Image caption: View of Flint Creek in Oklahoma, courtesy of USDA Natural Resources Conservation Service.

Stoneroller Fish (*Campostoma spp.*) Influence on Dose-Response Relationship Between Nutrients and Algae in Summer 2016 and Winter 2017

Kayla R. Sayre and Michelle A. Evans-White*

Department of Biological Sciences, University of Arkansas
*Corresponding author

Abstract: Elevated nitrogen (N) and phosphorous (P) in streams can cause nutrient pollution leading to instream and downstream problems of excess algal growth which can constrain the recreational use of streams and reduce stream biodiversity (Dodds and Welch, 2000). The United States Environmental Protection Agency (USEPA) provided national numeric nutrient criteria standards based on ecoregion, and states and tribes can adopt these criteria or develop their own standards. The objective of this project was to examine how stonerollers (Campostoma spp.) may modify the dose-response relationship between nutrients and algal biomass in wadeable Ozark Highland streams seasonally. Grazers tended to reduce algal biomass measured as chlorophyll a (chl *a*) in each stream, but most of the differences between grazer excluded and grazer present treatments were not statistically significant at $p<0.05$; grazer chl *a* effect sizes tended to be positively related to TP ($p>0.05$) and were greater in the summer compared to the winter (ANCOVA $F=59.85$, $p=0.0163$). This suggests that seasonality plays a role in stoneroller's influence on stream algae and it should be considered when examining dose-response relationships between nutrients and algae.

Key Points:

- Increased nutrient concentrations can stimulate benthic algal biomass; grazers, like the stoneroller (*Campostoma spp.*), may dampen the effect of nutrients on benthic algal biomass,
- But grazers are often not considered when constructing nutrient-algal relationships for the development of numeric nutrient criteria.
- Grazers appeared to reduce algal biomass measured as chlorophyll a (chl *a*) although differences were not significant.
- Grazer chl *a* effect sizes tended to be positively related to TP ($p>0.05$) and were greater in the summer compared to the winter (ANCOVA $F=59.85$, $p=0.0163$).
- Our results suggest that nutrient and grazer effects on benthic algae can be variable and seasonal.

Introduction

Elevated nitrogen (N) and phosphorous (P) in streams can cause excess algal growth, which can constrain the recreational use of streams and reduce stream biodiversity (Dodds and Welch, 2000). The United States Environmental Protection Agency (USEPA) provided national numeric nutrient criteria standards based on ecoregion and states and tribes can adopt these criteria or develop their own. Therefore, many states have decided to develop regional numeric nutrient criteria standards based on scientific methods, which can include assessment of algal biomass (USEPA, 2017). The Arkansas Department of Environmental Quality (ADEQ) is currently working toward federal TN and TP standards by assessing dose-response relationships between algae (chlorophyll a and ash-free dry mass), but does not currently have published federal total nitrogen (TN) or total phosphorus (TP) numeric nutrient criteria in accordance with the EPA (USEPA, 2017). Arkansas currently has algae narrative criteria for all water bodies and TP point source criteria for streams.

Arkansas currently has narrative standards for algae in waterbodies, according to Regulation No. 2 from the Arkansas Pollution Control and Ecology Commission (APCEC), which states that "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" (APCEC, 2015). The state intends to develop numeric nutrient criteria from dose-response relationships between nutrient levels and stream benthic algae; ADEQ is leading that effort. Relationships between nutrient concentrations and algae can be variable in Arkansas and Oklahoma (Stevenson et al., 2012, Haggard, 2013) since other factors in addition to nutrient concentrations can affect benthic algal concentrations. Specifically, some of the variation in the relationship between nutrients and benthic algae may be explained by macrograzer activity (Stevenson et al., 2012).

