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# Effect of Macrograzers (Campostoma spp. and Faxonius spp.) on Periphyton in Ozark Streams with Considerations Given to Macrograzer Biomass, Phosphorus, and Season: Mensurative and Manipulative Studies

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Effect of Macrograzers (*Campostoma spp. and Faxonius spp.*) on Periphyton in Ozark Streams  
with Considerations Given to Macrograzer Biomass, Phosphorus, and Season: Mensurative and  
Manipulative Studies

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Biology

by

Kayla Sayre  
University of New Mexico  
Bachelor of Science in Biology, 2012

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University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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**Abstract:**

Nutrient and benthic algal biomass relationships can guide numeric nutrient criteria development in lotic systems. However, herbivorous macrograzers may confound this relationship by reducing the slope of the positive relationship between nutrients and periphyton biomass in streams. I conducted a mensurative field study to determine if stoneroller and crayfish abundance related to algal biomass at varying nutrient concentrations and manipulated macrograzer presence with electrical exclosures in streams to examine macrograzer effects on algal biomass and understand whether these effects on periphyton varied with total phosphorus (TP) or season. Macrograzer density was quantified across a TP gradient (n=15 streams; range = 0.009-0.100 mg TP/L) in August 2015 and manipulative exclosure experiments were completed in a subset of these streams in late summer 2016 (n=5, range = 0.0025-0.140 mg TP/L) and winter 2017 (n=3, range 0.001-0.49 mg TP/L). Multiple linear regression of mensurative field data revealed a statistically-significant positive relationship between macrograzer abundance, nutrient levels, and algal biomass. In the manipulative experiments, macrograzer presence did not influence algal biomass. Benthic algal biomass did vary over time in 3 of the 5 stream, which may be due to stream flow permanence or intermittence. Macrograzer effect on algal biomass was not significant and did not depend on TP or season. The positive relationship between macrograzers on algal biomass measures in the mensurative abundance study was not corroborated by the manipulative experiment, but indirect positive effects of macrograzers may have been equal to direct negative consumptive effects making grazed and ungrazed algal biomass equal. Many previous studies observed a negative influence of macrograzers on filaments lengths of benthic algae with mixed effect on chlorophyll *a* and ash-free dry mass measures (algal biomass measures), but no or positive relationships were found in the present

study. Effects abundance and exclosure study may indicate indirect stimulatory effects of macrograzers on stream algal biomass where high abundances of macrograzers and TP may produce systems dominated by autotrophic processes. These studies highlight the importance of considering indirect effects of macrograzers on periphyton biomass.

Keywords: macrograzer, phosphorus, benthic algae, stoneroller, crayfish, stressor-response

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All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted and approved under University of Arkansas Institution of Animal Care and Use Committee (IACUC) permit 15050 and all fish were collected under permits through Arkansas Game and Fish Commission (license #042020151) and Oklahoma Department of Wildlife Conservation (license # 6382).

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## **Introduction**

The Ozark Highlands is a transboundary ecoregion located in Oklahoma, Arkansas, Kansas, and Missouri with variable stream nutrient concentrations. Nutrient concentrations in the Ozark Highlands related to land use practices, such as agriculture, poultry farming, and cattle farming (Stevenson et al., 2012), but urban point-source pollutants, such as sewage treatment plants (Haggard, 2010), also contribute to elevated concentrations. Algae can often be limited by N, P, or sometimes both (Dodds et al., 2002). Local studies have suggested that nutrients can be a determining factor of algal biomass in Ozark Highland streams and have suggested that algal growth is limited by N in Ozark streams (Power et al., 1988; Lohman et al., 1991; Lohman and Jones 1999), but other studies suggest algae is P-limitation (Stevenson et al., 2012). Therefore, increasing concentration of N, P, or both may result in increased algal biomass (Lohman et al., 1991; Lohman and Jones, 1999; Dodds and Welch, 2000; Dodds et al., 2002), which can shift the algal community composition towards taxa that are a concern for public health, cause anoxic conditions, and ultimately reduce the recreational and environmental value of the stream (Dodds and Welch, 2000).

The US Environmental Protection Agency (USEPA) requires US states and tribal nations to develop freshwater numeric nutrient criteria for nitrogen (measured as Total Nitrogen) and phosphorus (measured as Total Phosphorus), since these nutrient measurements are more highly correlated with algae biomass and nuisance algae composition than other nutrient measures alone (nitrate, nitrite, soluble reactive phosphorus etc., Evans-White et al., 2013). In 2000, the United States Environmental Protection Agency (USEPA) provided national guidance numeric nutrient criteria for 13 aggregate ecoregions based on the 25th percentile of all nutrient concentrations recorded (Evans-White et al., 2013). Recently, a stressor-response study was conducted between



algae and phosphorus in designated scenic streams and rivers in Arkansas and Oklahoma (Joint Scenic Rivers Study) which recommended the 6-month TP average should not exceed 0.035 mg/L in water samples collected during the critical season ( King, 2016). Relationships between nutrient concentrations and algae can be variable in lotic systems (Stevenson et al., 2012; Haggard, 2010) 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).

Stonerollers and crayfish are important to consider when understanding the relationship between nutrients and algal biomass in Ozarks streams since they are found in high densities in Ozark streams (Matthews et al., 1987) and are thought to alter the ecosystem structure and function as they are a major aquatic consumer and source of food to both aquatic and terrestrial predators (Evans-White et al., 2003; Momot et al., 1995). Stonerollers are omnivorous minnows whose diet is primarily composed of algae and any associated aquatic macroinvertebrates and microbes. Functionally, they serve as grazers since they feed by scraping their sub-terminal mouth across benthic biofilms of algae, diatom, and associated organic debris and invertebrates (Evans-White et al., 2009). High stoneroller densities may elicit grazing pressures that affect algal biomass (Ludlam and Magoulick, 2009; Gelwick and Matthews, 1992), filament length, and community composition (Power et al., 1988). Crayfish reach high abundances in Ozark streams and can act as keystone species since they are a major aquatic consumer and food source, so can influence populations at several trophic levels (Momot, 1995; Creed, 1994; Hart, 1992). Crayfish are important consumer in Ozark streams and are important components of energy flow within streams (Whitledge and Rabeni, 1997; Flinders and Magoulick, 2007) who's diet can be composed of up to 30% periphyton (Evans-White et al., 2003). Additionally, crayfish

influence algal communities indirectly through changes in sedimentation (Ludlam and Magoulick, 2009) and consumption of invertebrate grazers (Charlebois and Lamberti, 1996). Stable isotope studies suggest crayfish diets may be more dependent on algae than stoneroller diet and an experimental manipulation suggests that each grazer can reduce stream benthic algal biomass at natural densities (Evans-White et al., 2001). Most studies examining the relationships between grazers, algae, and nutrients have used snails and caddisflies as the study organism while less is known about the influence of algivorous fish, such as stonerollers, and crayfish on the relationship between nutrient enrichment and algae (Cattaneo and Mousseau, 1995).

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. *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 greatly reduced due to grazing (Matthews et al., 1987), but little is known about the potential of grazers to affect algal biomass in winter. Seasonal variation in *Campostoma* spp. grazing could explain variation in algal biomass across seasons and sites in Ozark streams with varying nutrient concentrations. Macrograzers and associated seasonal behavior may modify the dose-response relationship between nutrients and algal biomass in Ozark Highland streams.

Mensurative and manipulative approaches in natural stream conditions were conducted to understand how macrograzer biomass and presence influence periphyton along a TP gradient and how macrograzer pressure varies seasonally. In the mensurative study I examined the abundance of stonerollers (*Campostoma* sp.) and crayfish (*Faxonius* spp. (formerly *Orconectes*)) in Ozark

streams along a phosphorus gradient (hereafter referred to as “mensurative abundance study”). I hypothesized stoneroller and crayfish abundance would explain the variation in regression models predicting algal biomass from nutrient concentrations. Specifically, nutrients will have a positive effect on algal biomass. Streams with greater crayfish and stoneroller abundances will have lower than expected algal biomass based on the estimated regression line with nutrients (Hypothesis 1; H1). For the second experiment, we manipulated macrograzer presence/absence and examined how macrograzers influenced benthic algae seasonally along a phosphorus gradient in natural field conditions (hereafter referred to as “manipulative exclosure study”). I hypothesized stonerollers and crayfish would have a significant negative effect on benthic algae within each stream during the summer (Hypothesis 2; H2), and that negative consumptive effects of stoneroller and crayfish on algae would increase with total phosphorus (TP; Hypothesis 3; H3). Finally, I expected that negative consumptive macrograzer effects would be greater in the summer than the winter with these terms interacting with more pronounced consumptive effects in the summer and close to 1:1 or little to no effect of stonerollers in the winter (Hypothesis 4; H4).

## **Methods:**

### *Site Description*

Study was conducted in the Illinois River watershed and Eucho-Spavinaw River watershed in Ozark Highland ecoregion of Arkansas and Oklahoma. Aggregate nutrient ecoregion XI consists of the Ozark highlands (OH) that is more forested than adjacent regions with less than one fourth of the region having been clear cut for pasture, cropland, and logging operations (Rohm et al., 2002). Streams in this region are characterized as highly influenced by groundwater and topography (Leasure et al., 2016). Ozark Highland streams are naturally clear,

with karst geology (Rohm et al., 2002), and dynamic flow characteristics (Leasure et al., 2016). However, high gradient streams with steep irregular slopes contribute to more erosion in areas affected by vegetation disturbance such as logging, road construction, and landslides (Rohm et al., 2002).

### *Mensurative Abundance Study*

Fifteen scenic river sites were sampled in the Ozark Highlands Nutrient Ecoregion of Eastern Oklahoma and Northwest Arkansas. Five of the sites were located in the Eucha-Spavinaw Watershed and ten were located in the Illinois River Watershed (Table 1). Sites designated as scenic rivers or major tributaries into scenic rivers (based on state designated use) were the first criteria used in site selection (King, 2016). Since total phosphorus (TP) was the main predictor, sites were selected along a phosphorus gradient as the second criteria. Presence of riffle channel units, prevalence of medium to large cobble substrate, and open canopy were remaining factors of site selection. After a list of sites was chosen along a TP gradient, sites were scouted prior to sampling to assess sizes that would allow for backpack electrofishing sampling (knee depth or lower). In the end, sites chosen were small to medium sized streams with catchment area ranging from 36-410 km<sup>2</sup> (Table 1), an average width ranging from 4-18 m, an average depth ranging from 0.11-.282 m, an average velocity ranging from 0.08-0.42 m/s, TP ranging from 0.009-0.100 mg/L, and Nitrite-Nitrate (NO<sub>2</sub>+NO<sub>3</sub>-N) ranging from 0.15-6.3 mg/L (n=15). The geometric mean for the scenic rivers studied in the Joint Scenic Rivers Study ranged from <0.002-0.140 mg/L of TP (King et al. 2016, n=35).

Sampling was conducted from 5-29 August 2015. The experimental unit was stream segment (n=15) with three spatially distinct riffles. Three riffles were sampled in each stream, and five quadrats (5 m<sup>2</sup>) were sampled within each riffle (Figure 1). A modified-quantitative

kick-net and backpack electrofishing (Smith-Root LR-24) method was used to sample grazer populations (Flinders and Magoulick, 2005; Magoulick and Lynch, 2015). Specifically, the methods were modified to increase the area sampled to an area of five meters-square. A three-person crew composed of one person equipped with a backpack electrofishing unit and two kickers, started five meters upstream of a seine (3mm mesh) held five meters in width by two people perpendicular to flow. The electrofishing crew slowly moved downstream to the seine while dislodging the substrate and actively electrifying the water which allowed fish and crayfish to be dislodged into the downstream seine. Greater lengths were covered in streams where the width was less than five meters to standardize the area sampled. All stonerollers and crayfish were collected from the seine and put into separate five-gallon buckets after each electrofishing pass. Raw count and standard length of stonerollers as well as species and carapace length of crayfish were recorded. Substrate, flow, depth, and width were taken at each quadrat while habitat length and electrofishing seconds were recorded at each riffle.

A subset of stonerollers was retained and used to estimate length-mass relationships to determine total population biomass (Evans-White et al., 2003). Specifically, stonerollers from four sites were used for the length-mass relationship (Ill1, Ball1, Beat1, and Sprg3, Table 1), which represented low, middle, and high phosphorus concentrations along the gradient (total N=246). Stonerollers were dried at 48°C for a minimum of 72 hours. Once removed from oven, fish were placed in a desiccator for minimum of 1 hour. Fish were then weighted to the nearest 0.1mg. Length-mass relationship between natural log transformed dried mass and standard length were then calculated and used to estimate total biomass of stonerollers per sample reach. ANCOVA was used to verify rate of change in mass with length was similar between the four streams. Assumptions of independence, linearity, and bivariate normality were assessed visually

via histograms and scatter plots. Mass and length data were natural log transformed to meet assumption of normality and linearity.

Crayfish biomass was calculated following method of Evans-White et al. 2003 where linear regression equations for *Faxonius neglectus* (formerly *Orconectes*) were used to calculate ash-free dry mass (AFDM) of all species (*Faxonius meeki*, *F. neglectus*, and *F. nana*) based on carapace length and converted to dry mass using equations from Benke et al. (1999).

Chlorophyll *a* (CHLA), ash free dry mass (AFDM), and nutrient measurements were taken once at each stream in June and August by a separate study group and corollary land cover data was also used from this study (Joint Scenic Rivers Study, King, 2016) . Water samples were collected using Baylor University for Reservoir and Aquatic Systems Research (CRASR) quality assurance/quality control protocols and analyzed using cadmium reduction and standard operating procedure (King, 2016). Chlorophyll *a*, AFDM, TP, and NO<sub>2</sub>NO<sub>3</sub>-N values taken in June and August were averaged and used in statistical analysis since values from both months have a cumulative influence on stoneroller and crayfish biomass.

Multiple linear regression (MLR) was used to examine the relationship between macrograzer biomass, TP, and algae biomass. Ash-free dry mass and CHLA values were natural log transformed to meet assumption of normality. Correlation and visual inspection of scatter-plot matrices, were used to assess assumptions of collinearity. If explanatory variables (percent forest, percent developed by homes and businesses, NO<sub>2</sub>+NO<sub>3</sub>-N, and TP) were highly correlated ( $r > 0.65$ ), then they were not used as an explanatory variable in the MLR. Total phosphorus and NO<sub>2</sub>+NO<sub>3</sub>-N were highly correlated (0.74), so TP was used in explanatory variable in MLR. Percent forest was highly correlated with TP ( $r = 0.88$ ), so was not included in MLR. Furthermore, percent forest was highly correlated with percent developed ( $r = -0.76$ ) so were not included in

MLR. Instead these variables individually regressed against TP to understand the influence of land-cover on nutrient inputs. In the end, TP and total macrograzer biomass were used in MLR to explain variation in periphyton (AFDM and CHLA).

