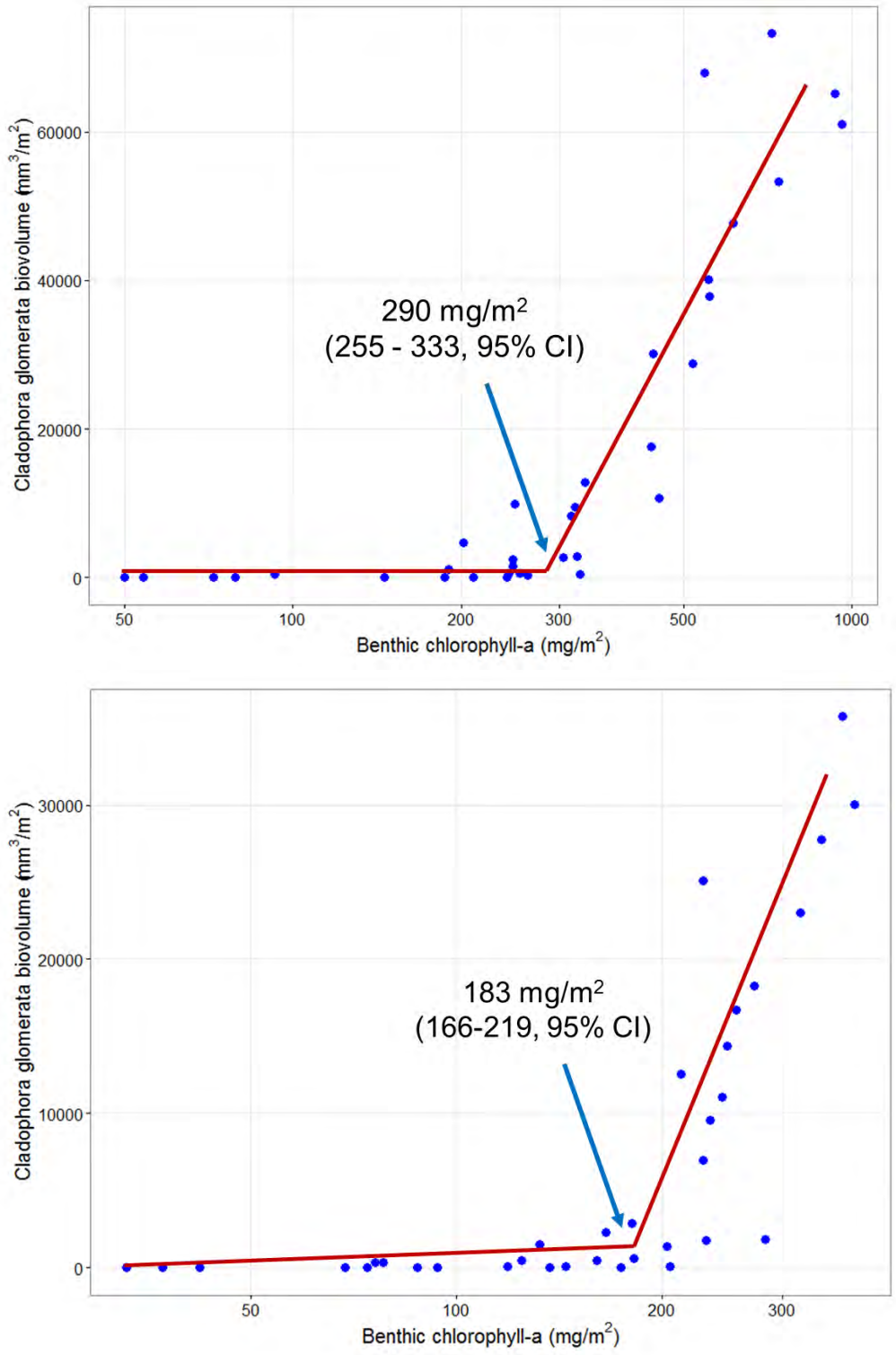


Figure 22. Results of segmented regression relating mean *Cladophora glomerata* biovolume to mean benthic chlorophyll-a during year 1 (upper panel) and year 2 (lower panel). Year 1 data represents a year with very stable flows overall, whereas year 2 represents a wet year with many scouring flows, including an historic flood.

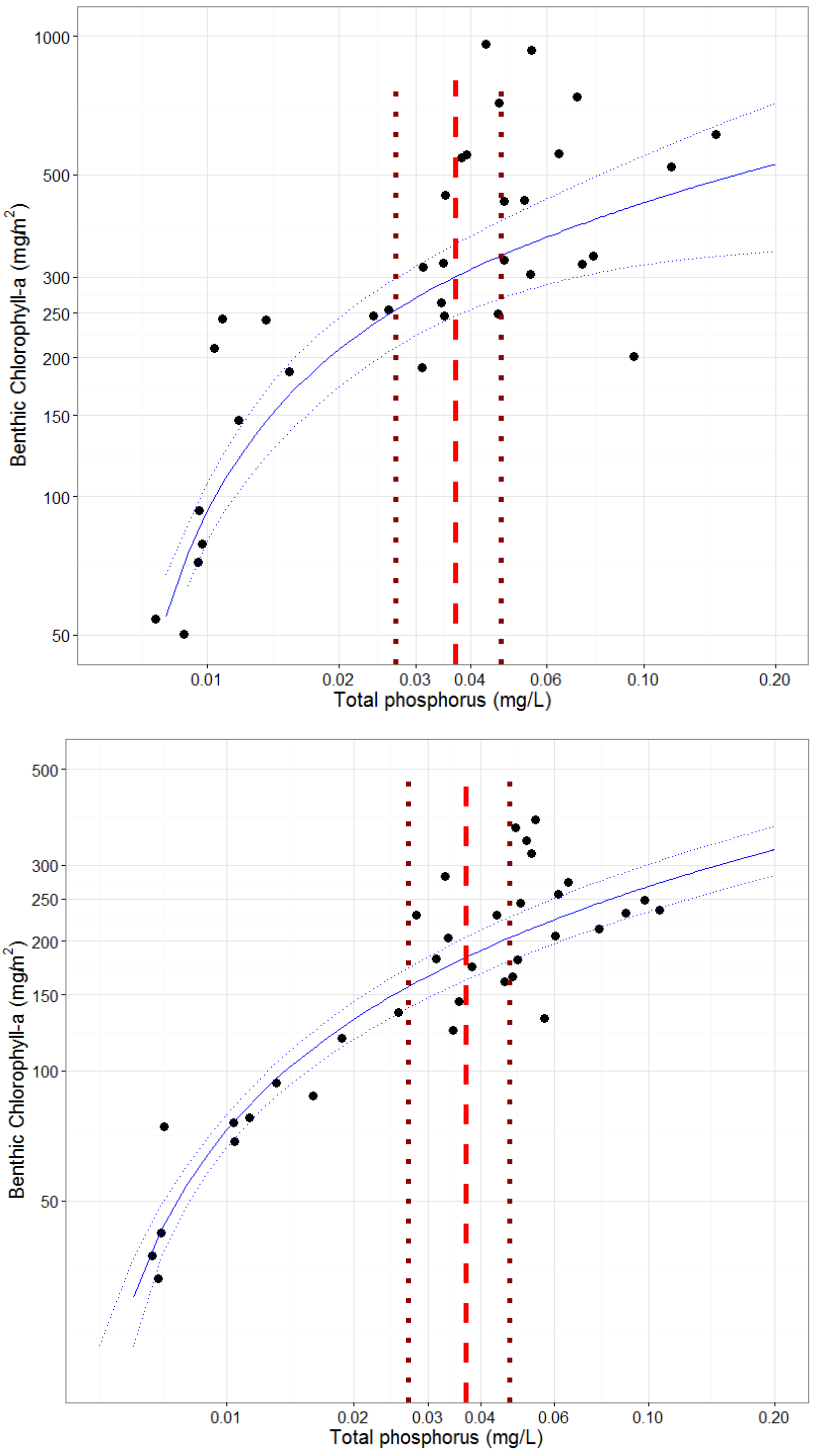


Figure 23. Mean benthic chlorophyll-a in year 1 (dry year, upper panel) and year 2 (wet year, lower panel) to annual mean total phosphorus. The fitted solid blue line is the result of a generalized additive model (GAM, deviance explained=88.5% and 89%, years 1 and 2 respectively) with 95% confidence limits shown as fine dotted lines around the fitted line. The mean chlorophyll-a values were weighted by inverse of the standard deviation to account for uncertainty. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

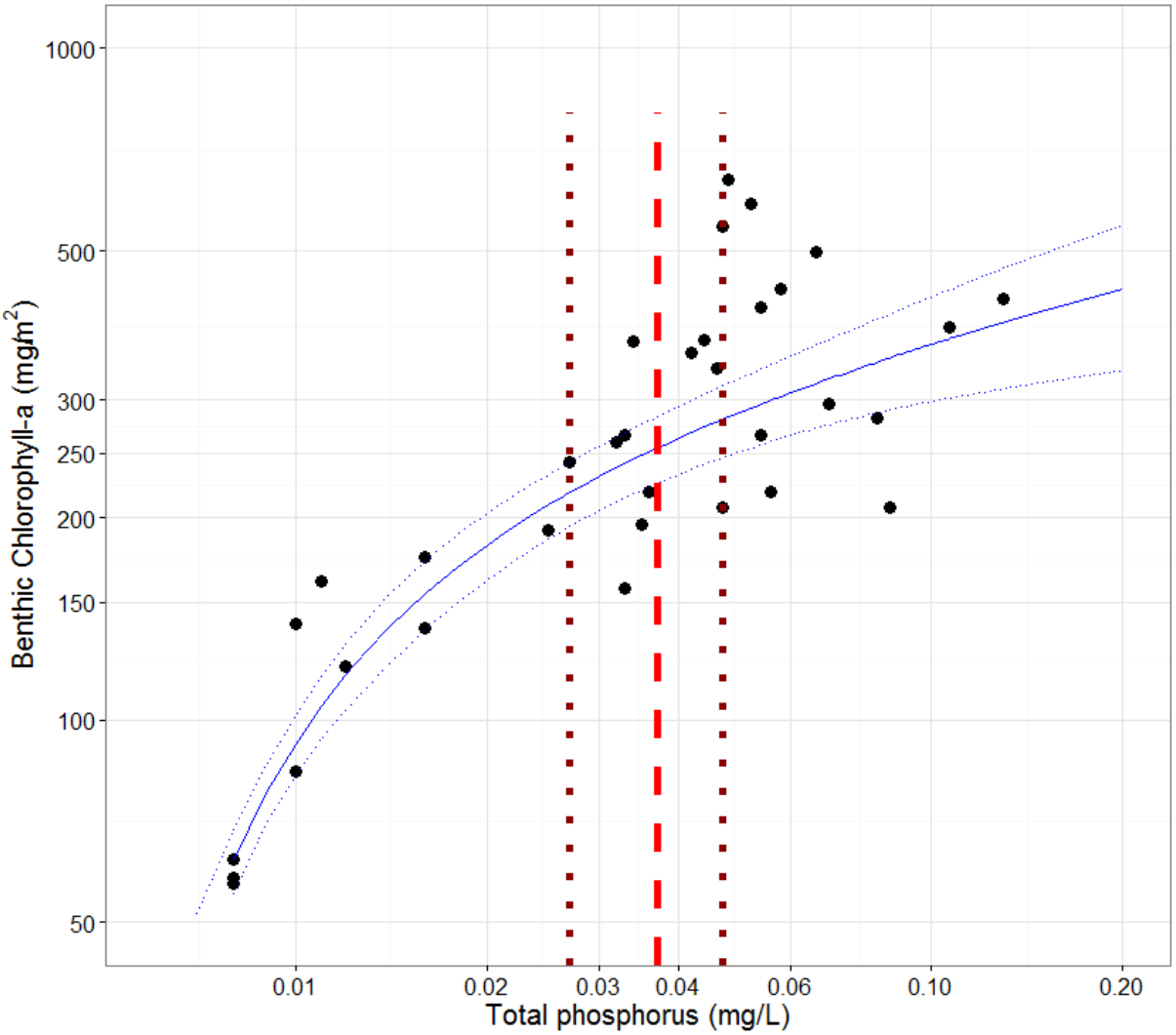


Figure 24. Mean benthic chlorophyll-a in response to mean 2-year total phosphorus. The fitted solid blue line is the result of a generalized additive model (GAM; deviance explained=90%, $p<0.00001$) with 95% confidence limits shown as fine dotted lines around the fitted line. The mean chlorophyll-a values were weighted by inverse of the standard deviation to account for uncertainty. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

Table 10. Predicted mean benthic chlorophyll-a in response year 1, year 2, and years 1 and 2-year mean total phosphorus at concentrations spanning 0.01 to 0.1 mg/L. The predictions are based on GAM models for each of the 3 data sets, with years 1 and 2 illustrated in the previous figure.

	Predicted benthic chlorophyll-a, mg/m^2								
	Year 1 (Dry, stable flows)			Year 2 (Wet, many storm flows)			Years 1 and 2, combined		
TP (mg/L)	Mean	5% CI	95% CI	Mean	5% CI	95% CI	Mean	5% CI	95% CI
0.010	93	80	107	73	67	79	92	83	102
0.020	209	173	244	131	118	145	182	161	203
0.027	255	211	298	157	140	174	218	194	242
0.030	270	224	317	166	148	184	230	205	256
0.037	300	247	354	183	163	204	254	225	283
0.040	311	255	368	190	168	212	263	232	293
0.047	333	269	397	204	180	227	280	246	314
0.050	342	275	409	209	184	233	287	251	323
0.060	366	289	444	224	197	251	307	265	348
0.075	396	304	488	243	213	273	331	281	381
0.100	435	319	550	267	234	301	362	298	427

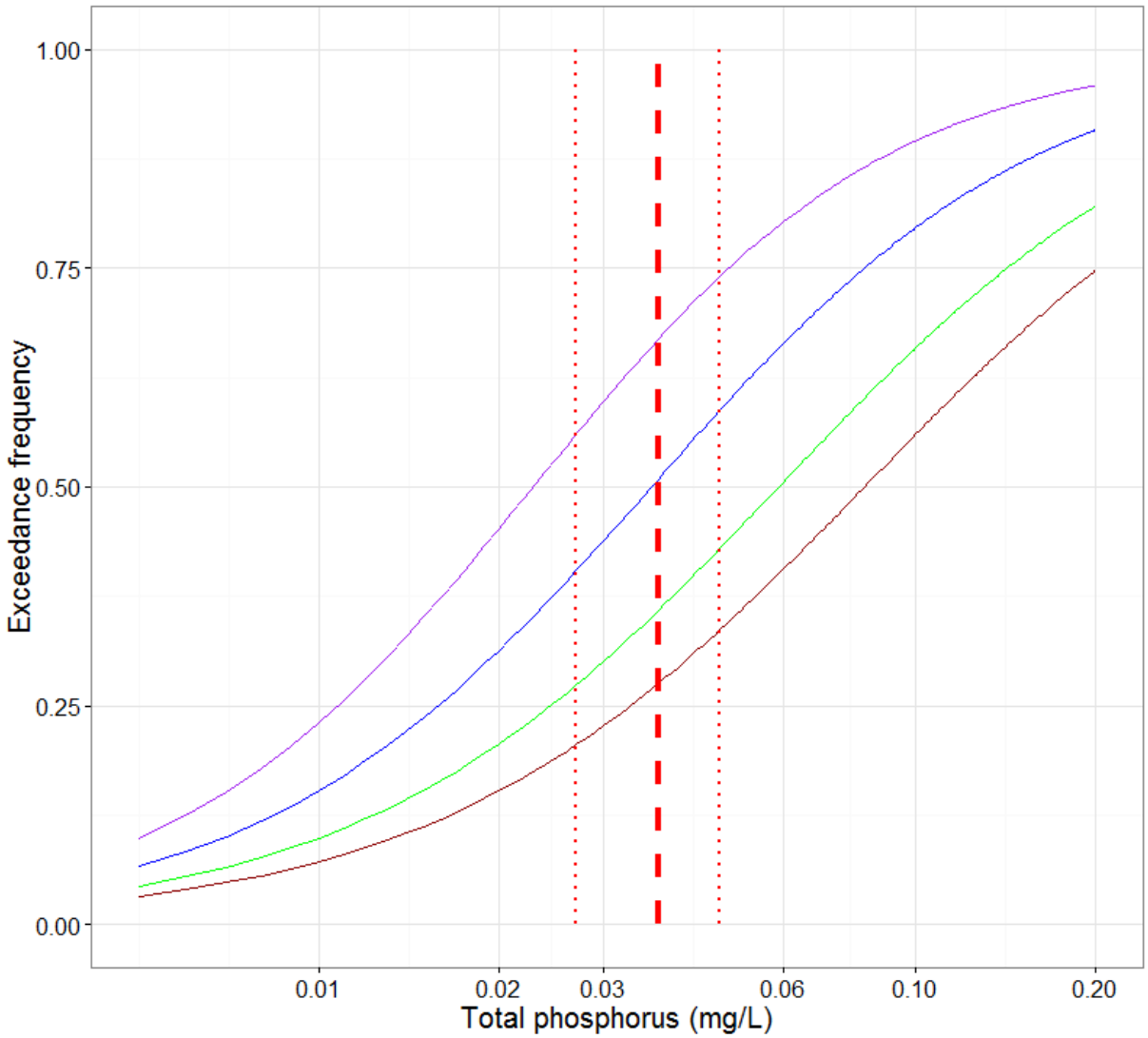


Figure 25. Exceedance frequencies of 150 (purple), 200 (blue), 250 (green), and 300 (dark red) mg/m^2 benthic chlorophyll-a in response to 2 year mean total phosphorus. GLM models for each response variable were fit using a binomial probability distribution and a logit link function. TP was a highly significant predictor in all 4 models ($p<0.00001$).

Table 11. Predicted exceedance frequencies of 150, 200, 250, and 300 mg/m^2 benthic chlorophyll-a for year 1 (dry year), year 2 (wet year), and years 1 and 2 combined in response to mean total phosphorus.

		Predicted exceedance frequencies of benthic chlorophyll-a vs. TP											
		150 mg/m2			200 mg/m2			250 mg/m2			300 mg/m2		
Year 1	TP (mg/L)	Mean	2.5%	97.%	Mean	2.5%	97.5%	Mean	2.5%	97.5%	Mean	2.5%	97.5%
	0.010	0.29	0.17	0.41	0.25	0.14	0.35	0.10	0.04	0.17	0.12	0.05	0.19
	0.020	0.68	0.59	0.77	0.47	0.38	0.56	0.21	0.14	0.29	0.22	0.15	0.30
	0.027	0.81	0.74	0.88	0.58	0.50	0.65	0.28	0.21	0.35	0.29	0.22	0.36
	0.030	0.84	0.78	0.91	0.61	0.54	0.69	0.30	0.23	0.37	0.31	0.24	0.38
	0.037	0.90	0.84	0.95	0.68	0.61	0.75	0.36	0.29	0.43	0.36	0.29	0.44
	0.040	0.91	0.86	0.96	0.71	0.63	0.78	0.38	0.31	0.45	0.39	0.31	0.46
	0.047	0.94	0.90	0.98	0.75	0.68	0.82	0.43	0.35	0.51	0.43	0.35	0.51
	0.050	0.95	0.91	0.98	0.77	0.70	0.84	0.45	0.37	0.53	0.45	0.37	0.53
	0.060	0.96	0.94	0.99	0.81	0.74	0.88	0.50	0.41	0.59	0.50	0.41	0.59
	0.075	0.98	0.96	1.00	0.86	0.79	0.92	0.57	0.46	0.67	0.56	0.45	0.66
	0.100	0.99	0.98	1.00	0.90	0.84	0.96	0.65	0.53	0.77	0.64	0.51	0.76
Year 2													
	0.010	0.14	0.06	0.22	0.06	0.01	0.11	0.09	0.03	0.16	0.03	0.00	0.06
	0.020	0.29	0.20	0.37	0.16	0.09	0.24	0.20	0.12	0.28	0.08	0.03	0.13
	0.027	0.37	0.29	0.45	0.24	0.16	0.31	0.27	0.20	0.34	0.12	0.06	0.18
	0.030	0.40	0.33	0.48	0.27	0.19	0.34	0.30	0.22	0.37	0.14	0.08	0.20
	0.037	0.47	0.40	0.55	0.33	0.26	0.41	0.36	0.29	0.43	0.18	0.12	0.24
	0.040	0.50	0.42	0.57	0.36	0.29	0.44	0.38	0.31	0.45	0.20	0.14	0.26
	0.047	0.55	0.47	0.63	0.42	0.34	0.50	0.43	0.35	0.51	0.24	0.18	0.31
	0.050	0.57	0.49	0.65	0.45	0.36	0.53	0.45	0.37	0.53	0.26	0.19	0.33
	0.060	0.62	0.54	0.71	0.52	0.42	0.61	0.51	0.42	0.60	0.32	0.23	0.40
	0.075	0.69	0.59	0.78	0.60	0.49	0.71	0.58	0.48	0.69	0.40	0.28	0.51
	0.100	0.89	0.80	0.97	0.87	0.77	0.98	0.83	0.72	0.95	0.75	0.55	0.95
Years 1 and 2													
	0.010	0.23	0.09	0.37	0.15	0.04	0.27	0.10	0.00	0.20	0.07	0.00	0.15
	0.020	0.45	0.33	0.57	0.31	0.20	0.43	0.21	0.11	0.30	0.15	0.07	0.23
	0.027	0.56	0.44	0.68	0.40	0.31	0.50	0.27	0.18	0.37	0.21	0.13	0.28
	0.030	0.60	0.50	0.70	0.44	0.34	0.54	0.30	0.20	0.40	0.23	0.13	0.33
	0.037	0.67	0.57	0.77	0.51	0.41	0.61	0.36	0.26	0.46	0.28	0.18	0.37
	0.040	0.69	0.60	0.79	0.54	0.44	0.63	0.38	0.28	0.48	0.29	0.20	0.39
	0.047	0.74	0.64	0.84	0.59	0.49	0.69	0.43	0.31	0.55	0.34	0.24	0.43
	0.050	0.76	0.68	0.84	0.61	0.51	0.71	0.45	0.33	0.57	0.35	0.26	0.45
	0.060	0.80	0.72	0.88	0.66	0.55	0.78	0.51	0.39	0.62	0.41	0.29	0.52
	0.075	0.85	0.77	0.93	0.73	0.61	0.84	0.57	0.44	0.71	0.47	0.34	0.61
	0.100	0.90	0.82	0.97	0.80	0.68	0.91	0.66	0.50	0.82	0.56	0.38	0.74

Diel dissolved oxygen and pH

Multiprobe data sondes were deployed for 48-h at a minimum of 25 sites during August 2014 (near median baseflow conditions, late summer) and September 2015 (high baseflow conditions). The following figures illustrate the relationship between 6 month mean TP and minimum dissolved oxygen and maximum pH recorded during each 48-h deployment.