Seasonal variations in algal density and associated determining factors, such as macrograzer activity, may cause some of the variation in dose-response relationship between nutrients and benthic algal biomass. Thus, these variations in dose-response relationships should be considered when developing numeric nutrient criteria for the Ozark Highland Ecoregion. Most studies examining the relationships between grazers, algae, and nutrients have used snails and caddisflies as the study organism while less in known about the influence of algivorous fish, such as stonerollers on algal biomass responses to nutrient enrichment (Cattaneo and Mousseau, 1995). Stonerollers (Campostoma spp.) are minnows that occur in high abundances in Ozark streams, and possess a sub-terminal mouth that makes them well-equipped grazers. Campostoma spp. grazing can be an important determining factor on algal biomass and community composition (Steward, 1987; Power et al., 1988) and they are thought to be grazing most actively during the warm season since they are ectotherms. During late summer, the standing stock of algae in pools can be nearly devoid of algae biomass due to grazing by Campostoma spp. (Matthews et al., 1987), but little is known about their potential to affect algal biomass in the winter. Seasonal variation in Campostoma spp. grazing could explain variation in algal biomass across seasons and sites in Ozark streams with varying nutrient concentrations.

The proposed study examining the seasonality of Campostoma spp. effects on benthic algae across streams with a gradient of total phosphorus concentrations can help the state understand how and why seasonality may result in variation in the relationship between nutrients and algae. The objective of this project is to examine how stonerollers (Campostoma spp.) may modify the dose-response relationship between nutrients and algal biomass in wadeable Ozark Highland streams seasonally. We hypothesized that stonerollers would have a significant negative effect on benthic algae within each stream during the summer (hypothesis 1; H1). Our second hypothesis was that stoneroller effects on algae would increase with total phosphorus (TP; hypothesis 2; H2). Finally, we expected that the stoneroller effect would be greater in the summer than the winter due to greater activity at greater stream temperatures (hypothesis 3; H3).

Methods

Our experiment was conducted in five Ozark Highland wadeable streams during the summer of 2016 (18 July- 3 October) and three streams during the winter of 2017 (24 January-6 March). Sites with a gradient of TP were selected (Table 1). Three blocks were set up in runs in the upper, middle, and lower sections of each stream reach (reach ≥ 200m) where each block was separated by at least one pool. Each block consisted of one treatment exclosure (stoneroller excluded) and one unelectrified control exclosure (stoneroller present) that were set up side-by side in equal flow conditions. Four unglazed tiles ($121 cm^2$) were zip-tied into each quadrate exclosure (31 X 5cm built from 19mm polyvinyl chloride pipe) to measure benthic algae. Treatment enclosures were set up with a 12 gauge insulated copper wire surrounding tiles and connected to a six volt ParMak solar fence charger (ParMak Precision Kansas City, MO) that sent an electrical pulse into the water deterring large-bodied organisms (> ~1cm) which exclude most crayfish and fish (Pringle and Blake, 1994). The charge extends about 10 cm outside the quadrate (Ludlam and Magoulick, 2009). Tiles were inoculated for 14 days in treatment and control conditions before they were collected on days 14, 21, and 28 in the summer and 14, 21, 28, and 35 in the winter. Algae was then measured for chlorophyll *a*, and ash-free dry mass (AFDM) was calculated using slurry from the whole tile. Water samples were taken throughout the experiment at each stream bi-weekly, placed in an iced cooler, and frozen upon returning to the laboratory to measure total phosphorus (TP) and total nitrogen (TN). Total phosphorus was measured in water samples by using a persulfate digestion and colorimetric analysis using the ascorbic acid method (American Public Health Association, 2005). Total

Table 1: Study information and nutrient concentrations, as measured on day 28 of the study in summer of 2016 (Sept 27-Oct 3) and winter of 2017 (15-16 February). Land use data from King et al., 2016. An asterisk (*) denotes values that were below the detection limit.

			Summer		Winter		
Stream	**State**	**Watershed**	**TP (mg/L)**	**TN (mg/L)**	**TP (mg/L)**	**TN (mg/L)**	**Land use**
Saline	OK	Eucha	0*	4.2	0*	0.7	60% Forest, 26% Pasture, 8% Grassland
Evansville	OK	Illinois	0.009	2.1	---	---	52% Forest, 40% Pasture, 3% Grassland
Beaty	OK	Eucha	0.027	1.9	0.029	1.7	30% Forest, 61% Pasture, 2% Grassland
Baron Fork	OK	Illinois	0.047	3.7	---	---	45% Forest, 48% Pasture, 2% Grassland
Flint	OK	Eucha	0.06	1.2	0.049	7.3	28% Forest, 58% Pasture, 3% Grassland

nitrogen was measured in water by using a sodium hydroxide digest to convert all nitrogen forms to nitrate and colorimetric (Hach DR 3900) analysis using Hach reagent powder pillows (Hach Permachem® Regant NitroVer© 5 nitrate reagent).