### *Manipulative Exclosure Study*

Exclosure experiments were conducted in five and three Ozark Highland Wadeable streams were studied during the summer of 2016 (18 July- 3 October) and winter of 2017 (24 January-6 March), respectively. Three, instead of 5, streams were measured in the winter due to drying in two of the streams (Baron Fork and Evansville Creek). Sites with similar macrograzer biomass measures were selected from mensurative experiment along a TP gradient. Three blocks were set up in riffle habitats in the upper, middle, and lower sections of each stream reach (reach  $\geq 200$  m) where each block was separated by at least one pool. Each block consisted of one treatment exclosure (macrograzer excluded) and one unelectrified control exclosure (macrograzer not excluded) that were set up side-by side about 24 cm apart in equal flow conditions. Four unglazed ceramic tiles (121 cm<sup>2</sup>) were zip-tied into each quadrat exclosure (31 X 5-cm built from 19-mm polyvinyl chloride pipe) to measure benthic algae (Figure 2). The frame was then elevated 6 cm from the streambed with rebar and zip ties since raising tiles above the benthic substrate has been shown to exclude poor swimming and dispersing macroinvertebrates which can graze on algae (Lamberti and Resh, 1983). Treatment exclosures 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 ( $> \sim 1$  cm), including most crayfish and fish (Pringle and Blake, 1994). The charge can extend about 10 cm outside the quadrat (Ludlam and Magoulick, 2009). Tiles were left for algal colonization for 14 days in treatment and control

conditions before they were collected on days 14, 21, 28, and 35 in the summer (Evansville and Baron Fork were not sampled on day 35) and winter. Algal biomass measures of CHLA and AFDM, were then measured using a slurry from the whole tile (American Public Health Association, 2005). Canopy cover was measured using a densiometer on last day of field sampling for each season. 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 colorimetric analysis using the ascorbic acid method (American Public Health Association, 2005) following a persulfate digest (American Public Health Association, 2007). Total nitrogen was measured in unfiltered-water samples by cadmium reduction (Hach Permachem® Regant NitroVer© 5 nitrate reagent, American Public Health Association, 2005) following a persulfate digest to covert all nitrogen forms to nitrate (American Public Health Association, 2007).

Statistical analysis was conducted in a hierarchal manner to understand the influence of macrograzers *within* each stream (H2), nutrients *across* streams (H3), and *across* season (H4). Repeated-measures, randomized-block analysis of variance (RM-RB-ANOVA) examined the main effects of macrograzers *within* each stream in summer and winter on CHLA and AFDM values (H2). CHLA and AFDM values were natural log plus 0.01 transformed to relax violation of normality. Even with transformation, Mauchly test revealed violation of the sphericity assumption, so Pillai Trace corrected F- and P-values were used to understand effects of treatment and day within each stream (Johnson and Field, 1993). Factors influencing grazer effects *across* streams were assessed using linear regression and analysis of covariance (ANCOVA, H3 and H4). First, the effect size was calculated in each block by dividing grazer-present by grazer-excluded algal biomass measures. Assumptions of normality, homogeneity of



variance and covariance were assessed using histograms and box plots and calculated CHLA and AFDM effect sizes were natural log plus 0.01 transformed to relax violations. To understand macrograzer effect along the TP gradient, natural log plus 0.01 transformed CHLA and AFDM effect size values were regressed against nutrient concentrations (TP) using linear regression in the summer. Last, analysis of covariance (ANCOVA) was used on streams sampled in both winter and summer (Beaty, Saline, and Flint) to understand how effect of macrograzers differs between the two seasons (H4). In the ANCOVA, the effect size was the dependent variable, nutrient concentrations were the independent variable, and season was the covariate. If ANCOVA among effect sizes, nutrients, and season was not significant, a regression was run between all algal biomass measures (with no regard to effect size) and TP and on day 21, 28, and 35 of the manipulation.

## **Results:**

### *H1-Abundance Study*

Analysis of covariance revealed a similar rate of change in stoneroller length and mass relationship among sites, but some differences in intercept between sites. ANCOVA between length and dry mass indicated differences among sites (site  $p \leq 0.001$ ) where the intercept differed between Illinois and all other sites ( $p \leq 0.001$ , Table 2). There was not a statistically-significant interaction between site (covariate) and mass (dependent variable) indicating similar slopes in length-mass relationships among sites. Therefore, I completed a regression between length (24-110 mm) and stoneroller dry mass across the four sites (dry mass (g)= $3.4[\text{length (mm)}]-13.9$ ,  $R^2=0.91$ ,  $p < 0.001$ ,  $n=246$ , Figure 3), which was used to calculate biomass as each site (Table 3).

Model fit was conducted from original data set where expected masses were regressed against observed mass. The modelled data ranged from 25mm-110mm (Figure 4). Once observed data was regressed against expected data, we found that stonerollers with lengths greater than 100 were greatly overestimated by the model regression (with modeled masses between 8-10 grams). Therefore, all lengths equal to or greater than 100 were assigned the mass of 6 in order to avoid overestimation of biomass at each site (Figure 4). Of the 1816 sampled individual stonerollers observed, 56 were at lengths 100 or above so were assigned a mass of 6 grams. Biomass was calculated by summing all weights of stonerollers and crayfish at each site.

Regression between TP and land-use variables percent forest and developed were significant. Percent forest was negatively associated with (p $\leq$ 0.001, R<sup>2</sup>=0.75) and percent developed was positively associated with TP (p=0.047, R<sup>2</sup>=0.21) while percent pasture shows no significant correlation (Figure 5). Sites associated with WWTP were observed to have higher CHLA and AFDM than sites without WWTP even at similar TP levels, and WWTP were associated with sites with more developed land-use (Figure 5).

Multiple linear regression revealed significantly positive relationships between predictor-TP and total macrograzer biomass and CHLA and AFDM (Table 4, Figure 6). Specifically, when residuals of the TP-CHLA and -AFDM relationships were output and regressed against total macrograzer biomass a positive relationship was revealed (Table 4, Figure 6) indicating that more CHLA and AFDM were found relative to that modeled by the line as macrograzer biomass increased.

#### *Exclosure Study: within-stream patterns (H2)*

Repeated-measures, randomized-block ANOVA with corrected Pillai Trace p-values, revealed no treatment affect for CHLA and AFDM measures taken in the summer or winter;

however, a significant effect of day was observed in most streams. Baron Fork and Evansvilles Creek did not have an influence of day in the summer for either CHLA or AFDM algal measures (Table 6, Figures 7 & 8). However, day influenced CHLA measures in Beaty, Flint, and Saline Creek in the summer and winter (Table 6, Figures 7,8,9,10). During the winter, Flint Creek did not experience an effect of day, but Beaty and Saline experiences a significant day affects when analyzing AFDM (Table 6, Figures 8 & 9). There were no significant day by treatment interactions at any site (Table 6).

*Exclosure Study: across stream patterns (H3 and H4):*

Total phosphorus and season did not significantly explain variation in effect size values of CHLA and AFDM, but single between TP and algal biomass measures revealed transient significant results (Table 7 & 8, Figures 10, 11, 12, & 13). Chlorophyll *a* and AFDM or CHLA effect sizes and stream TP were not significantly correlated in the summer (Table 7, Figure 10; H3). ANCOVA that included the three study sites sampled in both the summer and winter revealed no statistically significant results on day 28 or day 35 (Table 8, Figure 11; H4). Single regression between unmodified (no regard to effect size) algal biomass (all sites sampled in summer and winter) measures and TP revealed transient and significant relationships. On day 21, CHLA and AFDM were significantly correlated ( $R^2=0.27$ ,  $p=0.0001$  and  $R^2=0.43$ ,  $p<0.0001$ , Figure 13). On day 28, the relationship between CHLA and TP was statistically significant ( $R^2=0.12$ ,  $p=0.014$ , Figure 13), but the relationship between AFDM and TP was not significant ( $R^2=0.052$ ,  $p=0.121$ , Figure 13). On day 35, the relationship between CHLA and TP was not significant ( $R^2=0.08$ ,  $p=0.095$ ), but the relationship between AFDM and TP was significant ( $R^2=0.20$ ,  $p=0.007$ , Figure 13). Total nitrogen was not correlated with algal biomass measures at any point during the experiment.

**Discussion:**

Relationships between nutrients and benthic algal biomass were observed in manipulative and mensurative studies, but the cumulative influence of nutrients and macrograzers was variable between the two study approaches. In the mensurative abundance study, there was a clear positive relationship benthic macrograzers on algal biomass measures along a TP gradient while in the manipulative experiment there was no net effect of macrograzers in individual stream or along a TP gradient. Lastly, there was no apparent effect of grazers within each stream and of season on algal response to macrograzers between streams.

*Patterns in Algal Biomass*

Nitrogen (Lohman et al., 1991) and phosphorus (Stevenson et al., 2012) or both nutrients (Rodman and Scott, 2017) have been implicated as limiting nutrients to benthic algal production in Ozark streams. Similarly, the Illinois River across Arkansas and Oklahoma may be P limited because of high molar N:P ratio (Stevenson et al., 2012) and strong response in algal biomass to point-source P pollution, such as sewage treatment plants (Haggard, 2010). In the mensurative abundance study,  $\text{NO}_2+\text{NO}_3\text{-N}$  and TP measures showed significant and positive correlations with algal biomass; however, TP showed slightly higher  $R^2$  and p-value associated with the regression (Figure 12) and results from the manipulative study were more related with TP than to TN. Specifically, algal biomass measures showed transient, statistically-significant results where TP explained variation in algal biomass measures some weeks but not others (Figure 13). There was no significant correlation between TN and any algal biomass measure at any point during the experiment. Therefore, results from the present study provide more support for P- than N-limitation in the present study watersheds. Future experiments using nutrient diffusing substrata

would more definitively determine algal growth limitation than the correlative methods used in the present study.

Experiment date affected algal biomass in some streams, but not others, which may be explained by differences in stream flow. Two of my stream reaches experienced low to intermittent flow (Evansville and Baron Fork) and were completely dry in riffle block units toward the end of the experiment (day 22, 28). Furthermore, these streams tended to experience more flashy stream-flows with higher peak-flows compared to the other study streams; specifically, Baron Fork tended to have the highest range of discharge with a peak discharge of 191 cfs toward the beginning of the experiment, which may also promote the high variance in algal biomass measures (Table 5). All other study streams experience flow throughout the whole experiment and did not experience intermittent drying (Saline, Beaty, and Flint). In the perennial streams, sample time was an important factor, but in intermittent streams (Baron Fork and Evansville) that experience drying and higher peak flows during the summer no time effect was found (Table 6). Disturbance such as drying frequency and peak flows can influence algal patch dynamics by influencing the resistance to displacement and species/community composition of algal communities (Ledger et al., 2008; Townsend, 1989) and macrograzers can influence these factors as well (Power, 1990; Gelwick and Matthews, 1992). Further consideration should be given to the interaction among stream permanence and grazing.

#### *Macrograzer Influence on Algal Biomass*

Meta-analyses indicate grazers tend to have negative consumptive effects on benthic algal that can override the influence of nutrients (Hillebrand, 2002; 2009). However, most experiments included in the meta-analyses were those that had manipulated smaller aquatic macroinvertebrate grazers; with macrograzer effects making up a small proportion of the data

points in these analyses (Hillebrand, 2009; Hillebrand, 2002). In turn, influence of macrograzers on algal biomass is much less studied but may be more variable than the effects of smaller grazing invertebrates. In the present manipulative enclosure study, Ozark macrograzers, stonerollers and crayfish, did not have a detectable influence on stream algal biomass measures throughout the four week experiment. Stonerollers and other functionally similar fishes have been documented as having neutral (Bertrand and Gido, 2007; Ludlam and Magoulick, 2009; Vaughn, 1993, Reisinger et al., 2011), positive (Taylor et al., 2012) or negative (Evans-White et al., 2001; Flecker et al., 2002; Ludlam and Magoulick, 2009; Taylor et al., 2012) effects on CHLA measures with neutral (Vaughn et al., 1993; Ludlam and Magoulick, 2009), positive (Power and Matthews, 1983), and negative (Gelwick and Matthews, 1992; Ludlam and Magoulick, 2009) influences on AFDM measures of benthic algal biomass. Similarly, crayfish have been documented as having positive (Charlebois and Lamberti, 1996, natural enclosure-exclosure experiment), negative (Evans-white et al., 2001; Ludlam and Magoulick, 2009, natural open enclosure), and neutral (Bengtson et al., 2008, stream mesocosm) influence on CHLA measured with neutral and negative influences on AFDM. Variation in grazer biomass and nutrient availability may be important to the context determining the sign and magnitude of grazer effects.

Grazers can have positive, negative, or neutral influence on algal biomass measures through indirect nutrient and direct physical consumptive mechanisms. First, nutrient mineralization occurs when organisms excrete waste that increases dissolved inorganic N and P availability to periphyton; with periphyton responses to grazer mineralization likely dependent on the most limiting nutrient to benthic algal growth in the system (Evans-White and Lamberti 2005, 2006). Second, grazers can physically consume periphyton, which can decrease overall

biomass or positively influence periphyton biomass by selecting for species with greater chlorophyll *a* content (e.g. filamentous algae or diatoms) or by removing dead and otherwise unproductive algal species thus increasing the autotrophic proportion of the matt. Consumptive influences of macrograzers can cause decreases in algal filament lengths and biomass (Power et al., 1988; Gelwick and Matthews, 1992), but can also increase algal production due to sloughing of sediment and dead algal material that may otherwise impinge growth (Flecker et al., 2002; Charlebois and Lamberti 1996). The positive effects of nutrient mineralization by grazers can help combat negative consumptive or encourage positive algal growth (Hillebrand, 2002; 2009), which may result in neutral or positive influences of grazers, respectively. Lastly, high external nutrients in the system may lead to increased consumptive effects of grazers because of increased food availability and decreased nutrient limitation (Evans-White and Lamberti 2006) leading to a net negative influence due to increase grazer biomass (Hillebrand, 2009) and direct consumptive effects (Gelwick and Matthews, 1992), or a net neutral influence due to nutrients masking grazer consumptive effects (McCormick and Stevenson, 1991).