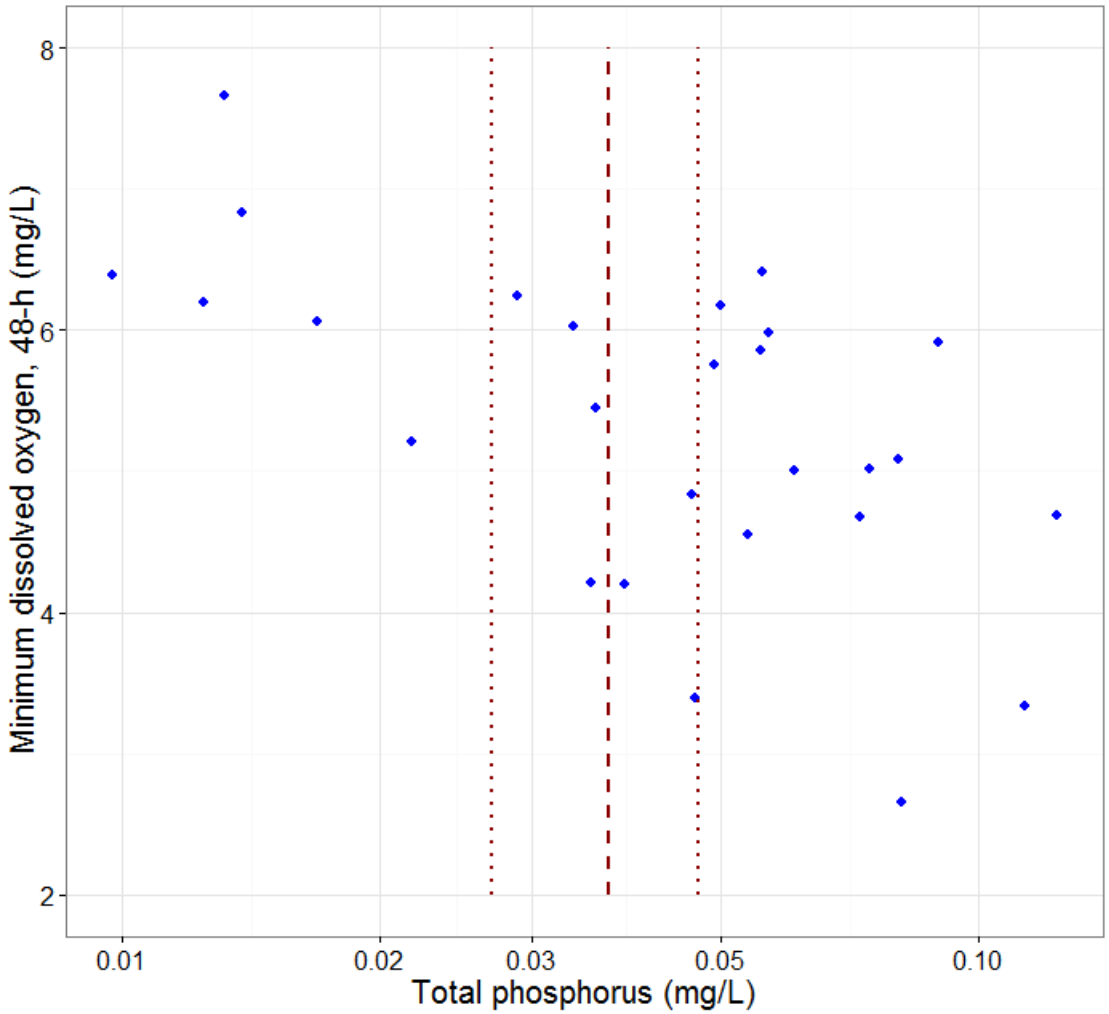


Figure 26. Minimum 48-h dissolved oxygen in August 2014 in response to mean 6-month total phosphorus. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

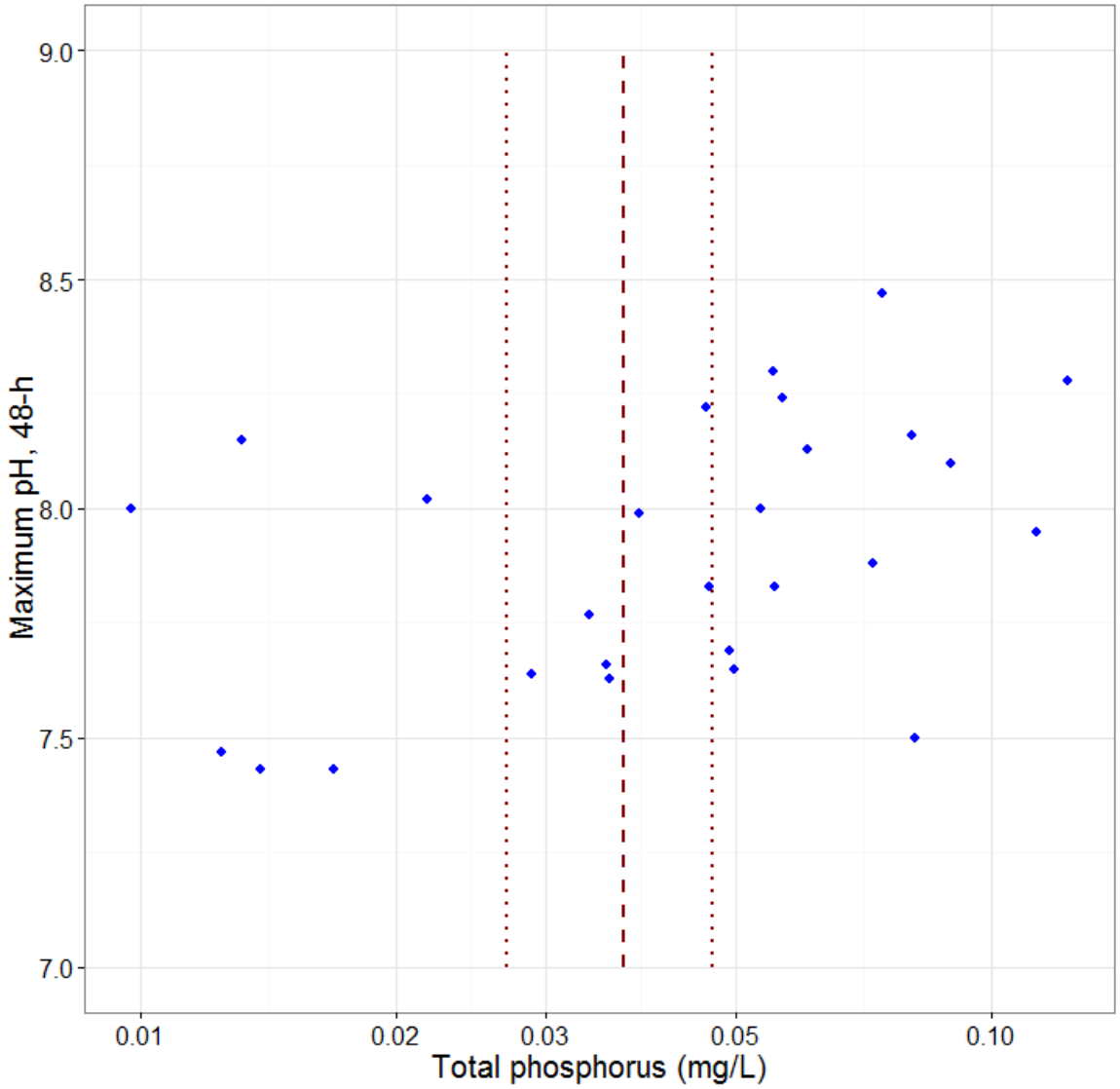


Figure 27. Maximum 48-h pH in August 2014 in response to mean 6-month total phosphorus. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

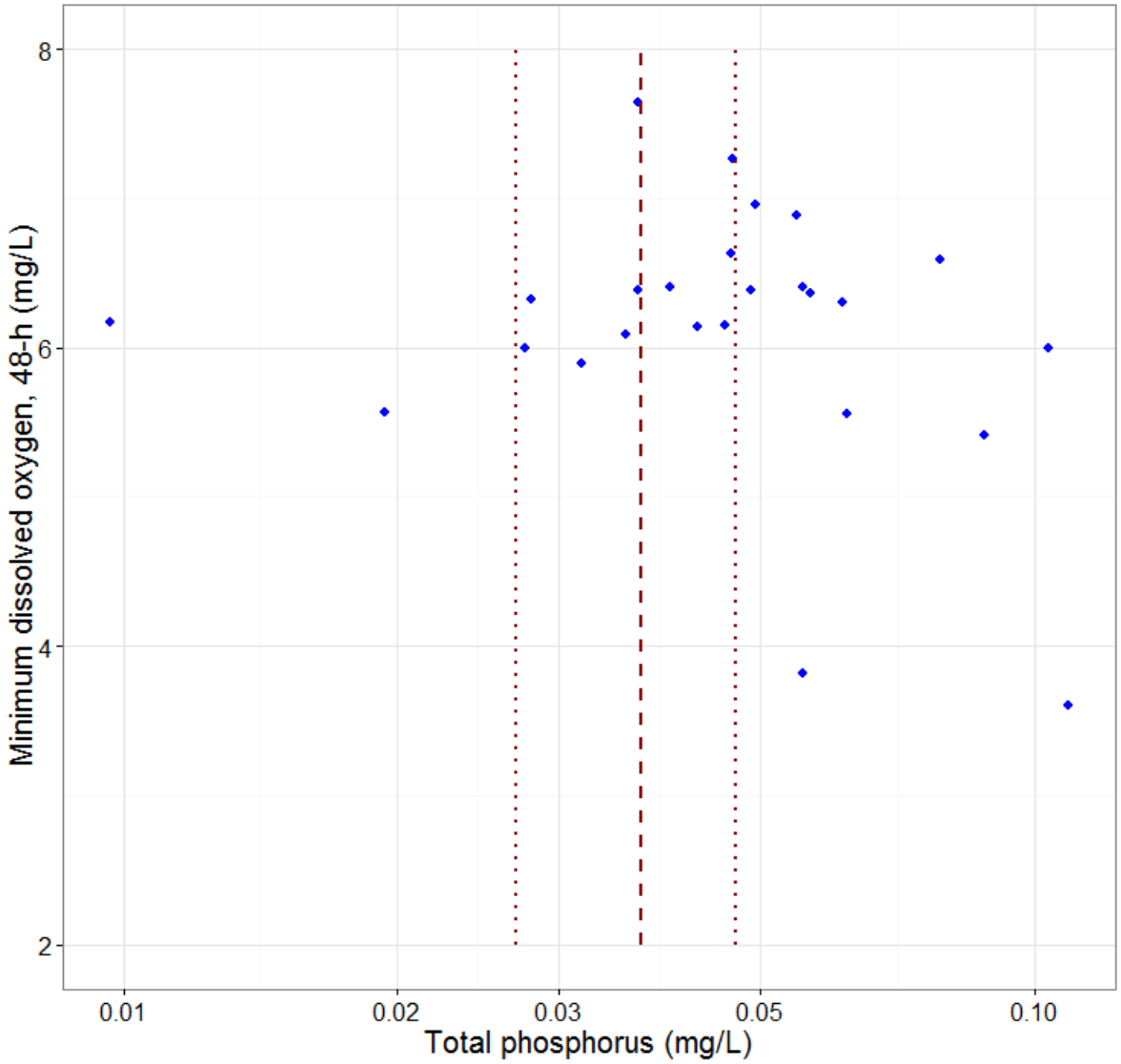


Figure 28. Minimum 48-h dissolved oxygen in September 2015 in response to mean 6-month total phosphorus. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

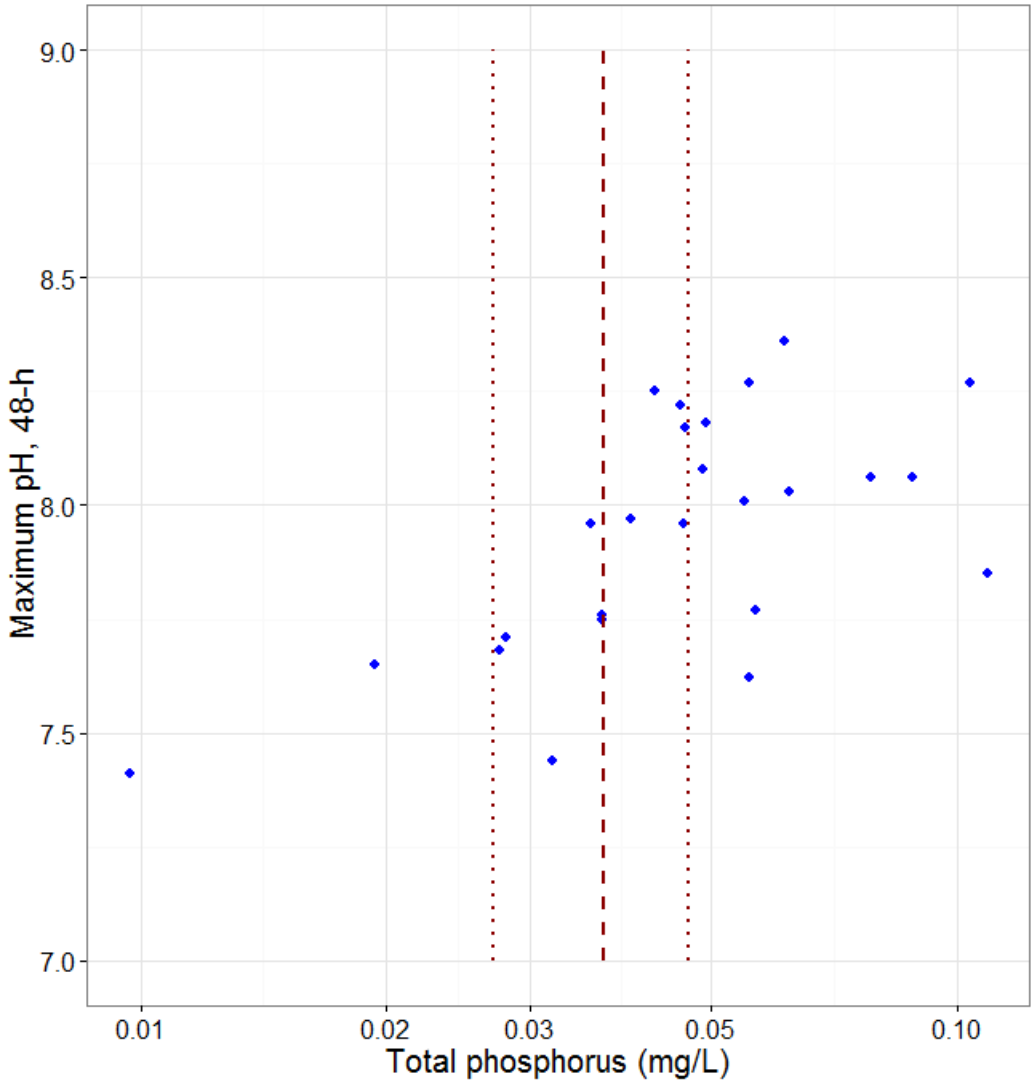


Figure 29. Maximum 48-h pH in September 2015 in response to mean 6-month total phosphorus. The red vertical dashed line corresponds to 0.037, whereas the dotted red vertical lines correspond to 0.027 and 0.047 mg/L, respectively.

Macroinvertebrates

The dominant grazing macroinvertebrate taxon in the study streams were snails in the family Pleuroceridae (Figure 30). Seasonally, pleurocerid densities varied considerably. Particular sites would have relatively few during one event but, by the next event, had exploded to levels such as those shown in the photograph.

Pleurocerids achieved densities up to 2000 individuals/m^2 based on estimates from Hess samples (Figure 31). The highest densities were observed in streams near the upper end of the phosphorus gradient, such as Spring Creek (AR; SPAR1), Osage Creek (OSAG1, OSAG2), Sager Creek (SAGE1), and Flint Creek (FLIN1, 2, and 3).

Figure 30. Photo of the stream bottom at Flint Creek (FLIN3) in late summer 2014, illustrating high densities of pleurocerid snails, the dominant algal grazing macroinvertebrate in the study streams.

Snails were abundant in at least a few streams during every event, regardless of season. Densities were likely underestimated during event 1 (June 2014) because snails would fall to the bottom of the stream bed when cobble and gravel were agitated to dislodge macroinvertebrates into the Hess sampler. Methods were adjusted during the following events such that rocks within the sampler were carefully lifted off the bottom and brushed directly into the net bag on the Hess sampler.

The only seasonal pattern evident was the nearly complete elimination of snails in events 11 and 12, which followed the historic flood of December 2015. This partially explains the very rapid growth of algae following the flood, as there was little to no grazing pressure by snails. Moreover, stonerollers (*Campostoma* spp.) were not actively grazing during the winter and early spring, thus February and April 2016 represented a nearly unrestricted growth response to nutrients.

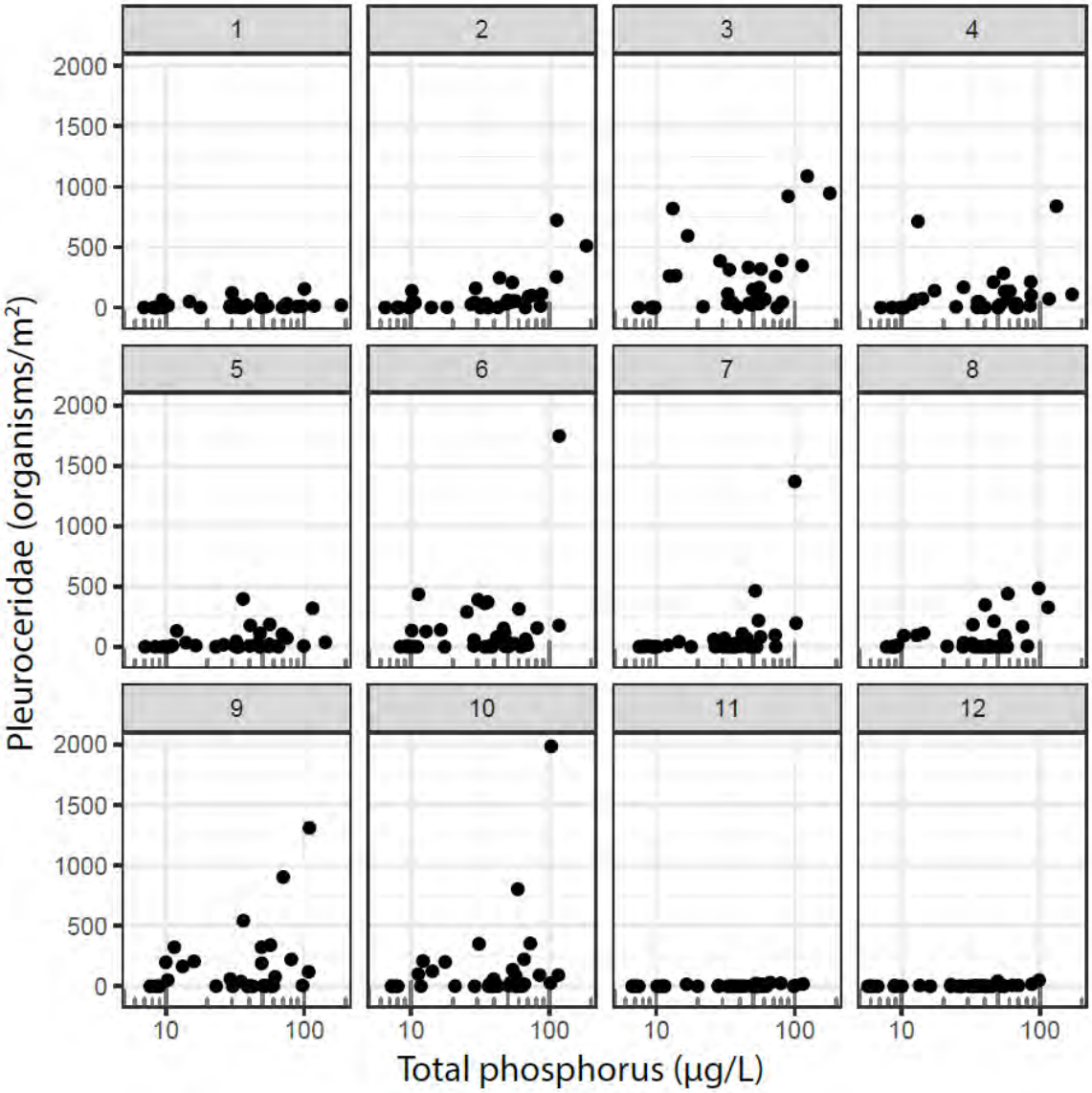


Figure 31. Densities of pleurocerid snails versus 6 month mean TP across the 2-year study period. Numbers in the upper panels are event numbers (1-12).