Statistical analysis was conducted in a hierarchal manner to understand the influence of grazers within each stream (H1), nutrients among streams (H2), and season among streams (H3). We addressed the grazing effect on benthic algal chlorophyll *a* and AFDM collected on day 28 within each stream during the summer and winter using a randomized-block analysis of variance (RB-ANOVA). Assumptions of variance, covariance, and normality were assessed visually using histograms and box plots. Interactions between environment and experiment were visually assessed using a line graph. The mean effect size was calculated per stream by averaging the effect size from each block (treatment: control, Grazer-excluded:Grazer-present) to address our second hypothesis. The mean effect size was regressed against nutrient concentrations (TP) to determine whether the grazer effect on benthic algae depended upon stream nutrient concentrations for the summer using all five stream reaches. Assumptions of normality and homogeneity of variance were assessed visually. Last, analysis of covariance (ANCOVA) was used on streams sampled in both winter and summer (Beaty, Saline, and Flint) to understand how effect of stonerollers differs between the two seasons. In the ANCOVA, the mean effect size (ratio Grazer-excluded:Grazer-present chlorophyll a and AFDM for each block averaged per stream) was the dependent variable, nutrient concentrations were the independent variable, and season was the covariate. Assumptions of linearity, homogeneity if variance, and relationship dependent and independent variable were assessed.

Results

As expected, stream TP ranged from below detection to 0.06 mg/L (Table 1). The TN concentration was high at all sites and varied less than TP. Grazers reduced benthic chlorophyll a in Saline and Beaty Creek in the summer (Table 2; Figure 1), but not in the winter (Table 3; Figure 2; H1). Grazers reduced