Top-down negative consumptive effects of grazers on algal biomass did not appear to dominate in the present study. The mensurative abundance study found a positive relationship between residuals of the benthic algal biomass- TP linear relationship and total macrograzer biomass, which indicates that there was more algae than expected at a given TP level as fish and crayfish biomass increased (Figure 6, Table 4). This positive relationship could suggest that the stimulatory effects of macrograzers on algal biomass increased with macrograzer biomass (i.e., top-down mechanisms). It is equally possible that greater algal biomass at a given TP concentration stimulated stoneroller biomass (i.e., bottom-up mechanisms). Results from this study may support the hypothesis that stonerollers and crayfish can have a positive influence on

algal biomass measures, which may be due to nutrient mineralization (Mulholland et al., 1991; Newbold et al., 1982; Rosemond et al., 1993; Evans-White and Lamberti, 2005; 2006) or promotion of algal growth through mechanical sloughing of dead algal cells (Lamberti et al., 1989). It can be challenging to separate the effects of nutrients and grazers on algae in correlative studies since both processes are occurring simultaneously (Flecker et al., 2002; Rosemond et al., 1993), so the present study cannot definitively prove which mechanism is dominant. The manipulative study was designed to directly test grazing effects, and found no effect of grazers. I found that benthic algal biomass changed over time in some streams but grazers did not affect the pattern over time. Similar to the present study, a natural circulating mesocosm experiment found AFDM accumulation over time increased, but grazers (stonerollers, snails, and crayfish) did not significantly influence this relationship overtime (Vaughn et al., 1993).

The interactive influence of nutrients and grazers (including small macroinvertebrates) can be variable in stream studies (Hillebrand et al., 2002) which may be due to the influence of abiotic, spatial, and temporal stream variables. The natural manipulative enclosure experiment showed no change in macrograzer effect along a TP gradient (Figure 10, Table 7), but to my knowledge no other study has compared macrograzer effects in natural streams with varying nutrient concentrations. Most studies that test the interactive influence of grazers and nutrients were done in manipulative outdoor recirculating stream mesocosms (Murdock et al., 2011; Taylor et al., 2012) or in one natural stream (Stewart, 1987; Flecker et al., 2002). These experiments found macrograzers may have a neutral influence at high P- (Taylor et al., 2012, 0.1 mg/L) or N-levels (Flecker et al., 2002), a positive influence at low N-levels (Murdock et al., 2011), a negative influence high N-levels (Stewart, 1987; Murdock, et al. 2011), or a negative



influence at low P-levels (Taylor et al., 2012). Macrograzer impact tends to vary with time (Ludlam and Magoulick, 2009; 2010; Murdock et al., 2011), space (Ludlam and Magoulick, 2009; Gelwick and Matthews, 1992), and nutrient concentration (Stewart, 1987; Flecker et al., 2002) in natural stream environments (Table 9). Therefore, these varying results may indicate that bottom-up and top-down controls by macrograzers are more context- (e.g. algal nutrient limitations of system, natural disturbance regime of system) and scale-dependent (e.g. year, day of the experiment) and sometimes interactive in natural systems. Further examination needs to be given to macrograzers' impact on stream algal growth and accumulation in nutrient-limited and nutrient-surplus conditions using natural stream experiments since habitat and between stream differences may explain macrograzer influence on algal biomass.

The result of the present experiment may be influenced by challenges and potential sources of error in experimental design. Experimental design can greatly influence the outcome of exclosure/enclosure studies with laboratory grazer studies tending to show stronger grazer effects than *in situ* grazer studies (Hillebrand, 2009). Although exclosures were raised to reduce macroinvertebrate colonization (Lamberti and Resh, 1983), I observed small drifting macroinvertebrates and snails within the quadrates, which 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. Personal observations showed that smaller stonerollers ( $\leq 50$  mm) may not have been excluded from electrical exclosure, which may have caused ungrazed and grazed tiles to have more similar algal biomass measure. Others have found that juveniles (ages 0-2 where age 1= 0-55 mm and age 2=55-81 mm) make up the bulk of stonerollers populations in prairie streams (Quist and Guy,

2001), while data from the mensurative abundance study found that 24%-66% of the fish collected from the 5 streams used in manipulative experiment were less than or equal to 50 mm (Figure 14). Lastly, there was very high variation in response variables even within the same stream. Increasing the number of replicates should reduce variation in algal biomass and may help tease apart these differences within a stream, but increasing replication in this present study would not have been feasible given human and economic constraints.

The present study may have also underestimated grazer impact due to the focus on riffle habitat units in the study design. Macrograzers may have a more predominant influence in pool environments compared to riffle environments. Natural macrograzer experiments have predominantly been done in pool habitats (Power et al., 1988; Ludlam and Magoulick, 2009; Stewart, 1987, Table 9), but few macrograzer experiments in natural stream environments have been conducted in riffle habitats (but see Power et al., 1989). While outdoor or greenhouse recirculating-stream experiments were conducted in riffle environments (Evans-white et al., 2001), pool (Vaughn et al., 1993; Taylor et al., 2012), or riffle and pool environments (Bertrand and Gido, 2007; Bengtson et al. 2008; Murdock et al. 2011, Reisinger et al., 2011; Pennock and Gido, 2016). Some mesocosm experiments show-case these differences and indicate that riffles have higher CHLA and algal filament lengths compared to pools with the same stoneroller density (Reisinger et al., 2011; Bengtson et al. 2008) but, to my knowledge, no experiment has compared the influence of macrograzers in natural streams between riffles and pools (Table 9). These results may be species-specific, since algal biomass was generally higher in riffles grazed by dace than in riffles grazed by crayfish (Bengtson et al., 2008). Therefore, my results may have been dampened by a possible interaction between flow and macrograzer activity; one would

expect non-grazed substrate to be less resistant to flow, which may encourage sloughing and, in turn, have led to the lack of difference between ungrazed and grazed substrate.

### *Synthesis*

While the correlative study suggested that macrograzers might stimulate the nutrient effect on benthic algae, the manipulative enclosure experiment found a neutral influence of macrograzer presence on algal biomass within streams, across a TP gradient, and between seasons. The abundance study and within-stream effects of macrograzers from the manipulative enclosure study may provide evidence for a stimulatory influence of macrograzers in open natural stream environments. While other studies have found negative, positive, neutral influence of macrograzers (Bentrland and Gido, 2007; Ludlam and Magoulick, 2009; Vaughn, 1993; Reisinger et al., 2011; Taylor et al., 2012; Charlebois and Lamberti, 1996; Bengtson et al., 2008), I found both positive and neutral effects in the present study. Furthermore, there was no apparent influence of macrograzer along a TP gradient in the manipulative experiment and a positive correlation in the mensurative study even though some studies have found that the combination of fish and nutrients produce neutral results on algal biomass (Flecker et al., 2002) and others have suggested that macrograzing fish (stonerollers) may shift systems toward autotrophy (Taylor et al., 2012). However, very few have studied the combined effect of nutrients and macrograzers in natural systems where migration between stream habitats is allowed. In the end, no other study to my knowledge has studied the influence of macrograzers along a nutrient gradient in streams. Combine results of enclosure and abundance study may indicate indirect stimulatory effects of macrograzers on stream algal biomass where high abundances of macrograzers may produce systems dominated by autotrophic processes. Correcting for macrograzer effects on relationships between algae and nutrients requires a lot of

man power and effort, and may not be viable to water managers or people examining stressor-response relationships. Further research can be done on macrograzer effects that may be linked to other more easily measured corollary factors such as stream flow, disturbance, and nutrient limitation of stream systems.

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## Tables and Figures

**Table 1: List of all study sites (stream) within the Ozark Highlands level III Nutrient Ecoregion sampled during mensurative study. Five streams were sampled from the Eucha-Spavinaw (Eucha), and ten in the Illinois River watershed (Illinois). Site ID will be used in all tables and figures that follow. Land-use data given by King et al. 2016.**

Stream	Site ID	State	Watershed	Catchment Area (km <sup>2</sup> )	Land Use
Illinois River	ILLI1	AR	Illinois	69	56% Forest, 67% Pasture, 3% Grassland, 4% Developed
Evansville Creek	EVAN1	OK	Illinois	154	52% Forest, 40% Pasture, 3% Grassland, 4% Developed
Spring Creek	SPRG3	OK	Illinois	297	50% Forest, 40% Pasture, 4% Grassland, 5% Developed
Saline	SALI1	OK	Eucha	270	60% Forest, 26% Pasture, 8% Grassland, 4% Developed
Little Saline	LSAL1	OK	Eucha	62	51% Forest, 35% Pasture, 8% Grassland, 3% Developed
Spavinaw Creek	SPAV1	AR	Eucha	174	39% Forest, 52% Pasture, 2% Grassland, 4% Developed
Barron Fork	BARR2	OK	Illinois	410	47% Forest, 45% Pasture, 3% Grassland, 5% Developed
Flint, Gentry	FLIN1	AR	Illinois	65	26% Forest, 62% Pasture, 3% Grassland, 9% Developed
Beaty Creek	BEAT1	OK	Eucha	153	30% Forest, 44% Pasture, 2% Grassland, 5% Developed
Goose Creek	GOOS1	AR	Illinois	36	26% Forest, 49% Pasture, <1% Grassland, 24% Developed
Osage Creek	OSAG2	AR	Illinois	337	11% Forest, 50% Pasture, <1% Grassland, 37% Developed
Ballard Creek	BALL1	OK	Illinois	90	23% Forest, 67% Pasture, 1% Grassland, 8% Developed
Osage Creek	OSAG1	AR	Illinois	101	11% Forest, 35% Pasture, <1% Grassland, 56% Developed
Flint Creek	FLIN3	OK	Illinois	245	28% Forest, 53% Pasture, 4% Grassland, 4% Developed
Spring Creek	SPAR1	AR	Illinois	91.7	12% Forest, 42% Pasture, <1% Grassland, 44% Developed

**Table 2: Analysis of co-variance revealed statistical differences between intercepts of Ballard Creek and the Illinois River [degrees of freedom (df), F-statistic values, and probability (p) values]. There was no difference among the slopes of the four sites (length x site >0.05), so data were lumped and linear regression was used to model masses for stonerollers sampled at all 15 sites.**

<b>Response</b>	<b>Predictor</b>	<b>df</b>	<b>F-Value</b>	<b>P-value</b>
	Length (mm)	1	2796.92	<0.0001
Mass (g)	Site	3	13.78	<0.0001
	Length x Site	3	2.37	0.0700

**Table 3: Biomass of stoneroller [biomass (g)] and crayfish [biomass (g)] was calculated and then summed for each site [total macrograzer biomass (g)]. Crayfish richness and species present (count) are also document for each site.**

Site	Stoneroller		Richness	Crayfish	Total Macrograzer Biomass (g)
	Biomass (g)	Biomass (g)		Species Present (count)	
BALL1	225.83	1.08	3	F. nana (39), F. meeki (12), F. neglectus(9)	226.91
BARR2	282.94	0.16	2	F. nana (2), F. meeki (0), F. neglectus(1)	283.10
BEAT1	148.06	6.38	3	F. macrus (9), F. meeki (4), F. neglectus(164)	154.44
EVAN1	89.35	2.24	2	F. nana (93), F. meeki (0), F. neglectus(39)	91.59
FLIN1	14.00	2.00	2	F. nana (73), F. meeki (14), F. neglectus(14)	16.00
FLIN3	62.85	0.49	2	F. nana (21), F. meeki (3), F. neglectus(3)	63.34
GOOS1	418.45	2.06	3	F. nana (8), F. meeki (21), F. neglectus(16)	420.51
ILLI1	92.12	1.19	3	F. nana (22), F. meeki (16), F. neglectus(25)	93.31
LSAL1	50.60	4.69	2	F. macrus (116), F. meeki (0), F. neglectus(214)	55.29
OSAG1	209.37	2.55	3	F. nana (218), F. meeki (25), F. neglectus(11)	211.92
OSAG2	89.01	1.32	2	F. nana (92), F. meeki (5), F. neglectus(0)	90.33
SAL1	10.87	1.92	2	F. macrus (10), F. meeki (0), F. neglectus(45)	12.79
SPAR1	348.39	1.83	3	F. nana (36), F. meeki (9), F. neglectus(25)	350.22
SPAV1	239.04	3.70	2	F. macrus (1), F. meeki (0), F. neglectus(165)	242.74
SPRG3	239.02	1.93	1	F. macrus (0), F. meeki (0), F. neglectus(39)	240.95

**Table 4: Multiple linear regression results [coefficients, standard errors (SE), F-statistic values and probability (p) values] for each predictor variable (total phosphorus and macrograzer biomass) and response variable [ash-free dry mass (AFDM) and chlorophyll a (CHLA)] . A positive and significant relationship was found between algal biomass (AFDM and CHLA) and predictors (TP, macrograzer biomass).**

<b>Response</b>	<b>Predictor</b>	<b>Coefficient</b>	<b>SE</b>	<b>F-value</b>	<b>p-value</b>
AFDM (g/m <sup>2</sup> )	Total Phosphorus (mg/l)	8.23	3.39	2.43	0.032
	Total Macrograzers Biomass (g)	0.002	0.0009	2.26	0.043
	<i>Overall</i>			8.54	<b>0.005</b>
	<i>R<sup>2</sup> =0.59</i>				
CHLA (mg/m <sup>2</sup> )	Total Phosphorus (mg/l)	10.99	4.205	2.61	0.023
	Total Macrograzers Biomass (g)	0.002	0.001	2.21	0.047
	<i>Overall</i>			9.07	<b>0.004</b>
	<i>R<sup>2</sup> =0.60</i>				

**Table 5: Ranges and means for total phosphorus (TP), total nitrogen (TN), percent canopy cover (canopy cover %), and discharge (cubic feet per second) across stream study sites during the late summer 2016 and winter 2017 manipulative experiment.**

Stream	Watershed	Season	TP (mg/L)			TN (mg/L)		Canopy Cover %		Discharge (cfs)	
			<i>n</i>	<i>range</i>	<i>mean</i>	<i>range</i>	<i>mean</i>	<i>Block 1;2;3</i>	<i>mean</i>	<i>Range</i>	<i>mean</i>
Saline	Eucha	Summer	3	0*-0.0025	0.0008	0.6-4.2	1.9	0%, 38%, 50%	29%	--	--
Evansville	Illinois		4	0.009-0.023	--	1.1-8.5	3.8	2%, 16%, 2%	6%	5.81-6.90	6.14
Beaty	Eucha		3	0.026-0.027	0.0267	1.9-4.0	2.6	7%, 3%, 32%	14%	1.98-10.40	4.42
Baron Fork	Illinois		2	0.047-0.140	--	3.7-4.5	4.1	12%, 19%, 6%	12%	1.42-191.00	4.69
Flint	Eucha		4	0.026-0.064	0.0520	1.2-2.0	1.5	22%, 18%, 13%	18%	5.00-24.00	12.00
Saline	Eucha	Winter	3	0*-0.001	0.0004	0.7-3.8	0.8	12%, 53%, 35%	14%	--	--
Evansville	Illinois		--	--	--	--	--	--	--	--	--
Beaty	Eucha		3	0.004-0.029	0.0173	1.6-3.9	1.7	4%, 4%, 38%	16%	--	--
Baron Fork	Illinois		--	--	--	--	--	--	--	--	--
Flint	Eucha		3	0.038-0.049	0.0453	1.9-7.3	2.4	13%, 12% 35%	17%	--	--