Responses of other macroinvertebrates varied but generally showed increases in density with increasing levels of TP. The following figure illustrates the mean response of each of the functional feeding groups of macroinvertebrates to TP over the 2-year study period.

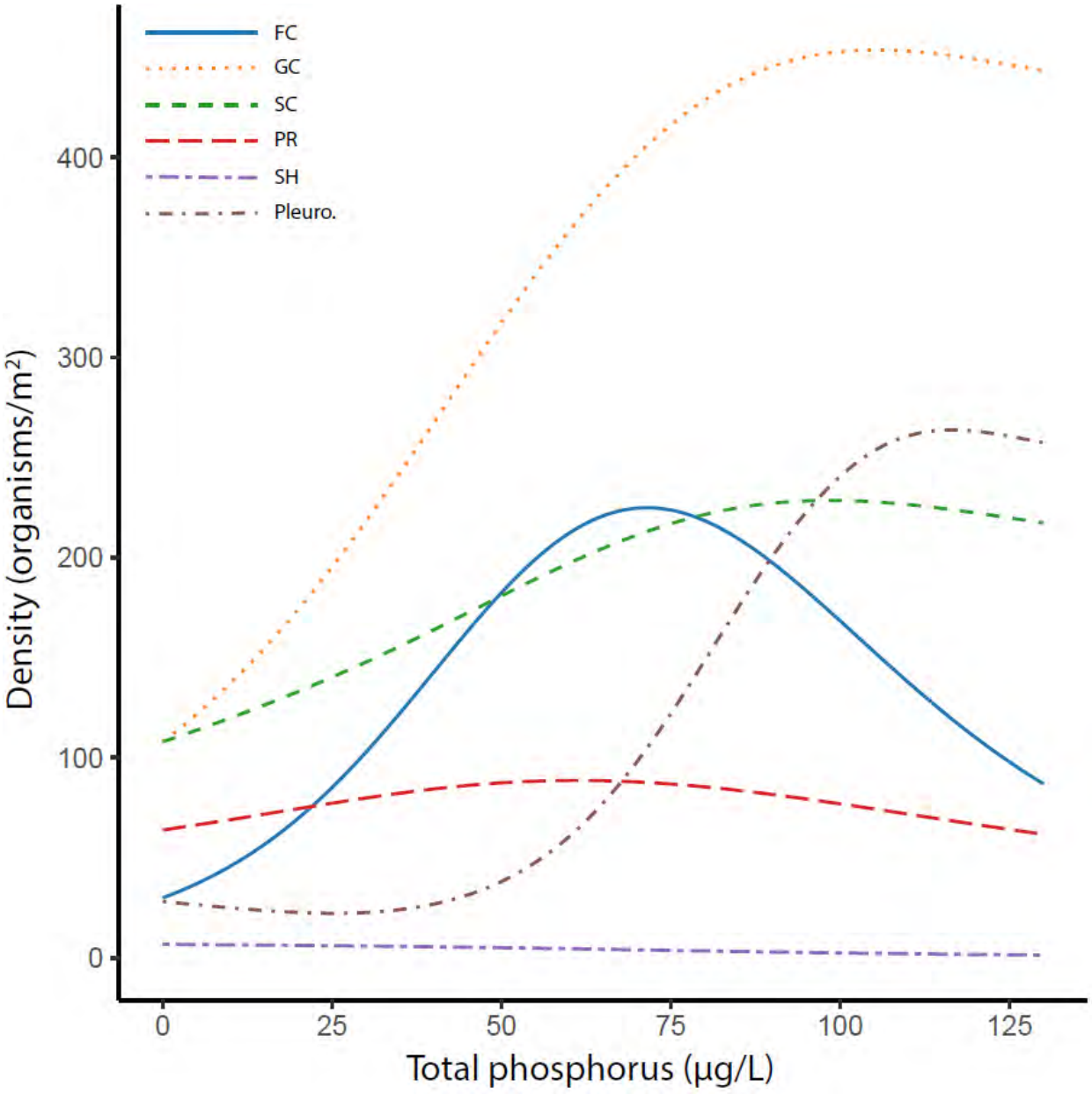


Figure 32. Mean responses of macroinvertebrate functional feeding groups to total phosphorus over the 2-year study period. FC=filtering collectors; GC=gathering collectors; SC=scrapers/grazers of algae, excluding pleurocerid snails; PR=predators; SH=shredders; Pleuro=pleurocerid snails.

Summary

The following histogram, which was requested by the Joint Study Committee, synthesizes the change points estimated by change point analysis and TITAN on all of the focal biological response variables analyzed using those techniques: benthic chlorophyll-a, *Cladophora* biovolume, nuisance taxa proportion, and community-level thresholds for negative and positive responding taxa (TITAN). Because analyses were conducted on several different TP durations, the 6 month duration was chosen for this summary because it was very similar to longer durations and was a stronger predictor than shorter durations in most cases.

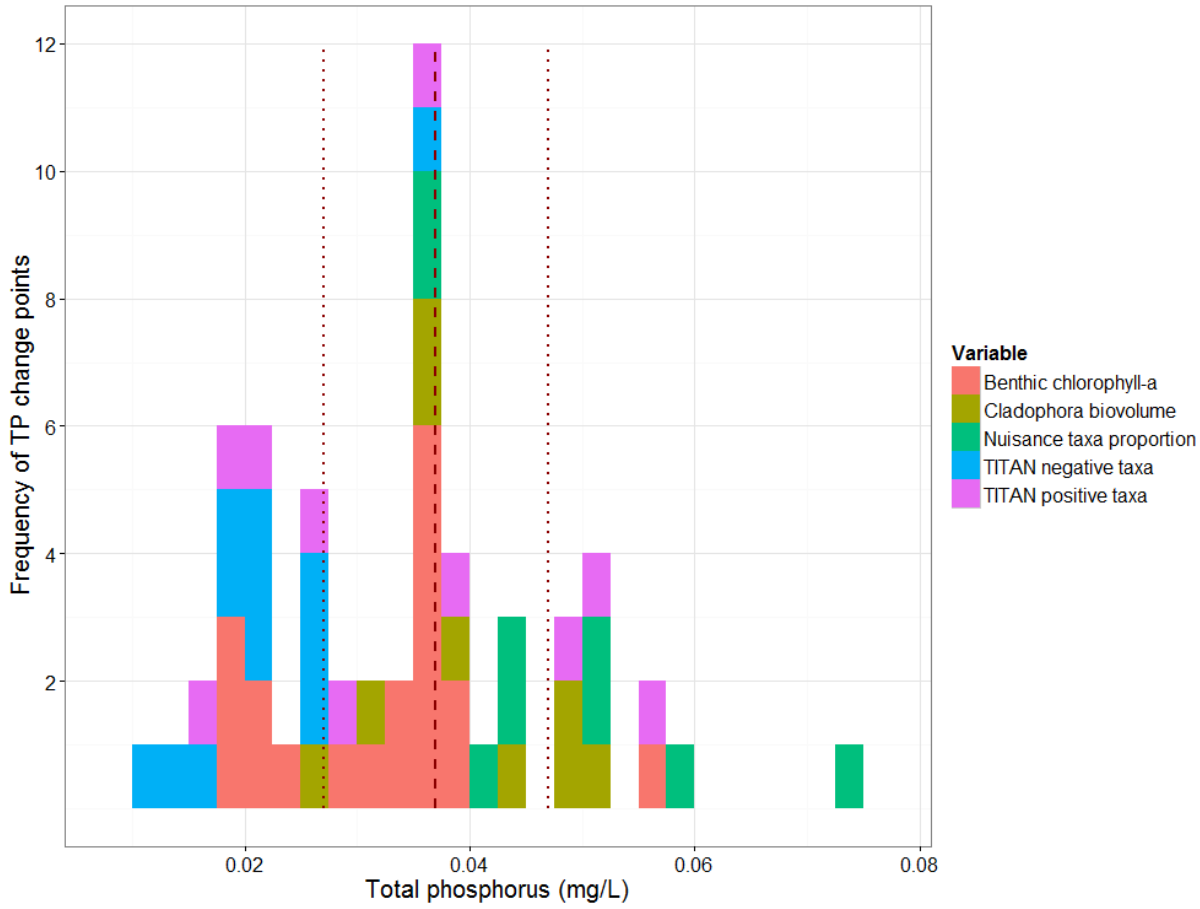


Figure 33. Histogram illustrating the distribution of total phosphorus change points across several response variables over the 2-year study period. Shown are change points associated with 6-month mean TP and instantaneous and mean responses that correspond to the TP data. The dashed red vertical line corresponds to 0.037, whereas the dotted vertical lines are 0.027 and 0.47, respectively.

Acknowledgments

This study was funded by the Arkansas-Oklahoma Joint Study Committee. Numerous individuals facilitated the execution of this study in accordance with Second Statement of Joint Principals and Actions and the approved work plan.

OWRB, OCC, and University of Arkansas provided historical data that helped guide site selection. Oklahoma Scenic Rivers Commission provided logistical support by showing the Baylor team access points on various rivers and helping us gain access on private lands. Days Inn of Tahlequah and Peyton's Place on the Illinois River provided greatly discounted accommodations to the Baylor team during field sampling trips that allowed the contractor to spend more time collecting data than would otherwise have been possible with the study budget.

Field sampling, sample processing, laboratory analyses, and data entry was completed by the outstanding efforts of Katherine Hooker, Morgan Bettcher, Stephen Elser, Stephen Cook, Caleb Robbins, and Lauren Housley. Dr. Jeffrey Back also participated in all aspects of the study, particularly performing water chemistry analyses and quality assurance oversight. Dr. Ryan King participated in all field sampling events, reviewed all data, performed data analyses and drafted the final report.

Drs. Stephen Porter and Barbara Winsborough performed taxonomic identifications and biovolume estimates of soft algae and diatoms, respectively.

Dr. Thad Scott served on the Joint Study Committee until early 2016. His service was greatly appreciated by the committee and the Baylor team.

Literature Cited

Baker, M. E. and R. S. King. 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods in Ecology and Evolution* 1:25-37

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

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. 1999. *Rapid bioassessment protocols for use in streams and wadeable rivers*. USEPA, Washington.

Biggs, B. J., & Kilroy, C. 2000. *Stream periphyton monitoring manual*. NIWA.

Biggs, B. J. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society* 19: 17-31.

Breiman, L., 2001. Random forests. *Machine learning* 45:5-32.

De'ath, G. and Fabricius, K.E., 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* 81:3178-3192.

Dodds, W.K. and Gudder, D.A., 1992. The ecology of *Cladophora*. *Journal of Phycology* 28:415-427.

Groffman, P.M., Baron, J.S., Blett, T., Gold, A.J., Goodman, I., Gunderson, L.H., Levinson, B.M., Palmer, M.A., Paerl, H.W., Peterson, G.D. and Poff, N.L., 2006. Ecological thresholds: the key to successful environmental management or an important concept with no practical application? *Ecosystems* 9:1-13.

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

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

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.

King, R.S. and M.E. Baker. 2014. Use, misuse, and limitations of Threshold Indicator Taxa Analysis (TITAN) for natural resource management. pp 231-254 In: G. Guntenspergen (editor), *Application of Threshold Concepts in Natural Resource Decision Making*, Springer.

Stevenson, R. J., B. J. Bennett, D. N. Jordan, and Ron D. French. 2012. Phosphorus regulates stream injury by filamentous green algae, DO, and pH with thresholds in responses. *Hydrobiologia* 695:25–42

Suplee, M.W., Watson, V., Teply, M. and McKee, H., 2009. How Green is Too Green? Public Opinion of What Constitutes Undesirable Algae Levels in Streams. *Journal of the American Water Resources Association* 45:123-140.

Taylor, J. M., J. A. Back, and R. S. King. 2012. Grazing minnows increase benthic autotrophy and enhance the response of periphyton elemental composition to experimental phosphorus additions. *Freshwater Science* 31:451-462.

Taylor, J. M., R.S. King, A. Pease, and K.O. Winemiller. 2014. Nonlinear response in stream ecosystem structure to low level phosphorus enrichment. *Freshwater Biology* 59:969-984.

Toms, J.D. and Lesperance, M.L., 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* 84:2034-2041.

USEPA (United States Environmental Protection Agency). 1998. *National strategy for the development of regional nutrient criteria*. EPA 822-R-98-002. Office of Water, Washington, DC

USEPA. 2010. *Using Stressor-response Relationships to Derive Numeric Nutrient Criteria*.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society* 24:706–723.

Zuur, A. F. 2009. *Mixed effects models and extensions in ecology with R*. Springer.

Zuur, A.F., Ieno, E.N. and Elphick, C.S. 2010. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1:3-14.

ARKANSAS DEPARTMENT OF ENERGY AND ENVIRONMENTAL, DIVISION OF ENVIRONMENTAL QUALITY

RE: FRL-comment on FRL–11994–01–R6

Exhibit C - *Justus, B.G. et al. 2010. A comparison of algal, macroinvertebrate, and fish assemblage indices for assessing low-level nutrient enrichment in wadeable Ozark streams. Ecological Indicators, May 2010, 627-638.*

A Comparison of Algal, Macroinvertebrate, and Fish Assemblage Indices for Assessing Low-Level Nutrient Enrichment in Wadeable Ozark Streams

Article *in* Ecological Indicators · May 2010

Impact Factor: 3.44 · DOI: 10.1016/j.ecolind.2009.10.007

5 authors, including:

Billy G. Justus
United States Geological Survey
19 PUBLICATIONS **60** CITATIONS
SEE PROFILE

J. Wallace
United States Geological Survey
4 PUBLICATIONS **39** CITATIONS
SEE PROFILE

Justus, B.G., Petersen, J.C., Femmer, S.R., Davis, J.V., and Wallace, J.E., in press, A comparison of algal, macroinvertebrate, and fish assemblage indices for assessing low-level nutrient enrichment in wadeable Ozark streams, Ecological Indicators, available online at http://dx.doi.org/10.1016/j.ecolind.2009.10.007

A Comparison of Algal, Macroinvertebrate, and Fish Assemblage Indices for Assessing Low-Level Nutrient Enrichment in Wadeable Ozark Streams

By B.G. Justus[a], James C. Petersen[a], Suzanne R. Femmer[b], Jerri V. Davis[b], and J.E. Wallace[a]

[a]*USGS, Arkansas Water Science Center, 401 Hardin Road, Little Rock, AR 72211, United States*

[b]*USGS, Missouri Water Science center, 1400 Independence Road, MS 100, Rolla, MO 65401 United States*

ABSTRACT

Biotic indices for algae, macroinvertebrate, and fish assemblages can be effective for monitoring stream enrichment, but little is known regarding the value of the three assemblages for detecting perturbance as a consequence of low-level nutrient enrichment. In the summer of 2006, we collected nutrient and biotic samples from 30 wadeable Ozark streams that spanned a nutrient-concentration gradient from reference to moderately enriched conditions. Seventy-three algal metrics, 62 macroinvertebrate metrics, and 60 fish metrics were evaluated for each of the three biotic indices. After a group of candidate metrics had been identified with multivariate analysis, correlation procedures and scatter plots were used to identify the four metrics having strongest relations to a nutrient index calculated from log transformed and normalized total nitrogen and total phosphorus concentrations. The four metrics selected for each of the three biotic indices were: algae—the relative abundance of most tolerant diatoms, the combined relative abundance of three species of *Cymbella*, mesosaprobic algae percent taxa richness, and the relative abundance of diatoms that are obligate nitrogen heterotrophs; macroinvertebrate—the relative abundance of intolerant organisms, Baetidae relative abundance, moderately tolerant taxa richness, and insect biomass; fish—herbivore and detritivore taxa richness, pool species relative abundance, fish catch per unit effort, and black bass (*Micropterus* spp.) relative abundance. All three biotic indices were negatively correlated to nutrient concentrations but the algal index had a higher correlation (rho = 0.89) than did the macroinvertebrate and fish indices (rho = 0.63 and 0.58, respectively). Biotic index scores were lowest and nutrient concentrations were highest for streams with basins having the highest poultry and cattle production. Because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity increases with poultry production. Studies are needed that address the synergistic effect of poultry and cattle production on Ozark streams in high production areas before ecological risks can be adequately addressed.

1. Introduction

In 2003, the U.S. Geological Survey (USGS) initiated several studies to evaluate the effects of nutrient enrichment on stream ecosystems in agricultural basins (Munn and Hamilton, 2003). These studies were initiated after the U.S. Environmental Protection Agency (USEPA) reported that nutrient enrichment was the cause of 40% of reported water-quality impairments (USEPA, 1998) and after results from studies conducted in the 1990s by the USGS National Water-Quality Assessment (NAWQA) Program demonstrated that high concentrations of both nitrogen (N) and phosphorus (P) were common in streams draining agricultural areas (Fuhrer et al., 1999). More recent USGS studies have indicated that agricultural streams can transport up to 50% of the N and 20% of the P applied annually to the land (Mueller and Spahr, 2006). USGS models indicate that manure may be a larger source of P to the Gulf of Mexico than are row-crop sources (Alexander et al., 2008), and USGS data indicate that manure sources of total nitrogen (TN) and total phosphorus (TP) are increasing in the Ozarks (Rebich and Demcheck, 2007).

Confined poultry and loosely confined beef cattle are often produced on the same or adjacent farms in the Ozarks and increases in animal production have resulted in increased nutrient runoff to streams. However, nutrient concentrations in most Ozark streams are relatively low compared to concentrations in other regions of the United States. Herlihy and Sifneos (2008) compared nutrient concentrations for wadeable streams across the United States and determined that TP and TN concentrations for reference streams in the nutrient ecoregion containing the Ozarks were typically lowest and second lowest (respectively) of the 11 nutrient ecoregions evaluated.

Interassemblage response to nutrients can vary because of differences related to trophic structure, mobility, and

longevity, and the biotic assemblage that is best suited for monitoring nutrients and other forms of ecological disturbance is frequently debated (Griffith et al., 2005; Hering et al., 2006; Resh, 2008). Algal indices have been shown to be effective for monitoring well-established nutrient gradients (Lavoie et al., 2004; Potapova and Charles, 2007; Porter et al., 2008), but indices using macroinvertebrate (King and Richardson, 2007; Haase and Nolte, 2008) or fish assemblages (Wang et al., 2007) have also been successful. Few, if any, studies, however, have compared the value of the three assemblages for detecting perturbance as a consequence of low-level nutrient enrichment.

Conducting biotic assessments when nutrient levels are low can be challenging because effects are often subtle and can appear to be positive in nature (Biggs and Smith, 2002; Stevenson et al., 2008), but also because low-level nutrient enrichment may influence biota less than other water-quality and habitat variables. It is important that relations between nutrient concentrations and biotic assemblages be investigated in this setting to ensure that assessment methods are capable of detecting ecosystem perturbation as a consequence of nutrient enrichment in areas that are relatively undisturbed.

The objectives of this paper are to (1) assess the value of algal, macroinvertebrate, and fish assemblage metrics and indices for assessing low-level nutrient enrichment, and (2) characterize relations between agricultural land use (livestock production) and the three biotic indices.

1.1. Study area

We sampled 30 wadeable streams along a nutrient-concentration gradient in the Ozarks. Sites were divided between the Springfield and Salem Plateau physiographic areas (Fig. 1), which contain most of northern Arkansas, southern Missouri, and extreme eastern Oklahoma, and overlap much of the Ozark Highlands Ecoregion. Topography of the Springfield and Salem Plateaus varies to some degree with gently rolling hills dominating the former and rugged hills dominating the latter; elevation above sea level ranges between 425 and 520 m (Fenneman and Johnson, 1946). The 30 streams generally are clear, with pool, riffle, and run sequences, and have moderate gradients with dominant substrates ranging in size from medium gravel to bedrock. Basin size ranges from 50 to 483 km^2 and streamflow measured at the time of sampling ranged from 0.01 to 0.55 m^3/s (Table S1 in Supplementary Material).