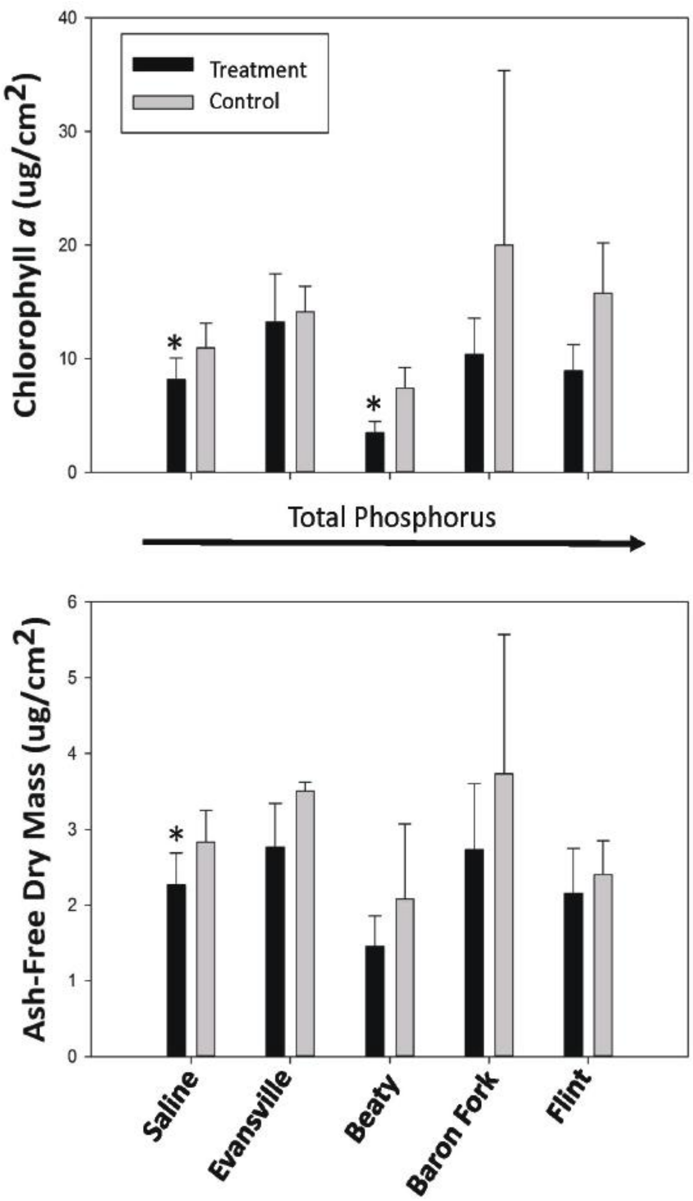

Figure 1: Algae collected from tiles on day 28 in late-summer of 2016, was measured for chlorophyll a and ash-free dry mass (AFDM, ug/cm2) values under treatment and control conditions. Mean and standard error (SE) were calculated for each stream (n=3). Significant differences are indicated with an asterisk (*).

Table 2: A Randomized block analysis of variance (ANOVA) was run on each stream to understand the influence on algae that was under grazer excluded or grazer present condition. There was a treatment effect in Saline creek on both chlorophyll a and ash-free dry mass (AFDM). Beaty Creek also had a significant treatment effect but only for chlorophyll a.

Stream	Variable	Factor	df	F-value	P-value
Saline	Chlorophyll a	Treatment	2	6.497	0.056*
		Block	2	20.952	0.006*
	AFDM	Treatment	2	7.003	0.049*
		Block	2	26.47	0.004*
Evansville	Chlorophyll a	Treatment	2	0.75	0.529
		Block	2	0.874	0.484
	AFDM	Treatment	2	2.668	0.184
		Block	2	0.55	0.615
Baron Fork	Chlorophyll a	Treatment	2	0.885	0.481
		Block	2	2.126	0.235
	AFDM	Treatment	2	0.947	0.461
		Block	2	1.786	0.279
Beaty	Chlorophyll a	Treatment	2	11.545	0.022*
		Block	2	7.365	0.046*
	AFDM	Treatment	2	0.287	0.765
		Block	2	1.404	0.345
Flint	Chlorophyll a	Treatment	2	1.836	0.272
		Block	2	0.017	0.983
	AFDM	Treatment	2	1.012	0.441
		Block	2	0.107	0.901

Table 3: Five streams were sampled on day 28 in winter 2017 (Feb 15-16). A Randomized block analysis of variance (ANOVA) was run on each stream understand the influence on algae that was under grazer excluded or grazer present conditions. There was no treatment or block effects.

Stream	Variable	Factor	df	F-value	P-value
Saline	Chlorophyll a	Treatment	2	1.98	0.252
		Block	2	0.33	0.735
	AFDM	Treatment	2	1.05	0.429
		Block	2	0.4	0.695
Beaty	Chlorophyll a	Treatment	2	2.96	0.234
		Block	2	3.85	0.117
	AFDM	Treatment	2	2.14	0.234
		Block	2	0.08	0.921
Flint	Chlorophyll a	Treatment	2	0.42	0.681
		Block	2	0.68	0.555
	AFDM	Treatment	2	0.88	0.482
		Block	2	0.22	0.81

benthic AFDM in the summer in Saline Creek only (Table 1; Figure 1). There was no statistically significant difference between treatment and control for either chlorophyll a or AFDM in any stream during the winter (Figure 2). Campostoma spp. abundance was measured in summer 2015, but we found that our abundance measurements did not influence the relationship between chlorophyll a and TP in this study (104b-Sayre and Evans-White 2016), and this data does not correlate with effect size for data taken in summer 2016 ($p=0.82$).

Chlorophyll *a* effect size and stream TP appeared to have a positive trend in the summer when all five study streams were included, but this trend was not statistically significant (Figure 3). However, there was no relationship between AFDM effect size and stream TP in the summer (Figure 3). The ANCOVA that included the three study sites sampled in both the summer and winter found no interaction between season and TP for either chlorophyll *a* or AFDM (Table 4; Figure 4). There was a season and a TP main effect for chlorophyll *a* (Table 4; Figure 4), but no interaction between those factors. Therefore, all six chlorophyll *a* effect sizes were combined into one regression, which was not statistically significant.