**Table 6: Randomized Block Analysis of Variance was run on summer (days 14, 21, and 28) and winter (days 14, 21, 28, 35) data [degrees of freedom (df), F- statistic values, and probability (p) values], to understand the influence of treatment [macrograzer excluded vs. present (TREAT)], day of the experiment (DAY), and block design (BLOCK) have on response variables [chlorophyll a (CHLA) and ash-free dry mass (AFDM)].**

Stream	Response	Factor	df	Summer		Winter	
				F-value	p-value	F-value	p-value
Saline	CHLA (mg/m <sup>2</sup> )	TREAT	3	0.61	0.493	5.02	0.111
		BLOCK	3	0.72	0.459	10.50	0.048
		DAY	9	64.94	<b>0.003</b>	10.50	<b>0.002</b>
		TREAT:DAY	9	0.68	0.471	42.71	0.267
		BLOCK:DAY	9	1.64	0.391	0.85	0.448
		TREAT:DAY	9	0.68	0.471	42.71	0.267
	AFDM (g/m <sup>2</sup> )	TREAT	3	0.77	0.445	3.72	0.149
		BLOCK	3	4.06	0.137	5.98	0.092
		DAY	9	42.93	<b>0.007</b>	74.16	<b>&lt;0.001</b>
		TREAT:DAY	9	0.82	0.432	4.73	0.0593
		BLOCK:DAY	9	6.73	0.080	0.26	0.78
		TREAT:DAY	9	0.82	0.432	4.73	0.0593
Beaty	CHLA (mg/m <sup>2</sup> )	TREAT	3	3.09	0.177	2.33	0.932
		BLOCK	3	1.08	0.373	0.01	0.932
		DAY	9	6.57	0.079	10.42	<b>0.024</b>
		TREAT:DAY	9	0.57	0.511	0.90	0.438
		BLOCK:DAY	9	0.09	0.799	0.52	0.574
		TREAT:DAY	9	0.57	0.511	0.90	0.438
	AFDM (g/m <sup>2</sup> )	TREAT	3	0.07	0.813	3.67	0.151
		BLOCK	3	0.01	0.951	1.05	0.381
		DAY	9	16.74	<b>0.02</b>	76.19	<b>&lt;0.001</b>
		TREAT:DAY	9	0.10	0.902	1.48	0.302
		BLOCK:DAY	9	0.43	0.576	0.67	0.538
		TREAT:DAY	9	0.10	0.902	1.48	0.302
Flint	CHLA (mg/m <sup>2</sup> )	TREAT	3	0.10	0.777	0.00	0.989
		BLOCK	3	0.01	0.927	0.68	0.469
		DAY	9	26.04	<b>0.007</b>	9.83	<b>0.051</b>
		TREAT:DAY	9	0.21	0.719	0.01	0.913
		BLOCK:DAY	9	1.51	0.304	0.10	0.773
		TREAT:DAY	9	0.21	0.719	0.01	0.913
	AFDM (g/m <sup>2</sup> )	TREAT	3	0.69	0.466	1.06	0.38
		BLOCK	3	0.01	0.917	2.55	0.209
		DAY	9	9.40	<b>0.525</b>	3.24	0.165
		TREAT:DAY	9	0.43	0.564	1.21	0.354
		BLOCK:DAY	9	0.06	0.827	0.92	0.416
		TREAT:DAY	9	0.43	0.564	1.21	0.354
Evansville	CHLA (mg/m <sup>2</sup> )	TREAT	3	0.92	0.408	--	--
		BLOCK	3	0.56	0.509	--	--
		DAY	9	0.57	0.555	--	--
		TREAT:DAY	9	1.73	0.268	--	--
		BLOCK:DAY	9	3.05	0.148	--	--
		TREAT:DAY	9	1.73	0.268	--	--
	AFDM (g/m <sup>2</sup> )	TREAT	3	0.11	0.767	--	--
		BLOCK	3	0.76	0.447	--	--
		DAY	9	1.99	0.241	--	--
		TREAT:DAY	9	2.06	0.234	--	--
		BLOCK:DAY	9	1.16	0.368	--	--
		TREAT:DAY	9	2.06	0.234	--	--
Baron Fork	CHLA (mg/m <sup>2</sup> )	TREAT	3	0.02	0.894	--	--
		BLOCK	3	3.59	0.154	--	--
		DAY	9	0.43	0.57	--	--
		TREAT:DAY	9	0.11	0.775	--	--
		BLOCK:DAY	9	0.21	0.691	--	--
		TREAT:DAY	9	0.11	0.775	--	--
	AFDM (g/m <sup>2</sup> )	TREAT	3	0.36	0.591	--	--
		BLOCK	3	2.73	0.197	--	--
		DAY	9	1.79	0.262	--	--
		TREAT:DAY	9	0.11	0.831	--	--
		BLOCK:DAY	9	1.67	0.278	--	--
		TREAT:DAY	9	0.11	0.831	--	--

**Table 7: Regression was run between algal biomass effect sizes [ash-free dry mass (AFDM), chlorophyll *a* (CHLA), grazed (G), ungrazed (UN)] were run against predictor [total phosphorus (TP)] in summer of 2016 on days 28 and 35. Effect size was calculated by dividing grazed by ungrazed tiles. No significant correlation was found [F-statistical values and probability (p) values].**

Day	Effect Size (G/UN)	Predictor	R <sup>2</sup>	F-value	p-value
28	CHLA	mean TP (mg/L)	0.057	0.78	0.393
	AFDM		0.003	0.03	0.856
35	CHLA	mean TP (mg/L)	0.019	0.13	0.726
	AFDM		0.014	0.10	0.762



**Table 8: An analysis of covariance (ANCOVA) was run between the effect size of algal biomass [ash-free dry mass (AFDM), chlorophyll *a* (CHLA), grazed (G), ungrazed (UN)] and predictors [total phosphorus (TP), season] on days 28 and 35. Effect size was calculated by dividing grazed by ungrazed tiles. Each block was put into the regression as a separate point. No significant results were found [degree of freedom (df), F-statistical values and probability (p) values].**

Day	Effect Size (G/UN)	Predictor	df	f-value	p-value
28	CHLA	Total Phosphorus (mg/l)	1	1.79	0.202
		Season	1	0.78	0.391
		TP:Season	1	1.11	0.31
	AFDM	Total Phosphorus (mg/l)	1	0.27	0.614
		Season	1	0.05	0.828
		TP:Season	1	0.74	0.403
35	CHLA	Total Phosphorus (mg/l)	1	2.16	0.164
		Season	1	0.68	0.423
		TP:Season	1	2.42	0.142
	AFDM	Total Phosphorus (mg/l)	1	2.25	0.156
		Season	1	0.66	0.431
		TP:Season	1	4.25	0.058

**Table 9: All macrograzer studies conducted with algal response to macrograzer biomass, presence, or nutrients being the main predictors. In study design [natural(nat.), mensurative (men), manipulative (man) ], enclosures are defined as cages when macrograzers are restricted to an area where enclosure are defined as cages or devices (e.g. electrical enclosures) that exclude macrograzers from a given area while they are allowed to roam free outside given enclosure space. Predictor variables [*Campostoma (C ) anomalum (anom.)*, *Faxonius (F)*, *Phoxiuns (P) erythrogaster (erythr)*, nitrogen (N), phosphorus (P), potassium (K), ammonium (NH<sup>4</sup>)] are compared to response variables [chlorophyll *a* (CHLA), ash-free dry mass (AFDM), algal filament length (AFL), particulate organic matter(POM), algal assemblage (ASSM), net primary production (NPP), gross primary production (GPP), denitrification(Dnit), algal nitrogen content (Alg.N), algal phosphorus content (Alg.P), water nitrogen content (W.N), water phosphorus content (W.P) ]. The symbol “+”, “-“, or “0” signifying a positive, negative, or neutral response, respectively, compared to “control” conditions when variables are categorical. When no “control” condition is listed look for individual comparisons [greater than (>), less than (<)] or assume gradient or regression response. Algal assemblage (ASSM) is either listed as changed (CHNG) or no change (0).**

Paper	Study Design	Study Region	Nutrient Range	Predictor(s)	CHLA	AFDM	AFL	AI	SED	POM	ASSM	NPP	GPP	Dnit	Alg.N	Alg.P	W.N	W.P
Power et al. 1985	nat. men.,	Brier Creek, OK	--	<i>C. spp.</i>							CHNG							
	fenced in pool		--	<i>C. spp.</i>								CHNG						
Steward 1987	nat. man.,	Brier Creek, OK	27:3:3	N:P:K (27:3:3)		+												
	fenced in pool with fish-exclosure pin	Temperate Ozark	--	<i>C. spp.</i> X Nutrients		-												
Power et al. 1988	nat. man.,	Baron Fork, OK	--	<i>C. spp.</i>							CHNG							
	flow-throuh enclosure, riffle	Temperate Ozark		control: <i>C. spp.</i> absent														
Gelwick & Matthews 1992	nat. man.,	Brier Creek, OK	--	high nat. density <i>C. spp.</i>							CHNG	+						
	cage enclosures, pools	Temperate Ozark		control: no <i>C. spp.</i>														
Vaughn et al. 1993	man.,	Greenhouse	--	<i>F. virilus</i>		0					CHNG							
	recirculating		<i>C. anom.</i> X <i>F. virilus</i>		0						CHNG							
	streams ,		<i>Physella</i> (snail)		0						0							
	pools		<i>C. anom.</i> X <i>Physella</i>		0						CHNG							
			control: non-grazed															

**Table 9 (cont.)**

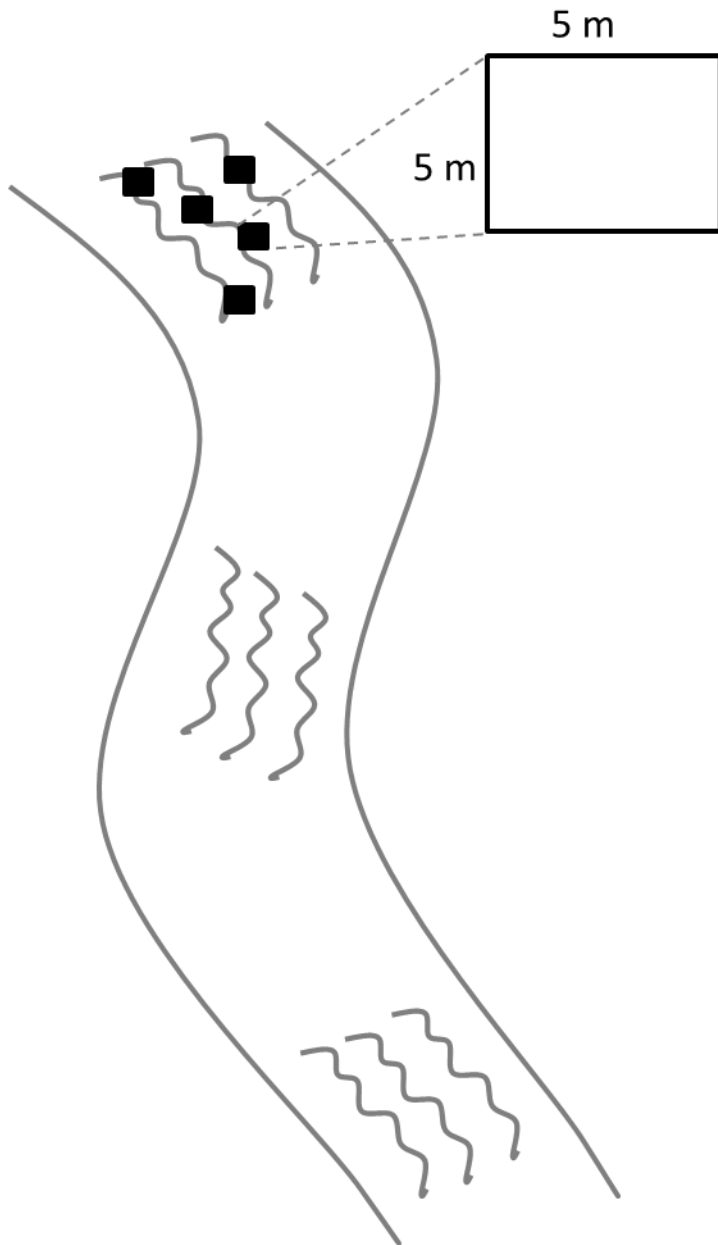
Paper	Study Design	Study Region	Nutrient Range	Predictor(s)	CHLA	AFDM	AFL	AI	SED	POM	ASSM	NPP	GPP	Dnit	Alg.N	Alg.P	W.N	W.P	
Creed 1994	nat. man., cage exclosures, pools	August Creek, MI Temperate	--	Crayfish present control: crayfish absent							CHNG								
	nat. man., cage enclosures, deep riffles	Middle Branch of Ontonagon River, MI Temperature	--	<i>F. rusticus</i> -density 5/m <sup>2</sup> <i>F. rusticus</i> -density 10/m <sup>2</sup> Control: <i>F. rusticus</i> - density 0/m <sup>2</sup>	0	0							0						+
Charlebois & Lamberti 1996	nat. men., 1 stream with nat. crayfish gradient			LS1- 3.1 <i>F. rusticus</i> /m <sup>2</sup>	+	0						0							
				LS2- 1.8 <i>F. rusticus</i> / m <sup>2</sup>	-	0			0										
				LS3- 0.93 <i>F. rusticus</i> /m <sup>2</sup>	-	0			0										
				LS4 - 0.18 <i>F. rusticus</i> /m <sup>2</sup>	-	0			0										
Evans-White et al. 2001	man. outdoor recirculating streams, riffle	Kings Creek, KS Temperate Pairie	--	<i>F. neglectus</i> <i>C. anom.</i> <i>F. neglectus</i> X <i>C. anom.</i> control: non-grazed	-														
Flecker et al. 2002	nat. man., cage exclosures vs. open cages , nutrient difusing substrate	Rio Las Maris Orinoco River System	P: 12 g NaH <sub>2</sub> PO <sub>4</sub> /L	Tropical grazing fish N fish x N	-				-										
		Tropical Andean Venezuela	agar, N:42 g NaNO <sub>3</sub> & 27 g N <sub>4</sub> Cl agar, N+P=10 N: 1P	control: non-grazed, unenriched	+			+											
Evans-White & Lamberti 2005	man., recirculating streams (0.053 m <sup>2</sup> )	Greenhouse	DIN: < 10 ug/L SRP: 9-20 ug/L	<i>F. propinquus</i> <i>Elimia livescens</i> (snail)	+	0									+	-	0	0	
Bertrand & Gido 2007	man., outdoor recirculating streams, riffle & pool	Kings Creek, KS Temperate Pairie		<i>P. erythr.</i> (dace) control: no fish	0		-	-	0	0	0	0	0						
				riffle (w/fish ) control: pool (w/fish)	0		-	0	0	0	0	0	0	0					