Land use in the 30 basins (Table 1) represented a gradient for pasture; urban land use was usually less than 5%, and no wastewater-treatment plants discharged into the streams. Poultry were produced in 17 of the 30 stream basins and cattle were produced in all basins. Agricultural intensity was greatest in basins of extreme northwestern Arkansas and southwestern Missouri, which have the highest poultry and cattle production of counties within the two states and Oklahoma (NASS, 2008a,b).

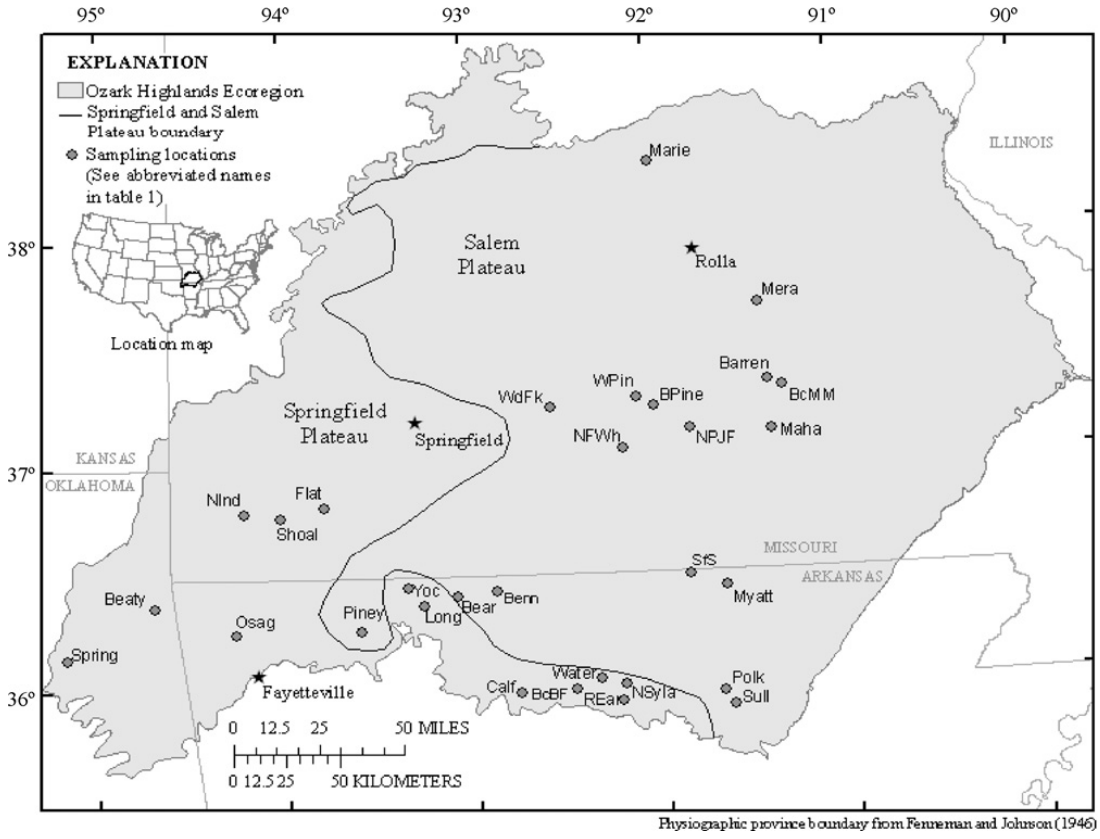


Fig. 1. Locations of 30 wadeable stream sites sampled in the Ozark Highlands in 2006 with a general border for the Springfield and Salem Plateaus.

Table 1. Nutrient and land-use characteristics for 30 wadeable streams sampled in the Ozark Highlands, 2006.

Site name	Abbreviated name (fig. 1)	Physiographic section	Mean total nitrogen (mg/L)	Mean total phosphorus (mg/L)	Nutrient index score	Pasture (percent)	Cattle produced (number per km²)	Poultry (houses per km²)
Barren Fork near Timber, Missouri	Barren	Salem	0.07	0.003	0.00	7	12	0.0
Big Creek near Big Flat, Arkansas	BcBF	Springfield	0.29	0.027	0.93	33	75	0.2
Big Creek at Mauser Mill, Missouri	BcMM	Salem	0.14	0.002	0.05	4	6	0.0
Bear Creek near Omaha, Arkansas	Bear	Salem	0.14	0.005	0.14	35	86	2.0
Beaty Creek near Sycamore, Oklahoma	Beaty	Springfield	1.56	0.047	2.27	71	259	9.0
Bennetts River near Vidette, Arkansas	Benn	Salem	0.37	0.010	0.47	56	80	0.0
Big Piney River at Simmons, Missouri	BPine	Salem	0.25	0.024	0.78	42	106	0.0
Calf Creek near Silver Hill, Arkansas	Calf	Springfield	0.41	0.029	1.08	32	73	0.0
Little Flat Creek near McDowell, Missouri	Flat	Springfield	2.51	0.031	2.15	58	184	3.3
Long Creek southeast of Denver, Arkansas	Long	Springfield	0.72	0.038	1.55	37	98	1.8
Mahans Creek at West Eminence, Missouri	Maha	Salem	0.39	0.011	0.53	7	11	0.0
Maries River Near Freeburg, Missouri	Marie	Salem	0.56	0.035	1.35	41	104	0.1
Meramec River above Cook Station, Missouri	Mera	Salem	0.10	0.004	0.05	17	29	0.0
Myatt Creek east of Salem, Arkansas	Myatt	Salem	0.39	0.011	0.54	42	52	0.0
North Fork White River near Cabool, Missouri	NFWh	Salem	0.23	0.007	0.27	32	80	0.0
North Indian Creek near Wanda, Missouri	NInd	Springfield	4.71	0.052	3.30	81	265	11.7
North Prong Jacks Fork below Arroll, Missouri	NPJF	Salem	0.22	0.006	0.24	21	52	0.0
North Sylamore Creek near Fifty Six, Arkansas	NSyla	Springfield	0.10	0.005	0.08	2	5	0.2
Little Osage Creek at Healing Springs, Arkansas	Osag	Springfield	3.33	0.051	2.95	76	284	8.5
Piney Creek near Cabanol, Missouri	Piney	Salem	0.56	0.009	0.61	31	94	4.0
Poke Bayou near Sidney, Arkansas	Poke	Salem	0.58	0.025	1.10	47	84	0.0
Roasting Ear Creek near Newnata, Arkansas	REar	Springfield	0.51	0.016	0.77	20	46	0.7
South Fork Spring River north of Moko, Arkansas	SfS	Salem	0.43	0.013	0.63	45	42	0.0
Shoal Creek near Wheaton, Missouri	Shoal	Springfield	2.02	0.062	2.88	81	258	10.9
Spring Creek near Locust Grove, Oklahoma	Spring	Springfield	0.25	0.010	0.38	44	93	2.6
Sullivan Creek near Sandtown, Arkansas	Sull	Salem	0.54	0.018	0.85	31	73	2.2
Water Creek near Evening Shade, Arkansas	Water	Springfield	0.14	0.004	0.10	18	71	0.3
Woods Fork near Hartville, Missouri	WdFk	Salem	0.27	0.035	1.12	55	142	0.2
West Piney Creek at Bado, Missouri	WPin	Salem	0.33	0.015	0.60	48	122	0.0
Yocum Creek near Oak Grove, Arkansas	Yoc	Springfield	2.37	0.047	2.57	71	217	8.4

2. Methods

2.1. Site selection

Geographic information system analysis and field reconnaissance were the primary methods used to select 30 streams that maximized the nutrient gradient across Ozark streams. Potential stream reaches were identified using the Elevation Derivatives for National Applications (USGS, 2005). Field reconnaissance was conducted at 54 candidate stream reaches that were selected from a larger group of reaches that met the basin size criterion (initially 90–300 km^2, however, 5 streams with basins outside this range but with a streamflow characteristic of the remaining streams were included). Nutrient concentrations were measured using a portable nutrient analyzer (Hach™ model DREL/2010) and dissolved oxygen, pH, specific conductance, temperature, and turbidity were measured in the field with water-quality monitors. Field forms were completed that documented observations for habitat quality and flow characteristics. Land use, geographic coverage, and spatial distribution were other factors considered as sites were selected.

2.2. Water-quality sampling

Water-quality samples were collected during base-flow conditions at the 30 sites in late June 2006 and again in July–August 2006 with the following exceptions. Flooding delayed the second round of water-quality sampling until early September at one site and drought conditions in the summer of 2006 resulted in 5 of the original 30 sites sampled in June being replaced for the July–August sampling effort. At the 25 sites sampled twice, nutrient concentrations for the two

samples were averaged to indicate nutrient enrichment for the month prior to biotic sampling; at the 5 remaining sites, the concentration from the single sample was used.

Standard USGS methods were used to collect and process water-quality samples. Water-quality samples were grabbed (because water velocities were <0.46 m/s) and were composited from three points that were equally distributed along the stream cross-section. Streamflow and field properties were measured at each site using a current meter (Rantz et al., 1982). Samples were analyzed for nutrient or nutrient-related (e.g. chlorophyll *a* and total organic carbon) constituents and all analyses were performed by the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado (Patton and Kryskalla, 2003; Fishman, 1993). Total nitrogen was determined by summing nitrogen species. For purposes of statistical analysis, all nondetect values were assigned one-half of the reporting limit. Quality-control samples were collected to assess bias and variability in the field and laboratory (Brightbill and Munn, 2008). The maximum difference between TP concentrations and TN concentrations in replicate samples was 0.0011 and 0.0260 mg/L, respectively. One of five blank samples had detections of TP (0.0029 mg/L) and TN (0.0350 mg/L).

2.3. Land use

Cattle density on pasture was estimated for each county contained in the stream basin by multiplying the amount of pasture in the county by county-level cattle density (the number of cattle produced in 2005 divided by the area of the county, NASS, 2008a). Cattle density on pasture then was combined for all counties in the stream basin, and that sum was divided by basin area to obtain an estimate of cattle density across the stream basin. Poultry production information was not available for 2005 (NASS, 2008a) and was not available for all counties in other years (NASS, 2008b). Consequently, poultry house density was used as a surrogate for poultry density. Poultry houses in each stream basin were counted using aerial photography (Center for Advanced Spatial Technologies, 2008) and were divided by the stream basin size to estimate the poultry houses per square kilometer of basin (Table 1).

2.4. Biotic sampling

Biotic sampling was conducted concurrently with the second water-quality sampling effort using NAWQA protocols (Moulton et al., 2002). Biotic samples were collected from a reach length that measured approximately 20 times the mean wetted channel width, with a minimum reach length of 150 m and a maximum of 300 m.

Algal assemblages were sampled using a cylinder surface area method. A quantitative algal subsample was collected from five cobbles at each of the five riffle locations (i.e. 25 subsamples were composited). The method involved placing a short cross section of PVC pipe (2.8- or 3.3-cm diameter) on each cobble, dislodging all algae outside of the pipe template with a wire brush or small knife, and rinsing the dislodged algae from the cobble with native water. Algae remaining inside the pipe template was dislodged with a wire brush or (scraped free) with a knife and rinsed into a sample bottle as the subsample. Sample area and total sample volume were recorded, and the sample was preserved with buffered formalin. Taxa were identified and enumerated at the Academy of Natural Sciences of Philadelphia (ANSP) Phycology Section in Philadelphia, Pennsylvania. The ANSP also determined cell density for each algal species using methods described in Charles et al. (2002). Chlorophyll *a* was determined at the USGS NWQL using methods described in Arar and Collins (1997).

A disturbance-removal process was used to collect macroinvertebrate samples from coarse-grained riffle substrates that were adjacent to locations where algal samples were collected. Five discrete samples were collected with a Slack sampler (50-cm 33-cm net frame, 500-mm Nitex™ net, and retrofitted with a 0.25-m^2 template) from riffles located throughout the reach. Macroinvertebrates were sampled from within the template as it was positioned on the stream bottom and immediately upstream from the Slack sampler. Substrate within the template was thoroughly disturbed using a small hand rake (or brushed if large cobble) and dislodged organisms were transported into the net by water current. All sample material was composited into a 20-L container and elutriated to remove sediment and larger particles. The material remaining on a 500-mm sieve after elutriation was preserved in 10% formalin and shipped to the USGS NWQL for identification and enumeration.

Fish were sampled at 29 sites using electrofishing and seining methods (fish were not sampled at Maries River because of potential occurrence of a federally listed threatened species). A backpack unit (Smith-Root model 12B) was used to electrofish all sites, and one pass was made along each bank. Electrofishing passes progressed from the downstream boundary of the sampling reach to the upstream boundary. Riffle habitats also were sampled by kick seining in conjunction with electrofishing. Most fish were identified and counted in the field and then were released. Fish that could not be positively identified in the field were preserved for laboratory identification. Fish were identified using taxonomic keys for Arkansas (Robison and Buchanan, 1988), Missouri (Pflieger, 1997), and Oklahoma (Miller and Robison, 2004), however, nomenclature follows Robins et al. (2004).

2.5. Metric sources

Two USGS software programs—the Macroinvertebrate Data Analysis System (IDAS; Cuffney, 2003) and the Algal Data Analysis System (ADAS; a derivative of the IDAS program)—were the primary means for calculating algal and macroinvertebrate metrics. Both programs process multiple levels of taxonomic resolution, resolve taxonomic ambiguities, and use attribute files to calculate assemblage and tolerance metrics common to the literature (Barbour et al., 1999; Porter,

2008). Also, some macroinvertebrate metrics used by local natural resource agencies were considered as potential metrics, as were all species—order level taxa for the macroinvertebrate and fish assemblages.

ADAS was used to calculate algal metrics using an attribute file of published values (Porter, 2008). A total of 73 algal metrics was calculated for soft algae and diatoms (Table S2 in Supplementary Material). Algal metrics were primarily indicative of trophic preferences (Van Dam et al., 1994) and pollution tolerance (Lange-Bertalot, 1979).

A total of 62 macroinvertebrate metrics was calculated (Table S3 in Supplementary Material) using data specific to the southeastern (Barbour et al., 1999; Lenat, 1993) and midwestern (Hilsenhoff, 1987) United States. Values for richness, percent richness, abundance, and percent relative abundance were evaluated for all but a few metrics where percentages were not beneficial to the analysis (e.g. diversity indices).

A total of 60 fish metrics used by local natural resource agencies or obtained from biotic indices developed for use in the Ozarks or adjacent areas (Dauwalter et al., 2003; Justus, 2003; Dauwalter and Jackson, 2004) were considered as candidates for the fish index (Table S4 in Supplementary Material). Fish metrics were calculated using fish traits from several sources (Robison and Buchanan, 1988; Pflieger, 1997; Petersen et al., 2008; USGS, 2008).

2.6. Statistical analysis

TN and TP were combined into a nutrient index to facilitate comparisons of nutrient enrichment and biotic metrics. TN and TP are commonly used by State monitoring agencies to characterize nutrient enrichment in the Ozarks and typically have close relations to livestock production in the Ozarks (Davis and Bell, 1998) and much of the United States (Alexander et al., 2008). Chlorophyll *a* also is used by State monitoring agencies to characterize nutrient enrichment and also was considered for the nutrient index but relations between chlorophyll *a* and TN and TP were poor (Spearman rho = 0.14 and 0.30, respectively).

A three-step process was used to calculate the nutrient index. First, mean values for TN and TP were normalized to a mean of 0 and a standard deviation of 1. Second, normalized values for TN and TP were averaged, and third, all normalized (average) values were standardized to positive numbers by adding the difference between the minimum value and zero. The resulting nutrient index ranged from 0 to 3.3 (Table 1, Fig. 2).

For each of the biotic indices, four nonredundant metrics were selected from the initial 195 (73 algal, 62 macroinvertebrate, and 60 fish) metrics aggregated for this study. Index robustness may sometimes be associated with increasing metric number, however, a decision was made to limit the number of metrics (to four) after preliminary analyses indicated that, for one or more assemblages, relations between the next best candidate metric(s) and the nutrient index were nonexistent. The decision to select a relatively small number of metrics for each index also reduced the risk that redundant metrics were included in the final indices.

Metrics that were the best candidates for the three biotic indices were identified with a process that included a combination of univariate and nonparametric multivariate methods. Prior to analysis, metrics were separated by guild (e.g. tolerance, behavior, feeding, or nesting traits) and scoring method (e.g. relative abundance, relative density, and richness). Pairs of metrics from respective metric guilds initially were evaluated using Spearman rank correlation to identify and eliminate redundant metrics. When two metrics that had taxa in common had rho > 0.70, the metrics were considered to be redundant and one metric was eliminated to avoid index bias and error. Scatter plot matrices also were used to visually identify outlying values or spurious correlations. Metric relevancy to nutrient enrichment (e.g. increasing biomass, a decrease in organisms intolerant of organic pollution, an increase in organisms tolerant of organic pollution) was the primary consideration that determined which of the redundant metrics was retained for further analysis.

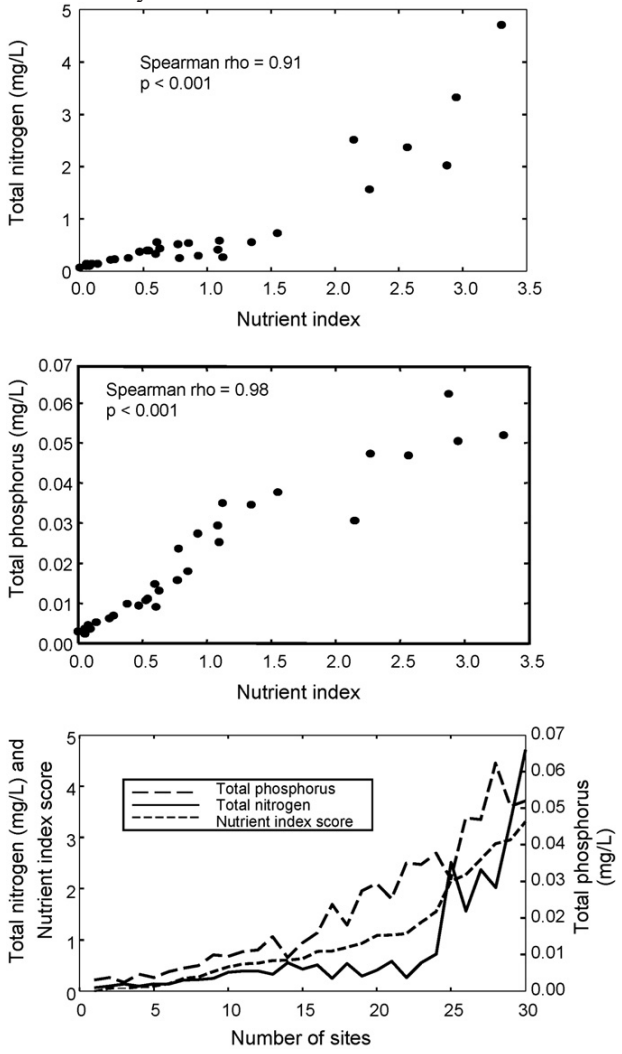


Fig. 2. Scatter plots and a line graph demonstrate relations of a nutrient index to total nitrogen and total phosphorus concentrations at 30 wadeable Ozark streams.

Once redundant metrics had been eliminated, BVSTEP, a nonparametric screening procedure in PRIMER v6 (Clarke and Warwick, 2001), was used to identify candidate metrics that "best" represented each of the three biotic assemblages. First, BVSTEP was used to compare the similarity matrices for an individual metric to the similarity matrix of all metrics in the same guild (group). This step helped identify individual metrics and metric combinations with the highest similarity to the metric guild (i.e. a multivariate sample pattern that matched that of the entire guild) and greatly reduced the number of metrics to be considered in further data reduction steps. The similarity matrix of the metric with the highest correlation to the similarity matrix of the entire guild was retained for further analysis. This step was repeated using an n–1 approach (once identified as an index candidate the metric was removed from the guild) until all metrics having a similarity matrix that was correlated (rho $\geq$ 0.25) to the similarity matrix of the parent guild had been identified. A rho value of 0.25 was selected because matrix correlations occur over a lower range than simple univariate correlations.

Metrics identified with the analytical step, above, were combined into a final "candidate metric subset" (generally 10–15 metrics). The BVSTEP process was used again, but on this occasion, similarity matrices of the candidate metrics were compared to the similarity matrix of the nutrient index. The candidate metrics that had similarity matrices with the highest correlations to the similarity matrix of the nutrient index were retained. Spearman rho was used again to evaluate for metric redundancy but this time for the small group of candidate metrics identified with the second round of BVSTEP. When pairs of redundant metrics with similar correlations to the nutrient index were identified, scatter plots were evaluated to determine which of the two redundant metrics had the best relation to nutrients and, ultimately, to identify the four candidate metrics that were selected for the respective assemblage index.