Conclusions, Recommendations and Benefits

Many studies have shown negative effects of stream grazers on benthic algae (Matthews et al., 1987; Steward, 1987; Power et al., 1988). Although grazer-exclosures tended to have greater benthic algal biomass than grazer-present treatments in the present study, these differences were only statistically significant in two streams with low to moderate TP concentrations during the summer (Table 2; Figure 1). A large amount of variation was observed in response variables across sites and increasing the number of replicates would help improve the power to address the interactive effects of grazers and nutrients on benthic algal biomass (Figures 1 and 2). Additionally, electrical exclosures did not exclude smaller macroinvertebrate grazers, like snails, that can negatively affect benthic algal biomass (Steinman et al., 1996). The electrical treatment should not have affected their presence, but the abundance and biomass of smaller benthic macroinvertebrates were not measured in this study and they could have added to the variability in effect sizes.

Our results suggest that macrograzers, such as Campostoma spp., can be more active and effective at grazing in the summer relative to the winter. The mean and variation in grazer chlorophyll *a* effect sizes tended to increase with TP concentrations in the summer, but not in the winter season (Figures 3 and 4). In addition, the mean grazer chlorophyll *a* effect size was greater in the summer than in the winter. Campostoma spp. were not seen during winter months except on a few occasions when the temperature was high in sunny runs. Other studies in Ozark streams suggest that Cam-

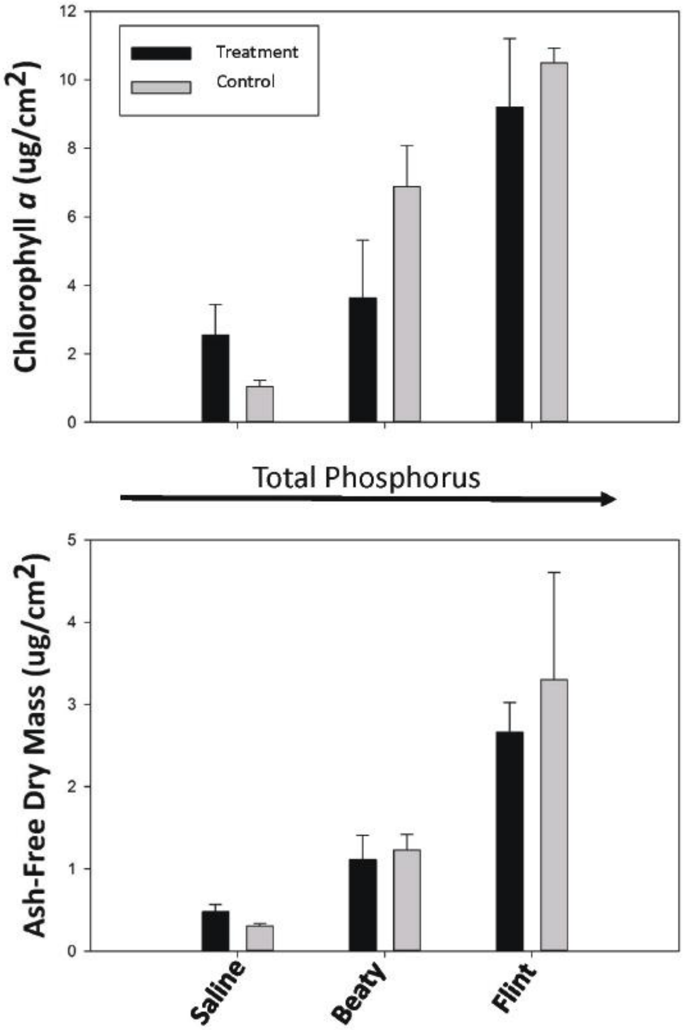

Figure 2: Algae collected from tiles on day 28 in winter of 2017, was measured for chlorophyll a and ash-free dry mass (AFDM, g/cm2) under treatment and control conditions. Mean and standard error (SE) were calculated for each stream (n=3). *RB-ANOVA indicated no statistically significant influence of grazer-exclusion for chlorophyll a or AFDM.

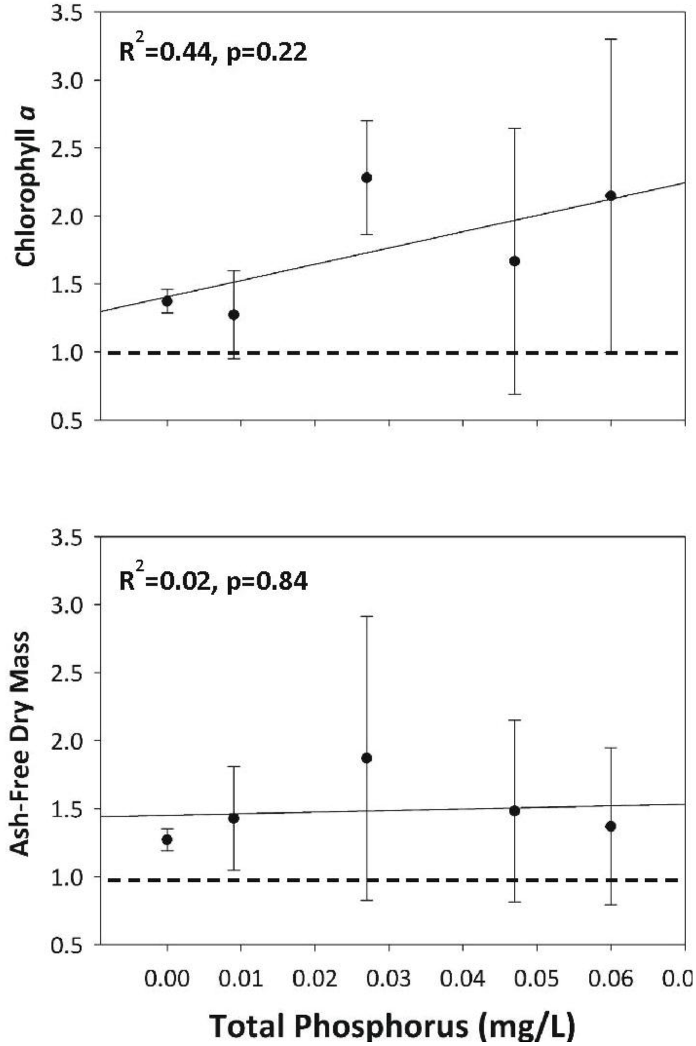

Figure 3: Mean effect size for algae collected from tiles on day 28 in late-summer of 2016, measured for chlorophyll a and ash-free dry mass (AFDM, ug/cm2) values under treatment and control (grazer-excluded and grazed) conditions. Bars represent the standard error of the effect size, but are not used in calculating regression statistics. The dashed-line indicates the 1:1 ratio at which treatment is equal to control where grazers do not have an influence.

postoma spp. influence can vary spatially and temporally within a single stream (Ludlam and Magoulick, 2009). The influence of grazers in these Ozark streams can depend on the presence of predators, stream conditions (e.g. drying), and depth (Ludlam and Magoulick, 2009) and our study suggests that their effects may also vary across nutrient levels. Grazer chlorophyll *a* and AFDM effect sizes were always greater than one suggesting that grazers tended to reduce benthic algal biomass across the stream TP gradient in the present study. A prior study that manipulated Campostoma and streamwater P levels in experimental streams found that stonerollers may stimulate benthic algal chlorophyll *a*, reduce benthic AFDM, and increase the autotrophic index even under P enriched conditions (Tayler et al., 2012). Taylor et al. (2012) focused on grazing effects in pools, and included a greater P enrichment up to 0.1 mg/L, and was completed in outdoor experimental streams in the early spring (March-April). All of these factors could result in the differences observed between these two studies and future experiments could manipulate temperature as well as nutrient concentrations in experimental streams to get at relative effects.

Dodds et al. (1997) proposed an oligotrophic-mesotrophic boundary at 2.0 μg/cm^2, and a mesotrophic-eutrophic boundary at 7.0 μg/cm^2 of chlorophyll *a*. Chlorophyll *a* measurements in the present study indicate that all streams were within the oligotrophic to mesotrophic range during the summer months. However, Flint became eutrophic in the winter, with Beaty on the border of eutrophic (Dodds et al., 1997). Therefore, adding in-stream manipulations in reaches with greater TP and benthic algal biomass would improve our understanding of the effects of grazers across nutrient gradients.

Overall, our data suggest the importance of seasonality with respect to macrograzer resource acquisition, macrograzer

Table 4: Three streams were sampled in both summer of 2016 and winter of 2017. Analysis of covariance (ANCOVA) was run where total phosphorus (TP) is the predictor, effect size chlorophyll a and ash-free dry mass (AFDM) was the response variables, and season (winter and summer) is the covariate. * Chlorophyll a effect size was significant for both TP and season. There was no interaction between TP and season.

Response	Predictor	df	F-Value	P-value
Effect Size Chlorophyll *a*	Total Phosphorus	1	47.84	0.0203*
	Season	1	59.85	0.0163*
	TP x Season	1	2.03	0.295
Effect Size AFDM	Total Phosphorus	1	0.1	0.101
	Season	1	0.26	0.258
	TP x Season	1	0.08	0.077
	Residuals	2	0.33	0.164

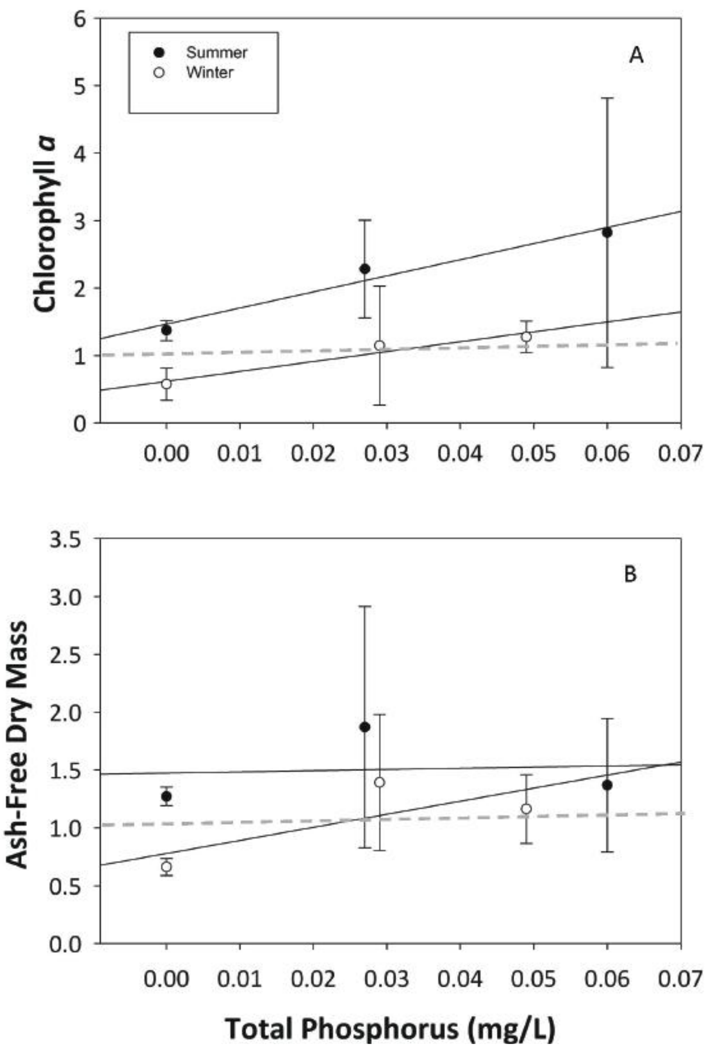

Figure 4: Mean effect size for algae collected from tiles on day 28 (27 September-3 October) in winter of 2017, measured for chlorophyll a and ash-free dry mass (AFDM, ug/cm2) values under treatment and control (grazer-excluded and grazed) conditions. Bars represent the standard error of the effect size, but are not used in calculating regression statistics. The dashed-line indicates the 1:1 ratio at which treatment is equal to control where grazers do not have an influence. There was a season and a TP main effect for chlorophyll a, but not for AFDM.

effect size, and dose-response relationship between nutrients and algae. A prior study in the Illinois River basin found that nutrients explained more variation in benthic algal biomass in the spring compared to the summer (Stevenson, 2012). The present study suggests that grazer effects are also lower in winter season and they may play a role in the observed relationship between nutrients and benthic algae. This seasonality effect on grazer influence should be considered when developing nutrient-algal dose response relationships and developing numeric nutrient criteria for the Ozark Highlands Ecoregion.

Acknowledgements

This material is based upon work supported by the U.S. Geological Survey under grant agreement No. G16AP00040 and administered by the Arkansas Water Resources Center. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey. Mention of trade names or commercial products does not constitute endorsement by the U.S. Geological Survey.

References

APCEC. 2017. Regulation no. 2- regulations establishing water quality startards for surface waters of the sate of Arkansas.

Cattaneo, A. and B. Mousseau .1995. Empirical Analysis of the Removal Rate of Periphyton by Grazers. Oecologia. 103:249-254.

Dodds, W.K., J.R. jones, and E.B. Welch. 1997. Suggested Classification of Stream Trophic State: Distributions of Temperate Stream Types by Chlorophyll, Total Nitrogen, and Phosphorus. Water Resources. 32(5): 455-1462

Dodds, W.K. and E.B. Welch. 2000. Establishing Nutrient Criteria in Streams. Journal of North American Benthological Society. 19(1): 186-196.

Haggard, B.E. 2010. Phosphorous Concentrations, Loads, and Sources within the Illinois River Drainage Area, Northwest Arkansas, 1997-2008. Journal of Environmental Quality. 39: 2113-2120.

Haggard, B.E., J.T. Scott, and S.D. Longing. 2013. Sestonic Chlorophyll-a Shows Hierarchical Structure and Thresholds with Nutrients across the Red River, USA. Journal of Environmental Quality. 42:437-445.

King, R.S. 2016. Fina Report Oklahoma-Arkansas Scenic Rivers Joint Phosphorus Study.

Ludlam, J.P. and D.D. Magoulick. 2009. Spatial and Temporal variation in Effects of Fish and Crayfish on Benthic Communities During Stream Drying. Journal of North American Benthological Society. 28 (2):317-382.

Matthews, W.J., A.J. Stewart, and M.E. Power.1987. Grazing Fishes as Components of the North American Stream Ecosystems: effects of Campostoma anomalum. Community and Evolutionary Ecology of North American Stream

Fishes. Mathews, W.J. and Heins, D.C. (eds).

Power, M.E., A.J. Stewart, and W.J. Mathews. 1988. Grazer control of algae in an Ozark mountainstream: effect of short-term exclusion. Ecology. 69(6): 1894-1898.

Pringle, C.M. and Blake, G.A. 1994. Quantitative Effects of Atyd Shrimp (Decapoda:Atyidae) on the Depositional Environment in Tropical Stream: Using Electricity for experiment exclusion. Canadian Journal of Fisheries and Aquatic Sciences. 51: 1443-1450.

Steinman, A.D. 1996. Effects of grazers on freshwater benthic algae. Algal Ecology: Freshwater Benthic Ecosystems. R.J. Stevenson, M.L. Bothwell, and R.L. Lowe (eds). Academic Press, San Diego, CA, USA.

Steward, A.J. 1987. Response of Streams to Grazing Minnows and Nutrients: a Field Test for Interactions. Oecologia. 72: 1-7.

Stevenson, R.J., B.J. Bennett, D.N. Jordan, and R.D. French. 2012. Phosphorous Regulates Stream Injury by Filamentous Green Algae, DO, and pH with Threshold Responses. Hydrobiologia.695: 25-42.

Taylor, J.M., J. A. Back, and R. S. King. 2012. Grazing minnows increase benthic increase benthic autotrophy and enhance the response of periphyton elemental composition to experimental phosphorus additions. Freshwater Science. 31(2):451-462.

USEPA .2017. State development of nutrient criteria for nitrogen and phosphorous pollution. http://www.epa.gov/nutrient-policy-data/state-developement-numeric-criteria-nitrogen-and-phosphorous-pollution#tabs-1. (accessed September 2014).