**Table 9 (cont.)**

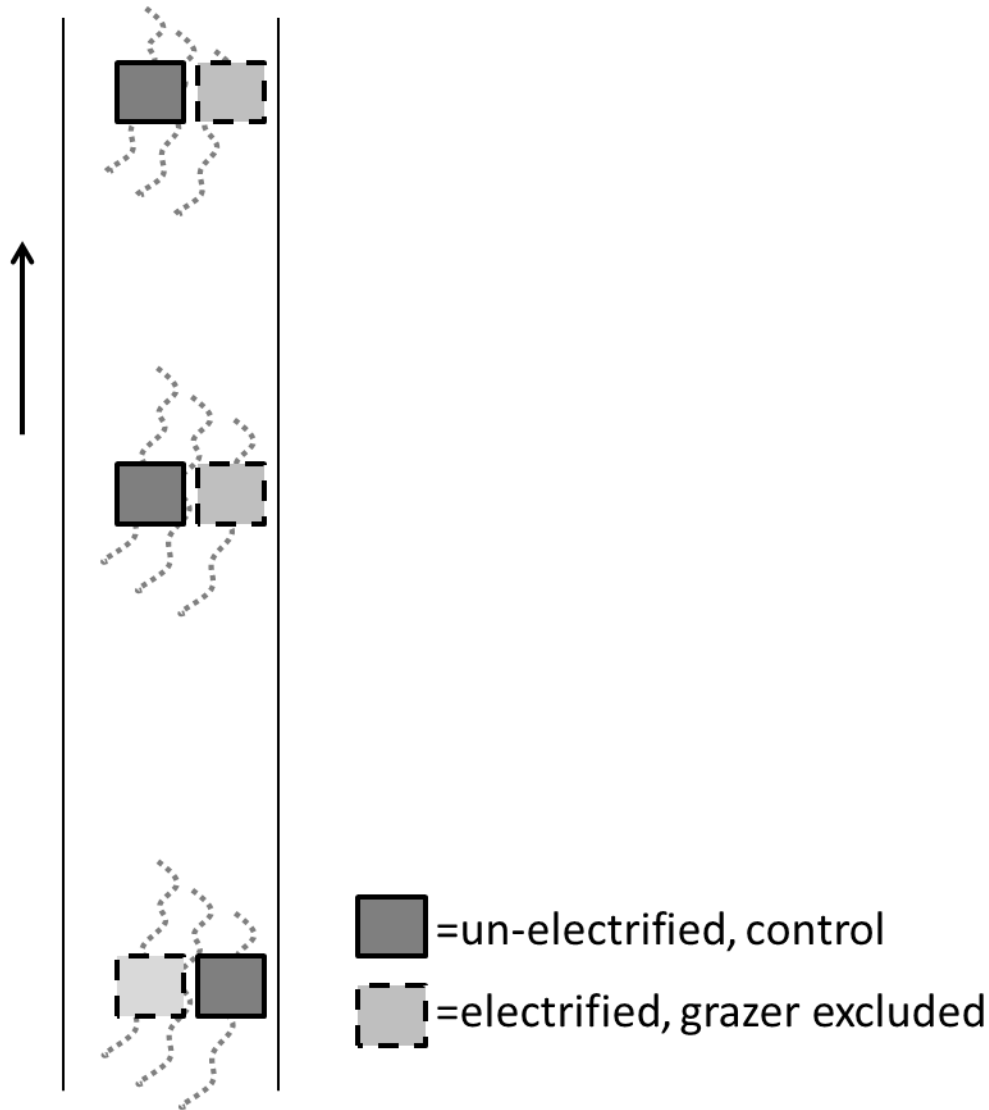
Paper	Study Design	Study Region	Nutrient Range	Predictor(s)	CHLA	AFDM	AFL	AI	SED	POM	ASSM	NPP	GPP	Dnit	Alg.N	Alg.P	W.N	W.P		
Bengtson et al. 2008	man., outdoor recirculating streams, riffle & pool	Kings Creek, KS Temperate Pairie	--	<i>P. erythr. vs. F. spp.</i>	0		>			0			0							
				riffle vs. pool	>		0		>											
				riffle dace vs. riffle <i>F. spp.</i>	>		0		0											
				with increased <i>F. spp.</i>	0		0		0						0					
				with increased <i>P. erythr.</i>	0		-			0			0							
Ludlam & Magoulick 2009	nat. man., electrical enclosure, pool	Little Mulberry Creek, AR Temperate Boston Mnt. Stream	--	Macrograzer present	-/0	-/0			-/0											
				control: absent																
				<i>C. spp.</i> biomass (August)	-	-			0											
				<i>F. meeki meeki</i> biomass (June)	0	0			-											
Ludlam & Magoulick 2010	nat. man., electrical enclosure, pool	Little Mulberry Creek, AR Temperate Boston Mnt. Stream	--	Macrograzer present (June)	0	0			0											
				control: absent (June)																
				Macrograzer present, (August)	+	0			0											
				control: absent (August)																
				<i>C. spp.</i> biomass	+	0														
				<i>F. meeki meeki</i> biomass	-	0														
Murdock et al. 2011	man., outdoor recirculating streams, riffle & pool, after disturbance	Kings Creek, KS Temperate Pairie	Nutrient treatment: NO <sub>3</sub> -N: 14-164 ug/L, TN: 207-291 ug/L, TP: 3.3-5.7 ug/L	nitrogen vs. <i>P. erythr.</i>	>		>						>				>			
				<i>P. erythr.</i> - low N-loading	+		-													
				<i>P. erythr.</i> - high N-loading	-		0													
				<i>P. erythr.</i> - day 1-14	-															0
				<i>P. erythr.</i> - day 14-35	0															0
				Fish treatment: N-loading- day 1-14	0															+
NO <sub>3</sub> -N: 61-71 ug/L, TN: 179-296 ug/L, TP: 2.7-5.6 ug/L	+															+				

**Table 9 (cont.)**

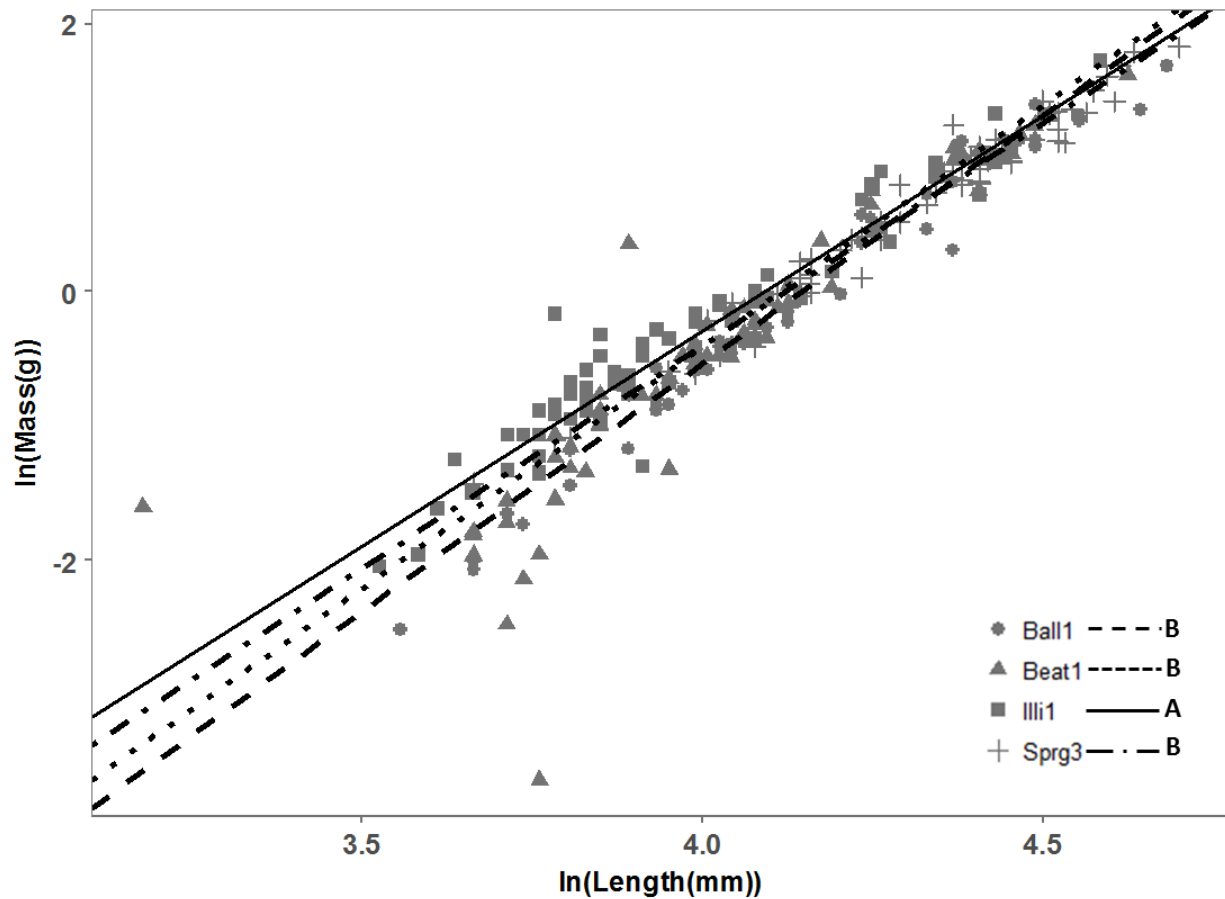
Paper	Study Design	Study Region	Nutrient Range	Predictor(s)	CHLA	AFDM	AFL	AI	SED	POM	ASSM	NPP	GPP	Dnit	Alg.N	Alg.P	W.N	W.P		
Reisinger et al. 2011	man., outdoor recirculating streams, riffle & pool	Kings Creek, KS Temperate Prairie	4,8,16, 32 X background	C. anom.	0	-	-						0	0			0			
				NH <sub>4</sub> <sup>+</sup>	0	0	0				0	*							+	
				Control: no grazing or NH <sub>4</sub> riffle vs. pool	<	<														
Taylor et al. 2012	man., outdoor stream mesocosms, fish exclusion cage, pools	North Bosque River Niels Creek	PO <sub>4</sub> P: 8, 20, 100 ug/L	Day 14: C. anom.- 8 ug/L	0	-			-						-			+		
				C. anom.- 20 ug/L	0	-			-							-			+	
		Baylor Experimental Aquatic Research Facility, TX	Temperate Wetland	C. anom.- 100 ug/L	0	-				-						-			+	
				control: no fish with 8, 20, or 100 ug/													0			0
		Day 28:	C. anom.- 8 ug/L	+	0					0						0			0	
			C. anom.- 20 ug/L	+	0					0						0			0	
			C. anom.- 100 ug/L	+	0					0										
			control: no fish with 8, 20, or 100 ug/																	
		Ungrazed, P-enrichment, day 14 &				0	0			0										0
Ludlam et al. 2015	manipulative mesocosm, cage enclosure vs. open cages	Greenhouse	PO <sub>4</sub> P: 0.001-0.013 mg/L NO <sub>3</sub> : 0.0089 mg/L	6 crayfish/m <sup>2</sup>	-	-														
				12 crayfish/m <sup>2</sup>	-	-														
				18 crayfish/m <sup>2</sup>	-	-														
				control: caged enclosure non-grazed																
Pennock & Gido 2016	manipulative outdoor recirculating streams, riffle & pool	Kings Creek, KS Temperate Prairie	--	C. anomalum density (3.7-24.6 g/m <sup>2</sup> )	0	0			0									+		



**Figure 1: Mensurative abundance study sampling design. We sampled three riffles total in each reach; each riffle was separated by at least one pool. Within each riffle, we used dual kick-net and electrofishing methods to sample for stonerollers and crayfish in 5 quadrats that were 5x5 meters in size.**

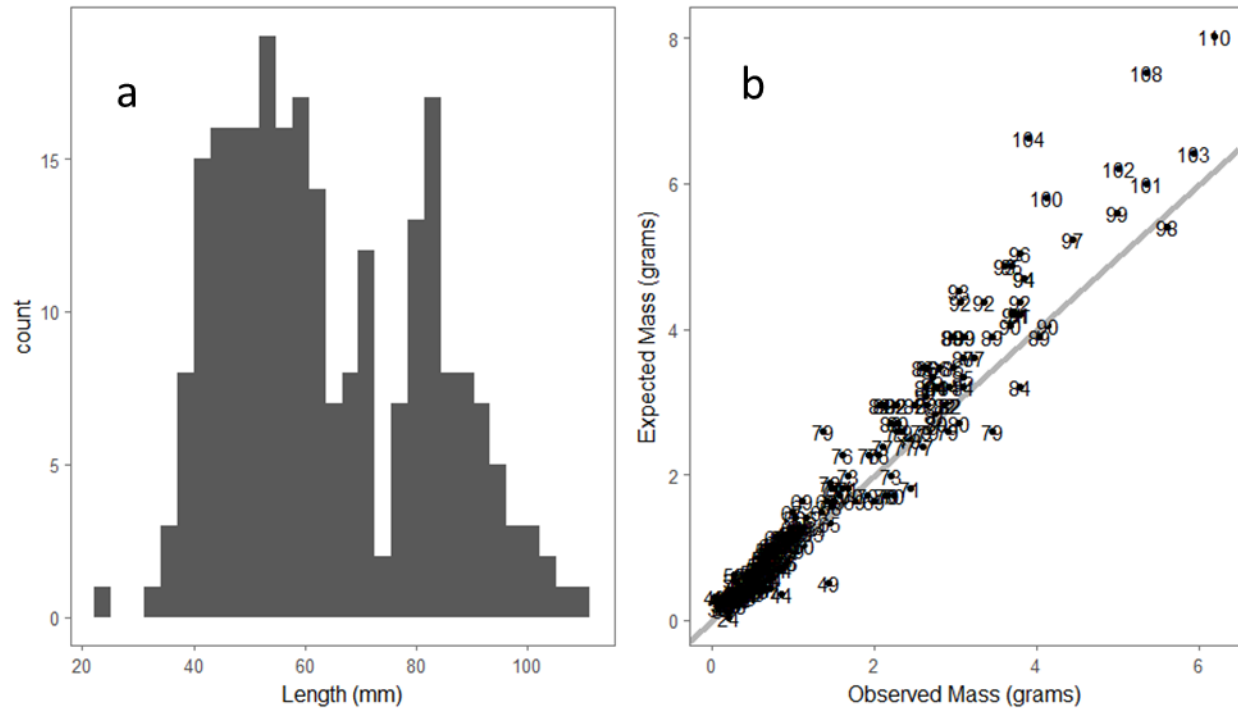


**Figure 2: Manipulative abundance study experimental design. Three blocks were set up in riffle habitats in the upper, middle, and lower sections of each stream reach (reach  $\geq 200$  m) where each block was separated by at least one pool. Each block consisted of one treatment enclosure (stoneroller excluded) and one un-electrified control quadrate (stoneroller present) that were set up side-by-side in equal flow conditions. Four unglazed ceramic tiles ( $121 \text{ cm}^2$ ) were zip-tied into each quadrate enclosure ( $31 \times 5\text{-cm}$  built from 19-mm polyvinyl chloride pipe) to measure benthic algae. The frame was then elevated 6cm from the streambed with rebar and zip ties since this has been shown to exclude poor swimming and dispersing macroinvertebrates (Lamberti and Resh, 1983). 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 ( $> \sim 1 \text{ cm}$ ), including most crayfish and fish (Pringle and Blake, 1994). The charge can extend about 10 cm outside the quadrate (Ludlam and Magoulick, 2009).**