Scores for each of the three biotic indices were calculated by combining values for the four respective metrics using a centering method (Justus, 2003). An advantage of the centering method is that it is more robust than other scoring methods (e.g. scores range from 0 to100 rather than tiered, preassigned metric classes of 1, 3, or 5). A disadvantage of the centering method is that it does not facilitate comparison of sites from independent data sets because metric scores are based on the range of sampling conditions that may not include least- or most-impaired sites.The centering method uses one of two scoring procedures depending if high or low metric values represent least-degraded conditions. If a high metric value indicated least-degraded conditions, the metric value was first divided by the maximum metric value (for all 30 sites), and the resulting quotient was multiplied by 100 to obtain a metric score. To obtain a metric score if low metric values indicated least degraded conditions, the metric value was again divided by the maximum metric value, but the resulting quotient was subtracted from 1 before being multiplied by 100. Scores for the four metrics were averaged to obtain an index score. Sites having the highest biotic index scores had the least-degraded-conditions. Relations between the three biotic indices and the nutrient index and TP and TN also were evaluated with correlation procedures and scatter plots. Scatter plots also were used to determine how poultry (houses) and cattle production varied for the 30 basins and to evaluate relations between the three biotic indices and the two forms of livestock production.

3. Results

3.1. Biotic metric/nutrient relations

Median concentrations of TN and TP were 0.393 mg/L (0.07–4.71 mg/L) and 0.015 mg/L (0.002–0.062 mg/L), respectively. Values for the nutrient index ranged from 0 to 3.3 and were highly correlated to TN and TP concentrations (rho = 0.91 and 0.98, respectively; Fig. 2). The 30 sites were equally divided above and below an index score of 0.75 (because TN and TP concentrations associated with that index score, 0.40 and 0.018 mg/L, respectively, are comparable to median concentrations).

Although, the four metrics selected for each of the three assemblage indices had the strongest relations to the nutrient index of all metrics evaluated for that assemblage, relations between a few of the 12 metrics and the nutrient index were weak (rho $\leq$ 0.36 and $p > 0.05$). In most cases, however, metric values above and below the nutrient index score of 0.75 had different distributions. The four biotic metrics selected for each index are reported in the order of the correlation of the metric to the nutrient index, which may also reflect or approximate each metric's relevance to nutrients (Table 2).

All four metrics selected for the algal index were associated with nutrient tolerance or dependence (Table 2). The four metrics were: relative abundance of most tolerant diatoms, a metric associated with tolerance to elevated nutrient concentrations; the combined relative abundance of *Cymbella delicatula*, C. *affinis*, and C. *hustedtii*, three species of diatoms that respond to low to moderate nutrient concentrations; mesosaprobic algae percent taxa richness, a metric associated with tolerance to moderately elevated nutrients; and lastly, the relative abundance of diatoms that are obligate nitrogen heterotrophs, a metric associated with nitrogen dependence. All but the second metric would be expected to have a positive relation to nutrient concentrations.

The algal index, calculated with the four metrics above, ranged from 20.9 to 94.7 (Table S5 in Supplementary Material) and had a high correlation to the nutrient index (rho = 0.89, Fig. 3). Correlations between the algal index and TP (rho = 0.91) were much higher than between the algal index and TN (rho = 0.72, Fig. 4).

3.3. Macroinvertebrate metric and index performance

The four metrics selected for the macroinvertebrate index included three metrics associated with organisms that are

Table 2. Algae, macroinvertebrate, and fish metrics selected for three indices, their expected response to nutrient exposure, correlation to a nutrient index, and a comparison of values above and below a median concentration.

Assemblage	Metric description	Expected response to nutrients	Rho	Distinction for sites above and below median concentrations
Algae	Most tolerant diatoms, relative abundance (percent)	Positive (Bahls, 1993)	0.80	Percent RA≥3% at 3 of 15 sites; percent RA≥3% at 12 of 15 sites
Algae	*Cymbella affinis*, C. *delicatula*, and C. *hustedtii* relative abundance (percent)	Negative (Potapova and Charles, 2007)	-0.71	Percent RA>10% at 11 of 15 sites; percent RA>10% at 2 of 15 sites
Algae	Mesosaprobic algae taxa richness (percent)	Positive (Lange-Bertalot, 1979)	0.65	Percent TR>10% at 5 of 15 sites; percent TR>10% at 11 of 15 sites
Algae	Obligate nitrogen heterotroph relative abundance (percent)	Positive (Leland, 1995)	0.57	Percent RA>1% at 1 of 15 sites; percent RA>1% at 8 of 15 sites
Macroinvertebrate	Intolerant relative abundance (percent)	Negative (Barbour et al., 1999)	-0.50	Percent RA>85% at 14 of 15 sites; percent RA>85% at 9 of 15 sites
Macroinvertebrate	Baetidae relative abundance (percent)	Positive (USEPA, 2008)	0.48	Percent RA>10% at 2 of 15 sites; percent RA>10% at 9 of 15 sites
Macroinvertebrate	Insect biomass (grams)	Positive (King and Richardson, 2007)	0.47	>2 g at 1 of 15 sites; >2 g at 7 of 15 sites
Macroinvertebrate	Moderately tolerant taxa richness	Positive (Barbour et al., 1999)	0.30	≥20 taxa at 6 of 15 sites; ≥20 taxa at 10 of 15 sites
Fish	Herbivore/detritivore taxa richness	Positive (Rashleigh, 2004)	0.41	≥4 taxa at 7 of 15 sites; ≥4 taxa at 10 of 14 sites
Fish	Pool species relative abundance (percent)	Indirect	-0.38	Percent RA>50% at 11 of 15 sites; percent RA>50% at 7 of 14 sites
Fish	Fish collected per meter	Positive (Pilati et al., 2009)	0.36	>2.5 fish/m at 5 of 15 sites; >2.5 fish/m at 7 of 14 sites
Fish	Black bass relative abundance (percent)	Indirect	-0.35	Percent RA>1% at 8 of 15 sites; percent RA>1% at 4 of 14 sites

intolerant or moderately tolerant of organic pollution, and a fourth metric associated with productivity. The three metrics evaluating tolerance included: the relative abundance of intolerant organisms, Baetidae (a family with several species that are moderately tolerant of nutrients) relative abundance, and moderately tolerant taxa richness. The fourth macroinvertebrate metric, and the metric related to productivity, was insect biomass. All but the first metric would be expected to have a positive relation to nutrient concentrations.

The macroinvertebrate index ranged from 36.3 to 85.7 (Table S6 in Supplementary Material) and decreased in relation to the nutrient index scores (rho = 0.63, Fig. 3). Correlations between the macroinvertebrate index and TN and TP concentrations were similar (0.64 and 0.60, respectively; Fig. 4).

3.4. Fish metric and index performance

The four fish metrics selected for the fish assemblage index were: herbivore and detritivore taxa richness, pool species relative abundance, fish catch per unit effort, and black bass (*Micropterus dolomieu*, *M. punctatus*, and *M. salmoides*) relative abundance. Two of the metrics—herbivore and detritivore taxa richness and fish catch per unit effort would be expected to have a positive relation to nutrient concentrations; however, the two remaining metrics—pool species relative abundance and black bass relative abundance—probably have indirect relations to nutrients.

The fish index ranged from 15.9 to 83.7 (Table S7 in Supplementary Material) and also decreased with increasing nutrient index scores (rho = 0.58, Fig. 3). The fish index had a stronger correlation to TN than to TP (rho = 0.68 and 0.54, respectively; Fig. 4).

3.5. Indices comparison

Of the three biotic indices, the algal index had a much higher correlation to the nutrient index (i.e. a rho of 0.89, compared to 0.63 and 0.58). Correlations to the nutrient index, for the algal, macroinvertebrate, and fish metrics ranged from 0.57 to 0.80, 0.30 to 0.50, and 0.35 to 0.41 (reported as absolute values, Fig. 3), respectively. All relations among the four algal metrics and the nutrient index were statistically significant ($p \leq 0.05$); however, relations for only 3 of 4 macroinvertebrate, and only 2 of 4 fish metrics were statistically significant to the nutrient index. Correlations of the three biotic indices to TN were similar (a range between 0.64 and 0.72, Fig. 4) but the algal index had a much higher correlation to TP (rho = 0.91)

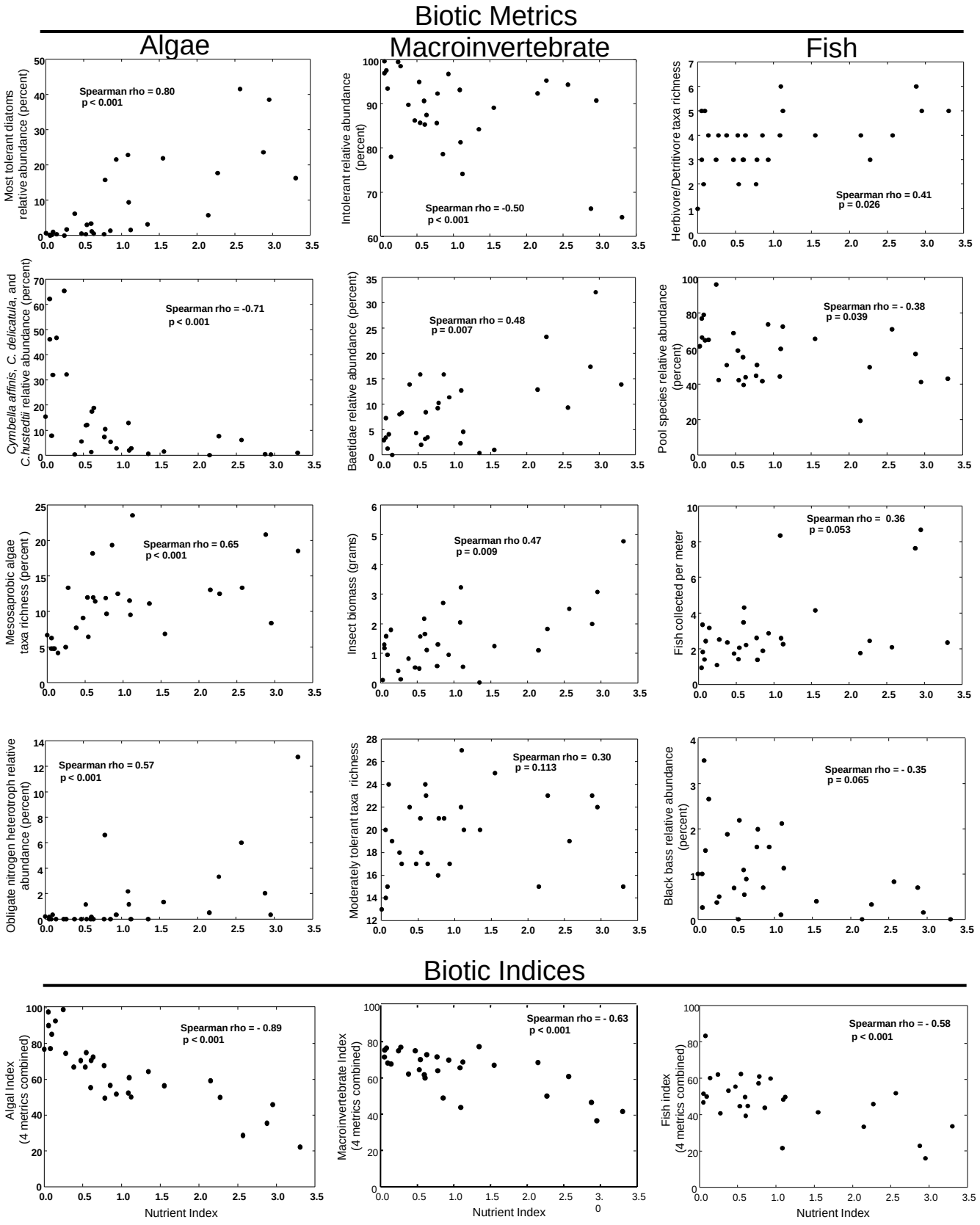


Fig. 3. Scatter plots and correlations comparing 12 biotic metrics and 3 biotic indices to a nutrient index (representing total nitrogen and total phosphorus concentrations) at 30 wadeable Ozark streams.

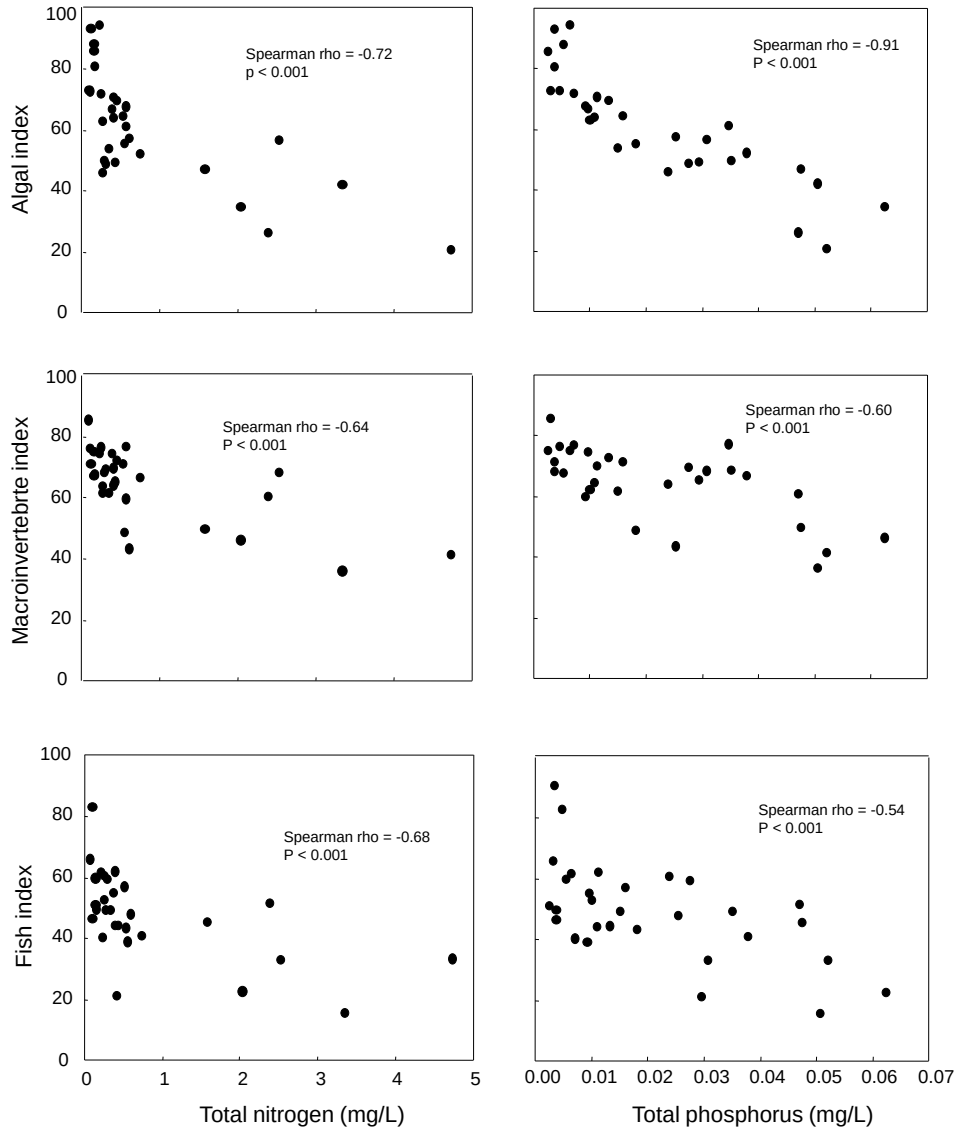


Fig. 4. Scatter plots and correlations comparing relations between three biotic indices and total nitrogen and total phosphorus concentrations at 30 wadeable Ozark streams.

than did the macroinvertebrate and fish indices (rho = 0.60 and 0.54, respectively).

3.6. Land use

Cattle were produced in all basins (a range of 5–284 cattle per km^2 of basin), but poultry were produced in only 17 of the 30 basins (the number of poultry houses ranged from 0 to 11.7 per km^2 of basin, Table 1). Cattle production generally was much higher in basins where poultry were produced than in basins where poultry were not produced, and was highest in basins with the highest poultry production (Fig. 5). The three biotic indices were negatively related to cattle production; correlations ranged from 0.46 to 0.76 (Fig. 6).

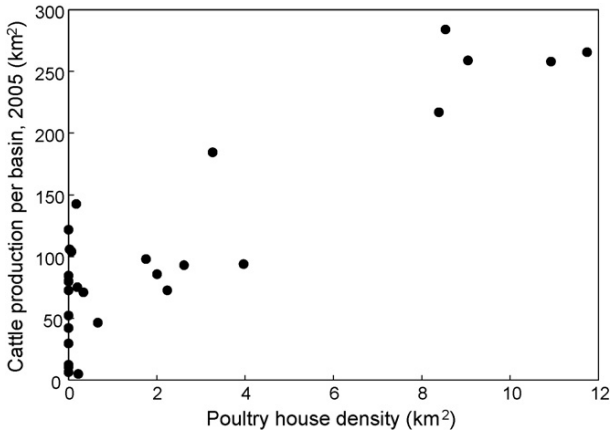


Fig. 5. A scatter plot comparing relations between cattle production and the number of poultry houses in 30 Ozark stream basins. Cattle production in the basins ranged from 5 to 125 cattle/km^2 when no poultry were produced but generally exceeded 75 cattle per km^2 when there was one or more poultry house in the basin.

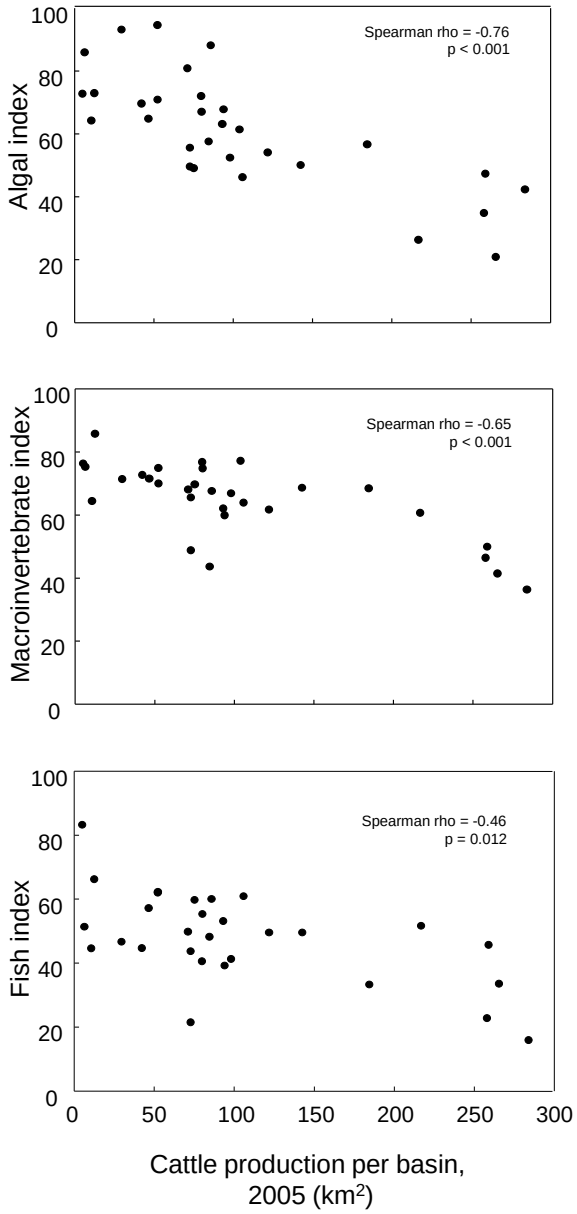


Fig. 6. Relations of three biotic indices to cattle density in 30 Ozark stream basins.

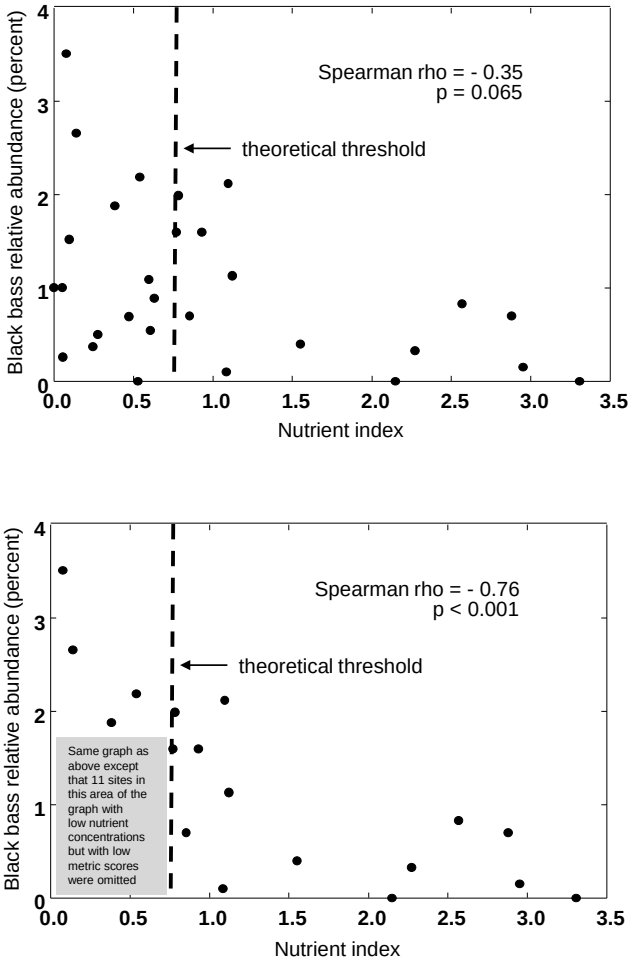


Fig. 7. Relations of black bass relative abundance to the nutrient index emphasize the relevance of the wedge-shaped scatter pattern. The correlations in the second plot doubles that of the previous plot after sites with low nutrient concentrations but with poor metric scores were omitted.

4. Discussion

4.1. Metric performance

Ten of the 12 metrics selected for the three biotic indices were measures of tolerance, biomass, or density that are known to fluctuate in response to stream productivity (e.g. Porter et al., 2008; Ortiz and Puig, 2007), and, thus, have an ecological relevance to nutrients. Correlations between metrics and the nutrient index generally declined across assemblages (from algae to macroinvertebrate to fish)—a probable consequence of the trophic level of the taxa targeted by the metrics and an associated decrease in dependence on inorganic nutrients. For the relative abundances of pool species and black bass, two fish metrics that are comprised of species of Centrarchidae which are known to be moderately tolerant of nutrients (Maceina and Bayne, 2001), relations may have been equal or stronger to variables associated with habitat quality than to nutrients.

Relations between the three biotic indices and the nutrient index were stronger than relations between the biotic metrics and the nutrient index, indication that even metrics that had weak relations to the nutrient index were beneficial to biotic indices. However, weak relations are to be expected between biotic metrics and nutrient enrichment when concentrations at some sites are below a threshold for which a biotic response occurs. Terrel et al. (1996) noted that wedge-shaped scatter plots are characteristic of the relation between a dependent variable and an independent [test] variable when some values for the independent variable are below the threshold for which a response occurs and when other unknown or unmeasured independent variables are influencing the dependent variable (see example in Fig. 7). Of the 12 metrics selected for the

three indices, wedge-shaped scatter plots are most apparent for the relative abundance of three *Cymbella* species and black bass relative abundance.

The small size of the data set limits our ability to identify thresholds for TN and TP, however, some literature indicate that TN and TP concentrations near median values for this study are near threshold concentrations that distinguish between reference streams and streams that are slightly enriched (i.e. near background, Table 3). Biotic metric scores were inversely related to nutrients and were generally highest when TN and TP concentrations were less than about 0.40 mg/L and about 0.018 mg/L (respectively), but were generally lowest when concentrations were higher. These TN and TP concentrations are comparable to background concentrations from sites across the United States (Clark et al., 2000; Smith et al., 2003; Herlihy and Sifneos, 2008). Other studies have indicated that substantial changes in macroinvertebrate assemblage structure (Smith et al., 2007) and algal biomass (Stevenson et al., 2006) may occur near these concentrations (Table 3).

Table 3. A comparison of median total nitrogen (TN) and total phosphorus (TP) concentrations at 30 wadeable Ozark streams to TN and TP concentrations that are equivalent to a nutrient index score of 0.75, and to concentrations suspected of distinguishing between reference streams and slightly enriched streams.

Description or data source	Total nitrogen (mg/L)	Total phosphorus (mg/L)
Median concentrations	0.39	0.015
Concentrations equivalent to a nutrient index score of 0.75	0.40	0.018
Dodds et al. (1998)[a]	0.70	0.025
Clark et al. (2000)[b]	0.26	0.022
Smith et al. (2003)[c]	0.26	0.020
Smith et al. (2007)	0.29	0.020
Herlihy and Sifneos (2008)[d]	0.31	0.017

[a] Concentrations are based on differences in chlorophyll *a* for oligotrophic and mesotrophic stream categories.

[b] Flow-weighted concentrations.

[c] Modeled values (not measured).

[d] 75th percentile of least-impaired sites sampled as part of the Environmental Protection Agency Wadeable Stream Assessment.

4.2. Index/nutrient relations

Of the three assemblages evaluated, the algal assemblage seems to be most appropriate for assessing effects of low-level nutrient enrichment in wadeable Ozark streams. These results are consistent with those of Lavoie et al. (2008) who found that algal diatoms were effective for monitoring low-level TN and TP concentrations similar to those observed in this study. Algae are primary producers and nutrient availability may be the most important variable influencing algae (Lowe and Pan, 1996; Borchardt, 1996; Porter, 2008). By contrast, variables other than nutrients may be of equal or greater importance to macroinvertebrates and fish because they are primary and secondary consumers. Other reasons why algae are effective for assessing low-level nutrient enrichment are related to motility and longevity. Most algae are sessile organisms that have a short life cycle that is completed in the sampling area (Lowe and Pan, 1996) and algae may be more resistant to hydrologic disturbance than macroinvertebrates or fish when benthic habitats are armored as they are in Ozark streams (Riseng et al., 2004). Even though algae seem to be well suited for assessing low-level nutrient enrichment, the increased assurance of an accurate assessment (Hering et al., 2006; Griffith et al., 2005) and public perception regarding the economic importance of macroinvertebrates and fish may justify costs associated with sampling multiple assemblages for some monitoring programs.

Algal indices may be an alternative to chlorophyll *a* for assessing the effects of nutrient enrichment in some regions. Relations between chlorophyll *a* and TN and TP were poor for our data set and have been found to be poor in the Midwest United States (Morgan et al., 2006; Lowe et al., 2008), possibly because of confounding factors (i.e. light intensity, degree of nutrient limitation, and habitat quality, Miltner and Rankin, 1998).

4.3. Biotic index/land-use relations

Poultry litter applications are a concern in the Ozarks and elsewhere because N and P application rates are difficult to quantify and because litter application rates may exceed commercial fertilizing rates when an abundance of litter is available (Knowlton et al., 2004). Ozark land-use data also indicate that because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity is increased in areas where poultry are produced.

Although the TN and TP contribution to Ozark streams from manure seems to be increasing in high poultry and cattle production areas (Rebich and Demcheck, 2007), we found no studies that have been designed to address the ecological risks to streams when high poultry and cattle production dominate basin land use. The combined influence of poultry litter and cattle manure on nutrient runoff has been simulated in field experiments (Sauer et al., 1999; Vadas et al., 2007), and several studies have addressed runoff loss from poultry litter (Pierson et al., 2001; Butler et al., 2008; Sistani et al., 2008) or cattle manure (Edwards et al., 2000; Capece et al., 2007; Butler et al., 2008) under various conditions (i.e. different application rates, precipitation rates, soil saturations, and grazing intensities), but the effects of cattle and litter applications are rarely considered in combination.

Cattle production can increase nutrient runoff to streams directly (i.e. fecal deposition) or indirectly (i.e. habitat alteration). Unrestricted cattle generally will spend a large part of the day in the riparian zone regardless of the season or the availability of water elsewhere (Zuo and Miller-Goodman, 2004; Bagshaw et al., 2008), and James et al. (2007) observed that fecal deposition was significantly higher near streams than

in other areas of the pasture. Cattle influence habitat variables that have indirect relations to nutrients and can confound relations between biotic integrity and nutrients (Miltner and Rankin, 1998; Maret et al., 2008). Nutrient runoff potential increases when the grass filter in the riparian zone is over grazed (Sistani et al., 2008) and can increase as much as 90% when cattle trample and compact soils (Nguyen et al., 1998). Streambank stability also declines when cattle graze banks and access streams which, in turn, can increase nutrient runoff, particularly for TP (Vidon et al., 2008; Zaimes et al., 2008).

4.4. Conclusions

Biotic assessment methods used to evaluate areas with little or no disturbance should be sensitive to low-level nutrient enrichment because changes in land use and associated effects on water quality and ecological condition often occur slowly and over extended periods. Some biotic metrics selected for the three indices had weak relations to nutrient enrichment probably because TN and TP concentrations were below a threshold to which a biological response occurs. Relations of the three biotic indices to nutrient enrichment, however, were much stronger than relations between the biotic metrics and nutrient enrichment. This observation indicates that metrics selected for the indices were beneficial to index development and provides some validation for the index approach.

The algal index had a much stronger relation to low- to moderate-level nutrient enrichment than did the macroinvertebrate or fish index but all three indices were negatively correlated to nutrient enrichment. Biotic index scores were lowest and nutrient concentrations were highest for streams with basins having the highest poultry and cattle production. Because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity increases with poultry production. The synergistic effect of poultry and cattle production on Ozark streams in high production areas has not been evaluated and additional studies are needed before ecological risks are adequately assessed.

Acknowledgments

This is one of the several studies supported by the USGS NAWQA program to evaluate the effects of nutrient enrichment on stream ecosystems. The project would not have been possible had it not been for a number of USGS employees who assisted with reconnaissance, sampling, data organization and compilation, maps and figures, and manuscript review. Special thanks are extended to Amy Beck, Kelly Brady, Brian Clark, Jimmy Clark, Rheannon Hart, Shannon Kelly, Dwight Lasker, and Dan Yeatts. Thanks are extended to Greg Kloxin, who provided metrics that originated from the Oklahoma Conservation Commission. Thanks are also extended to two students from the University of Arkansas at Pine Bluff, Avian Wright and Byron Burns, who assisted with biotic sampling. Colleague reviews by Terry Maret, Mark Munn, Ian Waite, and two anonymous reviewers improved the quality of the report.

References

Alexander, R.B., Smith, R.A., Schwarz, G.E., Boyer, E.W., Nolan, J.V., Brakebill, J.W., 2008. Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. Environ. Sci. Technol. 42, 822–830.

Arar, E.J., Collins, G.B., 1997. In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence, Method 445.0, Revision 1.2. U.S. Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, Cincinnati, OH.

Bagshaw, C.S., Thorrold, B., Davison, M., Duncan, I.J.H., Matthews, L.R., 2008. The influence of season and of providing a water trough on stream use by beef cattle grazing hill-country in New Zealand. Appl. Animal Behav. Sci. 109, 155–166.

Bahls, L.L., 1993. Periphyton Bioassessment Protocols for Montana Streams. Montana Department of Health and Environmental Sciences, Water Quality Bureau, Helena, Montana.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Algal, Benthic Macroinvertebrates, and Fish, EPA 841-B-99-002, 2nd ed. U.S. Environmental Protection Agency, Office of Water.

Biggs, B.J.F., Smith, R.A., 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnol. Oceanogr. 47, 1175–1186.

Borchardt, M.A., 1996. Algal ecology. In: Stevensen, R.J., Bothwell, M.L., Lowe, R.L. (Eds.), Nutrients. Academic Press, San Diego, pp. 183–227.

Brightbill, R.A., Munn, M.D., 2008. Environmental and biological data of the nutrient enrichment effects on stream ecosystems project of the National Water Quality Assessment Program, 2003–04: U.S. Geological Survey Data Series 345, Tacoma, Washington, 12 p., http://wa.water.usgs.gov/neet/products.html (accessed April 2009).

Butler, D.M., Franklin, D.H., Cabrera, M.L., Tasistro, A.S., Xia, K., West, L.T., 2008. Evaluating aeration techniques for decreasing phosphorus export from grasslands receiving manure. J. Environ. Qual. 37, 1279–1287.

Capece, J.C., Campbell, K.L., Bohlen, P.J., Graetz, D.A., Portier, K.M., 2007. Soil phosphorus, cattle stocking rates, and water quality in subtropical pastures in Florida, USA. Rangeland Ecol. Manag. 60 (1), 19–30.

Center for Advanced Spatial Technologies, 2008. University of Arkansas Spatial Library, http://watersheds.cast.uark.edu (accessed November 2008).

Charles, D.F., Knowles, C., Davis, R.S. (Eds.), 2002. Protocols for the analysis of algal samples collected as part of the U.S. Geological Survey National Water-Quality Assessment Program. Report No. 02-06. Patrick Center for Environmental Research, The Acad. of Natural Sci., Philadelphia, PA, 124 pp.

Clark, G.M., Mueller, D.K., Mast, M.A., 2000. Nutrient concentrations and yields in undeveloped stream basins of the United States. J. Am. Water Res. Assoc. 36, 849–860.

Clarke, K.R., Warwick, R.M., 2001. Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation. PRIMER_E Ltd., Plymouth, UK, 174 pp.

Cuffney, T.F., 2003. User's manual for the National Water-Quality Assessment Program macroinvertebrate data analysis system (IDAS) software: Version 3. U.S. Geological Survey Open-File Report 03-172, 103 pp.

Dauwalter, D.C., Jackson, J.R., 2004. A provisional fish index of biotic integrity for assessing Ouachita Mountains streams in Arkansas, USA. Environ. Monit. Assess. 91, 27–57.

Dauwalter, D.C., Pert, E.J., Keith, W.E., 2003. An index of biotic integrity for fish communities in Ozark Highland streams of Arkansas. Southeastern Nat. 2, 447–468.

Davis, J.V., Bell, R.W., 1998. Water-quality assessment of the Ozark Plateaus study unit, Arkansas, Kansas, Missouri, and Oklahoma—analysis of information on nutrients, suspended sediment, and suspended solids, 1970–92. U.S. Geological Survey Water-Resources Investigations Report 95-4042, 112 pp.

Dodds, W.K., Jones, J.R., Welch, E.B., 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Water Res. 32 (5), 1455–1462.

Edwards, D.R., Hutchens, T.K., Rhodes, R.W., Larson, B.T., Dunn, L., 2000. Quality of runoff from plots with simulated grazing. J. Am. Res. Assoc. 36 (5), 1063–1073.

Fenneman, N.M., Johnson, D.W., 1946. Physical Divisions of the United States (Map). U.S. Geological Survey, Washington, DC.

Fishman, M.J., 1993. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—determination of inorganic and organic constituents in water and fluvial sediments. U.S. Geological Survey Open-File Report 93-125, 217 pp.

Fuhrer, G.J., Gilliom, R.J., Hamilton, P.A., Morace, J.L., Nowell, L.H. Rinella, J.F., Stoner, J.D., Wentz, D.A., 1999. The quality of our nation's water: nutrients and pesticides. U.S. Geological Survey Circular 1225, 82 pp.

Griffith, M.B., Hill, B.H., McCormick, F.H., Kaufmann, P.R., Herlihy, A.T., Selle, A.R., 2005. Comparative applications of indices of biotic integrity based on periphyton, macroinvertebrates, and fish to southern Rocky Mountain streams. Ecol. Indicators 5, 117–136.

Haase, R., Nolte, U., 2008. The invertebrate species index (ISI) for streams in southeast Queensland. Aust. Ecol. Indicators 8, 599–613.

Hering, D., Johnson, R.K., Dram, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates, and fish: a comparative metric-based analysis of organism response to stress. Freshwater Biol. 51, 1757–1785.

Herlihy, A.T., Sifneos, J.C., 2008. Developing nutrient criteria and classification schemes for wadeable streams in the conterminous US. J. North Am. Benthological Soc. 27, 932–948.

Hilsenhoff, W.L., 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20, 31–39.

James, E., Kleinman, P., Veith, T., Stedman, R., Sharpley, A., 2007. Phosphorus contributions from pastured dairy cattle to streams of the Cannonsville Watershed. J. Soil Water Conserv. 62, 40–47.

Justus, B.G., 2003. An index of ecological integrity for the Mississippi Alluvial Plain Ecoregion: index development and relations to selected landscape variables. U.S. Geological Survey Water-Resources Investigations Report 03-4110, 32 pp.

King, R.S., Richardson, C.J., 2007. Subsidy–stress response of macroinvertebrate community biomass to a phosphorus gradient in an oligotrophic wetland ecosystem. J. North Am. Benthological Soc. 26, 491–508.

Knowlton, K.F., Radcliffe, J.S., Novak, C.L., Emmerson, D.A., 2004. Animal management to reduce phosphorus losses to the environment. J. Anim. Sci. 82, 173–195.

Lange-Bertalot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. Nova Hedwigia 64, 285–304.

Lavoie, I., Vincent, W.F., Pienitz, R., Painchaud, J., 2004. Benthic algae as bioindicators of agricultural pollution in the streams and rivers of southern Que´bec (Canada). Aquat. Ecosyst. Health Manag. 7, 43–58.

Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., Dillon, P.J., 2008. Are diatoms good integrators of temporal variability in stream water quality. Freshwater Biol. 53, 827–841.

Leland, H.V., 1995. Distribution of phytobenthos in the Yakima River basin, Washington, in relation to geology, land use, and other environmental factors. Can. J. Fish. Aquat. Sci. 52, 1108–1129.

Lenat, D.R., 1993. A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water quality ratings. J. North Am. Benthological Soc. 12, 279–290.

Lowe, R.L., Pan, Y., 1996. Benthic algal communities as biological monitors. In: Stevenson, R.J., Bothwell, M.L., Lowe, R.L. (Eds.), Algal Ecology—Freshwater Benthic Ecosystems. Academic Press, San Diego, pp. 705–739.

Lowe, B.S., Leer, D.R., Frey, J.W., Caskey, B.J., 2008. Occurrence and distribution of algal biomass and its relation to nutrients and basin characteristics in Indiana streams. U.S. Geological Survey Scientific Investigations Report 2008-5203, 146 pp.

Maceina, M.J., Bayne, D.R., 2001. Changes in the black bass community and fishery with oligotrophication in West Point Reservoir, Georgia. North Am. J. Fish. Manag. 21 (4), 745–755.

Maret, T.R., MacCoy, D.E., Carlisle, D.M., 2008. Long-term water quality and biological responses to multiple best management practices in Rock Creek, Idaho. J. Am. Water Res. Assoc. 44, 1248–1269.

Miller, R.J., Robison, H.W., 2004. Fishes of Oklahoma. University of Oklahoma Press, Norman, p. 450.

Miltner, R.J., Rankin, E.T., 1998. Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biol. 40, 145–158.

Morgan, A.M., Royer, T.V., David, M.B., Gentry, L.E., 2006. Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. J. Environ. Qual. 35 (4), 1110–1117.

Moulton, S.R., II, Carter, J.L., Grotheer, S.A., Cuffney, T.F., Short, T.M., 2002. Revised protocols for sampling algal, macroinvertebrate, and fish communities as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 02-150, 87 pp.

Mueller, D.K., Spahr, N.E., 2006. Nutrients in streams and rivers across the Nation—1992–2001. U.S. Geological Survey Scientific Investigations Report 2006-5107, 44 pp.

Munn, M.D., Hamilton, P.A., 2003. New studies initiated by the U.S. Geological Survey—effects of nutrient enrichment on stream ecosystems. U.S. Geological Survey Fact Sheet FS-118-03, 4 pp.

National Agriculture Statistical Service, 2008. http://www.nass.usda.gov/ QuickStats (accessed 30 April 2008).

National Agriculture Statistical Service, 2008. 2002 Census of Agriculture, http://www.nass.usda.gov (accessed May 2008).

Nguyen, M.L., Sheath, G.W., Smith, C.M., Cooper, A.B., 1998. Impact of cattle treading on hill land. 2. Soil physical properties and contaminant runoff. N. Z. J. Agric. Res. 41 (2), 279–290.

Ortiz, J.D., Puig, M.A., 2007. Point source effects on density, biomass, and diversity of benthic macroinvertebrates in a Mediterranean stream. River Res. Appl. 23, 155–170.

Patton, C.J., Kryskalla, J.R., 2003. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water. Water-Resources Investigations Report 03-4174, 33 pp.

Petersen, J.C., Justus, B.G., Dodd, H.R., Bowles, D.E., Morrison, L.W., Williams, M.H., Rowell, G.A., 2008. Methods for monitoring fish communities of Buffalo National River and Ozark National Scenic Riverways in the Ozark Plateaus of Arkansas and Missouri: Version 1. U.S. Geological Survey Open-File Report 2007-1302, 94 pp.

Pflieger, W.L., 1997. The Fishes of Missouri, revised ed. Missouri Department of Conservation, Jefferson City, Missouri, 372 pp.

Pierson, S.T., Cabrera, M.L., Evanylo, G.K., Kuykendall, H.A., Hoveland, C.S., McCann, M.A., West, L.T., 2001. Phosphorus and ammonium concentrations in surface runoff from grasslands fertilized with broiler litter. J. Environ. Qual. 30 (5), 1784–1789.

Pilati, A., Vanni, M.J., Gonza´ lez, M.J., Gaulke, A.K., 2009. Effects of agricultural subsidies of nutrients and detritus on fish and plankton of shallow-reservoir ecosystems. Ecol. Appl. 19 (4), 942–960.

Porter, S.D., 2008. Algal attributes: an autecological classification of algal taxa collected by the National Water-Quality Assessment Program. U.S. Geological Survey Data Series 329, http://pubs.usgs.gov/ds/ds329/ (accessed May 2009).

Porter, S.D., Mueller, D.K., Spahr, N.E., Munn, M.D., Dubrovsky, N.M., 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biol. 53, 1036–1054.