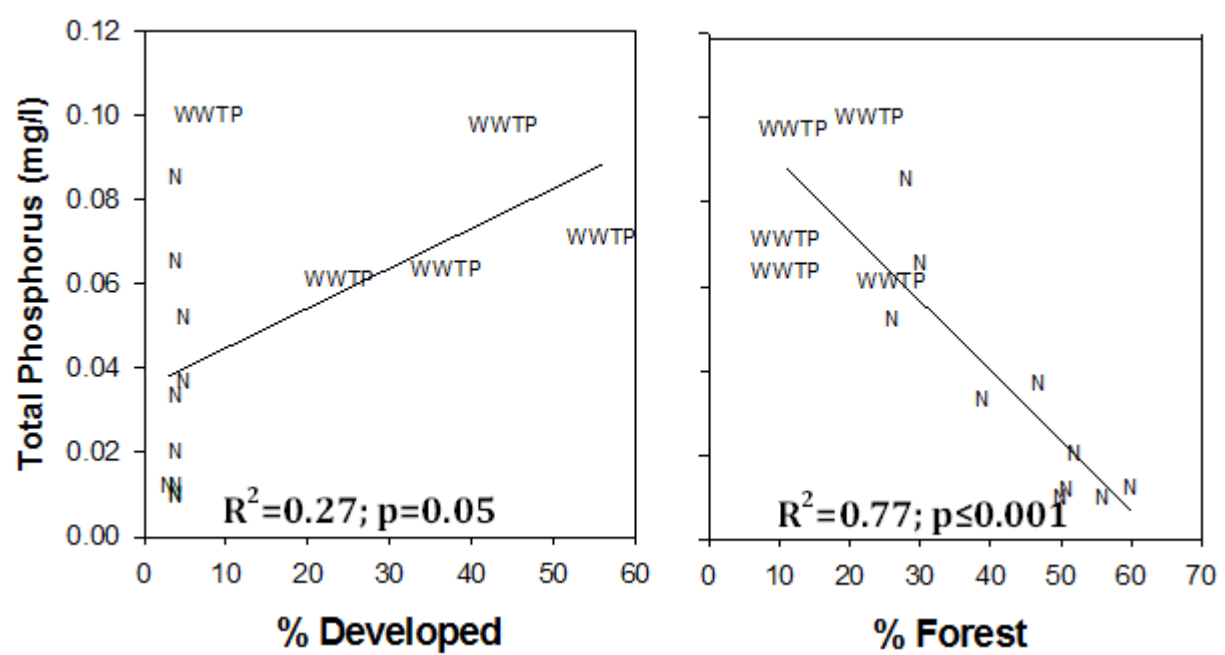


**Figure 3: Tukey's HSD test on ANCOVA model (Table 2) revealed statistical differences among intercepts between Illinois Creek and all other measured streams. There was no difference among the slopes of the four sites, so data were lumped and linear regression was used to model masses for stonerollers sampled at all 15 sites. Lengths ranged from 24-110(mm) and dry-masses ranged from 0.026-6.18(g).**

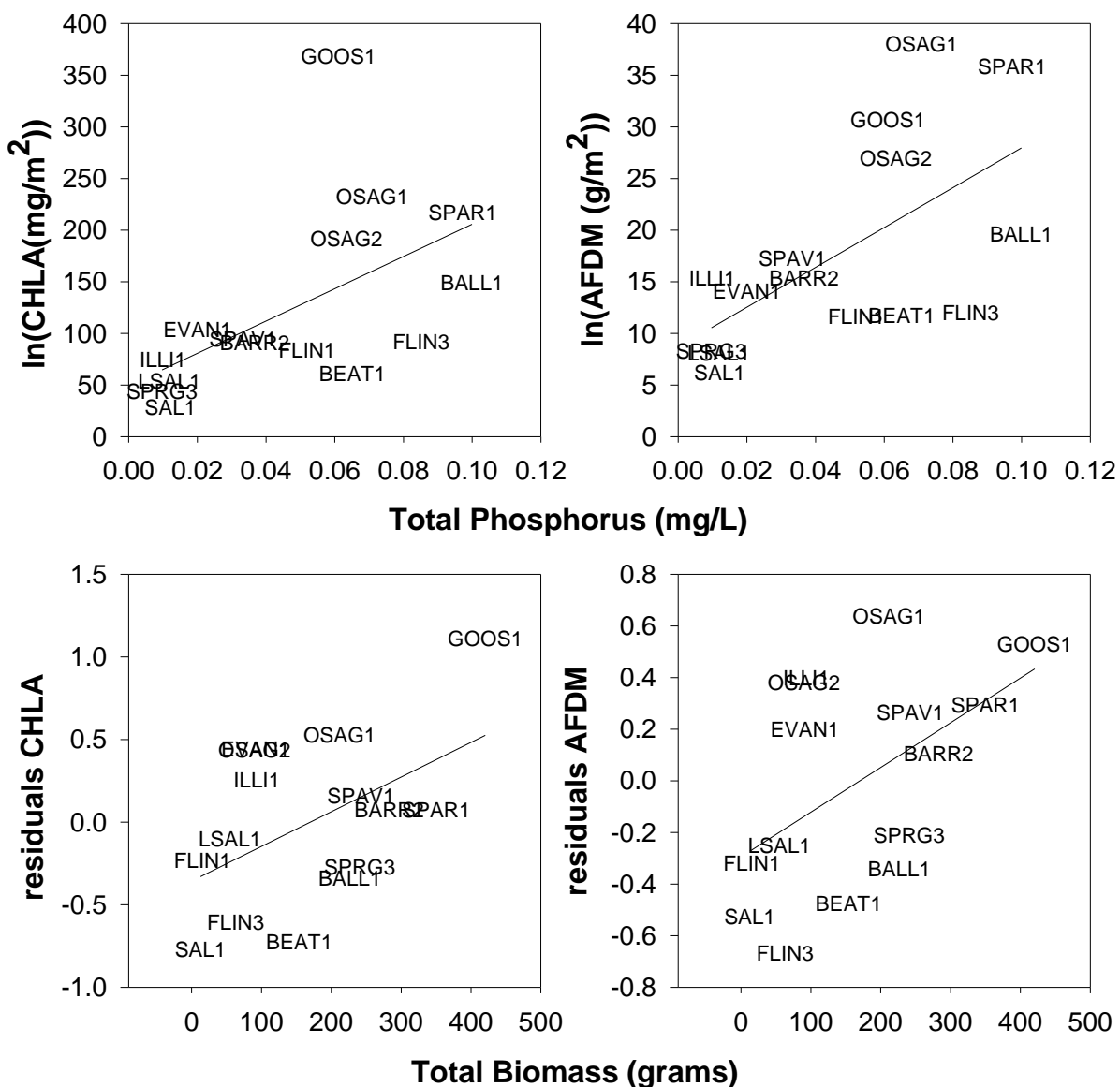




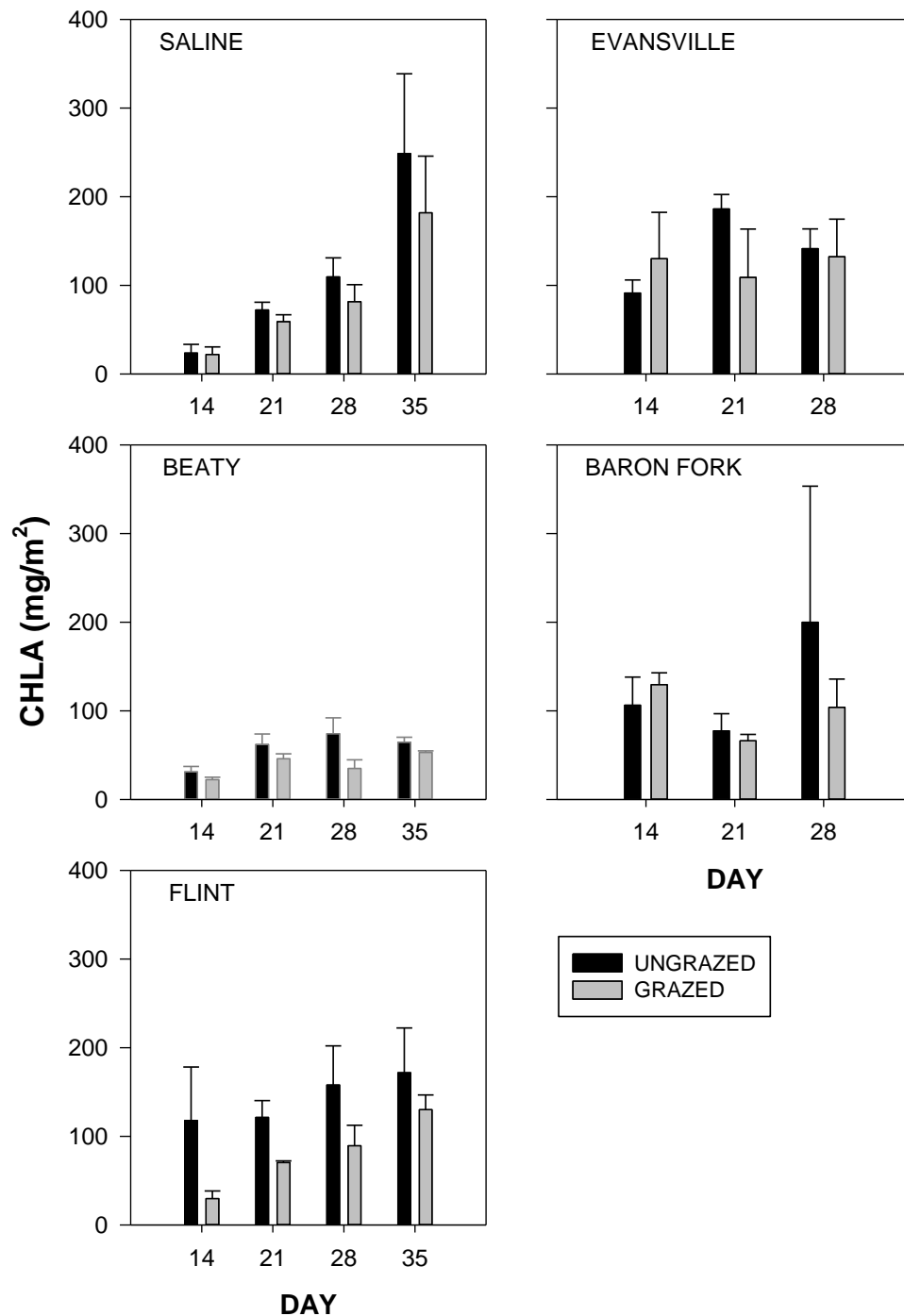
**Figure 4: Observed data ranged from 25-110 mm (a). Panel “b” gives the observed masses (taken in the field), expected masses (calculated from regression), and grey 1:1 line representing perfect model fit. Each point on panel “b” is labeled with its length in millimeters. Overall the regression model tended to overestimate masses. Some masses at lengths above 100 mm tended to be vastly overestimated, so a value of 6 grams was used from all length values at or above 100mm for future calculations.  $R^2$  between observed and expected was 0.95 showing high correlation between values.**