Potapova, M., Charles, D.F., 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecol. Indicators 7, 48–70.

Rantz et al., 1982. Measurement and computation of streamflow: volume 1. Measurement of stage and discharge. U.S. Geological Survey. Water Supply Paper 2175, v.1, 284 pp.

Rashleigh, B., 2004. Relation of environmental characteristics to fish assemblages in the upper French Broad River basin, North Carolina. Environ. Monit. Assess. 93 (1–3), 139–156.

Rebich R.A, Demcheck, D.K., 2007. Trends in nutrient and sediment concentrations and loads in major river basins of the South-Central United States, 1993–2004. U.S. Geological Survey Scientific Investigations Report 2007-5090, 112 pp.

Resh, V.H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environ. Monit. Assess. 138, 131–138.

Riseng, C.M., Wiley, M.J., Stevenson, R.J., 2004. Hydrologic disturbance and nutrient effects on benthic community structure in midwestern US streams: a covariance structure analysis. J. North Am. Benthological Soc. 23, 309–326.

Robins, C.R., Bailey, R.M., Bond, C.E., Brooker, J.R., Lachner, E.A., Lea, R.N., Scott, W.B., 2004. Common and Scientific Names of Fishes from the United States, Canada and Mexico, vol. 29. American Fisheries Society, Bethesda, MD, 386 pp. (Special Publication).

Robison, H.W., Buchanan, T.M., 1988. Fishes of Arkansas, 5th ed. The University of Arkansas Press, Fayetteville, Arkansas, p. 536.

Sauer, T.J., Daniel, T.C., Moore Jr., P.A., Coffey, K.P., Nichols, D.J., West, C.P., 1999. Poultry litter and grazing animal waste effects on runoff water quality. J. Environ. Qual. 28 (3), 860–865.

Sistani, K.R., Brink, G.E., Oldham, J.L., 2008. Managing broiler litter application rate and grazing to decrease watershed runoff losses. J. Environ. Qual. 37 (2), 718–724.

Smith, R.A., Alexander, R.B., Schwarz, G.E., 2003. Natural background concentrations of nutrient in streams and river of the conterminous United States. Environ. Sci. Technol. 37, 3039–3047.

Smith, A.J., Bode, R.W., Kleppel, G.S., 2007. A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities. Ecol. Indicators 7, 371–386.

Stevenson, R.J., Rier, S.T., Riseng, C.M., Schultz, R.E., Wiley, M.J., 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia 561, 149–165.

Stevenson, R.J., Hill, B.H., Herlihy, A.T., Yuan, L.L., Norton, S.B., 2008. Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. J. North Am. Benthological Soc. 27, 783–799.

Terrel, J.W., Cade, B.S., Carpenter, J., Thompson, J.M., 1996. Modeling stream fish habitat limitations from wedge-shaped patterns of variation in standing stock. Trans. Am. Fish. Soc. 125, 104–117.

U.S. Environmental Protection Agency, 1998. National strategy for the development of regional nutrient criteria. U.S. Environmental Protection Agency Office of Water Fact Sheet EPA–822–F–98-002, http://epa.gov/waterscience/criteria/nutrient/ (accessed February 2009).

U.S. Environmental Protection Agency, 2008. Biological Indicators of Watershed Health, http://www.epa.gov/bioiweb/html/invertclass.html (accessed October, 8, 2008).

U.S. Geological Survey, 2005. Elevation Derivatives for National Applications, http://edna.usgs.gov (accessed June 2009).

U.S. Geological Survey, 2008. NationalWater Quality Assessment Program, Ecological National Synthesis Project, Fish Traits and Tolerances, U.S. Geological Survey, http://water.usgs.gov/nawqa/ecology/pubs/FishSppTraits_PublicWebpage_011404 (accessed February 2009).

Vadas, P.A., Harmel, R.D., Kleinman, P.J.A., 2007. Transformations of soil and manure phosphorus after surface application of manure to field plots. Nutrient Cycling Agroecosyst. 77 (1), 83–99.

Van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Neth. J. Aquat. Ecol. 28, 117–133.

Vidon, P., Cambell, M.A., Gray, M., 2008. Unrestricted cattle access to streams and water quality in till landscape of the Midwest. Agric.Water Manag. 95, 322–330.

Wang, W., Robertson, A.E., Garrison, P.J., 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. Environ. Manag. 39, 194–212.

Zaimes, G.N., Schultz, R.C., Isenhart, T.M., 2008. Total phosphorus concentrations and compaction in riparian areas under different riparian land-uses of Iowa. Agric. Ecosyst. Environ. 127, 22–30.

Zuo, M.H., Miller-Goodman, M.S., 2004. Landscape use by cattle affected by pasture developments and season. J. Range Manag. 57, 426–434.

Supplementary Material

Table S1. General basin and reach characteristics (at the time of sampling) of 30 sites sampled in the Ozark Highlands, 2006.

Site name	USGS station ID	Basin size (km^2)	Mean stream-flow (m^3/s)	Reach length (m)	Lati-tude	Long-itude	Datum
Barren Fork near Timber, Missouri	07064780	132.5	0.46	215	372046	912327	NAD83
Big Creek near Big Flat, Arkansas	07057100	235.8	0.20	253	355843	922853	NAD27
Big Creek at Mauser Mill, Missouri	07065040	108.0	0.11	248	371847	911900	NAD27
Bear Creek near Omaha, Arkansas	07054410	344.6	0.03	262	362650	925600	NAD27
Beaty Creek near Sycamore, Oklahoma	071912219	132.8	0.08	215	362156	944339	NAD83
Bennetts River near Vidette, Arkansas	07058970	155.6	0.06	190	362540	920457	NAD27
Big Piney River at Simmons, Missouri	06928730	275.8	0.55	255	371431	920035	NAD83
Calf Creek near Silver Hill, Arkansas	07055893	116.7	0.03	230	355801	924632	NAD27
Little Flat Creek near McDowell, Missouri	07052790	115.1	0.33	235	364919	934740	NAD83
Long Creek southeast of Denver, Arkansas	07053203	256.0	0.15	230	362151	931614	NAD83
Mahans Creek at West Eminence, Missouri	07065950	140.3	0.29	180	370850	912242	NAD27
Maries River Near Freeburg, Missouri	06926900	483.3	0.01	165	382001	915934	NAD27
Meramec River above Cook Station, Missouri	07010335	243.2	0.10	200	374120	912531	NAD83
Myatt Creek east of Salem, Arkansas	070692655	286.0	0.26	160	362521	913928	NAD83
North Fork White River near Cabool, Missouri	07057280	49.9	0.03	168	370318	921116	NAD83
North Indian Creek near Wanda, Missouri	07188855	113.2	0.19	249	364840	941236	NAD27
North Prong Jacks Fork below Arroll, Missouri	07065160	144.7	0.54	163	370513	914500	NAD83
North Sylamore Creek near Fifty Six, Arkansas	07060710	151.7	0.11	224	355930	921250	NAD27
Little Osage Creek at Healing Springs, Arkansas	07194947	110.9	0.20	300	361513	941612	NAD27
Piney Creek near Cabanol, Missouri	07050228	110.0	0.03	215	361605	933806	NAD27
Poke Bayou near Sidney, Arkansas	07060890	86.0	0.08	200	355715	914155	NAD27
Roasting Ear Creek near Newnata, Arkansas	07060661	162.5	0.10	217	355519	921351	NAD27
South Fork Spring River north of Moko, Arkansas	07069267	242.5	0.09	150	362903	915048	NAD27
Shoal Creek near Wheaton, Missouri	07186670	112.4	0.17	204	364637	940127	NAD83
Spring Creek near Locust Grove, Oklahoma	07192100	297.6	0.08	204	360838	950955	NAD83
Sullivan Creek near Sandtown, Arkansas	07060894	75.0	0.18	227	355315	913830	NAD83
Water Creek near Evening Star, Arkansas	07056695	99.2	0.07	217	360259	923434	NAD27
Woods Fork near Hartville, Missouri	06927590	116.4	0.05	185	371443	923404	NAD27
West Piney Creek at Bado, Missouri	06928750	92.8	0.09	152	371653	920610	NAD83
Yocum Creek near Oak Grove, Arkansas	07053250	136.1	0.19	287	362716	932122	NAD83

Table S2. Algal metrics evaluated for an algal index at 30 wadeable Ozark streams.

Taxonomic metrics[1]	**Tolerance metrics**[2]
Diatom taxa	Benthic algal taxa
Non-diatom taxa	Sestonic algal taxa
Green algal taxa	Nitrogen-fixing algal taxa
Blue-green algal taxa	Non-nitrogen fixing algal taxa
Red algal taxa	Algal taxa in nitrogen uptake category 1: N autotroph (low organic N)
Yellow-green algal taxa	Algal taxa in nitrogen uptake category 2: N autotrophic (high organic N)
Cryptophyte algal taxa	Algal taxa in nitrogen uptake category 3: N heterotroph (high organic N, facultative)
Euglenoid algal taxa	Algal taxa in nitrogen uptake category 4: N heterotroph (high organic N, obligate)[2]
Dinoflagellate algal taxa	Organic N index (diatoms): nitrogen heterotrophs
Total taxa richness (all algae)	Algal taxa in oxygen requirements category 1: high oxygen requirements (~ 100% saturation)
Total number of *Cymbella* sp. (richness only)	Algal taxa in oxygen requirements category 2: fairly high oxygen requirements (> 75% saturation)
Sum of *Cymbella affinis* Kutzing, *Cymbella delicatula* Kutzing, and *Cymbella hustedtii* Krasske (relative abundance only) [2]	Algal taxa in oxygen requirements category 3: moderate oxygen requirements (> 50% saturation)
	Algal taxa in oxygen requirements category 5: very low oxygen requirements (~ 10% saturation)
Motility metrics	Oxygen tolerant: algae with an unknown oxygen tolerance
Benthic-sestonic algae: unknown or not classified	Saprobien index: oligosaprobous (diatoms)
Motile algae (all algae)	Algal taxa in saprobic category 2: b - mesosaprobic
Non-motile algae (all algae)	Algal taxa in saprobic category 3: a - mesosaprobic[2]
Motility: unknown or not classified	Algal taxa in saprobic category 4: a - meso/polysaprobic
	Algal taxa in saprobic category 5: polysaprobic
Biomass metrics	Algal taxa in Bahls (1993) pollution class 1, most tolerant taxa[2]
Ash-free biomass (g/m^2)	Algal taxa in Bahls (1993) pollution class 2, less tolerant taxa
Chlorophyll *a* (mg/m^2)	Algal taxa in Bahls (1993) pollution class 3, most sensitive taxa
Total cells/cm^2 (all algae)	Algal taxa in pollution tolerance category 1: very tolerant (polysaprobic)
Total biovolume/cm^2 (all algae)	Algal taxa in pollution tolerance category 2a: tolerant (a-meso/polysaprobic)
	Algal taxa in pollution tolerance category 2b: tolerant (a-mesosaprobic)
Trophic metrics[1]	Algal taxa in pollution tolerance category 3a: less tolerant (b-mesosaprobic)
Oligotrophic	Algal taxa in pollution tolerance category 3b: less tolerant (oligosaprobic)
Oligo-mesotrophic	Pollution tolerance (Lange-Bertalot, 1979): unknown or not classified
Mesotrophic	Algal taxa that are nuisance benthic bloom producers
Meso-eutrophic	Algal taxa that are nuisance sestonic bloom producers
Eutrophic	Algal taxa not categorized as nuisance algae
Hypereutrophic	Algal taxa categorized as eutrophic soft algal taxa
Trophic: polytrophic (diatoms)	Algal taxa not categorized as eutrophic soft algae
Trophic: eurytrophic (diatoms)	Algal taxa classified as eutrophic soft algae
	Dominant taxa
	Percentage of total abundance represented by the most abundant taxon
	Percentage of total abundance represented by the two most abundant taxa
	Percentage of total abundance represented by the three most abundant taxa
	Percentage of total abundance represented by the four most abundant taxa
	Percentage of total abundance represented by the five most abundant taxa
	Number of taxa in the most abundant class
	Number of taxa in the two most abundant classes
	Number of taxa in the three most abundant classes
	Number of taxa in the four most abundant classes
	Number of taxa in the five most abundant classes

[1]Richness, percent richness, density, and percent density were calculated for diatoms and for all algae unless otherwise specified.

[2]Metrics selected for the algal index

Table S3. Macroinvertebrate metrics evaluated for a macroinvertebrate index at 30 wadeable Ozark streams.

General community[1]	**Tolerance metrics**
Amphipoda	North Carolina biotic index (abundance-weighted)
Baetidae[2, 3]	North Carolina biotic index (tolerant richness)
Bivalvia	
Chironomidae	**Dominant taxa (percent total abundance)**
Coleoptera	Most abundant taxon
Corbicula (abundance and percent abundance)	Two most-abundant taxon
Crustacea and Mollusca	Three most-abundant taxon
Diptera	Four most-abundant taxon
Ephemeroptera	Five most-abundant taxon
Elmidae[2]	
Elmidae and Psephenidae[2]	**Functional feeding group**[1]
Ephemeroptera, Plecoptera, and Trichoptera (EPT)	Collector-gatherer
Gastropoda	Filtering collector
Isonychia and *Leuctra* (abundance and percent abundance)	Omnivore
Isopoda	Parasite
Non-insects	Piercer
Non-midge Diptera	Predator
Non-midge Diptera and non-insects[3]	Scraper
Odonata	Shredder
Oligochaeta	
Orthocladinae	**Diversity**
Plecoptera	Brillouin diversity
Pteronarcys (abundance and percent abundance)	Brillouin evenness
Ratio of EPT to Chironomidae[3]	Margalef diversity
Ratio of Orthocladinae to Chironomidae	Menhinick diversity
Ratio of Tanytarsini to Chironomidae	Shannon diversity
Tanytarsini	Shannon evenness
Total taxa	Simpson diversity
Trichoptera	Simpson dominance
Number of rare taxa	Simpson evenness
Total biomass[2]	
Crayfish	**Other**[4]
Insect[3]	Percent Chironomidae, Naidae, and Tubificidae
Mollusc	Percent of insect taxa
Total abundance	Number of insect taxa

[1]Richness, percent richness, abundance, and percent relative abundance were calculated for all "general community" and "functional feeding group" metrics unless otherwise specified

[2]Metrics calculated manually outside of the IDAS program

[3]Metrics selected for the macroinvertebrate index. Three metrics were calculated using relative abundance; however, "Insect biomass" was a weight calculation

[4]All "Other" metrics originated from the Oklahoma Conservation Commission (Greg Kloxin, Oklahoma Conservation Commission, written communication, September 2008)

Table S4. Fish metrics considered for a fish index at 29 wadeable Ozark streams.

Taxa abundance and taxa richness values were calculated for all metrics except catch per unit effort, which was reported as the number of fish collected per meter.

Tolerance	**Sensitive taxa**
Tolerant	*Ambloplites* and *Lepomis* spp.[6]
Moderately tolerant	*Ambloplites*[6]
Intolerant	Catostomidae
	Catostomidae and Cyprinidae
Feeding habitats	Catostomidae, Cottidae, and Percidae[6]
Grazer	Catostomidae, Cottidae, Cyprinidae, *Noturus*, and Percidae[6]
Herbivore	*Campostoma*[6]
Planktivore	Centrarchidae
Detritivore	Cottidae[6]
Invertivore	Cyprinidae
Carnivore	*Gambusia*
Primary[2]	*Lepomis*
Herbivore and grazer[6]	*Lepomis cyanellus*
Herbivore and detritivore[1,6]	*Lepomis megalotis*[6]
Insectivorous cyprinid[5]	*Gambusia* and *Lepomis*
	Micropterus and *Ambloplites*[6]
Spawning preference	*Micropterus*[1, 4]
Broadcasting	Percidae
Simple-nesting	Key species[2]
Complex-nesting	Sensitive species[2]
Migratory	
Nesting unknown	**Dominance**
	Number of species comprising 75 pecent of the abundance[5]
Distribution	
Endemic[6]	**Species association**
Exotic	Sedentary
	Schooling
Substrate preference	
Cobble or rubble	**Habitat preference**
Gravel	Riffle
Cobble-gravel (combined)[3]	Pool[1]
Sand	Run or main channel
Mud (silt, clay, detritus)	Backwater
Vegetation	Benthic
Substrate generalist	Surface-loving
	Headwater
Density	Habitat generalist
Catch per unit effort[1]	Pool and benthic

[1]Metrics selected for the fish index

[2]Metrics originated from the Arkansas Department of Environmental Quality (Jim Wise, Arkansas Department of Environmental Quality, written communication, August 2008)

[3]Metrics originated from Dauwalter et al., 2003

[4]Metric originated from Justus, 2003

[5]Metric originated from the Oklahoma Conservation Commission (Greg Kloxin, Oklahoma Conservation Commission, written communication, September 2008)

[6]Metric calculated by the authors to characterize taxa considered key to Ozark ecosystems

Table S5. Algal metric values, metric scores, and index scores for 30 wadeable Ozark streams. Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

Site name	Abbreviated name (fig. 1)	Most tolerant diatoms		Sum of *Cymbella affinis*, C. *delicatula*, and C. *hustedtii*		Mesosaprobic algae taxa richness		Diatoms as nitrogen heterotrophs		Algal index score	Nutrient index score
		Relative abundance (%)	Metric score	Relative abundance (%)	Metric score	Taxa richness (%)	Metric score	Relative abundance (%)	Metric score		
Barren Fork near Timber, Missouri	Barren	0.6	98.5	15.3	23.5	6.7	71.7	0.2	98.4	73.0	0.00
Meramec River above Cook Station, Missouri	Mera	0.2	99.6	62.1	95.0	4.8	79.8	0.2	98.7	93.3	0.05
Big Creek at Mauser Mill, Missouri	BcMm	0.0	100.0	46.1	70.4	6.3	73.4	0.0	100.0	86.0	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	0.2	99.6	7.8	11.9	4.8	79.8	0.0	100.0	72.8	0.08
Water Creek near Evening Star, Arkansas	Water	1.0	97.6	31.9	48.8	4.8	79.8	0.3	97.4	80.9	0.10
Bear Creek near Omaha, Arkansas	Bear	0.3	99.2	46.7	71.3	4.2	82.3	0.0	100.0	88.2	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	0.0	100.0	65.4	100.0	5.0	78.8	0.0	100.0	94.7	0.24
North Fork White River near Cabool, Missouri	NFWh	1.7	96.0	32.1	49.1	13.3	43.3	0.0	100.0	72.1	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	6.2	85.1	0.2	0.4	7.7	67.3	0.0	100.0	63.2	0.38
Bennetts River near Vidette, Arkansas	Benn	0.5	98.8	5.4	8.3	9.1	61.4	0.0	100.0	67.1	0.47
Mahans Creek at West Eminence, Missouri	Maha	0.3	99.2	11.8	18.0	12.0	49.0	1.2	90.8	64.3	0.53
Myatt Creek east of Salem, Arkansas	Myatt	3.0	92.8	12.0	18.4	6.5	72.6	0.0	100.0	70.9	0.54
West Piney Creek at Bado, Missouri	Wpin	3.3	92.0	1.2	1.9	18.2	22.7	0.0	100.0	54.1	0.60
Piney Creek near Cabanol, Missouri	Piney	1.2	97.2	17.4	26.5	12.0	49.0	0.2	98.7	67.9	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	0.5	98.8	18.7	28.6	11.4	51.4	0.0	100.0	69.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	0.3	99.2	7.2	11.0	11.9	49.4	0.0	100.0	64.9	0.77
Big Piney River at Simmons, Missouri	Bpine	15.7	62.1	10.4	15.8	9.7	58.9	6.6	48.3	46.3	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	1.3	96.8	5.3	8.2	19.4	17.7	0.0	100.0	55.7	0.85
Big Creek near Big Flat, Arkansas	BcBF	21.5	48.2	2.7	4.2	12.5	46.9	0.3	97.4	49.2	0.93
Calf Creek near Silver Hill, Arkansas	Calf	22.8	45.1	12.8	19.5	11.5	51.0	2.2	83.0	49.6	1.08
Poke Bayou near Sidney, Arkansas	Poke	9.4	77.4	1.9	2.9	9.5	59.5	1.2	90.8	57.7	1.10
Woods Fork near Hartville, Missouri	WdFk	1.5	96.3	2.7	4.2	23.5	0.0	0.0	100.0	50.1	1.12
Maries River Near Freeburg, Missouri	Marie	3.2	92.4	0.5	0.7	11.1	52.8	0.0	100.0	61.5	1.35
Long Creek southeast of Denver, Arkansas	Long	21.8	47.4	1.4	2.2	6.8	71.0	1.3	89.5	52.5	1.55
Little Flat Creek near McDowell, Missouri	Flat	5.7	86.3	0.0	0.0	13.0	44.6	0.5	96.1	56.7	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	17.7	57.4	7.5	11.5	12.5	46.9	3.3	73.8	47.4	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	41.5	0.0	6.0	9.2	13.3	43.3	6.0	52.9	26.3	2.57
Shoal Creek near Wheaton, Missouri	Shoal	23.6	43.2	0.4	0.6	20.8	11.5	2.0	84.1	34.8	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	38.5	7.2	0.2	0.4	8.3	64.6	0.3	97.4	42.4	2.95
North Indian Creek near Wanda, Missouri	NInd	16.2	60.8	0.9	1.4	18.5	21.3	12.7	0.0	20.9	3.30

Table S6. Macroinvertebrate metric values, metric scores, and index scores for 30 wadeable Ozark streams. Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

Site name	Abbreviated name (fig. 1)	Intolerant organisms		Baetidae		Insect biomass		Moderately tolerant taxa		Macroinvertebrate index score	Nutrient index score
		Relative abundance (%)	Metric score	Relative abundance (%)	Metric score	Grams	Metric score	Taxa richness	Metric score		
Barren Fork near Timber, Missouri	Barren	99.66	100.0	2.8	91.4	0.01	99.7	13	51.9	85.7	0.00
Meramec River above Cook Station, Missouri	Mera	96.95	97.3	3.4	89.4	1.29	73.0	20	25.9	71.4	0.05
Big Creek at Mauser Mill, Missouri	BcMm	99.64	100.0	7.2	77.4	1.17	75.6	14	48.1	75.3	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	97.52	97.9	1.3	96.0	1.58	67.0	15	44.4	76.3	0.08
Water Creek near Evening Star, Arkansas	Water	93.46	93.8	4.0	87.4	0.95	80.1	24	11.1	68.1	0.10
Bear Creek near Omaha, Arkansas	Bear	78.01	78.3	0.0	100.0	1.79	62.5	19	29.6	67.6	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	99.49	99.8	8.0	75.0	0.41	91.5	18	33.3	74.9	0.24
North Fork White River near Cabool, Missouri	NFWh	98.52	98.9	8.3	74.0	0.13	97.3	17	37.0	76.8	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	89.78	90.1	13.9	56.8	0.82	82.8	22	18.5	62.1	0.38
Bennetts River near Vidette, Arkansas	Benn	86.24	86.5	4.3	86.6	0.52	89.1	17	37.0	74.8	0.47
Mahans Creek at West Eminence, Missouri	Maha	94.96	95.3	15.9	50.5	0.49	89.7	21	22.2	64.4	0.53
Myatt Creek east of Salem, Arkansas	Myatt	85.69	86.0	2.0	93.7	1.58	67.0	18	33.3	70.0	0.54
West Piney Creek at Bado, Missouri	Wpin	90.65	91.0	3.2	90.1	2.17	54.6	24	11.1	61.7	0.60
Piney Creek near Cabanol, Missouri	Piney	85.32	85.6	8.4	73.8	1.65	65.5	23	14.8	59.9	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	87.50	87.8	3.4	89.3	1.12	76.7	17	37.0	72.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	85.68	86.0	9.2	71.4	0.57	88.0	16	40.7	71.5	0.77
Big Piney River at Simmons, Missouri	Bpine	92.31	92.6	10.2	68.0	1.30	72.9	21	22.2	63.9	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	78.61	78.9	15.9	50.5	2.70	43.6	21	22.2	48.8	0.85
Big Creek near Big Flat, Arkansas	BcBF	96.76	97.1	11.4	64.6	0.94	80.3	17	37.0	69.7	0.93
Calf Creek near Silver Hill, Arkansas	Calf	93.14	93.5	2.2	93.0	2.05	57.2	22	18.5	65.6	1.08
Poke Bayou near Sidney, Arkansas	Poke	81.28	81.6	12.7	60.3	3.23	32.5	27	0.0	43.6	1.10
Woods Fork near Hartville, Missouri	WdFk	74.09	74.3	4.6	85.7	0.55	88.6	20	25.9	68.6	1.12
Maries River Near Freeburg, Missouri	Marie	84.22	84.5	0.4	98.8	0.02	99.5	20	25.9	77.2	1.35
Long Creek southeast of Denver, Arkansas	Long	89.09	89.4	1.0	96.9	1.24	74.0	25	7.4	66.9	1.55
Little Flat Creek near McDowell, Missouri	Flat	92.34	92.6	12.9	59.8	1.11	76.9	15	44.4	68.4	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	95.26	95.6	23.2	27.5	1.82	61.9	23	14.8	49.9	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	94.34	94.7	9.3	70.9	2.50	47.6	19	29.6	60.7	2.57
Shoal Creek near Wheaton, Missouri	Shoal	66.26	66.5	17.3	45.9	1.99	58.4	23	14.8	46.4	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	90.73	91.0	32.0	0.0	3.07	35.8	22	18.5	36.3	2.95
North Indian Creek near Wanda, Missouri	NInd	64.33	64.5	13.9	56.7	4.78	0.0	15	44.4	41.4	3.30

Table S7. Fish metric values, metric scores, and index scores for 29 wadeable Ozark streams (one site was not sampled for fish because of the potential occurrence of a federally-listed threatened species). Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

		Herbivore/ Detritivore		Pool species		Catch per unit effort		Black bass			
Site name	Abbrevi-ated name (fig. 1)	Taxa rich-ness	Metric score	Relative abun-dance (%)	Metric score	Fish per meter	Metric score	Relative abun-dance (%)	Metric score	Fish index score	Nutrient index score
Barren Fork near Timber, Missouri	Barren	1	83.3	61.2	63.8	0.9	89.2	1.00	28.5	66.2	0.00
Meramec River above Cook Station, Missouri	Mera	5	16.7	76.9	80.1	3.3	61.4	1.00	28.5	46.7	0.05
Big Creek at Mauser Mill, Missouri	BcMm	3	50.0	66.2	68.9	1.8	79.1	0.26	7.4	51.4	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	2	66.7	79.0	82.3	1.4	83.8	3.51	100.0	83.2	0.08
Water Creek near Evening Star, Arkansas	Water	5	16.7	64.6	67.3	2.4	72.0	1.52	43.3	49.8	0.10
Bear Creek near Omaha, Arkansas	Bear	4	33.3	64.9	67.6	3.2	63.5	2.66	75.8	60.1	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	3	50.0	96.0	100.0	1.1	87.4	0.37	10.5	62.0	0.24
North Fork White River near Cabool, Missouri	NFWh	4	33.3	42.1	43.9	2.5	70.9	0.50	14.2	40.6	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	4	33.3	50.6	52.7	2.4	72.8	1.88	53.6	53.1	0.38
Bennetts River near Vidette, Arkansas	Benn	3	50.0	68.7	71.5	1.7	80.1	0.69	19.7	55.3	0.47
Mahans Creek at West Eminence, Missouri	Maha	4	33.3	58.8	61.3	1.4	83.8	0.00	0.0	44.6	0.53
Myatt Creek east of Salem, Arkansas	Myatt	2	66.7	42.0	43.8	2.1	76.2	2.19	62.4	62.3	0.54
West Piney Creek at Bado, Missouri	Wpin	3	50.0	55.1	57.4	3.5	59.9	1.09	31.1	49.6	0.60
Piney Creek near Cabanol, Missouri	Piney	3	50.0	39.4	41.1	4.3	50.4	0.54	15.4	39.2	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	4	33.3	43.7	45.5	2.2	74.6	0.89	25.4	44.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	2	66.7	44.6	46.5	2.6	70.0	1.60	45.6	57.2	0.77
Big Piney River at Simmons, Missouri	Bpine	3	50.0	50.7	52.8	1.4	84.1	1.99	56.7	60.9	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	4	33.3	41.6	43.3	1.9	78.2	0.70	19.9	43.7	0.85
Big Creek near Big Flat, Arkansas	BcBF	3	50.0	73.5	76.6	2.9	66.9	1.60	45.6	59.8	0.93
Calf Creek near Silver Hill, Arkansas	Calf	4	33.3	44.1	45.9	8.3	3.8	0.10	2.8	21.5	1.08
Poke Bayou near Sidney, Arkansas	Poke	6	0.0	59.8	62.3	2.6	70.2	2.12	60.4	48.2	1.10
Woods Fork near Hartville, Missouri	WdFk	5	16.7	72.3	75.4	2.3	74.0	1.13	32.2	49.6	1.12
Long Creek southeast of Denver, Arkansas	Long	4	33.3	65.4	68.1	4.1	52.2	0.40	11.4	41.3	1.55
Little Flat Creek near McDowell, Missouri	Flat	4	33.3	19.2	20.0	1.7	79.8	0.00	0.0	33.3	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	3	50.0	49.4	51.5	2.4	71.9	0.33	9.4	45.7	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	4	33.3	70.8	73.7	2.1	75.9	0.83	23.6	51.7	2.57
Shoal Creek near Wheaton, Missouri	Shoal	6	0.0	56.9	59.3	7.6	11.9	0.70	19.9	22.8	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	5	16.7	41.1	42.8	8.7	0.0	0.15	4.3	15.9	2.95
North Indian Creek near Wanda, Missouri	NInd	5	16.7	42.9	44.7	2.3	72.9	0.00	0.0	33.6	3.30

ARKANSAS DEPARTMENT OF ENERGY AND ENVIRONMENTAL, DIVISION OF ENVIRONMENTAL QUALITY

RE: FRL-comment on FRL–11994–01–R6

Exhibit D - *Email to EPA on February 21, 2024, providing DEQ's assessment of Springs Creek, associated data, and narrative explanation.*

Basil Hicks [redacted]

From: Stacie Wassell [redacted]
Sent: Thursday, February 22, 2024 11:07 AM
To: Joe Martin [redacted] Basil Hicks [redacted] Bryan Leamons [redacted]
Subject: Fw: 303(d) narrative and associated data
Attachments: Spring Creek short term continuous assessment.xlsx; Spring Creek Fish Data.xlsx; Ozark Highlands Fish Biocriteria.pdf; 303(d) Supplemental Data Narrative.pdf

FYI

Stacie R. Wassell | Associate Director
Arkansas Energy and Environment
Division of Environmental Quality | **Office of Water Quality**
[redacted]

From: Stacie Wassell [redacted]
Sent: Wednesday, February 21, 2024 9:47 PM
To: 'Jones, Curry'
Cc: Bailey Taylor [redacted]
Subject: 303(d) narrative and associated data

Curry,
I have attached the data and associated narrative of the data to this email for your review and consideration. Please let me know if you would like to schedule a call or Teams meeting with our team to discuss the data.

Kind regards,

Stacie R. Wassell | Associate Director
Arkansas Energy and Environment
Division of Environmental Quality | **Office of Water Quality**
[redacted]

Arkansas Department of Energy & Environment's Division of Environmental Quality (DEQ) sampled streams in the Illinois River basin as part of DEQ's ecoregion project for the Ozark Highlands and has collected the required data to assess Arkansas Pollution Control and Ecology Commission's (APC&EC) Rule 2, Water Quality Standards for Surface Waters of the State of Arkansas's narrative nutrient criterion for Spring Creek.

1. Total Phosphorus Analysis

The APC&EC Rule 2.509 states,

> Materials stimulating algal growth shall not be present in concentrations sufficient to cause objectionable algal densities or other nuisance aquatic vegetation or otherwise impair any designated use of the waterbody.

While Rule 2 does not specify concentrations in the form of a numeric standard, DEQ does have a process for assessing waterbodies for the narrative nutrient criterion. This process has been reviewed by EPA and is reflective of APC&EC Rule 2.509, which states,

> Because nutrient water column concentrations do not always correlate directly with stream impairments, impairments will be assessed by a combination of factors such as water clarity, periphyton or phytoplankton production, dissolved oxygen (D.O.) values, D.O. saturation, diurnal D.O. fluctuations, pH values, aquatic-life community structure and possibly others.

EPA stated in their Record of Decision (ROD) that their evaluation focused on multiple lines of evidence, consistent with APC&EC Rule 2, but EPA did not provide any evidence relating to periphyton production, diurnal D.O. fluctuations, pH values, or aquatic life community structure.

DEQ collected data for Spring Creek throughout 2023 and assessed the data according to DEQ's Assessment Methodology. Due to the data being collected in the summer of 2023, an equivalent period of record was developed for comparison starting in September 2023 and going back five years. The mean total phosphorus concentration was greater than the 75th percentile for the ecoregion so the next step in the flow chart is required (see table below). The 48-hour D.O. and pH datasets do not exceed applicable criteria and, therefore, the stream is supporting the narrative nutrient criteria for the stream. Although not required by the assessment methodology due to D.O. and pH attaining, the fish assemblage was also assessed and was also supporting the aquatic life use. In addition to supporting the use, 10 of the 23 species captured were sensitive species. **DEQ used multiple lines of evidence from empirical data collected on Spring Creek and determined that there was no impairment of DEQ's EPA-approved narrative nutrient criterion using DEQ's Assessment Methodology.**

Nutrient Assessment	**Spring Creek**	**Decision**
Are mean TP and/or TN concentrations > 75% for the ecoregion?	Yes	Move to next step
Do continuous datasets for D.O. or pH exceed criteria?	No	Support
Are biological assemblages impaired?	No (fish only)	Support

DEQ's use of its own EPA-approved narrative criterion and assessment methodology is appropriate for assessing waters in the state of Arkansas and demonstrates that there is no impairment due to nutrients in Spring Creek. Spring Creek also had the highest geometric mean total phosphorus of all the assessment units (AU) EPA proposed to promulgate and was determined to not be impaired by DEQ's assessment of the narrative nutrient criterion. If EPA was incorrect about Spring Creek, the stream with the highest total phosphorus concentration, they are likely wrong about the other six assessment units proposed for promulgation in the EPA Record of Decision (ROD).

2. Periphyton Growth

EPA evaluated periphyton results from a McGoodwin, Williams and Yates study titled *Water Quality and Ecological Assessment of Osage and Spring creeks in the Illinois River Basin*. EPA's reason for citing this study appears to be due to the passive diffusion periphytometers lack of ability to find statistically significant results with nutrient limitation in the streams. Therefore, if nitrogen or phosphorus are not limiting, the concentrations must be high and the stream must be impaired. This is flawed logic. Not only are nutrient bioassays difficult for statistical significance due to sample size and variability of chlorophyll *a*, the study points out that something other than nutrients such as light, temperature, or turbidity is limiting periphyton growth. The study states,

> The conclusion is that there is no evidence that discharge of wastewater from the Rogers WWTP to Osage Creek or the Springdale WWTP to Spring Creek results in any violation of water quality standards according to the criteria of ADEQ Reg. 2. There appears to be no justification from this data for placing Spring and Osage Creeks on the 303(d) list of impaired waters for impairment by nutrients.

Oklahoma's Scenic River phosphorus criterion is based on the Joint Study by Dr. Ryan King, which states that the phosphorus criterion is "based on empirical stressor-response relationships between total phosphorus and response variables related to nuisance levels of algae." DEQ's narrative nutrient criterion is based on the prevention of "objectionable algal densities or other nuisance aquatic vegetation." With nuisance algae being the condition that leads to impairment, it would be helpful to review Dr. King's study to determine what those conditions were during the Joint Study. Previous literature values have stated that 150–200 mg/m^2 represent nuisance conditions, yet Dr. King states that these values are subjective and need context. He further stated that "some of our sites with low phosphorus consistently yielded benthic chlorophyll *a* levels that approached or exceeded literature values for 'nuisance' conditions (>150–200 mg/m^2), yet virtually none of this algal biomass was Cladophora or other nuisance species of filamentous green algae." Dr. King ultimately stated, "150–200 mg/m^2 likely represented the lower end of potential nuisance levels of algal biomass in the Designated Scenic Rivers during a wet year, whereas levels above 300 mg/m^2 should be considered nuisance levels under most conditions." Spring Creek was sampled for periphyton in the summer of 2023, considered abnormally dry/moderate drought by the National Oceanic and Atmospheric Administration's drought monitor. Benthic chlorophyll *a* for Spring Creek was 211 mg/m^2, well below the 300 mg/m^2 threshold Dr. King developed in his stressor-response study.

EPA stated that the total phosphorus concentrations measured during the MWY study were of similar magnitude to those measured during EPA's analysis that was used to propose promulgation of 303(d) listings on seven AUs in the Illinois River basin. If so, then the corresponding benthic chlorophyll *a* values should also demonstrate nuisance levels of algae that would cause an impairment. As exhibited in the MWY study, this was not the case in Osage Creek sites 1, 2, and 3 corresponding to AU AR_11110103_930, or

Osage Creek sites 4 and 5 corresponding to AU AR_11110103_030. Mean benthic chlorophyll *a* for all Osage Creek sites during the first critical season were never above 55 mg/m^2. Mean benthic chlorophyll *a* for all Osage Creek sites during the second critical season were never above 128 mg/m^2 and four of five sites were below 100 mg/m^2. Mean benthic chlorophyll *a* for all Osage Creek sites during the third critical season were never above 180 mg/m^2 and four of the five sites were below 150 mg/m^2. None of the Osage Creek sites during the study ever approached the 300 mg/m^2 nuisance condition that Dr. King described and on only one occasion did any site reach over 150 mg/m^2. The data from this study demonstrates that nuisance levels of algae, under total phosphorus concentrations of similar magnitude as EPA's analysis, did not occur in Osage Creek according to thresholds derived by Dr. King's study of streams in the Illinois River basin.

The sampling sites in the USGS paper, *A comparison of algal, macroinvertebrate, and fish assemblage indices for assessing low-level nutrient enrichment in wadeable Ozark streams*, had land use that was usually less than 5% urban and no wastewater treatment plants discharged to any of the streams, certainly not comparable to the heavily urbanized streams with wastewater discharges on which EPA is proposing to promulgate nutrient impairments. The USGS paper states, "the small size of the data set limits our ability to identify thresholds for TN and TP, however, some literature indicates that TN and TP concentrations near median values for this study are near threshold concentrations that distinguish between reference streams and streams that are slightly enriched (i.e. near background, Table 3)." The 0.018 mg/L total phosphorus concentration EPA used in their ROD was not derived through developing thresholds for nutrient enrichment, rather, it happens to correspond to *some* literature that distinguishes between reference streams and streams that are slightly enriched or near background concentrations. Further, the description of Table 3 in the USGS paper states that the total phosphorus concentrations are "suspected of distinguishing between reference streams and slightly enriched streams." The term "suspected" is used because the indices EPA cites have not been validated to determine if they can accurately differentiate between reference and test streams. The streams in the USGS study are not similar to the streams on which EPA proposes to promulgate nutrient impairments, have nothing to do with Rule 2's narrative nutrient criteria, do not speak to nuisance algae levels, had no reported amount of benthic algae per unit area (even though it was collected), and had poor relationships between nutrients and chlorophyll *a*. EPA's title for this comment was "linking aquatic life community structure to nutrients." When DEQ sampled Spring Creek's aquatic life, the sample demonstrated that 43% of fish sampled were sensitive species and none of the criteria to protect the aquatic life use were impaired.

EPA stated in their Basis for Decision to Disapprove and Add Waters to the Arkansas 2020 Section 303(d) List that the seven AUs are not attaining the narrative nutrient criteria, which states, "Materials stimulating algal growth shall not be present in concentrations sufficient to cause objectionable algal densities or other nuisance aquatic vegetation or otherwise impair any designated use of the waterbody." EPA failed to produce any evidence that objectionable algal densities or other nuisance aquatic vegetation was present or that any designated use of the waterbody was impaired. EPA stated that they focused on multiple lines of evidence, but EPA provided no evidence in regards to water clarity, periphyton production, diurnal D.O. fluctuations, pH values, or aquatic life community structure—all factors EPA cited in their ROD. When those factors were taken into consideration, as in the case of Spring Creek being assessed with Arkansas's approved assessment methodology, it was clear that there was no violation of the narrative nutrient criterion and that no designated uses were impaired. Further, EPA cited a study on Spring and Osage Creeks that concluded that there appears to be no justification from this data for placing Spring and Osage Creeks on

the 303(d) list of impaired waters for impairment by nutrients. EPA's analysis is flawed and DEQ demonstrated above that the AUs in the Illinois River basin should not be listed as impaired.

FISH COMMUNITY BIOCRITERIA

Ozark Highlands Streams (All Watersheds)

METRIC	5	3	1†
% Sensitive Individuals	>31	31 – 20	<20
% Cyprinidae (Minnows)	>48 – 64	39 – 48 **or** >64 – 73	<39 **or** >73
% Ictaluridae (Catfishes)	>2 **and** ≤3% bullheads from total catch	1 – 2 **and** ≤3% bullheads from total catch	<1 **or** >3% bullheads from total catch
% Centrarchidae (Sunfishes)	4 – 15 **and** ≤2% Green sunfish from total catch	<4 **or** > 15 – 20 **and** ≤ 2% Green sunfish from total catch	>20 **or** >2% Green sunfish from total catch
% Percidae (Darters)	>11	5 – 11	<5
% Primary Feeders	<42	42 – 49	>49
% "Key" Individuals	>23	23 – 16	<16
Diversity	>2.77	2.77 – 2.37	<2.37
# Species	>(wtrshd*0.034)+16.45	(wtrshd*0.034)+16.45 – (wtrshd*0.034)+12.26	<(wtrshd*0.034)+12.26

†if a raw metric score is zero, score as zero, except for Primary Feeders

Total Score

37-45 Mostly Similar
25-36 Generally Similar
13-24 Somewhat Similar
12-0 Not Similar