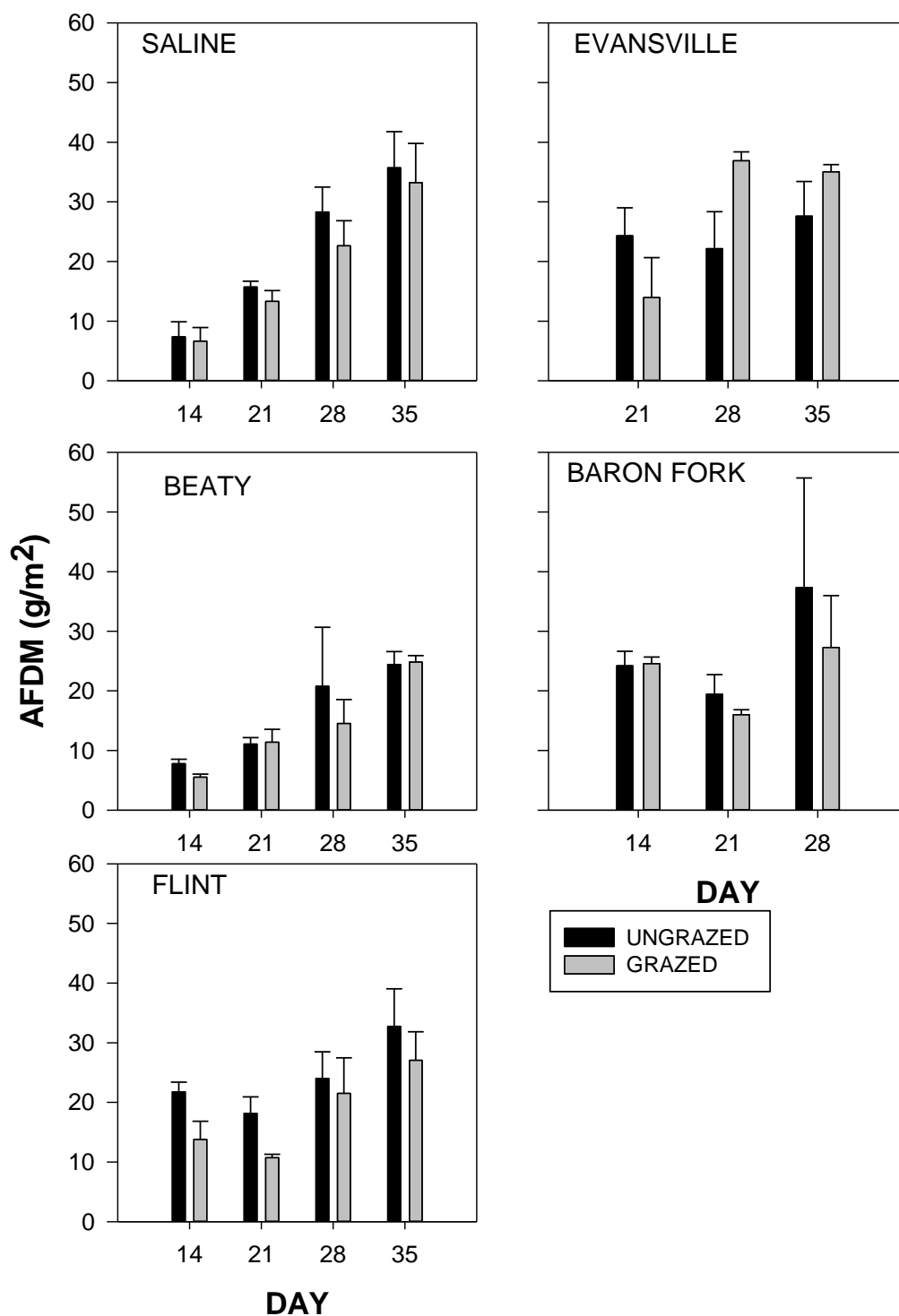
**Figure 5: Percent forest is significantly correlated with total phosphorus in the sites sampled (right panel) while percent developed is negatively correlated (left panel). Sites with waste water treatment plants (WWTP) effluents (Goos1, Osag2, Osag1, Spar1) were found in watersheds with highly developed urban areas.**



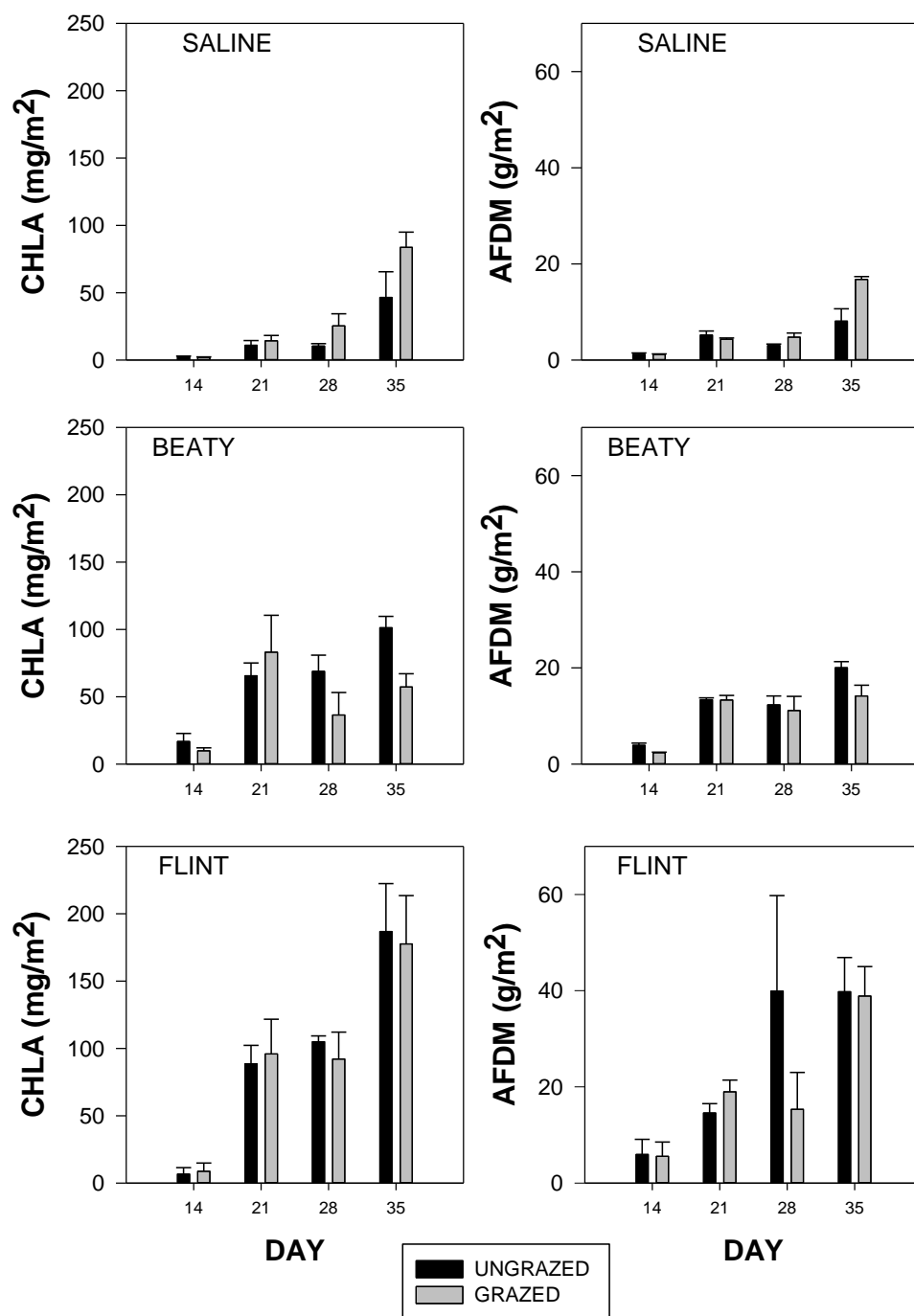
**Figure 6: Multiple linear regression was run to understand the influence of nutrient (total phosphorus) and macrograzer biomass on ash-free dry mass (AFDM) and chlorophyll *a* (CHLA) measurements (H1, Table 4). Regression was conducted simultaneously, with model 1 being AFDM (mg/m<sup>2</sup>) regressed against TP (mg/L and total macrograzer biomass (g) and model two being CHLA (mg/m<sup>2</sup>) regressed against TP(mg/L) and total macrograzer biomass (g). Table 4 shows the output for these regressions. The above figure separates out the predictors to visualize the individual effects. As expected there is a positive relationship between TP and algal measures, but counter to our hypothesis there is a positive relationship between the residuals of algal measure to TP and total macrograzer biomass (g).**



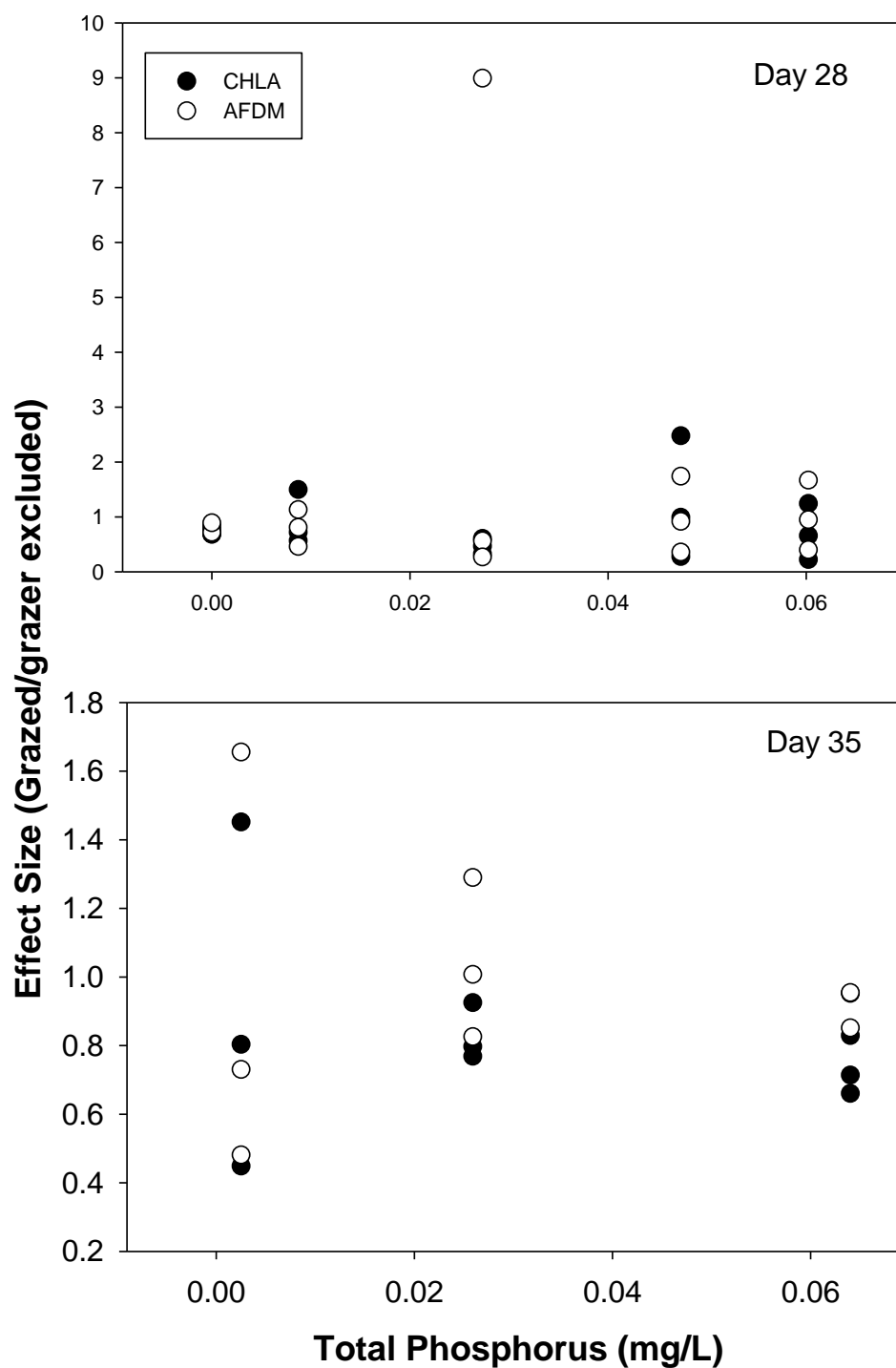
**Figure 7: A randomized block- repeated measures ANOVA (RM-RB-ANOVA) was used to understand the treatment effect on days 14,21,28,35 of chlorophyll a (CHLA) data collected in summer of 2016 (H2). Repeated measures ANOVA examined the influence of day and block on each stream. A significant effect of day was found in in Saline, Beaty, and Flint Creeks (Table 6).**



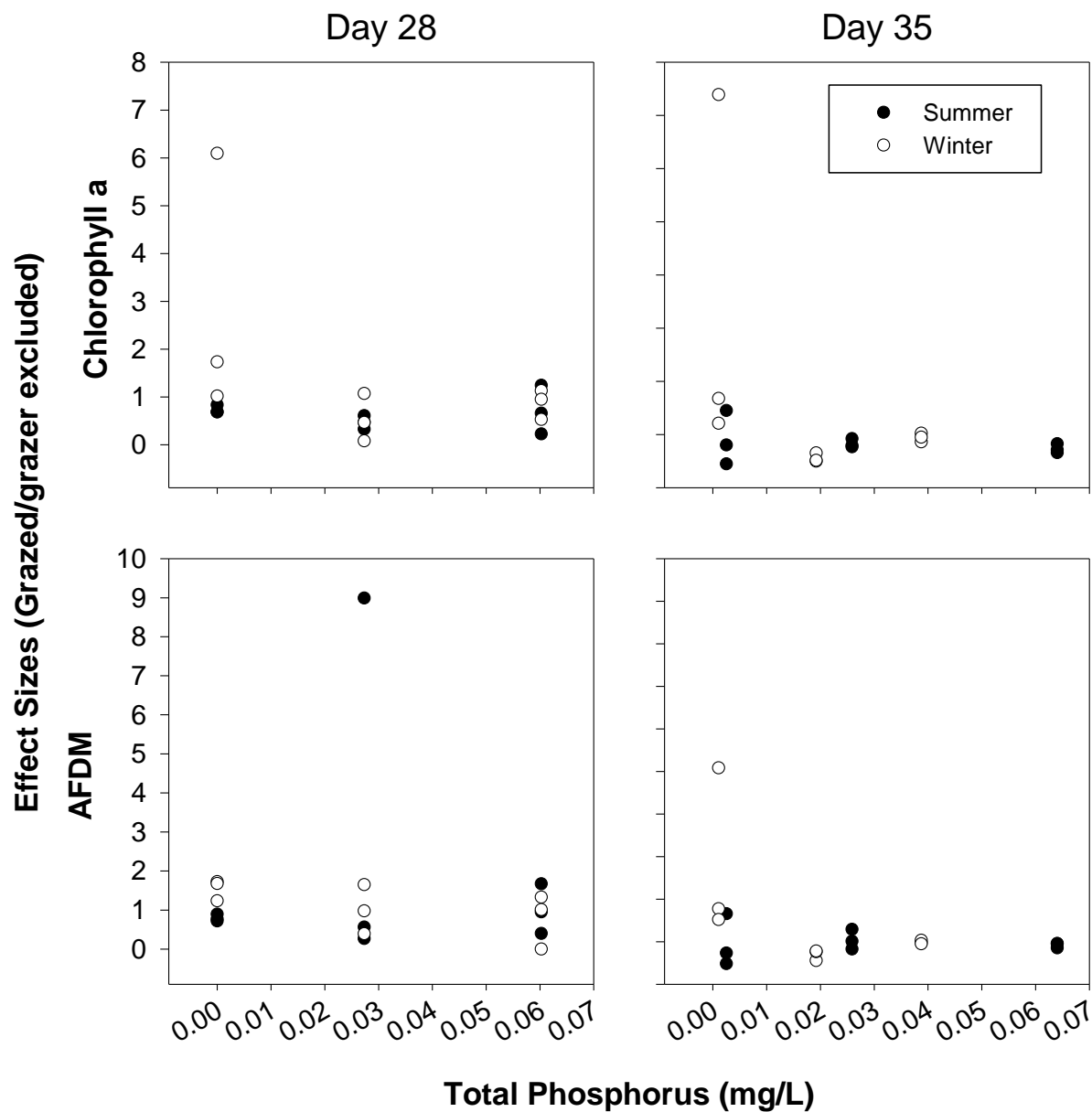
**Figure 8:** A randomized block- repeated measures ANOVA (RM-RB-ANOVA) was used to understand the treatment effect on days 14,21,28,35 of ash-free dry mass (AFDM) data collected in summer of 2016 (H2). Repeated measures ANOVA examined the influence of day and block on each stream. A significant effect of day was found in in Saline, Beaty, and Flint Creeks (Table 6).



**Figure 9:** A randomized block- repeated measures ANOVA (RM-RB-ANOVA) was used to understand the treatment effect on days 14,21,28,35 of chlorophyll a (CHLA) and ash-free dry mass(AFDM) data collected in late winter of 2017 (H2). Repeated measures ANOVA examined the influence of day and block on each stream. A significant effect of day was found in in Saline, Beaty, and Flint Creeks with response variable CHLA and Saline and Beaty Creeks with response variable AFDM (Table 6).

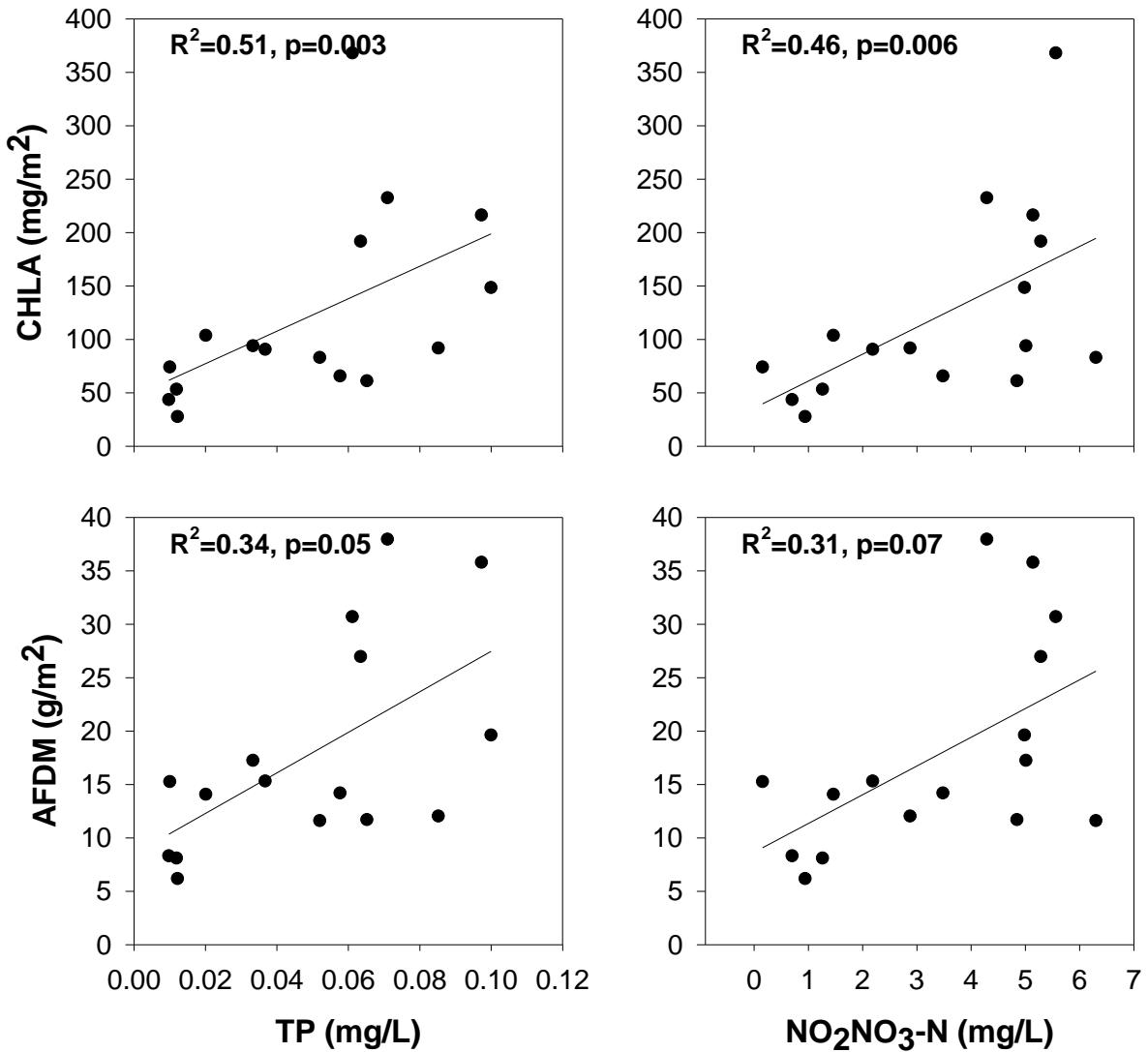


**Figure 10: Regression results for CHLA and AFDM effect sizes across a mean TP (mg/L) gradient for days 28 (top) and 35 (bottom). No significant correlation was found with either measure on either day.**



**Figure 11: An analysis of covariance (ANCOVA) was used to understand the treatment effect on day 28 and day 35 (H3). Total phosphorus (TP) levels ranged from below detection to 0.07 mg/L of TP during both seasons on day 28. On day 35, TP levels were lower on during the winter at Flint Creek, but summer TP levels were consistent. ANCOVA test revealed no statistically significant main effects between TP and effect sizes of algal biomass, no significant difference between seasons, and no significant interaction between seasons.**





**Figure 12: The relationship between natural log transformed chlorophyll  $a$  ( $\text{mg}/\text{l}$ ) on total phosphorus (TP, left) and Nitrate-Nitrite ( $\text{NO}_2\text{NO}_3\text{-N}$ , right). All measurements were taken within two weeks of macrograzer sampling by a separate study group.**

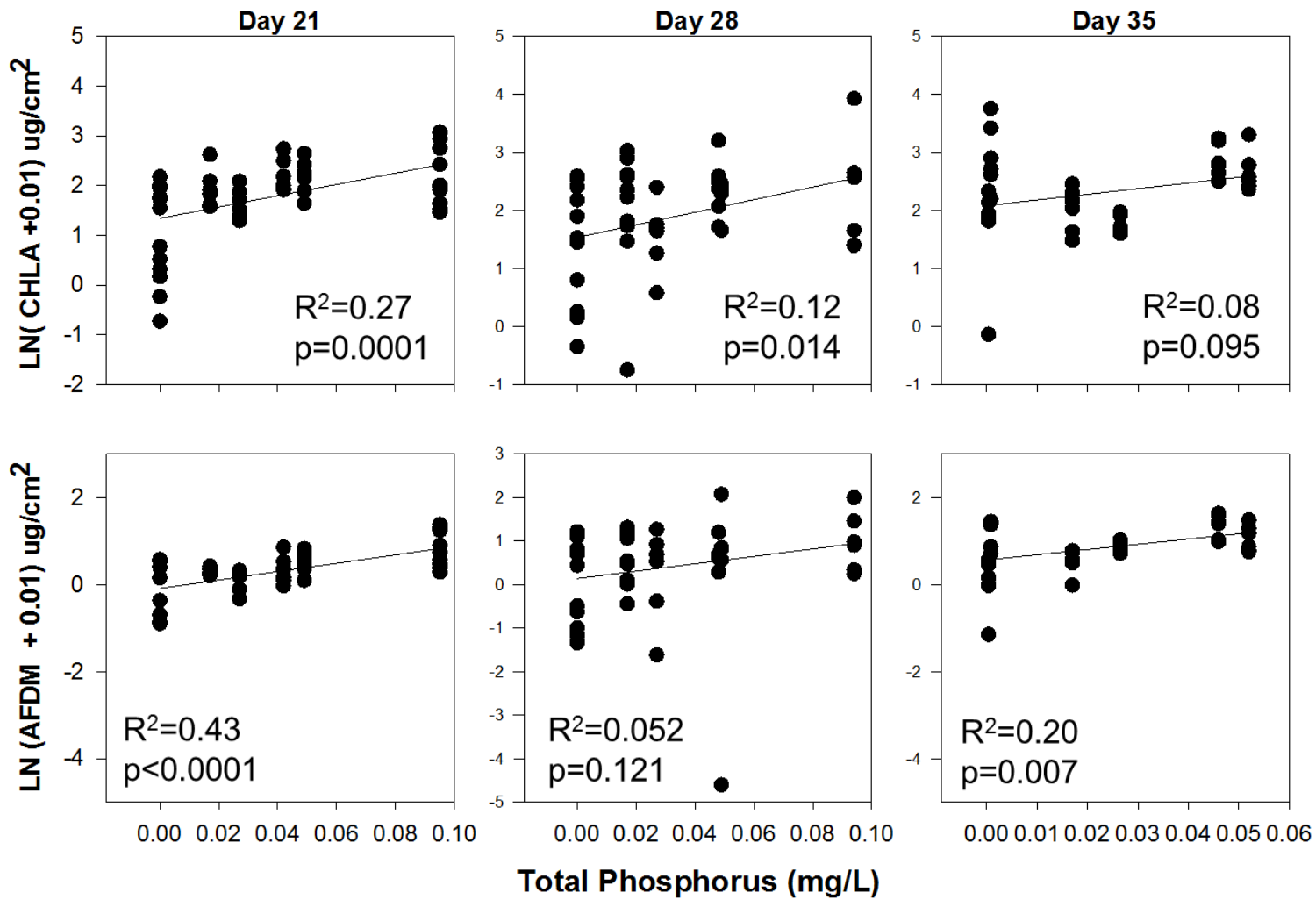
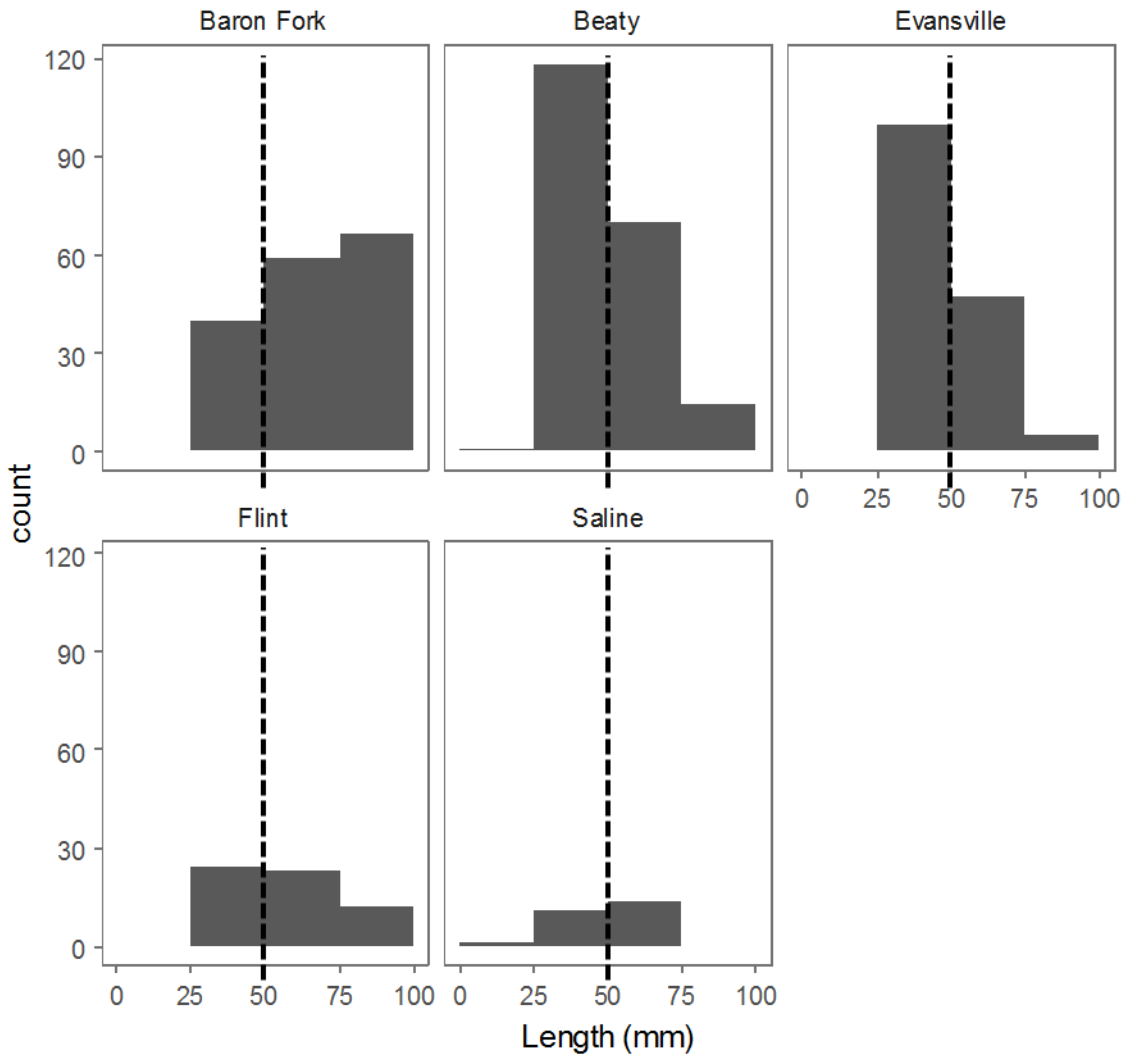


Figure 13: Linear regression was run between all algal biomass measures (without regard to effect size) to understand the effects of TP on algal in manipulative enclosure experiment. Single regressions revealed transient- statistically significant related.



**Figure 14: Histograms of length distribution taken during the summer of 2015 in mensurative abundance study. Five sites in diagram were used in manipulative abundance study. Overall the fraction of sampled that was  $\leq 50$  was 24% for Baron Fork, 58% for Beaty, 66% for Evansville, 50% for Flint, and 50% for Saline Creek.**

**Appedices**

Office of Research Compliance

## MEMORANDUM

TO: Michelle Evans-White  
FROM: Craig N. Coon, Chairman  
DATE: May 1, 2015  
SUBJECT: IACUC Approval  
Expiration Date: May 31, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Protocol: 15050 Relationship between nutrients, macrograzer abundance (Central Stonerollers and crayfish), and algae on Ozark Streams

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 31, 2017 you must submit a modification or new protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian