5-2016

A Method Comparison and Stressor-Response Experimental Study of Nitrogen and Phosphorus Impacts to Periphyton in Ozark Streams

Ashley Renee Rodman
University of Arkansas, Fayetteville

A Method Comparison and Stressor-Response Experimental Study of Nitrogen and Phosphorus Impacts to Periphyton in Ozark Streams

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

By

Ashley R. Rodman
University of Arkansas
Bachelor of Science in Environmental, Soil, and Water Science, 2014

May 2016
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

_____
Dr. J. Thad Scott
Thesis Director

_____
Dr. Jason M. Taylor
Committee Member

_____
Dr. Kristofor R. Brye
Committee Member

_____
Dr. Michelle A. Evans-White
Committee Member

_____
Dr. Andrew N. Sharpley
Committee Member

ABSTRACT

Stream bioassessment is important for understanding algal-nutrient relationships and the development of scientifically defensible numeric nutrient criteria. However, multiple methods of periphyton data collection are currently used, and little is known about the comparability of resulting datasets. Literature also suggests other factors besides nutrients (i.e. variable grazing, light, and flow) can confound algal-nutrient relationships. A one-year method comparison study and 31-day algal biomass-nutrient manipulative experiment were conducted in the southern Ozarks of Arkansas. The method comparison study was implemented using two common bioassessment procedures (whole-surface and delimiter-reduced periphyton removal) to assess the potential for combining datasets. During the manipulative experiment, cobbles from the Buffalo River Watershed were exposed to a range of phosphorus (P) and nitrogen (N) concentrations during P-only and N + P enrichment periods to evaluate algal biomass responses using recirculating streamside mesocosms. Results of the method comparison study showed no statistical difference between bioassessment procedures for chlorophyll-a (chl-a) and ash-free dry mass (AFDM) ($p = 0.123$ and $p = 0.550$, respectively) or any interaction between method and season. Differences in chl-a and AFDM from both methods were detected ($p < 0.001$ and $p = 0.012$, respectively) when comparing warmer versus cooler seasons. Temperature-dependent grazing pressures were a potential explanation for the observed seasonal variability in biomass. The experiment revealed a positive linear relationship between benthic chl-a and increasing P and N addition up to 0.2 mg/L P ($p < 0.001$), with apparent N-limitation observed during the P-only enrichment period. After 17 days of P-only enrichment, chl-a increased with increasing P concentrations ($p < 0.001$), ranging from 4.4 to 57.9 mg/m^2. After 14 additional days of N + P enrichment, mean chl-a had almost tripled across respective treatments, ranging from 13.3 to

171.1 mg/m^2. Results support the need for controlling N and P in freshwater systems to avoid excessive algal biomass accrual and provide insight into how possible increases in nutrient loading may influence the Buffalo River Watershed, disregarding confounding factors. Overall, both studies further scientific understanding of algal-nutrient relationships and verify the combining of both bioassessment methods for developing regional nutrient criteria and protecting stream designated uses.

ACKNOWLEDGEMENTS

Thank you to my committee members, Drs. Thad Scott, Jason Taylor, Kristofor Brye, Michelle Evans-White, and Andrew Sharpley, for their support and guidance throughout my time in the Crop, Soil, and Environmental Sciences Graduate Program. I appreciate the statistical assistance and insights provided for this thesis by Drs. Thad Scott and Jason Taylor. My gratitude goes out to those who provided countless hours of field and laboratory assistance to make this thesis possible, including: Amie West, Shannon Speir, Matthew Rich, Toryn Jones, Justin Woodruff, Sara Hallett, Delaney Hall, Nathan Sorey, Tim Glover, April Price, Harrison Smith, Kayla Sayre, Jessalyn Kohn, Byron Winston, and Deanna Mantooth. I would like to acknowledge the Natural Resource Conservation Service and the State of Arkansas for providing funding for this study and the National Park Service for allowing me to conduct research within the Buffalo River National Park.

TABLE OF CONTENTS

1. INTRODUCTION

Shifts to large-scale crop and animal productions, as well as growing urban populations, have led to cultural eutrophication of lotic systems mainly through non-point source loading of excess nutrients (Carpenter et al. 1998; Sharpley et al. 1994). Cultural eutrophication is another term given to this type of man-made pollution in aquatic systems. According to the latest United States Environmental Protection Agency's (USEPA) National Water Quality Assessment Report, 53.7% of assessed rivers and streams are impaired, and nutrients are the third highest cause of impairment (USEPA 2014a). Nitrogen (N) and phosphorus (P) are the main nutrients of concern because they are the primary nutrients limiting, and often colimiting, autotrophic production (Dodds et al. 2002; Pringle and Bowers 1984; Tank and Dodds 2003). Autotrophic production in streams is driven by periphyton, which is primarily composed of algae and is important to water quality because of the detrimental impacts excessive amounts can have on aquatic systems (Minshall 1978; Stevenson 2014; Stevenson and Sabater 2010).

The intensified pace of stream alteration through nutrient enrichment can negatively influence ecological communities, as well as society as a whole (Dodds et al. 2013; Smith 2003). Consequences of eutrophication of surface waters may include economic losses (Dodds et al. 2009; Michael et al. 1996), low dissolved oxygen levels (Sabater et al. 2000), potential taste and odor changes to drinking water (WHO 2008), increased exposure to higher levels of NO_3^- (Zhang et al. 2014), the potential for production of harmful algal toxins (USEPA 2010), and trophic shifts in ecosystems and impacts to stream functions (Clapcott et al. 2010; Meyer et al. 2005; Singer and Battin 2007). Increased nutrient concentrations in streams have been found to increase food quality, shifting macroinvertebrate communities toward lower diversity, particular for shredder and collector-gatherer functional groups (Evans-White et al. 2009).

1.1 Nutrient Criteria Development in the U.S.

The realization that governmental regulation of pollution discharge into waterways was necessary led to the passage of the Clean Water Act (CWA) in 1972. The CWA has been very successful at regulating point source pollution, mainly derived from industrial and municipal entities. The main implications of the CWA are that each state is required to develop, monitor, assess, and enforce nutrient standards for all surface waters in said state, and any entity wishing to discharge waste into a waterway is required to obtain a discharge permit through the National Pollution Discharge Elimination System (USEPA 2014b). Unfortunately, the CWA did not address non-point source pollution as stringently as point source pollution.

Water quality standards can either be numeric, meaning that a certain concentration of pollutant is tolerated in a waterbody, or narrative, meaning there is a written description of impairment. Narrative standards often cite a waterbody as impaired when a certain visual change occurs that is not aesthetically pleasing. More recently, narrative nutrient standards have been scrutinized due to difficulty in understanding the exact correlation between the definition of impairment and the visual appearance of a waterbody. For example, narrative nutrient criteria in Arkansas states, "Materials stimulating algal growth shall not be present in concentrations sufficient to cause objectionable algal densities or other nuisance aquatic vegetation or otherwise impair any designated use of the waterbody (APCEC 2014)." Confusion about the actual meaning of words like "objectionable" often makes narrative nutrient standards difficult to quantify and enforce.

In 1998 the USEPA created the Clean Water Action Plan (CWAP) to address non-point source pollution in the United States. The CWAP required the USEPA to gather and evaluate data for 14 designated nutrient ecoregions, based from Omernik (1987) ecoregions, to estimate

potential numeric nutrient standards in the United States by 2001 (USEPA 1998). According to the plan, states were supposed to develop their own numeric nutrient standards for rivers, lakes, and wetlands by 2003 using the USEPA nutrient standards as guidelines; however, many states still only have narrative standards for nutrients as of 2014 according to recent data from the USEPA (USEPA 1998; USEPA 2014c). Debates surrounding lack of data, the uncertainty of the scale used to derive data, and the proper methods for developing numeric nutrient standards have caused setbacks for states (Evans-White et al. 2013). Sound scientific data that can accurately derive numeric nutrient standards for surface waterbodies is warranted (Dodds and Welch 2000).

Several studies, ranging in methods of data collection, analyses, and spatial scales, have been conducted to aid in determining numeric nutrient standards for different areas of the U.S. A recent review of nutrient criteria development in the U.S. described the two primary methods for deriving numeric nutrient standards that have been used in many of these studies– percentile analysis and stressor-response relationships (Evans-White et al. 2013).

1.1.1 *Percentile Analysis*

The USEPA has recommended two ways to use percentile analysis for aiding in the development of numeric nutrient standards using reference streams and non-reference streams (USEPA 2000). The USEPA's reference nutrient standards were developed for each of the 14 nutrient ecoregions by calculating the 25th percentile of the distribution of N and P concentrations found in all streams (reference and non-reference) sampled in each ecoregion, ranging from minimally impacted to highly impacted streams (USEPA 2000). This method assumes that many of the streams sampled are already impaired. Results from the 25th percentile analysis method are suggested upper guidelines derived to aid states and tribes in the process of furthering scientific investigations that refine numeric nutrient criteria. Calculating the 75th

percentile of the distribution of N and P concentrations found only in reference streams, streams with negligible anthropogenic impacts, in a designated area (e.g. nutrient ecoregion) has also been recommended by the USEPA to aid in developing suggested lower numeric nutrient criteria guidelines for further investigation by states and tribes (UESPA 2000). However, finding minimally impacted sites is often difficult. Even streams appearing to be insignificantly disturbed by anthropogenic activities are often impacted (e.g. through atmospheric deposition of various contaminants) and not necessarily representative of nutrient concentrations found in reference streams (Smith et al. 2003). Dodds and Oakes (2004) indicated that when establishing waterbodies as reference streams, land use impacts by humans are often overlooked. The 75th percentile analysis also assumes that 25% of all reference streams are impaired. Additionally, percentile-analysis does not take into consideration nutrient impacts to designated uses but merely evaluates the ranges of nutrient concentrations present in reference and non-reference streams (Smith and Tran 2010).

The 25th and 75th percentile analyses were originally expected to offer similar results; however, Herlihy and Sifneos (2008) and Suplee et al. (2007) observed that results fluctuated between the two analyses. Evans-White et al. (2013) observed that data derived using the 75th percentile analysis method often allowed for the development of more lenient regulations than data from the 25th percentile analysis method. Nevertheless, the percentile analysis method can give investigators a starting point for initiating development of numeric nutrient standards.

The sites used in this study were located within USEPA nutrient ecoregion XI, the Central and Eastern Forested Uplands (CEFU). USEPA suggested nutrient standards for the CEFU are as follows: total phosphorus (TP) 5.63-10.47 µg/L, total nitrogen (TN) 0.21-0.58 mg/L, sestonic chlorophyll-a (chl-a) 0.25-3.26 µg/L (Evans-White et al. 2013).

1.1.2 *Stressor-Response Relationships*

USEPA and leading scientists in the field have recommended that stressor-response data relating to nutrients and ecological impacts be collected for nutrient criteria development (Dodds and Welch 2000; Stevenson 2014; USEPA 2010). The idea behind the stressor-response method is that input of a certain concentration of some resource (e.g. N and P) will cause a dramatic change to occur in an indicator organism's population, often resulting in a linear or threshold response (e.g. increased production or shifts in community structure) (Dodds et al. 2010). Unlike percentile analysis, stressor-response relationships can be used to derive meaningful numeric nutrient criteria that relate to designated uses of streams (Smith and Tran 2010). Phosphorus and chl-a relationships in lakes have been found to exhibit linear responses (Dillon and Rigler 1974), however, linear responses are not always evident in streams. For example, the abundance of two fish species (*Etheostoma spectabile* and *Campostoma anomalum*) in central Texas streams decreased at TP concentrations of 28 and 34 µg/L, while more tolerant fish species (i.e. *Cyprinus carpio* and *Cyprinella lutrensis*) were observed increasing in abundance with increases in TP concentration (Taylor et al. 2014). Groffman et al. (2006) referred to this point of dramatic shift as an ecological threshold. Many statistical programs have been developed to identify ecological thresholds, as discussed by Dodds et al. (2010).

The most prominent ecological indicators used in stressor-response studies include benthic macroinvertebrates, fishes, and algae (i.e. periphyton). Many studies (e.g. Chambers et al. 2012; Dodds et al. 1997; Taylor et al. 2014) have specifically used nutrient impacts to algae metrics to derive stressor-response data, mainly in cobble or gravel dominated streams, due to periphyton's high sensitivity to nutrient enrichment as a primary producer (King et al. 2009). Increased periphyton productivity often results in negative impacts to fishes and benthic

macroinvertebrates as well through large fluctuations in diel dissolved oxygen, changes to habitat, and impacts to quantity and quality of food resources (Heiskary and Markus 2003; Sabater et al. 2000). Van Nieuwenhuyse and Jones (1996) established that there is a positive relationship between sestonic chlorophyll and TP. Lohman and Jones (1999) observed this relationship in Ozark streams, as well as found a positive curvilinear relationship between sestonic chl-a and mean TN and TP using data from 23 study sites. Seventeen sites without known direct sources of nutrient inputs exhibited a linear relationship (Lohman and Jones 1999). Dodds et al. (1997) suggested TN and TP better represented periphyton biomass than dissolved inorganic N or P. Taylor et al. (2014) observed that small increases in TP concentration (≥ 0.021 mg/L) shifted algal communities to more tolerant species in central Texas streams. Stevenson et al. (2012) detected shifts to nuisance algae species in the Illinois River Watershed at TP concentrations of 0.027 mg/L. Results of a study conducted in western U.S. streams suggested TN and TP thresholds of 0.59 to 1.79 mg/L and 0.03 to 0.28 mg/L, respectively, elicited shifts in algal communities to more nutrient tolerant species (Black et al. 2011). Dodds and Welch (2000) suggested TP and TN concentrations of < 0.4 and < 3.0 mg/L, respectively, would control benthic chlorophyll from becoming unsightly or impacting recreational activities. Miltner (2010) observed changes in benthic chl-a at 0.435 and 0.038 mg/L inorganic nitrogen and TP, respectively.

1.2 Stressor-Response Approach to Understanding Periphyton-Nutrient Relationships

1.2.1 *Influence of Spatial Scale on Criteria Development*

Wickham et al. (2005) and Robertson et al. (2006), among others, have suggested that the spatial scale numeric nutrient standards are derived from can impact results. These studies advise against using the nutrient ecoregion scale for developing numeric nutrient standards. Stevenson

(2014) discussed the need for stressor-response studies that take into account regional differences that may impact nutrient concentrations and ecological responses. Longing and Haggard (2010) analyzed watershed-specific nutrient criteria for TP and TN data from the Red River Basin and found that the more spatially restrictive area often yielded different results compared to using the nutrient ecoregion scale. Lohman and Jones (1999) established that incorporating a watershed's land use and size, along with nutrient concentrations, increased the accuracy of predicting sestonic chl-a.

1.2.2 *Periphyton Biomass Limiting Factors*

Dodds and Welch (2000) supported the need for data derived from stressor-response studies using periphyton and nutrients, but cautioned that other factors may limit or impact periphyton biomass responses. Some studies have found variation in algal-nutrient relationships, but they acknowledge other possible limiting factors were not accounted for (Stevenson et al. 2006).

Vannote et al. (1980) described many of the natural factors influencing algal production in streams, such as light availability, slope of the stream, and changes in community structure, with the formation of the River Continuum Concept. Seasonal patterns in algal biomass have been observed, possibly due to changes in light intensity and fluxes in nutrient inputs (Biggs 2000). Hill et al. (2009) observed that after ~ 60 µmol photos/m^2/s algal biovolume stopped increasing linearly and reached photoinhibition at ~ 100 µmol photos/m^2/s. Grazers, such as *Campostoma anomalum*, can have both positive and negative impacts to periphyton through direct consumption, accidental sloughing, and fertilization (Power et al. 1985; Taylor et al. 2012). Flow and flood intensity and frequency can impact algal species composition, grazers, and algal biomass (Biggs 2000; Biggs and Close 1989; Ceola et al. 2013). Biggs and Close

(1989) discovered that flow and nutrients combined explained ~ 63% of the variability in periphyton biomass. Geology and climate are two factors that impact the amount of light, natural background concentration of nutrients, temperature, as well as community composition found in an area (Dodds and Welch 2000; Rohm et al. 2002; Stevenson 1997). Changes in land use to predominately urban and agriculture generally increase nutrient concentrations in streams and shift streams that were historically controlled by heterotrophic production to be dominated by autotrophy (photosynthesis:respiration > 1) (Busse et al. 2006; Broussard and Turner 2009; Bernot et al. 2010).

1.2.3 *Trophic State*

Stevenson et al. (2006) observed that relatively high nutrient concentrations in their study streams led to greater occurrences of the filamentous algae, *Cladophora*. Increased biomass, especially from filamentous algae, was found to decrease the aesthetic appeal of water bodies when maximum benthic chl-a levels reached 100 to 150 mg/m^2 (Welch et al. 1988). Data from surveys conducted by Suplee et al. (2009) found that mean benthic chl-a levels > 200 mg/m^2 were unattractive to the general public, while levels < 150 mg/m^2 were considered more appealing. Investigators have used lentic trophic states (i.e. oligotrophic, mesotrophic, and eutrophic) as guidelines for setting nutrient standards (Dodds et al. 1998). Dodds et al. (1998) initially established a fundamental way to classify streams based on trophic state by using cumulative frequency distributions; however, the authors admitted more empirical data was needed to refine the trophic classification system. Biggs (2000) further suggested that trophic boundaries could shift depending on the days between disturbance and inorganic N and soluble reactive phosphorous (SRP) levels.

1.2.4 *Local Stressor-Response Studies*

The Arkansas Department of Environmental Quality (ADEQ) and the Arkansas-Oklahoma Scenic Rivers Joint Study (SRJS) Committee are conducting two separate stressor-response studies in northern Arkansas and northeast Oklahoma, respectively, to aid in developing regional numeric nutrient standards. Unfortunately, both studies are using different sampling methodologies for collecting data. ADEQ's sampling methodology is modified from the USGS National Water Quality Assessment method (NAWQA; Moulton et al. 2002), while the Arkansas-Oklahoma SRJS sampling methodology is modified from the USEPA Rapid Bioassessment Protocol (Barbour et al. 1999). To my knowledge, no studies have been conducted comparing results from the two commonly used sampling methodologies. Method comparison is warranted to ensure that numeric nutrient standards are developed from the most accurate datasets and to possibly offer a way to link results from studies that use different methods for data collection.

Arkansas Department of Environmental Quality is in the process of gathering physical, chemical, and biological data for Extraordinary Resource Waters (ERW) in the six Level III Ecoregions found in Arkansas to aid in understanding stressor-response relationships for regional numeric nutrient criteria development. Specifically, ADEQ began collecting data for 10 ERW in the Ozark Highlands in 2013 and finished with data collection in 2014 and began sampling 11 ERW in the Boston Mountains in 2014 and finished sampling in 2015. The sampling method used collects periphyton biomass data (i.e. ash-free dry mass (AFDM) and chl-a) by gathering five haphazardly selected rocks each from the head and toe of two riffles at individual stream sites (ADEQ 2014, unpublished). Closed canopy reaches are not avoided. Known areas from the

top surface of each rock are scraped to retrieve the periphyton using a rubber delimiter, scoopula, brush with plastic bristles, and stream water.

The Arkansas-Oklahoma SRJS is located in the Illinois River Basin in Northeast Oklahoma and Northwest Arkansas and focuses on the Oklahoma designated scenic rivers. The goal of the study is to collect chemical, physical, and biological data in 35 wadeable streams to determine possible TP ecological thresholds for the Illinois River Basin. The study began in April 2014 and is set to conclude July 2016. Five rocks (10-20 cm^2) are systematically selected each from three minimally shaded transects in riffle-glide habitat. The periphyton from the top surface of each rock is collected with metal bristled brushes and stream water, and the top surface area of each rock is recorded with aluminum foil for later surface area calculations. Periphyton samples are analyzed for AFDM and chl-a content.

1.2.5 *Need for Experimental Studies*

When nutrient gradients are small, such in the ERW sampled by ADEQ, observing algal biomass responses may be challenging. Studies have also discussed the importance of controlling environmental variables known to confound algal biomass-nutrient relationships (Biggs and Close 1989; Hill et al. 2009; Rosemond et al. 2000). Experimental algal biomass-nutrient studies can remedy these issues by allowing researchers to observe algal biomass responses across chosen nutrient gradients with the option to control for other environmental variables (e.g. variation in light, temperature, grazing, and flow). Watershed-specific studies have also been recommended (Smith et al. 2003; Smucker et al. 2013). Although experimental nutrient manipulation studies have been done, no watershed-specific studies have been conducted within the Buffalo River Watershed, which is valued for its aesthetic, economic, and biological attributes (Panfil and Jacobson 2001).

1.3 Study Objectives

The objectives of this thesis were (1) to compare two common periphyton sampling methodologies, the modified USEPA Rapid Bioassessment and the modified NAWQA protocols, and (2) to conduct a nutrient enrichment experiment in streamside mesocosms to better understand periphyton biomass-nutrient relationships within a select southern Ozarks watershed. Physical, chemical, and biological data, including biomass and nutrient data, were collected in selected Ozark streams and in streamside mesocosm for this thesis.

1.4 References

APCEC (Arkansas Pollution Control and Ecology Commission) (2014). *Regulation No. 2*. Arkansas Department of Environmental Quality. Little Rock, AR.

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish*. EPA 841/B-99/022. Office of Water, US Environmental Protection Agency, Washington, D.C.

Bernot, M. J., Sobota, D. J., Hall, R. O., Mulholland, P. J., Dodds, W. K., Webster, J. R., Tank, J. L., et al. (2010). Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology, 55*, 1874–1890.

Biggs, B. J. F., & Close, M. E. (1989). Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology, 22*, 209–231.

Biggs, B. J. F. (2000). Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of North American Benthological Society, 19*(1), 17–31.

Black, R. W., Moran, P. W., & Frankforter, J. D. (2011). Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams. *Environmental Monitoring and Assessment, 175*, 397–417.

Broussard, W., & Turner E. R. (2009). A century of changing land-use and water-quality relationships in the continental US. *Frontiers in Ecology and the Environment, 7*, 302–307.

Busse, L. B., Simpson, J. C., & Cooper, S. D. (2006). Relationships among nutrients, algae, and land use in urbanized southern California streams. *Canadian Journal of Fisheries and Aquatic Sciences, 63*, 2621–2638.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*, 559–568.

Ceola, S., Hödl, I., Adlboller, M., Singer, G., Bertuzzo, E., Mari, L., Botter, G., Waringer, J., Battin, T. J., & Rinaldo, A. (2013). Hydrologic variability affects invertebrate grazing on phototrophic biofilms in stream microcosms. M. R. Liles (Editor). *PLoS ONE, 8*, 1–11.

Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A. (2012). Development of environmental thresholds for nitrogen and phosphorus in streams. *Journal of Environment Quality, 41*, 7–20.

Clapcott, J. E., Young, R. G., Goodwin, E. O., & Leathwick, J. R. (2010). Exploring the response of functional indicators of stream health to land-use gradients. *Freshwater Biology, 55*, 2181–2199.

Dillon, P. J. & Rigler, F. H. (1974). The phosphorus-chlorophyll relationship in lakes. *Limnology and Oceanography, 19*(5), 767-773.

Dodds, W. K., Smith, V. H., & Zander, B. (1997). Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. *Water Research, 31*, 1738–1350.

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

Dodds, W. K., & Welch, E. B. (2000). Establishing nutrient criteria in streams. *Journal of North American Benthological Society, 19*, 186–196.

Dodds, W. K., Smith, V. H., & Lohman, K. (2002). Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences, 59*, 865–874.

Dodds, W. K., & Oakes, R. M. (2004). A technique for establishing reference nutrient concentrations across watersheds affected by humans. *Limnology and Oceanography, 2*, 333–341.

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser, J. T., & Thornbrugh, D. J. (2009). Eutrophication of U. S. freshwaters: analysis of potential economic damages. *Environmental Science & Technology, 43*, 12–19.

Dodds, W. K., Clements, W. H., Gido, K., Hilderbrand, R. H., & King, R. S. (2010). Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. *Journal of the North American Benthological Society, 29*, 988–97.

Dodds, W. K., Perkin, J. S., & Gerken, J. E. (2013). Human impact on freshwater ecosystem services: a global perspective. *Environmental Science & Technology, 47*, 9061–9068.

Evans-White, M. A., Dodds, W. K., Huggins, D. G., & Baker, D. S. (2009). Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. *Journal of the North American Benthological Society, 28*, 855-868.

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality, 42*, 1002–14.

Groffman, P. M., Baron, J. S., Blett, T., Gold, A. J., Goodman, I., Gunderson, L. H., Levinson, B. M., et al. (2006). Ecological thresholds: the key to successful environmental management or an important concept with no practical application? *Ecosystems, 9*, 1–13.

Heiskary, S., & Markus, H. (2003). *Establishing relationships among in-stream nutrient concentrations, phytoplankton abundance and composition, fish and macroinvertebrate indices, and biochemical oxygen demand in Minnesota USA rivers*. Minnesota Pollution Control Agency, Environmental Outcomes Division, St. Paul, MN.

Herlihy, A. T., & Sifneos, J. C. (2008). Developing nutrient criteria and classification schemes for wadeable streams in the conterminous US. *Journal of the North American Benthological Society, 27*, 932–948.

Hill, W. R., Fanta, S. E., & Roberts, B. J. (2009). Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography, 54*, 368–80.

King, R. S., & Winemiller, K. O. (2009). Development of biological indicators of nutrient enrichment for application in Texas streams. 106 Water Pollution Control Grant#98665304, Baylor University, Waco, Texas, USA.

Lohman, K., & Jones, J. R. (1999). Nutrient-sestonic chlorophyll relationships in northern Ozark streams. *Canadian Journal of Fisheries and Aquatic Sciences, 56*, 124–130.

Longing, S. D., & Haggard, B. E. 2010. Distributions of median nutrient and chlorophyll concentrations across the Red River Basin, USA. *Journal of Environmental Quality, 39*, 1966–1974.

Meyer, J. L., Paul, M. J., & Taulbee ,W. K. (2005). Stream ecosystem function in urbanizing landscapes. *Journal of North American Benthological Society, 24*, 602–12.

Michael, H. J., Boyle, K. J., & Bouchard, R. (1996). *Water quality affects property prices: a case study of selected Maine lakes*. Miscellaneous Report 298. University of Maine, Main Agricultural and Forest Experiment Station, ME.

Miltner, R. J. (2010). A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. *Environmental Management, 45,* 842-855.

Minshall, G. W. (1978). Autotrophy in stream ecosystems. *Bioscience, 28*, 767–770.

Moulton II, S. R., Kennen, J. G., Goldstein, R. M., & Hambrook, J. A. (2002). *Revised protocols for sampling algal, invertebrate, and fish communities as part of the national water-quality assessment program*. Open-File Report 02-150. U.S. Department of the Interior, U.S. Geological Survey, Reston, Virginia.

Omernik, J. M. (1987). Ecoregions of the conterminous United States. *Ann. Assoc. Am. Geogr*., *77*, 118–125.

Panfil, M. S., & Jacobson, R. B. (2001). *Relations Among Geology, Physiography, Land Use, and Stream Habitat Conditions in the Buffalo and Current River Systems, Missouri and Arkansas*. ISSN 1081-292X. US Department of the Interior, US Geological Survey, Biological Resources Division, Columbia Environmental Research Center, Columbia, Missouri.

Power, M., Matthew, W., & Stewart, A. (1985). Grazing minnows, piscivorous bass, and stream algae : dynamics of a strong interaction. *Ecology, 66*, 1448–1456.

Pringle, C. M., & Bowers, J. A. (1984). An in situ substratum fertilization technique: diatom colonization on nutrient-enriched, sand substrata. *Canadian Journal of Fisheries and Aquatic Sciences, 41*, 1247–1251.

Robertson, D. M., Saad, D. A., & Heisey, D. M. (2006). A regional classification scheme for estimating reference water quality in streams using land-use-adjusted spatial regression-tree analysis. *Environmental Management, 37*, 209–229.

Rohm, C. M., Omernik, J. M., Woods, A. J., & Stoddard, J. L. (2002). Regional characteristics of nutrient concentrations in streams and their application to nutrient criteria development. *Journal of American Water Resources Association, 38*, 213–39.

Rosemond, A. D., Mulholland, P. J., & Brawley, S. H. (2000). Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(1), 66–75.

Sabater, S., Armengol, J., Comas, E., Sabater, F., Urrizalqui, I., & Urrutia, I. (2000). Algal biomass in a disturbed Atlantic river: water quality relationships and environmental implications. *The Science of the Total Environment, 263*, 185–195.

Sharpley, A. N., Chapra, S. C., Wedepohl, R., Sims, J. T., Daniel, T. C., & Reddy, K. R. (1994). Managing agricultural phosphorus for protection of surface waters: issues and options. *Journal of Environment Quality, 23*, 437–451.

Singer, G. A., & Battin, T. J. (2007). Anthropogenic subsidies alter stream consumer-resource stoichiometry, biodiversity, and food chains. *Ecological Applications, 17*, 376-389.

Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems: a global problem. *Environmental Science and Pollution Research International, 10*(2), 126–139.

Smith, R. A., Alexander, R. B., & Schwarz, G. E. (2003). Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology, 37*, 3039–3047.

Smith, R. A., & Tran C. R. (2010). A weight-of-evidence approach to define nutrient criteria protective of aquatic life in large rivers. *Journal of the Northa American Benthological Society, 29*, 875-891.

Smucker, N. J., Becker, M., Detenbeck, N. E., & Morrison, A. C. (2013). Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. *Ecological Indicators*, *32*, 51–61.

Stevenson, R. J. (1997). Scale-dependent determinants and consequences of benthic algal heterogeneity. *Journal of the North American Benthological Society, 16*(1), 248–262.

Stevenson, R. J., & Sabater, S. (2010). Understanding effects of global change on river ecosystems: science to support policy in a changing world. *Hydrobiologia, 657*, 3-18.

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

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

Stevenson, J. (2014). Ecological assessments with algae: a review and synthesis. L. Graham (Editor). *Journal of Phycology, 50*, 437–461.

Suplee, M. W., Varghese, A., & Cleland, J. (2007). Developing nutrient criteria for streams: an evaluation of the frequency distribution method. *Journal of the American Water Resources Association, 43*, 453–472.

Suplee, M. W., Watson, V., Teply, M., & McKee, H. (2009). How Green is too green? Public opinion of what constitutes undesirable algae levels in streams. *Journal of the American Water Resources Association, 45*, 123–140.

Tank, J. L., & Dodds,W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology, 48*, 1031–1049.

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

Taylor, J. M., King, R. S., Pease, A. A., & Winemiller, K. O. (2014). Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment. *Freshwater Biology, 59*, 969–984.

Van Nieuwenhuyse, E. E., & Jones, J. R. (1996). Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area. *Canadian Journal of Fisheries and Aquatic Sciences, 53*, 99–105.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences, 37*, 130-137.Welch, E. B., Jacoby, J. M., Horner, R. R., & Seeley, M. R. (1988). Nuisance biomass levels of periphytic algae in streams. *Hydrobiologia, 157*, 161–168.

Wickham, J. D., Riitters, K. H., Wade, T. G., & Jones, K. B. (2005). Evaluating the relative roles of ecological regions and land-cover composition for guiding establishment of nutrient criteria. *Landscape Ecology, 20*, 791–798.

WHO (World Health Organization). (2008). Acceptability aspects. Pages 210-220. In *Guidelines for drinking-water quality*. 3rd edition.

USEPA (US Environmental Protection Agency). (1998). *EPA National Strategy for the Development of Regional Nutrient Criteria*. EPA-822-R-98-002. US Environmental Protection Agency, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2000). *Nutrient Criteria Technical Guidance Manual Rivers and Streams*. EPA-822-B-00-002. US Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2010). *Using Stressor-response Relationships to Derive Numeric Nutrient Criteria*. EPA-820-S-10-001. US Environmental Protection Agency, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2014a). National water quality assessment report. http://ofmpub.epa.gov/waters10/attains_nation_cy.control (Accessed 11 December 2014).

USEPA (US Environmental Protection Agency). (2014b). Clean water act. http://www.epa.gov/agriculture/lcwa.html#National%20Pollutant (Accessed 11 December 2014).

USEPA (US Environmental Protection Agency). (2014c). State development of numeric criteria for nitrogen and phosphorus pollution. http://cfpub.epa.gov/wqsits/nnc-development/ (Accessed 11 December 2014).

Zhang, Y., Zhang, Q., Li, F., Liu, Q., & Li, J. (2014). Tracing nitrate pollution sources and transformation in surface- and ground-waters using environmental isotopes. *The Science of the Total Environment, 490*, 213-222.

2. COMPARING TWO PERIPHYTON COLLECTION METHODS COMMONLY USED FOR STREAM BIOASSESSMENT AND THE DEVELOPMENT OF NUMERIC NUTRIENT STANDARDS

2.1 Introduction

Coping with the consequences of living in an anthropogenically-induced greener world due to excess nitrogen (N) and phosphorus (P) inputs into waterways is a reality that scientists and the public alike must acknowledge (Carpenter et al. 1998; Sharpley et al. 1994; Dodds et al. 2013). Indeed, a survey of US streams found 42% of assessed stream lengths to be in relatively poor condition, with N and P being two of the top four causes of impairment (Paulsen et al. 2008). The United States Environmental Protection Agency (USEPA) has requested states and tribes use empirically generated numeric nutrient criteria to assess accelerated eutrophication of streams, lakes, and estuaries (USEPA 1998). Even though the USEPA charged states and tribes with developing these criteria by 2003, as of December 2015, Hawaii is the only state that the USEPA recognizes as having both N and P criteria for all waterbody types (USEPA 2015). Specifically pertaining to streams, five states have partial N and P criteria (Arizona, California, Florida, Montana, and Nevada), three states have partial P criteria (New Mexico, Oklahoma, and Vermont), four states have statewide P criteria (Wisconsin, New Jersey, Minnesota, and Hawaii), and only Hawaii has statewide N criteria (USEPA 2015). Lack of data availability and issues related to the spatial scale of studies, and proper data collection methods, have contributed to delays in criteria development (Evans-White et al. 2013).

One way the USEPA recommends states and tribes develop nutrient criteria for streams is through the use of predictive relationships that may be identified using bioassessment (USEPA 2000). Predictive relationships, also known as stressor-response analyses, rely on a stressor variable (e.g. N and P concentrations) and a response variable (e.g. fish, macroinvertebrate,

and/or microbial assemblages) for determining potential thresholds that contribute to impairment of particular designated uses (USEPA 2010). Periphyton is a complex assemblage of algae, cyanobacteria, heterotrophic microorganisms, and detritus that are attached to solid submersed substrates in aquatic ecosystems (Stevenson et al. 1996). Periphyton are of particular interest in stream bioassessment for developing nutrient water quality criteria because primary production should be directly influenced by nutrient availability (Dodds et al. 2002). Periphyton assemblage and biomass (e.g. mean and maximum chlorophyll-a (chl-a) and mean and maximum ash-free dry mass (AFDM)) have been used extensively in the literature as response variables to N or P as stressor variables (Evans-White et al. 2013). However, confounding factors, such as canopy cover, grazing, watershed land use, and stream flow can influence nutrient-periphyton relationships and must also be taken into consideration during bioassessment (Biggs 2000; Rosemond et al. 2000; Wickham et al. 2005).

Multiple methods have been used throughout the United States for collecting periphyton biomass in the field (Aloi 1990; Stevenson and Bahls 1999; Lowe and LaLiberte 2007). The most common differences between methods include: 1) whether artificial or natural substrates are used, 2) how the substrate are selected, 3) the stream habitat (riffle, run, glide, pool, margin) from which periphyton are collected, and 4) the size of the periphyton removal area. Unfortunately, no consensus exists about which method, if any, is most appropriate to use. Problems may arise if and/or when datasets are combined from multiple studies that use different data collection methods because the various methods could be biased differently relative to confounding factors. For example, periphyton biomass would be highly variable if site selection did not control for canopy cover because some stream reaches can be heavily shaded and others not. A comparative study of periphyton collection methods could benefit stream bioassessment

and nutrient criteria development by clarifying the appropriateness of combining measurements derived from different sampling protocols.

Two stressor-response studies using different data collection methods in the southern Ozarks of Oklahoma and Arkansas were conducted between 2013 and 2016 and highlight three of the four previously mentioned differences among common methods. The first study was conducted by the Arkansas Department of Environmental Quality (ADEQ) to develop nutrient criteria for Arkansas' Extraordinary Resource Waters of Arkansas' Ozark Highlands and Boston Mountains. ADEQ used a modified version of the USGS National Water Quality Assessment method (NAWQA; Moulton II et al. 2002) for sampling periphyton, which has also been adopted by other state agencies such as the New Mexico Environment Department (NMED 2014). Periphyton is collected from a known area using a delimiter from haphazardly selected rocks for this sampling method. The second study, overseen by the Arkansas-Oklahoma Scenic Rivers Joint Study (SRJS) Committee, focused on the Oklahoma-designed scenic rivers located in northeastern Oklahoma and northwestern Arkansas. The Arkansas-Oklahoma SRJS used a modified version of the USEPA periphyton sampling methods, which collects periphyton from the entire top surface of systematically selected rocks (Barbour et al. 1999; King 2014). The goal of both studies was to develop stressor-response relationships between total P (TP) in stream water and periphyton biomass. We were interested in determining if the different methods used in these two studies would yield similar results so the data could potentially be combined into a regional stressor-response in the future (Smith et al. 2003; Smucker et al. 2013). Thus, the objective of this study was to compare the two periphyton sampling methodologies to determine if and how results varied with known confounding factors. Due to the potential for greater experimenter error with the periphyton sampling protocol used by the ADEQ Extraordinary

Resource Waters Study (i.e. potential experimenter bias in the rock selection process and during delimiter placement on rocks) and other differences in collection procedures between the two methods, we hypothesized biomass results would be different between methods.

2.2 Materials And Methods

2.2.1 *Study Area*

The Ozarks, a level II Omernik ecoregion located in the United States, is composed of two level III ecoregions, the Boston Mountains to the south and the Ozark Highlands to the north (Omernik 1987). The dominant geology of the Ozark Highlands is limestone and dolomite, while the Boston Mountains is mainly composed of sandstone, shale, and siltstone (The Nature Conservancy 2003). Oak (*Quercus*), hickory (*Carya*), and pine (*Pinus*) forests dominate the Ozarks. Mean temperatures range from 8.7 to 22 °C, and mean precipitation is 115 cm per year (U.S. Climate Data 2016).

Seven southern Ozark streams were designated study sites due to similarities in geology, drainage area, land use, and proximity to the two Ozark stressor-response studies (Table 2.1; Fig. 2.1). Drainage areas ranged from 130 km^2 at Cave Creek to 368 km^2 at the Little Buffalo River (USGS 2015). Land use was predominantly forested in all watersheds, ranging from 75.4% to 94.3% at Bear Creek and the White River, respectively (Table 2.1). Sampled stream sections were primarily gravel and cobble dominated, which is a common stream condition throughout the Ozarks (Panfil and Jacobson 2001). All sample streams originate in the Boston Mountains and drain north into the Ozark Highlands (Fig. 2.1). Big Creek, Cave Creek, Little Buffalo River, and Bear Creek are also tributaries of the Buffalo River, which is designated as an Extraordinary Resource Water by the state of Arkansas. The study streams were centrally located between the Arkansas-Oklahoma SRJS field sites and the ADEQ Extraordinary Resource Waters field sites

(Fig. 2.1). We originally sought study streams along an anthropogenic nutrient concentration gradient, but preliminary analysis of stream water samples revealed similar ranges in total N (TN) and TP across all sample sites throughout the study (Table 2.1). Thus, the relationships between algal biomass and nutrient concentrations were not evaluated in this study. Instead, the primary goal was to investigate potential periphyton biomass variability across methods.

2.2.2 *In-Stream Sampling and Data Collection*

The modified NAWQA protocol used by ADEQ is referred to here as "Method A". This method involved collecting periphyton from small delimited areas on various sized cobble substrate from streams (Aloi 1990). Alternatively, the study overseen by the Arkansas-Oklahoma SRJS, referred to here as "Method B", involved collecting periphyton from the entire top surface of systematically selected cobble substrate and estimating the surface area from which periphyton were removed (Barbour et al. 1999; King 2014). Other major differences between the two methods include the type of stream habitat sampled, cobble selection method, cobble size, number of cobbles collected, and canopy cover preference (Table 2.2). Methods A and B were used to collect periphyton from each study stream over a one-year period and specific details of each sampling method are provided below. Periphyton sampling commenced in mid-August 2014 with consecutive sampling occurring roughly every three months thereafter (i.e. November 2014, February 2015, June 2015, and August 2015) to capture seasonal changes. Flow, habitat characteristic data, and water chemistry data were collected during each sampling event. We used both methods to collect periphyton on the same day in each stream. All seven streams were sampled within a two-week time period during each season, unless unforeseen weather-related circumstances prevented sampling due to safety issues or periphyton scouring events. When major scouring events were observed, sampling was postponed two weeks to allow periphyton

regeneration. We measured flow in a single location during each quarterly sampling event using the mid-point method with a Marsh-McBirney Inc. Flo-mate (model #2000, Frederick, MD). Water samples were collected from each site monthly for nutrient analysis.

Method A

We selected riffles by visual observation of relatively turbulent water velocity and retrievable substrate and avoided areas were periphyton had been scoured or vegetation covered the streambed. No preference to canopy cover was given when making riffle selections. Two riffles were selected at each stream, with the riffle at the lowest downstream location sampled for periphyton first. An attempt was made to sample upstream of bridges; however, this was often unavoidable due to study site characteristics. The head and toe of each riffle were identified using best professional judgment. Five evenly spaced rocks representative of the streambed environment were collected in an approximate straight line from the right to left bank across the head and toe of each riffle. Rocks were placed in clean white containers as they were collected in the stream and covered with stream water to prevent periphyton drying out. We avoided selecting rocks that had obviously been scoured or were embedded in the streambed. A total of 20 primarily cobble-sized rocks (10 rocks/riffle) were collected per stream for this method. Size was not a main consideration when making rock selections; however, as mentioned previously, the stream reaches were generally dominated by gravel to cobble sized rocks.

A rubber gasket (i.e. delimiter) with a 6.35 cm outer diameter and a 2.54 cm inner diameter was placed on the top surface of each rock (Fig. 2.2a). The periphyton inside the inner area of the gasket was removed (Fig. 2.2b) and placed into a clean white container using a metal scoopula and/or a small brush with plastic bristles using as little stream water as possible for rinsing. Care was taken to minimize detachment of periphyton outside of the designated removal

area. Periphyton removed from the 10 rocks collected from each riffle were combined into a single sample so that a periphyton sample was obtained for each of the two riffles. Periphyton samples from each riffle were stored separately in labeled one-liter acid washed dark bottles and placed on ice until transported to the laboratory at the University of Arkansas, Fayetteville, AR.

Habitat characteristic data were recorded for each riffle. We recorded cross-sectional area at 10 evenly spaced points along the total riffle length by measuring the stream depth at mid channel and near the left and right banks. We estimated mean seasonal velocity for Method A habitat in each stream using the cross-sectional area measurements and stream flow. Percent closed canopy cover was measured using a concave densiometer at the lower, middle, and upper sections of each riffle.

Method B

We selected riffle-run habitat at each site with relatively open canopy cover and cobble substrate. We avoided areas in streams that exhibited obvious periphyton scouring, any rooted vegetation, and depths greater than 30 cm. The number of riffles selected for sampling varied depending on the availability of ideal sampling habitat. For example, sometimes one long riffle-run was selected, but other times three separate riffles were selected and sequentially sampled from downstream to upstream. As with Method A, attempts were made to reduce the number of samples collected directly downstream of bridges; however, this was often unavoidable. Three transects were placed along the wetted width of the selected riffle-run habitat, with the first transect placed farthest downstream. Transects were positioned a minimum distance apart, which was no less than the length of the longest transect. Starting with the most downstream transect and progressing to the consecutive upstream transects, five large washers with flagging tape (referred to as “markers”) were systematically placed in the stream equal distances apart along

each transect. One cobble-sized rock (10-20 cm^2) was collected within a 1 m^2 area of each marker that best represented periphyton growth in that respective part of the stream. Embedded rocks were avoided. Selected rocks were placed in clean white containers organized by transect number, and containers were filled with stream water to avoid periphyton drying out. Rocks were photographed with identifying information to visually document periphyton biomass.

Periphyton were removed from the entire top surface of each rock using a small brush with wire bristles and a minimal volume of stream water. The resulting slurry was compiled for all transects into one-liter acid washed dark bottles and placed on ice until transported to the laboratory at the University of Arkansas, Fayetteville, AR. Aluminum foil was trimmed to match the area scraped on each rock. A linear regression of area by weight of the aluminum foil was used to convert foil weight to area of each rock scraped.

Percent canopy cover and velocity were recorded at the center marker of each transect using a concave densitometer and Marsh-McBirney Inc. Flo-mate, respectively. Depth and qualitative measurements, such as dominant substrate, sedimentation on a scale of 1 to 20, percent embeddedness, and percent filamentous cover, were recorded at each marker. We estimated mean seasonal velocity for Method B habitat in each stream using measured mean wetted width and mean depth from the riffle habitats and the stream flow measured as described previously.

2.2.3 *Laboratory Analyses*

Periphyton samples from both sampling methods were individually homogenized with a handheld blender, and total slurry volumes were recorded for each sample per stream within 48 hours of sample collection. Each slurry was separately mixed on a magnetic stirring plate, and subsamples of the mixed slurries were individually filtered onto 25 mm Whatman glass fiber

filters (GF/F) for analysis of periphyton biomass as chl-a. Subsamples were also filtered onto preweighed 47 mm Whatman GF/F that had been combusted at 450 °C for AFDM analysis. All samples were then frozen at -20 °C for analysis on subsequent dates. A Turner Designs Trilogy Fluorometer (model #7200-000, Sunnyvale, CA) was used to analyze samples for periphtyon chl-a following the acetone extraction method (APHA 2005; #10200 H). Ash-free dry mass was estimated following sample drying and ashing at 450 °C (APHA 2005; #10300 C). Subsample chl-a and AFDM results and the calculated area of periphyton removal were used to compute biomass collected per m^2 for slurries amassed using each method. Water samples were analyzed for TN and TP on a Skalar San++ Continuous Flow Analyzer (Skalar Inc., The Netherlands) (APHA 2005; #4500-N C and #4500-P F) at the Arkansas Water Resource Center's Certified Laboratory, Fayetteville, AR, following persulfate digestion (APHA 2005; #4500-P J).

2.2.4 *Data Analysis and Statistics*

Samples collected using Method A yielded two periphyton biomass measurements (one per riffle) for each stream reach sampled on each date, while method B yielded just one periphyton biomass measurement for each reach on each date. Preliminary data analysis using Welch two sample t-tests revealed no significant difference in periphyton biomass (chl-a or AFDM) between upstream and downstream riffles sampled using Method A ($p = 0.638$, $t = 0.474$, and $df = 63.9$ and $p = 0.559$, $t = 0.587$, and $df = 55.8$, respectively). Thus, the average periphyton chl-a and AFDM from both riffles were used to represent results from Method A.

To compare results from the two methods, the Mixed Procedure in SAS (Version 9.4; Cary, NC) was used to run a repeated measures analysis of variance (ANOVA) with a random block design for both periphyton AFDM and chl-a. Because there was little variation in TP among streams, we had no a-priori hypothesis about potential differences in periphyton biomass

among streams. We considered stream to be a random effect for this reason and to account for potential autocorrelation. Method was the main effect, and time was treated as a repeated measure. Potential method and time interactions were taken into consideration using an unstructured covariance matrix. The model assumed that all correlations and variances could be different. Two chl-a datapoints (War Eagle Creek in August 2014 and White River in August 2015) and three AFDM datapoints (War Eagle Creek in June 2015, White River in June 2015 and August 2015) were excluded in the final data analysis because insufficient material was collected onto filters, which caused measurement errors near the level of detection for the instrumentation.

In addition to the statistical comparison of methods, we also computed paired chl-a, AFDM, velocity, and canopy cover differences between methods by subtracting Method B results from Method A results. This was done to graphically examine the degree of difference between methods across seasons and explore any possible relationships between numerical differences in periphyton biomass and numerical differences in variables that we expected to potentially confound the direct nutrient control of periphyton biomass in streams. We also calculated the absolute value of each biomass difference and the 5^{th} and 25^{th} percentiles of all chl-a and AFDM measurements collected throughout the study from all streams and methods. From these calculations, the percentage of absolute values below each respective percentile of all chl-a and AFDM measurements was determined.

2.3 Results

2.3.1 *Method and Time Comparisons*

Estimated chl-a and AFDM did not differ between Method A and Method B ($p = 0.123$ and $p = 0.550$, respectively). Additionally, neither chl-a nor AFDM differed between methods

over time ($p = 0.270$ and $p = 0.200$, respectively). However, chl-a and AFDM differed through time, regardless of sampling method ($p < 0.001$ and $p = 0.012$, respectively). Results of the repeated measures ANOVA are shown in Table 2.3.

The seasonal variability in periphyton biomass created a range in chl-a and AFDM that demonstrated the proportionality of Methods A and B (Figs. 2.3a,b). Interestingly, the chl-a results from Method A were often greater than the chl-a results from method B (i.e. many data < 1:1 line in Fig. 2.3a). The trend in numerical differences between methods was primarily driven by observations in November, February, and June (Fig. 2.4a,b). However, there were no numerical differences in AFDM (Fig. 2.4c,d), and the repeated measures ANOVA indicated that the numerical differences in chl-a between methods were not statistically significant (Table 2.3).

Because periphyton biomass did not differ between methods, results from both methods were combined into a one-way ANOVA to compare specific differences among seasons (i.e. August as summer, November as fall, February as winter, and June as spring) using least squares means differences. Mean chl-a collected from all streams using both methods was greatest in November and February (116.7 and 108.8 mg/m^2, respectively) and least in June and August (60.0 and 70.6 mg/m^2, respectively) (Fig. 2.5a). Mean AFDM collected from all streams using both methods was also greatest in November and February (69.8 and 58.4 g/m^2, respectively) and least in June and August (40.0 and 38.7 g/m^2, respectively) (Fig. 2.5b). However, AFDM measured in February did not differ from that measured in June and August 2015. February AFDM was greater than AFDM measured in August 2014 by 23.6 g/m^2.

2.3.2 *Relationships with Confounding Factors*

Mean discharge throughout the study was lowest in September 2014 (0.20 m^3/s) and peaked in May 2015 (23.3 m^3/s) before the June sampling event (Table 2.4). Mean velocity

differences between methods across all sites, calculated by subtracting Method B velocity values from Method A velocity values, ranged from -1.4 m/s in June to 0.5 m/s in November (Fig. 2.6a). The most variability in numerical velocity differences between methods was in June, but numerical velocity differences were minimal throughout the study (Fig. 2.6a). A paired t-test showed there was no difference in velocity of the habitats sampled for either method ($p = 0.511$, $t = -0.665$, and $df = 32$). We also observed similarities in percent closed canopy cover between habitats sampled using both methods in each stream throughout the study (Fig. 2.6b). Specifically, mean percent closed canopy cover from each stream across all seasons ranged from 17.5% in February to 22.9% in August 2015 and 16.6% in November to 26.1% in August 2014 for habitats sampled using Method A and Method B, respectively. Mean differences in percent closed canopy cover between habitats sampled using both methods across all streams, calculated by subtracting Method B canopy cover values from Method A canopy cover values, ranged from -1.5% in November to 4.5% in August 2014 (Fig. 2.6b). A paired t-test showed no difference in canopy cover of the habitats sampled for either method ($p = 0.588$, $t = 0.547$, and $df = 33$). Thus, mean percent canopy cover of habitats sampled using both data collection methods did not explain small numerical differences between methods either in chl-a or AFDM data collected from each stream across seasons.

2.4 Discussion

Methods that quantify periphyton biomass are extremely important in stream bioassessment and in developing numeric nutrient standards. However, existing methods for quantifying periphyton are diverse and may not address common confounding factors equally. Results of this study indicated the whole-surface and the delimiter-reduced periphyton removal methods used by the Arkansas-Oklahoma SRJS and ADEQ, respectively, yielded similar results

at the level II ecoregion scale and across seasons. The lack of difference between methods was notable because there was significant seasonal variability in periphyton biomass, regardless of which method was used.

2.4.1 *Were methods comparable throughout the study and temporally?*

Results suggested chl-a and AFDM datasets collected using Method A and Method B provided comparable results across sample sites and seasons. Although numerical variation in measurements were observed between methods, the magnitude of these differences was small compared to the magnitude of variability in periphyton biomass over the course of the study. For example, 45% of the absolute value of all chl-a difference values and 63% of the absolute value of all AFDM difference values were equal to or less than the 5th percentiles of all chl-a and AFDM measurements collected from all streams throughout the entire study. Variations in chl-a and AFDM values between methods were relatively small when considering overall chl-a and AFDM measurements. Seventy percent of the absolute value of all chl-a difference values and 75% of the absolute value of all AFDM difference values were equal to or less than the 25th percentiles of all chl-a and AFDM measurements collected from all streams throughout the study, respectively. Thus, the variation between methods was not statistically significant, and the numerical variation among methods was small compared with the numerical (and statistically significant) variation in periphyton biomass among seasons.

2.4.2 *Seasonal Influences on Periphyton Biomass*

Temporally changing biological processes are known to influence periphyton biomass accrual in streams (e.g. temperature, grazing, flow, light and nutrient availability) and have been well documented in the literature (Ceola et al. 2013; Lange et al. 2011; Winkelmann et al. 2014). Periphyton biomass was greatest during the cool seasons when water temperatures were less than

or equal to 10 °C. Periphyton growth rates typically increase with water temperature up to approximately 30 °C (DeNicola, 1996). Specifically, increased sloughing and reduced biomass of filamentous algae, primarily *Cladophora*, has been observed with temperature increases between 23.5 and 30 °C and may have contributed to the decreasing trend in periphyton biomass during the warmer months of June and August (Dodds and Gudder 1992; Whitton 1970). However, the effect of temperature was probably indirect because temperatures greater than or equal to 30 °C were minimal during our study. Thus, greater periphyton biomass in November and February was perhaps caused by decreased grazing pressures or variable light availability due to changing deciduous tree canopies (Hillebrand 2009; Quinn et al. 1997).

Riparian vegetation is known to influence the amount of photosynthetically active radiation (PAR) available to periphyton communities, which can control biomass accumulation (Lowe et al. 1986; Hill 1996). The Ozarks are dominated by deciduous forest (The Nature Conservancy 2003), which can translate to seasonally dynamic light conditions in streams (Halvorson et al. in-press). Although operational guidelines for collecting canopy cover measurements were less strict for Method A, canopy cover results happened to be similar between Method A and Method B throughout the entire study. This result, along with the fact that we targeted open-canopy environments with Method B, suggests canopy cover was not the primary factor influencing seasonal differences in periphyton biomass in this study. Nutrient concentrations remained relatively similar for each stream throughout the duration of the study, but the availability of reactive nutrients was not measured (Dodds et al. 2002). Flow conditions also varied seasonally (Table 2.4), and increased flow has been shown to increase periphyton biomass (Biggs et al. 2005). However, a conflicting pattern was observed in June when chl-a and AFDM values were relatively lower even though flow rates were greatest in June. Furthermore,

any quantitative threshold in flow that could induce scouring remains unknown. Thus, seasonal grazing pressure appears to be a logical explanation for the variation observed in periphyton biomass in this study.

Grazer and algal community structures often display seasonal changes, and edibility of algae can vary throughout the year (Vanni and Temte 1990). For example, algal community composition changes can be coupled with changes in grazer or scraper community composition (DeNicola et al. 1990). In this study, increased filamentous algae biomass was visually observed in streams during the cooler months, which may have been caused by, or resulted in, shifts in macroinvertebrate functional feeding groups due to preferential feeding (Dodds and Gudder 1992; Hawkins and Sedell 1981). In contrast to this study, Rosemond (1994) did not detect seasonal trends in periphyton accrual and attributed the observation to large quantities of grazing snails. Thus, the dynamic nature of grazing as a potential confounding factor must be explicitly considered in stream bioassessment methods that involve periphyton biomass quantitation.

Two crayfish species, *Cambarus chasmodactylus* and *Orconectes cristavarius*, and three benthic fish species, northern hogsuckers (*Hypentelium nigricans*), white suckers (*Catastomus commersoni*), and central stonerollers (*Campostoma anomalum*), exhibited reduced feeding activities during winter when the mean temperature was less than 6 °C in a temperate North Carolina river (Fortino 2006). Increased feeding activities of the same crayfish species were previously documented in the same river during August when mean temperatures were relatively warmer (Helms and Creed 2005). Dewey (1981) reported seasonal changes in small fish abundances in a southern Ozark stream, implying grazing pressures also varied throughout the year. Specifically, the central stoneroller (*C. anomalum*) is one of the most abundant fish species in Ozark streams, making them an important control on periphyton biomass due to their strong

grazing influences (Gelwick and Matthews 1992; Robison and Buchanan 1988; Matthews et al. 1987). Temperature-mediated impacts on trophic interactions observed in a boreal stream mesocosm experiment showed increased predatory fish (*Salvelinus malma*) feeding activity at 12 °C led to decreased caddisfly (*Glossosoma* spp.) grazing and increased periphyton biomass accrual (Kishi et al. 2005). Decreased feeding activity of the predacious fish at 21 °C allowed caddisfly grazing to reduce periphyton biomass (Kishi et al. 2005). Evans-White et al. (2003) observed preferential seasonal feeding on algae by two crayfish known to occur in Arkansas, *Orconectes nais* and *O. neglectus*, with greater grazing occurring in spring compared to summer or fall. Results from these studies support the idea that grazing pressures may have been reduced during November and February in all streams during our study when the mean temperature was 8 °C.

2.4.3 *Study Implications*

The large-scale implication of this study is that periphyton biomass collected from cobbles using the whole-surface periphyton removal method versus the delimiter-reduced periphyton removal method resulted in no measureable differences. Variations in velocity and light between the methods may have minimally influenced comparability of periphyton biomass. However, these variations were not statistically significant and were small relative to the seasonal changes in periphyton biomass. More specifically, these results suggest managers should use discretion, but may combine data from these different methods in order to generate sufficiently large databases to support nutrient and periphyton biomass relationships. For example, the Arkansas-Oklahoma SRJS data and the ADEQ Extraordinary Resource Waters data may be linked together in regional stressor-response analyses for the ultimate goal of deriving regional nutrient criteria for streams and rivers of the southern Ozarks. Although there was no

method by season interaction observed in this study, the data exhibited heteroscedasticity (i.e. the magnitude of difference between methods and the measured periphyton biomass was positively correlated). Thus, fair caution may be to only combine datasets collected in seasons with similar temperature ranges so as to avoid any seasonally dynamic confounding factors.

The limited spatial scale and small number of sample sites in this study provide a brief glimpse into the possibilities of combining datasets collected with Method A and Method B. Canopy cover in this study was similar between habitats sampled using both methods. Thus, more extreme variations in canopy cover may have greater implications for methodological differences. Additionally, grazing macroinvertebrates and fish are known to influence standing stocks of periphyton in aquatic habitats, especially where large numbers of grazers reside (Hillebrand 2008; Hillebrand et al. 2008; Taylor et al. 2012). We were unable to quantitatively sample for grazers in this study due to time constraints. However, anecdotal evidence suggests grazing pressure may strongly control periphyton biomass in Ozark streams (Power et al. 1988). Different types of algae also are better suited for certain habitats and variable temperatures and can produce varying amounts of biomass (Biggs and Hickey 1994; Lange et al. 2011). Taxonomic identification of periphyton communities was not conducted for samples in this study. Quantitatively identifying the impacts of differences between habitats sampled, primarily variations in periphyton community composition, canopy cover, and grazing influences are important considerations for future method comparison studies.

In an ideal situation, scientists and natural resource managers would agree to use one method of data collection and hold true to that decision. In reality, existing datasets that used different methods of data collection already exist and each investigator has reasons why one method may be better to use over another method (Aloi 1990). Consensus about which method to

use is unlikely to happen in the foreseeable future, so using the best available information to justify combining datasets is pertinent because combining datasets increases the availability of data for use in bioassessment and nutrient criteria development. Although these findings suggest there was no significant difference between AFDM and chl-a results collected using the whole-surface and the delimiter-reduced periphyton removal methods, future studies with larger sample sizes may yield other results, and caution should be applied when combing data from different studies.

2.5 Conclusion

The goal of this study was to assess the comparability of two common periphyton sampling protocols for the purpose of advancing nutrient criteria development. Results demonstrated that there were no discernable differences in data based on the method of data collection. Sharp differences in periphyton biomass were observed between the warmer (i.e. August and June) and cooler months (i.e. November and February) of this study, with the cooler months yielding more variable results. Evidence from measurements of confounding factors indicate that the seasonal difference was possibly caused by variable grazing. Although results of this study suggest estimates of chl-a and AFDM from each method are comparable, managers must make the ultimate decision about whether results are similar enough to justify combining datasets. The current study provides an important starting point for combining datasets for bioassessment and to inform numeric nutrient criteria development for streams of the southern Ozarks.

2.6 References

APHA (American Public Health Association). (2005). Standard methods for the examination of water and wastewater. 21st ed. Easton, A. D., Clesceri, L. S., Rice, E. W., and Greenburg, A. E. (Editors). American Public Health Association, American Water Works, Water Environment Federation, Washington D.C.

Aloi, J. (1990). A critical review of recent freshwater periphyton field methods. *Canadian Journal of Fisheries and Aquatic Sciences*, *47*, 656–670.

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish. EPA 841/B-99/022. Office of Water, US Environmental Protection Agency, Washington, D.C.

Biggs, B. J. F. (2000). Eutrophication of streams and rivers : dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of North American Benthological Society*, *19*(1), 17–31.

Biggs, B. J. F., & Hickey, C. W. (1994). Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. *Freshwater Biology*, *32*, 49–59.

Biggs, B. J. F., Nikora, V. I., & Snelder, T. H. (2005). Linking scales of flow variability to lotic ecosystem structure and function. *River Research and Applications*, *21*, 283–298.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, *8*(3), 559–568.

Ceola, S., Hödl, I., Adlboller, M., Singer, G., Bertuzzo, E., Mari, L., et al. (2013). Hydrologic variability affects invertebrate grazing on phototrophic biofilms in stream microcosms. *PLoS ONE*, *8*(4), 1–11. doi:10.1371/journal.pone.0060629

DeNicola, D. M. (1996). Periphyton responses to temperature at different ecological levels. In: *Algal Ecology: Freshwater Benthic Ecosystems*. R.J. Stevenson, M.L. Bothwell, and R.L. Lowe (Editors). Academic Press, San Diego, California, USA, 149–181.

DeNicola, D., McIntire, C. D., Lamberti, G. A., Gregory, S. V., & Ashkenas, L. R. (1990). Temporal patterns of grazer-periphyton interactions in laboratory streams. *Freshwater Biology*, *23*(3), 475–489.

Dewey, M. R. (1981). Seasonal abundance, movement, and diversity of fishes in an Ozark stream. *Arkansas Academy of Science Proceedings, 35*, 33–39.

Dodds, W. K., & Gudder, D. A. (1992). The ecology of Cladophora. *Journal of Phycology*, 415–427.

Dodds, W. K., Perkin, J. S., & Gerken, J. E. (2013). Human impact on freshwater ecosystem services: a global perspective. *Environmental Science & Technology*, *47*, 9061–9068. doi:10.1021/es4021052

Dodds, W. K., Smith, V. H., & Lohman, K. (2002). Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(5), 865–874. doi:10.1139/f02-063

Evans-White, M. A., Dodds, W. K., & Whiles, M. R. (2003). Ecosystem significance of crayfishes and stonerollers in a prairie stream: functional differences between co-occurring omnivores. *Journal of the North American Benthological Society*, *22*(3), 423–441. doi:10.2307/1468272

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality*, *42*(4), 1002–1014. doi:10.2134/jeq2012.0491

Fortino, K. (2006). Effect of season on the impact of ecosystem engineers in the New River, NC. *Hydrobiologia*, *559*(1), 463–466. doi:10.1007/s10750-005-5325-5

Gelwick, F. P., & Matthews, W. J. (1992). Effects of an algivorous minnow on temperate stream ecosystem properties. *Ecological Society of America*, *73*(5), 1630–1645.

Hawkins, C., & Sedell, J. (1981). Longitudinal and seasonal changes in functional organization of macroinvertebrate communities in four Oregon streams. *Ecology*, *62*(2), 387–397. doi:10.2307/1936713

Helms, B. S., & Creed, R. P. (2005). The effects of 2 coexisting crayfish on an Appalachian river community. *Journal of the North American Benthological Society*, *24*(1), 113–122.

Hill, W. R., 1996. Factors Affecting Benthic Algae. In: *Algal Ecology: Freshwater Benthic Ecosystems*. R.J. Stevenson, M.L. Bothwell, and R.L. Lowe (Editors). Academic Press, San Diego, California, USA, pp. 31-56.

Hillebrand, H. (2008). Grazing regulates the spatial variability of periphyton biomass. *Ecological Society of America*, *89*(1), 165–173.

Hillebrand, H. (2009). Meta-analysis of grazer control of periphyton biomass across aquatic ecosystems. *Journal of Phycology*, *45*(4), 798–806. doi:10.1111/j.1529-8817.2009.00702.x

Hillebrand, H., Frost, P., & Liess, A. (2008). Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia*, *155*, 619–630. doi:10.1007/s00442-007-0930-9

King, R. S. (2014). Oklahoma Scenic Rivers Joint Phosphorus Study Work Plan. https://www.ok.gov/conservation/documents/Baylor%20University%20Contract%20and%20Workplan.pdf (Acessed 11 January 2016)

Kishi, D., Murakami, M., Nakano, S., & Maekawa, K. (2005). Water temperature determines strength of top-down control in a stream food web. *Freshwater Biology*, *50*(8), 1315–1322. doi:10.1111/j.1365-2427.2005.01404.x

Lange, K., Liess, A., Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2011). Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, *56*(2), 264–278. doi:10.1111/j.1365-2427.2010.02492.x

Lowe, R. L., & LaLiberte, G. D. (2007). Benthic stream algae: distribution and structure. In: *Methods in Stream Ecology* (Eds F.R. Hauer and G.A. Lamberti). Academic Press, San Diego.

Lowe, R. L., Golladay, S. W., & Webster, J. R. (1986). Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *Journal of the North American Benthological Society*, *5*(3), 221–229.

Matthews, W. J., Stewart, A. J., & Power, M. E. (1987). Grazing fishes as components of North American stream ecosystems: Effects of Campostoma anomalum. *Community and Evolutionary Ecology of North American Stream Fishes*, 128–135.

Moulton II, S. R., Kennen, J. G., Goldstein, R. M., & Hambrook, J. A. (2002). *Revised protocols for sampling algal, invertebrate, and fish communities as part of the national water-quality assessment program*. Open-File Report 02-150. U.S. Department of the Interior, U.S. Geological Survey, Reston, Virginia.

NMED (New Mexico Environment Department). (2014). Periphyton sampling. SOP 11.2. Surface Water Quality Bureau. https://www.env.nm.gov/swqb/SOP/11.2SOP-PeriphytonSampling2014.pdf (Accessed 3 March 2016).

Omernick, J. M. (1987). Ecoregions of the conterminous United States. *Ann. Assoc. Am. Geogr*. 77:118–125.

Panfil, M. S., & Jacobson, R. B. (2001). *Relations Among Geology, Physiography, Land Use, and Stream Habitat Conditions in the Buffalo and Current River Systems, Missouri and Arkansas*. ISSN 1081-292X. US Department of the Interior, US Geological Survey, Biological Resources Division, Columbia Environmental Research Center, Columbia, Missouri.

Paulsen, S. G., Mayio, A., Peck, D. V., Stoddard, J. L., Tarquinio, E., Holdsworth, S. M., et al. (2008). Condition of stream ecosystems in the US: an overview of the first national assessment. *Journal of the North American Benthological Society*, *27*(4), 812–821.

doi:10.1899/08-098.1

Power, M. E., Stewart, A. J., & Matthews, W. J. (1988). Grazer control of algae in an Ozark mountain stream: effects of short-term exclusion. *Ecology*, *69*(6), 1894–1898. doi:10.2307/1941166

Quinn, J. M., Cooper, A. B., Stroud, M. J., & Burrell, G. P. (1997). Shade effects on stream periphyton and invertebrates: An experiment in streamside channels. *New Zealand Journal of Marine and Freshwater Research*, *31*(5), 665–683. doi:10.1080/00288330.1997.9516797

Robison, H. W., & Buchanan, T. M. (1988). *Fishes of Arkansas*. University of Arkansas Press, Fayetteville, Arkansas.

Rosemond, A. D. (1994). Multiple factors limit seasonal variation in periphyton in a forest stream. *Journal of the North American Benthological Society*, *13*(3), 333–344.

Rosemond, A. D., Mulholland, P. J., & Brawley, S. H. (2000). Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(1), 66–75. doi:10.1139/f99-181

Sharpley, A. N., Chapra, S. C., Wedepohl, R., Sims, J. T., Daniel, T. C., & Reddy, K. R. (1994). Managing agricultural phosphorus for protection of surface waters: issues and options. *Journal of Environment Quality*, *23*, 437–451. doi:10.2134/jeq1994.00472425002300030006x

Smith, R. A., Alexander, R. B., & Schwarz, G. E. (2003). Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology*, *37*, 3039–3047.

Smucker, N. J., Becker, M., Detenbeck, N. E., & Morrison, A. C. (2013). Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. *Ecological Indicators*, *32*, 51–61. doi:10.1016/j.ecolind.2013.03.018

Stevenson, R. J., Bothwell, M. L., & Lowe, R. L. (1996). *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, San Diego, California, USA.

Stevenson, J. R., & Bahls, L. L. (1999). Periphyton Protocols. In *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (pp. 1–23).

Taylor, J. M., Back, J. A., & King, R. S. (2012). Grazing minnows increase benthic autotrophy and enhance the response of periphyton elemental composition to experimental phosphorus additions. *Freshwater Science*, *31*(2), 451–462. doi:10.1899/11-055.1

The Nature Conservancy, Ozarks Ecoregional Assessment Team. (2003). Ozarks Ecoregional Conservation Assessment. Minneapolis, MN: The Nature Conservancy Midwestern Resource Office. 48 pp. + 5 appendices.

U.S. Climate Data. 2016. Climate Ozarks-Arkansas. http://www.usclimatedata.com/climate/ozark/arkansas/united-states/usar0428 (Accessed 11 January 2016).

USEPA (US Environmental Protection Agency). (1998). *EPA National Strategy for the Development of Regional Nutrient Criteria*. EPA-822-R-98-002. US Environmental Protection Agency, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2000). *Nutrient Criteria Technical Guidance Manual Rivers and Streams*. EPA-822-B-00-002. US Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2010). *Using Stressor-response Relationships to Derive Numeric Nutrient Criteria*. EPA-820-S-10-001. US Environmental Protection Agency, Office of Water, Washington, D.C.

USEPA (US Environmental Protection Agency). (2015). State Development of Numeric Criteria for Nitrogen and Phosphorus Pollution. US Environmental Protection Agency, Washington, D.C. http://cfpub.epa.gov/wqsits/nnc-development/ (Accessed 28 December 2015).

USGS (US Geological Survey). (2015). Streamstats. Version 3.0. http://water.usgs.gov/osw/streamstats/arkansas.html (Accessed 28 December 2015).

Vanni, M. J., & Temte, J. (1990). Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology and Oceanography*, *35*(3), 697–709. doi:10.4319/lo.1990.35.3.0697

Wickham, J. D., Riitters, K. H., Wade, T. G., & Jones, K. B. (2005). Evaluating the relative roles of ecological regions and land-cover composition for guiding establishment of nutrient criteria. *Landscape Ecology*, *20*(7), 791–798. doi:10.1007/s10980-005-0067-3

Winkelmann, C., Schneider, J., Mewes, D., Schmidt, S. I., Worischka, S., Hellmann, C., & Benndorf, J. (2014). Top-down and bottom-up control of periphyton by benthivorous fish and light supply in two streams. *Freshwater Biology*, *59*, 803–818. doi:10.1111/fwb.12305

Whitton, B. A. (1970). Biology of Cladophora in freshwaters. *Water Research, 4*, 457-476. doi: 10.1016/0043-1354(70)90061-8

2.7 Tables

Table 2.1. Select Arkansas Ozarks study area characteristics

Site	Drainage Area (km^2)	% Land Use			Latitude (°)	Longitude (°)	Mean TN (mg/L)	Mean TP (mg/L)	Mean Flow (m^3/s)
		Pasture	Forest	Urban					
Bear Creek	237	21.4	75.4	2.9	35.9929	-92.7015	0.69	0.034	5.7
Cave Creek	130	21.4	77.5	0.8	35.9679	-92.9636	0.36	0.023	2.2
War Eagle Creek	271	12.8	86.1	0.8	36.0420	-93.7041	0.49	0.028	5.9
Little Buffalo River	368	10.7	88.5	1.1	36.0333	-93.1174	0.38	0.027	9.6
Big Creek	232	10.3	88.8	0.4	35.9775	-93.0434	0.44	0.029	6.5
Kings River	165	6.5	91.8	0.5	36.0196	-93.5387	0.32	0.023	4.4
White River	232	4.4	94.3	1.3	35.8290	-93.8330	0.47	0.030	14.6

TN- Total Nitrogen; TP- Total Phosphorus

Table 2.2. Major differences in periphyton biomass collection between Method A and Method B

	Method Type	
Method Characteristic	Method A	Method B
Habitat	Turbulent riffles	Riffle-run
Rock selection method	Haphazardly	Systematically
Rock size	Representative of habitat	10-20 cm^2
Periphyton collection area	50.7 cm^2/riffle	Variable
Number of rocks collected	20	15
Canopy cover	No preference	Open

Table 2.3. Unstructured covariance matrix results for chlorophyll-a (chl-a) and ash-free dry mass (AFDM)

Variable	Effect	df	f	p-value
chl-a	Method	1	2.46	0.123
	Time	4	7.74	<0.001
	Method x Time	4	1.34	0.270
AFDM	Method	1	0.36	0.550
	Time	4	3.58	0.012
	Method x Time	4	1.56	0.200

Table 2.4. Mean stream flow from July 2014 through August 2015 from U.S. Geological Survey, Arkansas Water Science Center stream flow gaging stations within the Buffalo River Watershed (07055660, Buffalo River at Ponca, AR; 07055780, Buffalo River at Carver access near Hasty, AR; and 07056515, Bear Creek near Silver Hill, AR)

Year	Month	Mean Flow (m^3/s)
2014	July	3.0
	August	0.9
	September	0.2
	October	2.6
	November	1.1
	December	2.9
2015	January	5.6
	February	2.1
	March	16.8
	April	8.3
	May	23.3
	June	18.4
	July	4.3
	August	0.4

2.8 Figure Legends

Figure. 2.1. Map of study stream locations (yellow triangles), Arkansas-Oklahoma (AR/OK) Scenic Rivers Joint Study range (green squares), and the Arkansas Department of Environmental Quality (ADEQ) Extraordinary Resource Waters study range (red circles). U.S. level III ecoregion GIS layer provided by USEPA. State boundaries GIS layer provided by ESRI, TomTom, U.S. Department of Commerce, and U.S. Census Bureau

Figure 2.2. (a) During and (b) after periphyton removal using a rubber delimiter following Method A sampling protocol

Figure 2.3. Seasonal and site specific (a) chl-a and (b) ash-free dry mass results collected using Method A and Method B. Dashed line represents 1:1 relationship. Colors represent seasons: August 2014 (red), November 2014 (green), February 2015 (blue), June 2015 (pink), and August 2015 (black). Symbols represent streams: Bear Creek (■), Big Creek (●), Cave Creek (▲), Kings River (◆), Little Buffalo River (⊠), War Eagle Creek (+), and White River (✳)

Figure 2.4. (a) Seasonal influence on chlorophyll-a (chl-a) differences between both methods for all streams, and (b) seasonal influence by site on chl-a differences between both methods. (c) Seasonal influence on ash-free dry mass (AFDM) differences between both methods for all streams, and (d) seasonal influence by site on AFDM differences between both methods. Differences were calculated by subtracting Method B biomass results from Method A biomass

results. Symbols represent streams: Bear Creek (□), Big Creek (○), Cave Creek (△), Kings River (◆), Little Buffalo River (⊠), War Eagle Creek (+), and White River (✳). The blue diamonds are the means of each response variable, and the solid middle lines are the medians of each response variable

Figure 2.5. Seasonal influence on (a) all chlorophyll-a (chl-a) and (b) all ash-free dry mass (AFDM) collected from all streams using both methods throughout the study. The blue diamonds are the means of each response variable, and the solid middle lines are the medians of each response variable. Significant differences are designated by letters (a, b, and c)

Figure 2.6. Seasonal influence on (a) differences in velocity between habitats sampled in all streams using both methods, and (b) percent closed canopy cover differences between methods. Differences were calculated by subtracting Method B results from Method A results. The blue diamonds are the means of each response variable, and the solid middle lines are the medians of each response variable

2.9 Figures

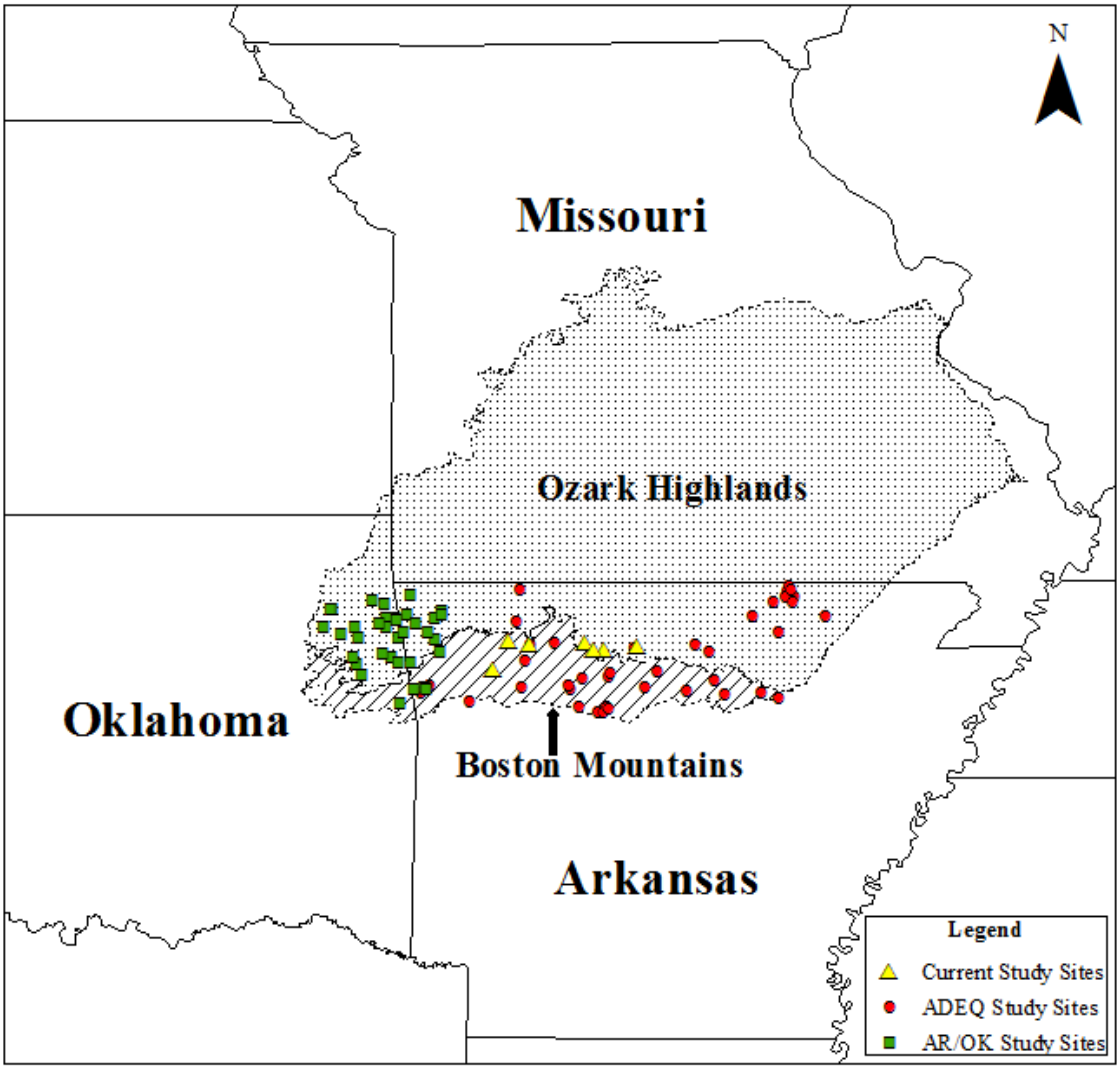

Figure 2.1

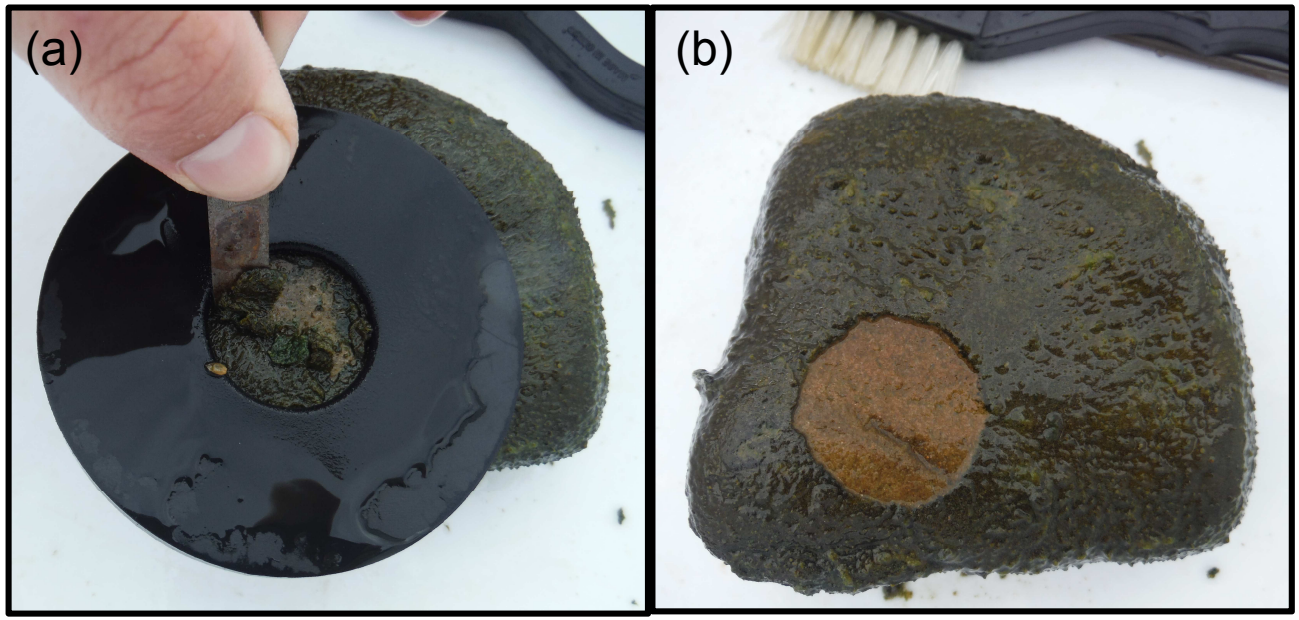

Figure 2.2

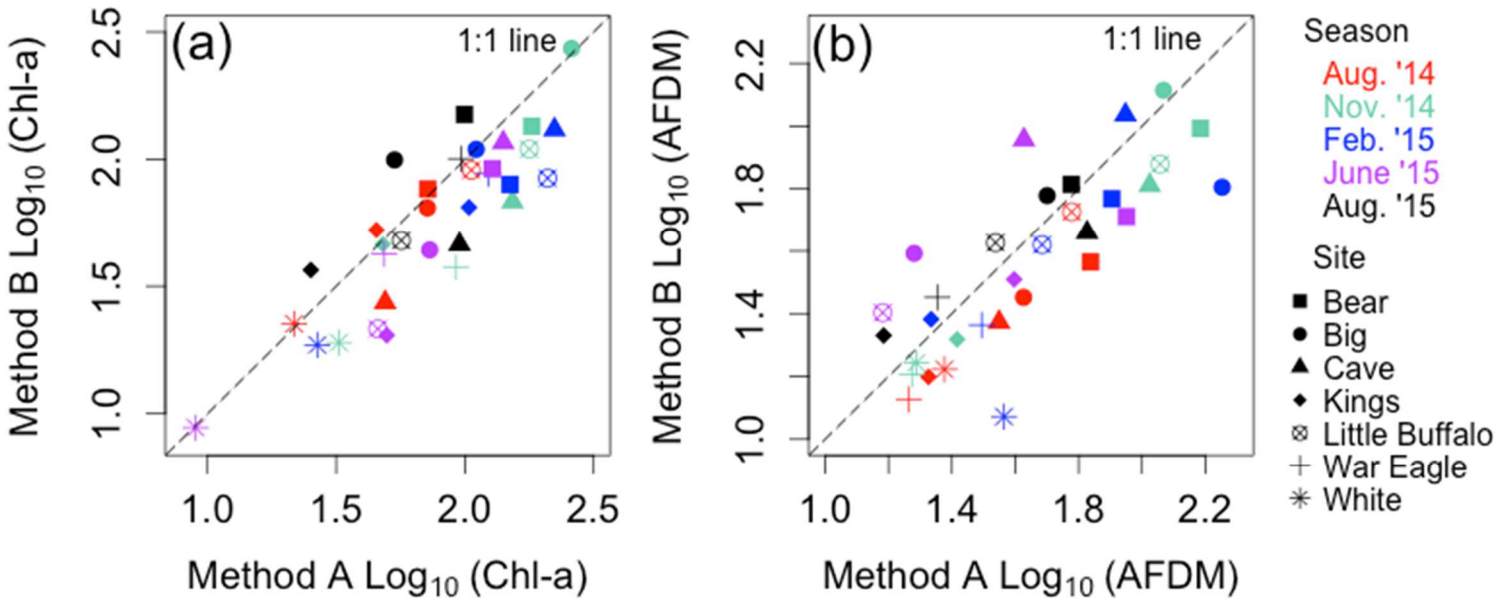
(a)
1:1 line
Method B Log10 (Chl-a)
Method A Log10 (Chl-a)
1.0
1.5
2.0
2.5
(b)
1:1 line
Method B Log10 (AFDM)
Method A Log10 (AFDM)
1.0
1.4
1.8
2.2
Season
Aug. '14
Nov. '14
Feb. '15
June '15
Aug. '15
Site
Bear
Big
Cave
Kings
Little Buffalo
War Eagle
White

Figure 2.3

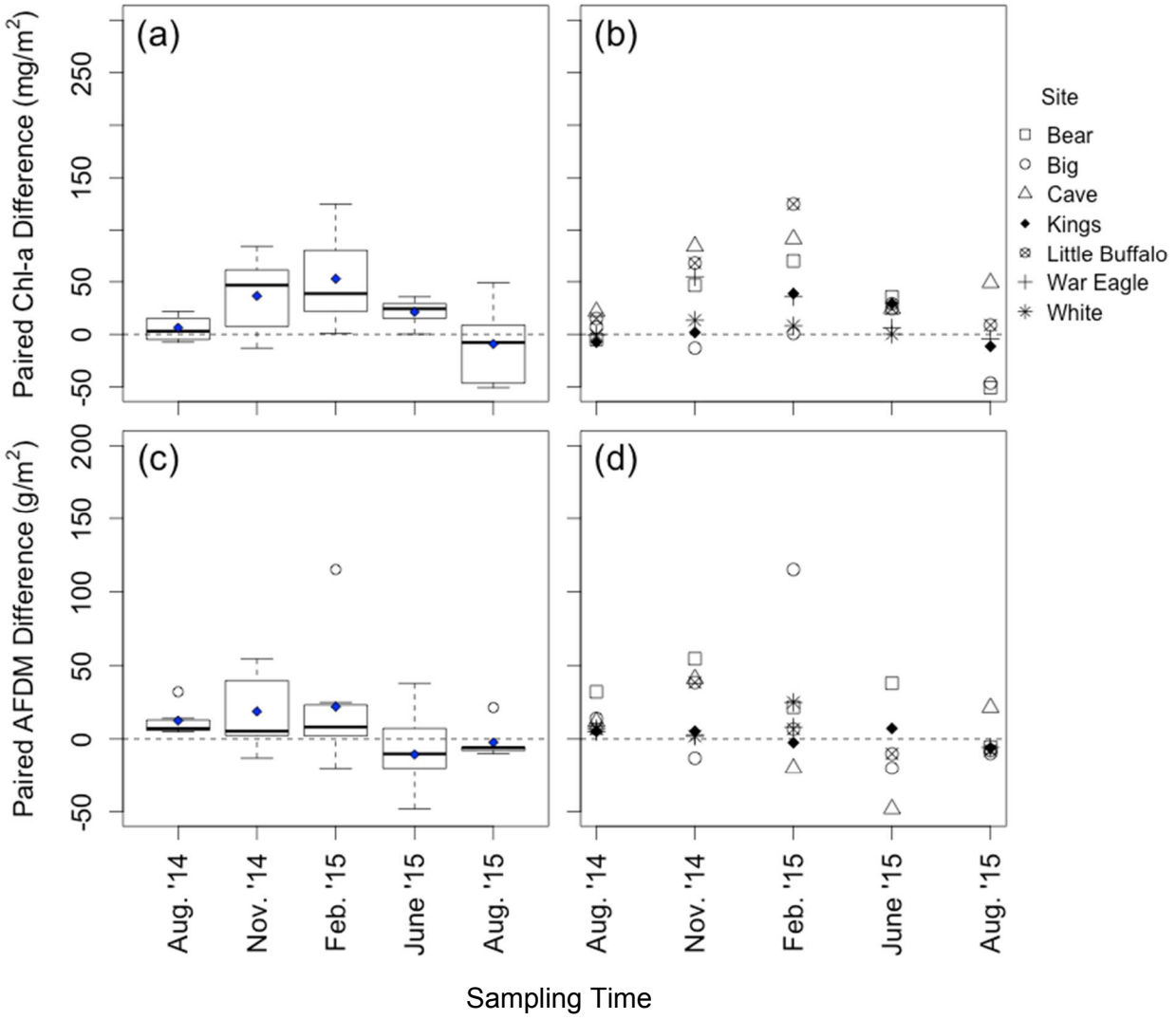
(a)
(b)
(c)
(d)
Paired Chl-a Difference (mg/m^2)
Paired AFDM Difference (g/m^2)
-50
0
50
150
250
-50
0
50
100
150
200
Aug. '14
Nov. '14
Feb. '15
June '15
Aug. '15
Sampling Time
Site
Bear
Big
Cave
Kings
Little Buffalo
War Eagle
White

Figure 2.4

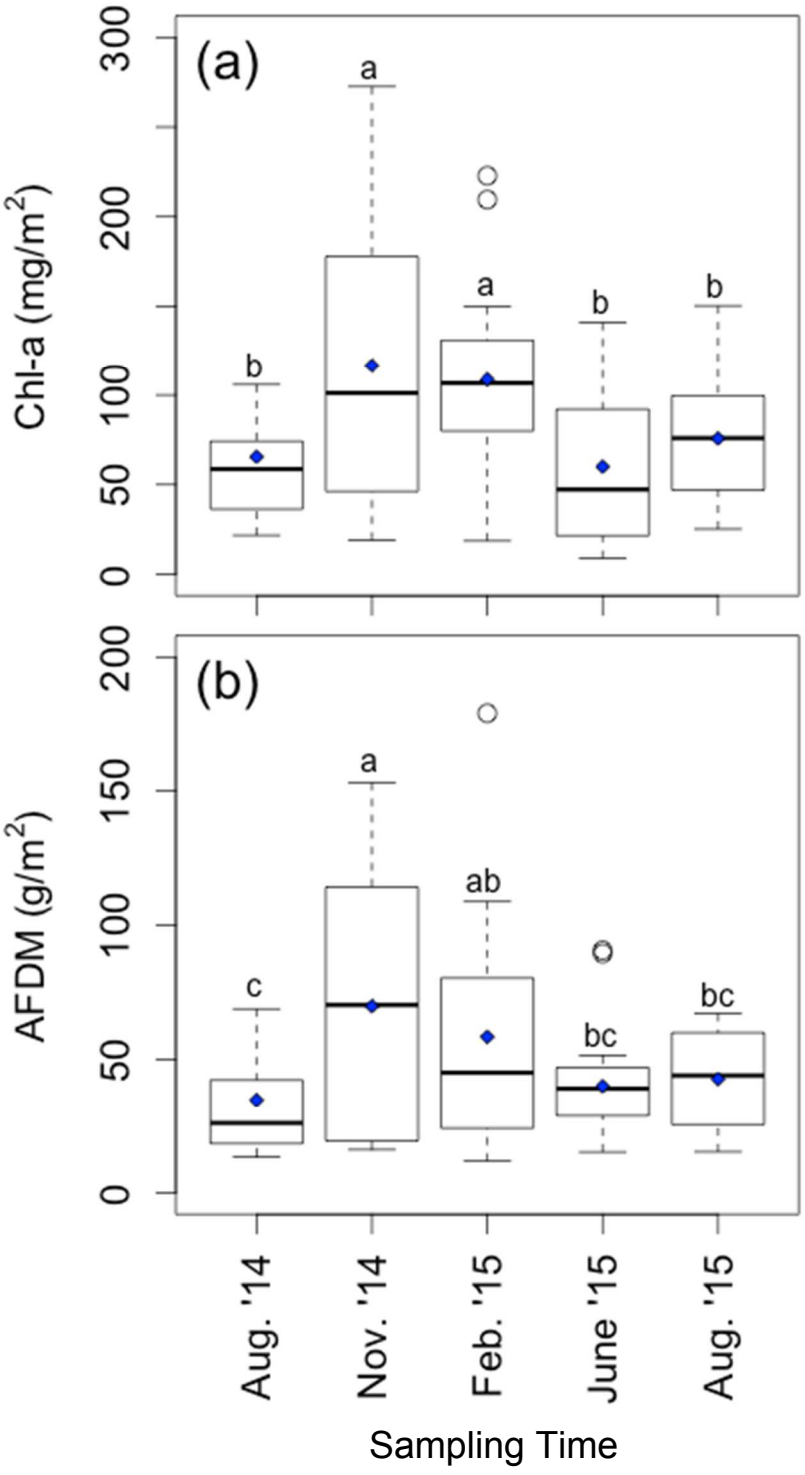
(a)
Chl-a (mg/m^2)
0
50
100
200
300
b
a
a
b
b
(b)
AFDM (g/m^2)
0
50
100
150
200
c
a
ab
bc
bc
Aug. '14
Nov. '14
Feb. '15
June '15
Aug. '15
Sampling Time

Figure 2.5

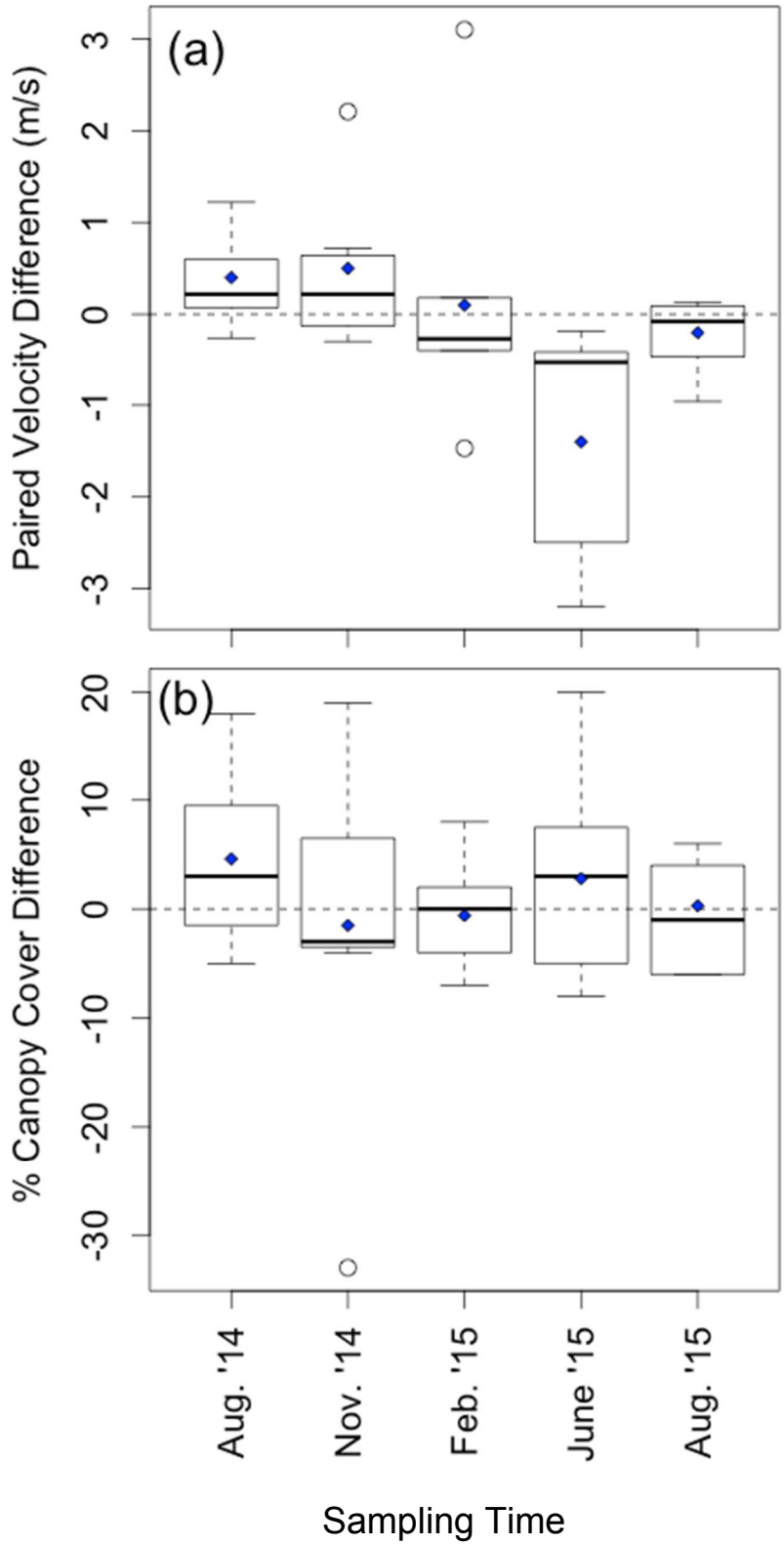
(a)
Paired Velocity Difference (m/s)
3
2
1
0
-1
-2
-3
(b)
% Canopy Cover Difference
20
10
0
-10
-20
-30
Aug. '14
Nov. '14
Feb. '15
June '15
Aug. '15
Sampling Time

Figure 2.6

3. EXPERIMENTAL ALGAL BIOMASS-NUTRIENT RELATIONSHIPS IN THE BUFFALO NATIONAL RIVER WATERSHED

3.1 Introduction

The Buffalo National River is a state designated Extraordinary Resource Water and Natural and Scenic Waterway located in the southern Ozarks and is known as a popular recreational river with pristine water conditions (APCEC 2014; Panfil and Jacobson 2001). The economies of the surrounding communities are intertwined with river tourism. The National Park Service attributed 56.6 million dollars to visitation to the Buffalo River in 2014 alone (Thomas et al. 2015). Sensitivity of the watershed to nutrient pollution is a primary concern to scientists and local communities because the river has seen increases in urban and agricultural land uses (Panfil and Jacobson 2001; Scott and Hofer 1995).

Autotrophic production in streams and rivers is primarily controlled by nitrogen (N) and phosphorus (P) availability (Dodds et al. 2002). Changing land uses can dynamically influence stream processes, such as community respiration and nutrient limitation (Johnson et al. 2009), and increased agricultural land use has been correlated with increased nutrient loading in streams (Allan 2004; Broussard and Turner 2009; Mallin et al. 2015). Specifically, Haggard et al. (2003) observed a positive relationship between increased stream nutrient concentrations and increased percent pasture associated with animal agriculture in a southern Ozark watershed. Potential shifts in animal production within the Buffalo River Watershed could lead to increased N and P loading, causing autotrophic production to increase (i.e. more algal blooms, reduced aesthetic appeal, and negative impacts to other designated uses). The watershed is primarily underlain by karst geology, which is traditionally believed to move nutrients more easily than other types of geology (Jarvie et al. 2014; Kaçaroğlu 1999). This suggests the Buffalo River Watershed may be more vulnerable to nutrient impacts than other watersheds without karst.

Under the guidance of the U.S. Environmental Protection Agency (USEPA), states have struggled with developing nutrient criteria to better protect aquatic environments (Evans-White et al. 2013). Directly investigating algal responses to nutrient inputs (i.e. stressor-response relationships) is one suggested method for refining nutrient criteria because increased nutrient concentrations can strongly influence algal biomass and community composition which, in turn, can impair stream designated uses (USEPA 2010). Watershed-specific studies have been suggested due to variations in land use and the influence of natural confounding factors on algal biomass in streams (Smith et al. 2003; Smucker et al. 2013). For example, catchment size has been shown to be an important predictor of sestonic chlorophyll-a in the Ozarks (Lohman and Jones 1999). However, algal biomass-nutrient relationships are often difficult to study in the field due to the aforementioned confounding factors, and relatively pristine watersheds, such as the Buffalo River Watershed, generally only represent small nutrient gradients (Larned 2010). This provides challenges when studying algal responses at relatively higher nutrient concentrations.

No prior studies have investigated algal biomass responses across a manipulated nutrient gradient in the Buffalo River Watershed, and few studies have examined periphyton growth across manipulated nutrient gradients in streamside mesocosms while controlling for other influences to periphyton biomass (Rier and Stevenson 2006). Rier and Stevenson (2006) observed peak biomass (86.5 mg/m^2) and growth (0.183 mg/cm^2/day) at 0.038 and 0.016 mg/L P as soluble reactive phosphorus (SRP), respectively. Studies investigating periphyton biomass in relation to nutrients and other factors in experimental streams have observed peak algal biomass (between ~ 100 to 250 mg/m^2) at 0.025 and 0.028 mg/L P as SRP (Bothwell 1989; Horner et al. 1983) and maximum growth (between 0.22 and 0.24 mg/cm^2/day) at 0.025 mg/L P as SRP and

between 0.022 and 0.082 mg/L P as SRP (Hill and Fanta 2008; Hill et al. 2009). Controlled manipulative experiments that investigate basin-specific periphyton biomass responses across increasing nutrient gradients are warranted for us to better understand the dynamic relationships of nutrients and periphyton and to prepare for potential future nutrient impacts to streams. P-limitation has traditionally been accepted as the main influence on freshwater production (Schindler 1977). However, N-limitation has been observed in the northern Ozarks, and other studies have reported N and P co-limitation in aquatic environments (Elser et al. 2007; Lohman et al. 1991; Tank and Dodds 2003). Thus, experimental nutrient studies should consider changes in algal biomass across both N and P gradients to better understand algal responses.

Proactive, instead of reactive, management has been suggested to combat potential nutrient impacts to aquatic systems (Mainstone and Parr 2002; Palmer et al. 2009). Understanding algal biomass-nutrient relationships in the Buffalo River Watershed is important for forecasting possible future algal responses to increased nutrient loading from changing land uses and would aid managers in making informed decisions when it comes to proactively protecting water resources within the basin (Jarvie et al. 2013; White et al. 2004). In this study, we subjected periphyton collected from within the Buffalo River Watershed to a gradient of N and P treatments in recirculating streamside mesocosms, while controlling for other factors that occur in situ (i.e. variability in shading, grazing, and flow) for 31 days. We aimed to: (1) observe trends in periphyton biomass accumulation and nutrient uptake across a gradient of P-only enrichments followed by N + P enrichments and (2) compare algal biomass-nutrient relationships in this study to a stressor-response field study in the southern Ozarks. We hypothesized: (1) periphyton biomass would be positively correlated with increased nutrient immobilization and would increase with increasing P treatment, but the greatest response would be in the N + P

enrichment because N would be a co-limiting factor with P (Lohman et al. 1991; Tank and Dodds 2003), and (2) based on previous studies (Hill et al. 2009; Rier and Stevenson 2006), we would observe a threshold response in periphyton biomass between the 0.012 and 0.05 mg/L P treatments.

3.2 Materials and Methods

3.2.1 *Study Area*

The Buffalo River drains approximately 3470 km^2 of the Boston Mountains and Ozark Highlands, both level III Omernik ecoregions (Omernik 1987; USGS 2015). Specifically, the Buffalo River at the Steel Creek Recreational Area is located in the upper portion of the watershed and has low nutrient concentrations year round. Mean SRP and nitrate (NO_3^-) in the Buffalo River at the Steel Creek Recreational Area throughout this experiment were < 0.014 mg/L P (i.e. detection limit) and 0.027 mg/L N (± 0.0024 standard error), respectively. The Steel Creek Field Station at the Steel Creek Recreational Area is adjacent to the river and served as the location of the transplant study due to accessibility to a low-nutrient water source (i.e. Buffalo River) and nearby streams where periphyton were initially collected. Big and Cave Creeks, which drain 130 and 232 km^2, respectively, of the Buffalo River Watershed provided substrate and periphyton for this study (USGS 2015). Periphyton from these streams were originally chosen to represent algal communities from relatively different background nutrient environments; however, analyzed water samples showed that nutrient concentrations were similar between streams, and a flooding event immediately preceding the experiment complicated this comparison.

3.2.2 *Transplant Experimental Design*

This experiment took place over a 31-day time period from July 7th through August 6th 2015 when mean air temperature was 25 °C. Eighteen circular recirculating streamside mesocosms were set up using a random-block design, consisting of three blocks, on a level area with no canopy cover at the Steel Creek Field Station near Ponca, AR (Fig. 3.1). Each block contained six mesocosms attached to a gear-driven paddle wheel system. The same source of power supplied all paddle wheel systems. Each mesocosm was considered an experimental unit, and there were three replicates per treatment. Replicates were placed in separate blocks, and mesocosms within each block were randomly assigned treatments. Treatments consisted of six P treatments in the form of KH_2PO_4 and no N treatments for 17 consecutive days (i.e. P-only enrichment) followed by 14 consecutive days of the same P treatments coupled with N as KNO_3 (i.e. N + P enrichment) added at a 10:1 mass ratio of N:P (Table 3.1). This N:P mass ratio represents ideal growing conditions for freshwater periphyton (Downing and McCauley 1992; Healey and Hendzel 1980; Hecky and Kilham 1988). Limited quantities of mesocosms prevented co-occurring P-only and N + P enrichments. Replicates that received treatments of 0 mg/L were considered controls. Centrally placed sensors (Quantum Sensor, LI-COR, model #LI-190R, Lincoln, NE; Multi-plate Radiation Shield, R. M. Young Company, model #41003, Traverse, MI; Relative Humidity and Temperature Probe, R. M. Young Company, model #41382LC2, Traverse, MI; Rain Gauge, Texas Electronics, Inc., model #TR-525M, Dallas, TX) mounted at 1.2 m above the land interface and connected to a datalogger (Campbell Scientific Inc., model #CR10X, Logan, UT) measured photosynthetically active radiation (PAR), air temperature and relative humidity, and precipitation at 1-hour intervals throughout the experiment (Fig. 3.1).

Water from the Buffalo River adjacent to the Steel Creek Field Station was pumped into a pre-rinsed polyethylene storage tank using a submersible water pump and water hose, and each mesocosm was filled with 75 L of stream water. Respective nutrient treatments were pipetted into the mesocosms, and the paddle wheel systems mixed the nutrients and stream water. One hundred and seventy-one cobbles (10-20 cm^2) were collected from riffle habitat of both Big and Cave Creeks, and nine rocks from each stream were placed into each mesocosm. Rocks were spatially separated in the mesocosms by stream to allow comparisons of periphyton biomass over time from rocks that originated from the two separate streams.

The water in each mesocosm was completely replenished and re-spiked with respective nutrient treatments on days 4, 8, 11, 15, 17, 21, 24, and 28. Evaporative losses and possible dilution effects caused by precipitation were assumed to affect mesocosms equally. Overflow pipes in each mesocosm allowed excess water to drain. Grazing pressures were negligible because macroinvertebrates were immediately removed, if observed. Throughout the study, water samples were separately collected from each mesocosm in 250 mL dark bottles prior to replenishing water in the experiment. To calculate nutrient immobilization and know background nutrient concentrations, water samples were also collected from the Buffalo River in 250 mL acid-washed dark bottles each time mesocosms were filled with water. Periphyton were individually collected from six rocks per mesocosm (i.e. three representative of each stream) on days 3, 9, 14, 17, 24, and 31 from within a 5.07 cm^2 area per rock using clean white plastic containers, rubber delimiters, small brushes with plastic bristles, metal scoopulas, and minimal water from each respective mesocosm. Periphyton biomass samples were compiled by original stream and nutrient treatment into 1 L acid-washed dark bottles. Water and periphyton samples

were transported on ice to the laboratory at the University of Arkansas, Fayetteville, AR for nutrient and biomass analysis, respectively.

3.2.3 *Laboratory Analysis*

All water and periphyton samples were processed within 48 hours of collection. A handheld blender was used to individually mix periphyton samples prior to recording slurry volumes. While slurries were individually mixing on a magnetic stir plate, subsamples were pipetted from each periphyton slurry onto 25 mm Whatman glass fiber filters (GF/F) and pre-weighed and pre-combusted 47 mm GF/F for later analysis of benthic chlorophyll-a (chl-a) and ash-free dry mass (AFDM), respectively. Ash-free dry mass filters were combusted at 450 °C prior to filtering. All water samples were individually filtered through acid washed and rinsed 47 mm GF/F, and the filtrate was collected for SRP and NO_3^- analyses. Periphyton filters and filtrate were stored frozen at -20 °C at the University of Arkansas, Fayetteville, AR until ready to be analyzed. Benthic chl-a was analyzed on a Turner Designs Trilogy Fluorometer (model #7200-000, Sunnyvale, CA) following acetone extraction, and AFDM was calculated following sample drying and ashing at 450 °C (APHA 2005). Following analysis, biomass per area was calculated. Soluble reactive phosphorus and NO_3^- were analyzed on a Turner Designs Trilogy Fluorometer using the ascorbic acid (APHA 2005) and cadmium reduction methods, respectively (HACH 2015).

3.2.4 *Data Analysis and Statistics*

Nitrogen and P immobilization (mg) were calculated for each treatment during the P-only and N + P enrichments using the following equation [1]:

$$Immoblization = ((N_B + N_T) - N_L) * 75 \quad [1]$$

where N_B is the mean background nutrient concentration of stream water added, N_T is the nutrient (i.e. N or P) treatment concentration, and N_L is the mean leftover nutrient concentration after being exposed to periphyton and directly before replenishing stream water and reapplying nutrient treatments. To convert to a mass of nutrients immobilized we multiplied by the volume of water in each mesocosm (i.e. 75 L).

R Statistical Software (R Core Team 2015) was used to analyze data using a two-way analysis of covariance (ANCOVA) with biomass as the response, P treatment as a continuous predictor variable, and stream and N added (yes/no) as factors. Alpha was set at 0.05. The models were simplified if no interactions or factorial effects were observed. A linear mixed-effects model ANOVA was used because the general linear model (GLM) test assumptions were violated due to repeated measures over time and heterogeneity in residuals. For example, when chl-a was the dependent variable, residuals exhibited heteroscedasticity as P treatment increased and the GLM ANOVA did not take lack of independence associated with repeated measures or blocking into consideration, so the data were analyzed with a linear mixed-effects model ANOVA. A random intercept model was used that included mesocosms nested within blocks (random = ~ 1|block/mesocosm) and a fixed variance structure (varFixed = P treatment) which allowed for larger residual spread with increasing P treatment. We used the nlme package to run linear mixed-effects models in R (Pinheiro et al. 2016).

3.3 Results

3.3.1 *Biomass Responses*

We observed notable increases in benthic chl-a with increasing P treatment throughout the study, and the highest benthic chl-a accumulation was achieved during the second half of the study after N was added to the mesocosms at a 10:1 ratio (Figs. 3.2a and 3.3a). An interaction

between P treatment and N addition was observed during the N + P enrichment period for benthic chl-a (Fig. 3.3a). These patterns suggested N-limitation may have occurred sometime before day 17 and was alleviated after the N + P enrichment period began. Phosphorus treatment did not, however, influence AFDM even though distinctions were seen with the addition of N ($p < 0.001$) (Figs. 3.2b and 3.3b). On day 31, after 14 days of N + P enrichment, benthic chl-a was significantly greater than on day 17 (last day of P-only enrichment) ($p < 0.001$), but stream did not have a significant effect ($p = 0.455$) (Fig. 3.4a). Stream significantly influenced AFDM accumulation during both enrichments ($p <0.001$), with rocks from Cave Creek developing more AFDM than Big Creek rocks (Fig. 3.4b).

Initially, mean benthic chl-a decreased until day 9 of the P-only enrichment, but thereafter, mean benthic chl-a increased with time and increasing P treatment (Fig. 3.2a). Differences in mean benthic chl-a across P treatments also became more pronounced with time, particularly during the N + P enrichment period (Fig. 3.2a). Benthic chl-a exhibited a significant linear response to increasing P treatment on days 17 (last day of P-only enrichment) and 31 (last day of N + P enrichment) ($p < 0.001$) (Fig. 3.3a). However, the slope of the chl-a response to P treatment relationship was significantly greater on day 31 (last day of N + P enrichment) ($p < 0.001$; Fig. 3.3a). On day 17 (P-only enrichment), mean benthic chl-a across both streams ranged from 12.07 mg/m^2 in the control treatments to 45.8 mg/m^2 in the 0.2 mg/L P treatments (Fig. 3.3a; Table 3.1). On day 31 (N + P enrichment), mean benthic chl-a across streams ranged from 13.75 mg/m^2 in the control treatments to 153.9 mg/m^2 in the 0.2 mg/L P treatments, which was almost triple the biomass compared to the same treatment on day 17 (Fig. 3.3a; Table 3.1). Mean AFDM increased throughout time (Fig. 3.2b) and was significantly greater on day 31 (N + P enrichment) compared to day 17 (P-only enrichment) ($p < 0.001$; Fig. 3.3b). However, P

treatment inadequately explained variation in mean AFDM on both days 17 and 31 ($p = 0.552$; Fig. 3.3b; Table 3.1).

3.3.2 *Nutrient Immobilization*

We observed strong linear relationships ($r^2 \geq 0.70$) between the masses of P and N immobilized and the amount of cumulative benthic chl-a present in the mesocosms during the N + P enrichment (Fig. 3.5; Table 3.1). A linear relationship was reported between cumulative benthic chl-a and mass of P immobilized during the P-only enrichment; however, the slope of the relationship was almost three times less than during the N + P enrichment (Fig. 3.5; Table 3.1). Specifically, the presence or absence of N available for immobilization profoundly influenced the extent of cumulative benthic chl-a observed throughout the experiment (Fig. 3.5a). During the P-only enrichment, when no N treatments were added, cumulative benthic chl-a across both streams was less than 25 mg/m^2 (Fig. 3.5a). However, more N was immobilized after N was added to the mesocosms during the N + P enrichment period, which resulted in increased mean cumulative benthic chl-a up to 153.9 mg/m^2 across streams (Fig. 3.5a). Although increasing mass of P immobilized was strongly correlated with increasing cumulative benthic chl-a during both the P-only and N + P enrichments for both Big and Cave Creeks ($r^2 \geq 0.70$), we observed the greatest cumulative benthic chl-a with increasing mass of P immobilized during the N + P enrichment when the mass of N immobilized was also the greatest (Fig. 3.5b). This supports the idea that N limitation occurred during the P-only treatment and was no longer limiting after N addition.

3.3.3 *Transplant Experiment and Field Study Comparison*

Periphyton from the field and transplant studies responded differently to nutrients, with more biomass accumulating at similar P concentrations in the field study than in the transplant

study (Tables 3.1 and 3.2). Mean benthic chl-a in the field study ranged from 15.4 to 99.7 mg/m^2 in response to a relatively small P gradient ranging from 0.019 to 0.037 mg/L total phosphorus (TP) (Table 3.2). Comparatively, benthic chl-a in the transplant study subjected to similar nutrient ranges were more than two times smaller than values observed in the field study. Transplant study benthic chl-a values ranged from 4.4 to 48.1 mg/m^2 across similar P treatments as those observed in the field study (0 to 0.05 mg/L SRP) when results were combined from both the P-only and N + P enrichments (Table 3.1). In the field study, mean benthic chl-a values from Big Creek and Cave Creek were 65.9 and 95.1 mg/m^2, respectively, at 0.033 and 0.025 mg/L TP. The mean ratio of N to P was 13:1 for the field study data, which was similar to the transplant study (N:P = 10:1). These results suggest one or more periphyton biomass-specific controls influenced benthic chl-a accumulation differently in the two studies.

3.4 Discussion

The purpose of this study was to experimentally investigate algal biomass-nutrient relationships within the Buffalo River Watershed to better understand the potential impacts of increased nutrient loading on streams within the drainage. We hypothesized biomass would exhibit positive relationships with increasing nutrient availability, and a threshold response would be observed below 0.05 mg/L P. We found that algae collected from within the Buffalo River Watershed exhibited a positive linear response to increasing P treatment up to 0.2 mg/L P throughout the study ($p < 0.001$), and N addition stimulated an even greater biomass response across P treatments ($p < 0.001$). We also observed a positive correlation between increasing biomass and increasing nutrient immobilization ($r^2 \geq 0.70$). However, no thresholds in periphyton biomass were detected across the P gradient, with or without N additions. Results give insight into the potential influence of increased nutrient loading within the Buffalo River

Watershed, support N and P colimitation literature, and suggest that lack of N can significantly limit periphyton biomass accumulation even when P may be plentiful.

3.4.1 *Periphyton Biomass and Nutrient Relationships*

Benthic chl-a increased with increasing P treatment throughout the study, but the greatest benthic chl-a accumulation occurred during the N + P enrichment period. Although scientists have traditionally thought freshwaters were limited by P availability (Schindler 1977), more modern literature supports co-limitation by both N and P (Elser et al. 2007; Scott et al. 2009; Lang et al. 2012). Results of our study confirm the possibility of colimitation within the Buffalo River Watershed. During the initial 17 days of P-only enrichment, periphyton communities within the mesocosms were N limited. Mean NO_3^- levels in Big Creek and Cave Creek, sites where original periphyton communities were collected, were 0.17 and 0.10 mg/L, respectively. Nitrogen limitation has already been documented in the Ozarks with NO_3^- levels up to 0.10 mg/L (Lohman et al. 1991), so it may be realistic to assume the periphyton communities were already naturally N limited before the study began. If not already N limited, periphyton became N limited during the first 17 days of the experiment because of lack of N addition and low background N in the Buffalo River. With the addition of N after day 17, N limitation was alleviated and periphyton responded through increased benthic chl-a accumulation up to 171 mg/m^2 on day 31. Phosphorus immobilization was similar between the P-only and N + P enrichments, and considerable increases in benthic chl-a were observed after N immobilization increased during the N + P enrichment (Fig. 3.3; Table 3.1). This further suggests that by increasing N availability we reduced nutrient constraints on the periphyton.

Other studies have similarly observed increasing benthic chl-across P gradients; however, contrary to results of our study, threshold responses in benthic chl-a below 0.05 mg/L SRP have

been reported in the literature (Bothwell 1989; Horner et al. 1983; Rier and Stevenson 2006). Bothwell (1989) reported peak benthic chl-a (between ~ 100 to 250 mg/m^2) in three stream trough experiments at 0.028 mg/L SRP and observed thresholds and then declines in biomass across P gradients after 30 to 40 days, suggesting we might have observed a threshold response if the study had continued past 31 days. Peak benthic chl-a of 86.5 mg/m^2 was reported at 0.038 mg/L SRP when N was not limiting and the N:P ratio was 18:1 (Rier and Stevenson 2006). In our study, benthic chl-a was half that observed by Rier and Stevenson (2006) in the 0.05 mg/L SRP treatment, and the N:P ratio was 10:1 during the N + P enrichment. Other nutrient manipulation studies supplied continuous nutrients to the periphyton and maintained set photosynthetically active radiation (PAR) levels, while our mesocosms were not continuously dosed with nutrients and were subjected to full sunlight conditions. All of the P (i.e. background and P treatment) may have been taken up between nutrient dosing days, which may have influenced periphyton biomass differently than if nutrients had been supplied continuously. Since our study took place during the summer months, PAR was very high (i.e. mean PAR was 709 μmol/m^2/s). Benthic chl-a has been reported to decrease with increasing PAR, and algal biovolume may saturate at PAR levels ~ 100 μmol/m^2/s (Hill et al. 2009). Differences in community composition may also have induced disparities in biomass accumulation (Chetelat et al. 1999).

3.4.2 *Transplant and Field Study*

One of the key reasons for conducting a streamside mesocosm experiment was to be able to control for variability in periphyton biomass caused by confounding factors. However, natural influences on periphyton biomass (i.e. variable light, flow, and accumulation time) may have created observable differences between biomass results of this study and the field study. For

example, the periphyton in the field study received a continuous supply of nutrients, but periphyton in the mesocosms were exposed to a large mass of nutrients over a short period of time every ~ 3 days whenever the water was changed and dosed. Although light is a known limiting factor on periphyton biomass accrual (Cashman et al. 2013), periphyton in the transplant study were purposely not light-limited, while periphyton in the field study were subjected to natural variability in light caused by canopy cover differences. Even though the closed canopy cover was less than 30% in the field study, enough shading from extreme temperatures may have been provided to reduce stress that could have otherwise lessened biomass accumulation (DeNicola, 1996). Alternatively, ambient temperatures ranging from 16.7 to 35.7 °C during the transplant study may have reduced the potential maximum biomass due to heat stress.

Velocity and accumulation time influence periphyton biomass and sloughing as well as community composition (Ahn et al. 2013; Biggs 2000; Hondzo and Wang 2002). Periphyton in the mesocosm study only had 31 days to acclimate to a different environment compared to the field study which already had established periphyton communities adapted to the environmental conditions present there. The continuous disruptive motion of the mesocosm paddle wheels might have further divided comparison of biomass between the two studies due to differential sloughing pressures. Additionally, the beginning of the transplant study coincided with a potential scouring event, and scouring was very evident on rocks from Big Creek. Thus, the initially lower biomass on rocks used in the transplant study, in combination with other differences in confounding factors, contributed to variations in biomass between the field and transplant studies. Because biomass increased with increasing nutrient treatment in the transplant study and was greater in the field study at comparable nutrient levels with confounding factors

present, we expect increased biomass accumulation within the Buffalo River Watershed if nutrient loading increases in the future.

3.4.3 *Future Outlook*

Benthic chl-a levels exceeding 100 mg/m^2 have been used to indicate the beginning of stream degradation (Dodds et al. 1998; Suplee et al. 2009; Welch et al. 1988). This biomass level was only surpassed in our study in the 0.2 mg/L P treatment during the N + P enrichment period when mean benthic chl-a was 153.9 mg/m^2 (Table 3.1); however, the field study data indicated 0.037 mg/L TP allowed biomass accumulation up to 100 mg/m^2 (Table 3.2). Dodds et al. (1998) suggested trophic boundaries of 20 mg/m^2 (i.e. oligotrophic to mesotrophic boundary) and 70 mg/m^2 (i.e. mesotrophic to eutrophic boundary) for benthic chl-a to better classify streams into oligotrophic, mesotrophic, and eutrophic categories, such as done with lakes. These stream classification boundaries are not definitive, but may be helpful in categorizing stream trophic state based on periphyton biomass. Mean benthic chl-a in the 0.1 and 0.2 mg/L P treatments during the P-only enrichment period were within the lower range of the mesotrophic boundaries (29.2 and 45.8 mg/m^2, respectively), while mean benthic chl-a in all other P treatments were considered oligotrophic (12.1, 8.8, 13.9, and 18.6 mg/m^2 in the 0, 0.012, 0.025, and 0.05 mg/L P treatments, respectively). During the N + P enrichment period the 0 and 0.012 mg/L P treatments and 0.025 and 0.05 mg/L P treatments were in the ranges of the oligotrophic (13.8 and 14.4 mg/m^2) and mesotrophic (21.3 and 44.2 mg/m^2) categories, respectively, while the 0.1 and 0.2 mg/L P treatments were both considered eutrophic (80.5 and 153.9 mg/m^2, respectively). Field data were more often classified as mesotrophic and eutrophic even though nutrient concentrations were lower in the actual streams than in the mesocosms (e.g. mean benthic chl-a was 65.5 mg/m^2 across streams) (Table 3.2). Thus, caution should be taken when

using experimental data for understanding algal biomass-nutrient relationships, especially because disparities in similar field study data have been observed in this study. Understanding the dynamic relationship of confounding factors on periphyton biomass needs further investigation, especially when comparing experimental and field studies.

3.5 Conclusion

Changes to watershed characteristics (i.e. land use) can cause considerable alterations within receiving streams that provide beneficial uses. Specifically, increased nutrient loading may lead to impaired designated uses due to excessive algal growth. Thus, understanding algal biomass-nutrient relationships at the watershed-specific scale will help managers better protect stream designated uses, but continued investigations about the dynamic role confounding factors play by influencing periphyton biomass accrual is essential, especially when understanding the relevancy of experimentally derived algal biomass-nutrient data. Results of our study give insight into algal biomass-nutrient relationships within the Buffalo River Watershed while reducing confounding factor impacts. Our results provide experimental evidence that suggests both N and P enrichment have the capacity to stimulate algal biomass and provide new insights into algal biomass-nutrient relationships within the watershed. However, disparities in environmental variability between the field and experimental data, mainly continuous nutrient loading at the field sites versus pulsed dosing in our experiment, prevented the identification of concentration-based thresholds.

3.6 References

Ahn, C. H., Song, H. M., Lee, S., Oh, J. H., Ahn, H., Park, J. R., et al. (2013). Effects of water velocity and specific surface area on filamentous periphyton biomass in an artificial stream mesocosm. *Water (Switzerland)*, *5*(4), 1723–1740. doi:10.3390/w5041723

Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *35*(1), 257–284. doi:10.1146/annurev.ecolsys.35.120202.110122

APCEC (Arkansas Pollution Control and Ecology Commission) (2014). *Regulation No. 2*. Arkansas Department of Environmental Quality, Little Rock, AR.

APHA (American Public Health Association). (2005). *Standard methods for the examination of water and wastewater*. 21st ed. Easton, A. D., Clesceri, L. S., Rice, E. W., and Greenburg, A. E. (Editors). American Public Health Association, American Water Works, Water Environment Federation, Washington D.C.

Biggs, B. J. F. (2000). Eutrophication of streams and rivers : dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of North American Benthological Society*, *19*(1), 17–31.

Bothwell, M. L. (1989). Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*, 1293–1301.

Broussard, W., & Turner, E. R. (2009). A century of changing land-use and water-quality relationships in the continental US. *Frontiers in Ecology and the Environment*, *7*(6), 302–307.

Cashman, M. J., Wehr, J. D., & Truhn, K. (2013). Elevated light and nutrients alter the nutritional quality of stream periphyton. *Freshwater Biology*, *58*(7), 1447–1457. doi:10.1111/fwb.12142

Chetelat, J., Pick, F., & Morin, A. (1999). Periphyton biomass and community composition in rivers of different nutrient status. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*, 560–569. doi:10.1139/cjfas-56-4-560

DeNicola, D. M. (1996). Periphyton responses to temperature at different ecological levels. In: *Algal Ecology: Freshwater Benthic Ecosystems*. R.J. Stevenson, M.L. Bothwell, and R.L. Lowe (Editors). Academic Press, San Diego, California, USA, 149–181.

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

Dodds, W. K., Smith, V. H., & Lohman, K. (2002). Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(5), 865–874. doi:10.1139/f02-063

Downing, J. A., & McCauley, E. (1992). The nitrogen : phosphorus relationship in lakes. *Limnol. Oceanogr., 37*(5), 936-945.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., et al. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality*, *42*(4), 1002–1014. doi:10.2134/jeq2012.0491

HACH. (2015). Cadmium reduction method. Method #8192. HACH Company, Loveland, CO.

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

Healey, F. P., & Hendzel, L. L. (1980). Physiological indicators of nutrient deficiency in lake phytoplankton. *Can. J. Fish. Aquat. Sci., 37*, 442-453.

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol. Oceanogr., 33*(4, part 2), 796-822.

Hill, W. R., & Fanta, S. E. (2008). Phosphorus and light colimit periphyton growth at subsaturating irradiances. *Freshwater Biology*, *53*(2), 215–225. doi:10.1111/j.1365-2427.2007.01885.x

Hill, W. R., Fanta, S. E., & Roberts, B. J. (2009). Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography*, *54*(1), 368–380.

Hondzo, M., & Wang, H. (2002). Effects of turbulence on growth and metabolism of periphyton in a laboratory flume. *Water Resources Research*, *38*(12), 1277. doi:10.1029/2002WR001409

Horner, R. R., Welch, E. B., & Veenstra, R. B. (1983). Development of nuisance periphyton algae in laboratory streams in relation to enrichment and velocity. *Internation Workshop on Periphyton of Freshwater Ecosystems*, 121–134.

Jarvie, H. P., Sharpley, A. N., Brahana, V., Simmons, T., Price, A., Neal, C., et al. (2014). Phosphorus retention and remobilization along hydrological pathways in karst terrain. *Environmental Science and Technology*, *48*(9), 4860–4868. doi:10.1021/es405585b

Jarvie, H. P., Sharpley, A. N., Withers, P. J. A., Scott, J. T., Haggard, B. E., & Neal, C. (2013). Phosphorus mitigation to control river eutrophication: murky waters, inconvenient truths, and "postnormal" science. *Journal of Environment Quality*, *42*(2), 295–304. doi:10.2134/jeq2012.0085

Johnson, L. T., Tank, J. L., & Dodds, W. K. (2009). The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. *Canadian Journal of Fisheries and Aquatic Sciences*, *66*(7), 1081–1094. doi:10.1139/F09-065

Kaçaroğlu, F. (1999). Review of groundwater pollution and protection in karst areas. *Water, Air, and Soil Pollution*, *113*(1-4), 337–356. doi:10.1023/A:1005014532330

Lang, D. A., King, R. S., & Scott, J. T. (2012). Divergent responses of biomass and enzyme activities suggest differential nutrient limitation in stream periphyton. *Freshwater Science*, *31*(4), 1096–1104. doi:10.1899/12-031.1

Larned, S. T. (2010). A prospectus for periphyton: recent and future ecological research. *Journal of the North American Benthological Society*, *29*(1), 182–206. doi:10.1899/08-063.1

Lohman, K., & Jones, J. R. (1999). Nutrient - sestonic chlorophyll relationships in northern Ozark streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*(1), 124–130.

Lohman, K., Jones, J. R., & Baysinger-Daniel, C. (1991). Experimental-evidence for nitrogen limitation in a northern Ozark stream. *Journal of the North American Benthological Society*, *10*(1), 14–23.

Mainstone, C. P., & Parr, W. (2002). Phosphorus in rivers - ecology and management. *Science of the Total Environment*, *282-283*(FEBRUARY 2002), 25–47. doi:10.1016/S0048-9697(01)00937-8

Mallin, M. A., McIver, M. R., Robuck, A. R., & Dickens, A. K. (2015). Industrial swine and poultry production causes chronic nutrient and fecal microbial stream pollution. *Water, Air, & Soil Pollution*, *226*, 1–13. doi:10.1007/s11270-015-2669-y

Omernik, J. M. (1987). Ecoregions of the conterminous United States. *Annals of the Association of American Geographers*, *77*(1), 118–125.

Palmer, M. A., Lettenmaier, D. P., Poff, N. L., Postel, S. L., Richter, B., & Warner, R. (2009). Climate change and river ecosystems: protection and adaptation options. *Environmental Management*, *44*, 1053–1068. doi:10.1007/s00267-009-9329-1

Panfil, M. S., & Jacobson, R. B. (2001). *Relations Among Geology, Physiography, Land Use, and Stream Habitat Conditions in the Buffalo and Current River Systems, Missouri and Arkansas*.

Pinheiro J., Bates D., DebRoy S., Sarkar D. & R Core Team (2016). *nlme: Linear and Nonlinear*

Mixed Effects Models. R package version 3.1-127, http://CRAN.R-project.org/package=nlme.

R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rier, S. T., & Stevenson, R. J. (2006). Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia*, *561*(1), 131–147. doi:10.1007/s10750-005-1610-6

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science (New York, N.Y.)*, *195*(4275), 260–262. doi:10.1126/science.195.4275.260

Scott H. D. and Hofer K. R. 1995. Spatial and temporal analyses of the morphological and land use characteristics of the Buffalo River watershed. Publication No. 170, University of Arkansas, Arkansas Water Resources Center, Fayetteville, AR.

Scott, J. T., Lang, D. A., King, R. S., & Doyle, R. D. (2009). Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton. *Journal of the North American Benthological Society*, *28*(1), 57–68. doi:10.1899/07-107.1

Smith, R. A., Alexander, R. B., & Schwarz, G. E. (2003). Natural background concentrations of nutrients in streams and rivers of the conterminous United States. *Environmental Science & Technology*, *37*, 3039–3047.

Smucker, N. J., Becker, M., Detenbeck, N. E., & Morrison, A. C. (2013). Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. *Ecological Indicators*, *32*, 51–61. doi:10.1016/j.ecolind.2013.03.018

Suplee, M. W., Watson, V., Teply, M., & McKee, H. (2009). How green is too green? public opinion of what constitutes undesirable algae levels in streams. *Journal of the American Water Resources Association*, *45*(1), 123–140. doi:10.1111/j.1752-1688.2008.00265.x

Tank, J. L., & Dodds, W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology*, *48*, 1031–1049. doi:10.1046/j.1365-2427.2003.01067.x

Thomas, C. C., Huber, C., & Koontz, L. (2015). *2014 National Park Visitor Spending Effects: Economic Contributions to Local Communities, States, and the Nation*. http://www.nature.nps.gov/socialscience/docs/NPSVSE2012_final_nrss.pdf

USEPA (US Environmental Protection Agency). (2010). *Using Stressor-response Relationships to Derive Numeric Nutrient Criteria*. EPA-820-S-10-001. US Environmental Protection Agency, Office of Water, Washington, D.C.

USGS (US Geological Survey). (2015). Streamstats. Version 3.0. http://water.usgs.gov/osw/streamstats/arkansas.html (Accessed 28 December 2015).

Welch, E. B., Jacoby, J. M., Horner, R. R., & Seeley, M. R. (1988). Nuisance biomass levels of periphytic algae in streams. *Hydrobiologia*, *157*, 161–168.

White, K. L., Haggard, B. E., & Chaubey, I. (2004). Water quality at the Buffalo National River, Arkansas, 1991-2001. *Transactions of the American Society of Agricultural Engineers*, *47*(2), 407–417. pdf

3.7 Tables

Table 3.1. Transplant study corresponding data

Enrichment Type	Treatment + Background NO_3 (mg/L)	Treatment + *Background SRP (mg/L)	Final NO_3 Concentration (mg/L)	Final SRP Concentration (mg/L)	Mass N Immobilized (mg)	Mass P Immobilized (mg)	Cave Chl-a (mg/m^2)	Big Chl-a (mg/m^2)	Cave AFDM (g/m^2)	Big AFDM (g/m^2)
P-Only	0 + 0.026	0 + 0.014*	0.0165*	0.014*	0.713	0.000	19.7 (±3.8)	4.44 (±0.4)	63.1 (±8.2)	19.4 (±5.0)
P-Only	0 + 0.026	0.012 + 0.014*	0.0165*	0.014*	0.713	0.900	9.09 (±3.7)	8.58 (±2.0)	56.3 (±4.5)	22.1 (±3.1)
P-Only	0 + 0.026	0.025 + 0.014*	0.0165*	0.014*	0.713	1.875	16.9 (±2.6)	10.8 (±2.7)	65.3 (±16.1)	16.1 (±0.4)
P-Only	0 + 0.026	0.05 + 0.014*	0.0165*	0.014*	0.713	3.750	15.3 (±6.8)	21.8 (±10.9)	57.6 (±13.0)	25.8 (±3.8)
P-Only	0 + 0.026	0.1 + 0.014*	0.0165*	0.014*	0.713	7.500	31.5 (±11.5)	26.9 (±12.6)	74.5 (±10.4)	19.3 (±2.9)
P-Only	0 + 0.026	0.2 + 0.014*	0.0165*	0.020	0.713	14.570	33.7 (±14.1)	57.9 (±50.1)	56.5 (±22.1)	21.4 (±0.9)
N + P	0 + 0.029	0 + 0.014*	0.0165*	0.014*	0.938	0.000	13.3 (±2.4)	14.2 (±6.7)	47.2 (±12.6)	24.8 (±4.3)
N + P	0.12 + 0.029	0.012 + 0.014*	0.0165*	0.014*	9.938	0.900	13.3 (±1.3)	15.5 (±5.4)	56.8 (±12.6)	43.4 (±15.1)
N + P	0.25 + 0.029	0.025 + 0.014*	0.0165*	0.014*	19.688	1.875	26.8 (±11.9)	15.7 (±3.3)	113.6 (±45.8)	48.5 (±16.4)
N + P	0.5 + 0.029	0.05 + 0.014*	0.0165*	0.014*	38.438	3.750	48.1 (±14.9)	40.2 (±9.3)	82.5 (±20.1)	66.4 (±18.8)
N + P	1.0 + 0.029	0.1 + 0.014*	0.0165*	0.014*	75.938	7.500	89.8 (±33.0)	71.1 (±6.1)	121.3 (±24.8)	45.6 (±12.1)
N + P	2.0 + 0.029	0.2 + 0.014*	0.0165*	0.014*	150.938	15.000	136.7 (±44.2)	171.1 (±10.5)	68.0 (±13.7)	53.5 (±7.6)

*Indicates nutrient concentration was below minimum detection level
Standard errors presented in parentheses

Table 3.2. Compiled data from algal biomass-nutrient relationship field study in the Ozarks

Stream	SRP (mg/L)	NO_3^-+NO_2^- (mg/L)	NH_3 (mg/L)	TP (mg/L)	TN (mg/L)	Chl-a (mg/m^2)	AFDM (g/m^2)
Bear	0.010 (±0.002)	0.269 (±0.050)	0.027 (±0.002)	0.037 (±0.005)	0.486 (±0.072)	99.7 (±16.2)	72.7 (±8.76)
Big	0.008 (±0.004)	0.169 (±0.031)	0.034 (±0.006)	0.033 (±0.003)	0.395 (±0.077)	65.9 (±6.33)	37.2 (±9.33)
Cave	0.008 (±0.002)	0.096 (±0.004)	0.013 (±0.002)	0.025 (±0.002)	0.261 (±0.012)	95.1 (±26.5)	48.3 (±9.61)
Kings	0.004 (±0.000)	0.114 (±0.025)	0.019 (±0.006)	0.020 (±0.002)	0.255 (±0.009)	40.1 (±7.54)	25.3 (±7.29)
Little Buffalo	0.003 (±0.002)	0.104 (±0.011)	0.027 (±0.008)	0.028 (±0.002)	0.324 (±0.025)	69.5 (±18.5)	36.6 (±13.0)
War Eagle	0.002 (±0.000)	0.101 (±0.051)	0.023 (±0.001)	0.027 (±0.002)	0.308 (±0.040)	72.6 (±24.0)	20.5 (±2.10)
White	0.002 (±0.001)	0.215 (±0.009)	0.030 (±0.021)	0.019 (±0.003)	0.340 (±0.006)	15.4 (±6.35)	13.3 (±10.5)

Standard errors presented in parentheses

3.8 Figure Legends

Figure 3.1. Diagram of transplant study experimental setup

Figure 3.2. (a) Mean benthic chlorophyll-a (chl-a) and (b) mean ash-free dry mass (AFDM) across soluble reactive phosphorus treatments throughout the transplant study. Dashed line represents when N + P enrichment began

Figure 3.3. (a) Cumulative benthic chlorophyll-a (chl-a) across soluble reactive phosphorus (SRP) treatments on days 17 and 31, and (b) ash-free dry mass (AFDM) across SRP treatments on days 17 and 31. Standard error represented by error bars. (a) (—●—) $y = 223.5474$ (SRP) + 3.8374 and $p < 0.001$; (- - ○ - -) $y = 647.3938$ (SRP) + 3.8374 and $p < 0.001$

Figure 3.4. (a) Cumulative benthic chloropyll-a (chl-a) and (b) cumulative ash-free dry mass (AFDM) across streams during the P-only and N + P enrichment periods. Standard error represented by error bars

Figure 3.5. Relationships of cumulative benthic chlorophyll-a (chl-a) to mass of (a) nitrogen (N) and (b) phosphorus (P) immobilized by periphyton from Big Creek and Cave Creek during the P-only and N + P enrichments. (a) (—■—) $y = 1.072$ (N Immobilized) + 1.749, $r^2 = 0.98$, and $p = 0.002$; (···●···) $y = 0.8665$ (N Immobilized) + 11.95, $r^2 = 0.98$, $p < 0.001$. (b) (—■—) $y = 10.72$ (P Immobilized) + 2.754, $r^2 = 0.98$, $p < 0.001$; (···●···) $y = 8.665$ (P Immobilized) + 12.77, $r^2 = 0.98$, and $p < 0.001$; (···□···) $y = 3.540$ (P Immobilized) + 4.871, $r^2 = 0.98$, and $p < 0.001$; (···○···) $y = 1.478$ (P Immobilized) + 13.99, $r^2 = 0.70$, and $p = 0.035$

3.9 Figures

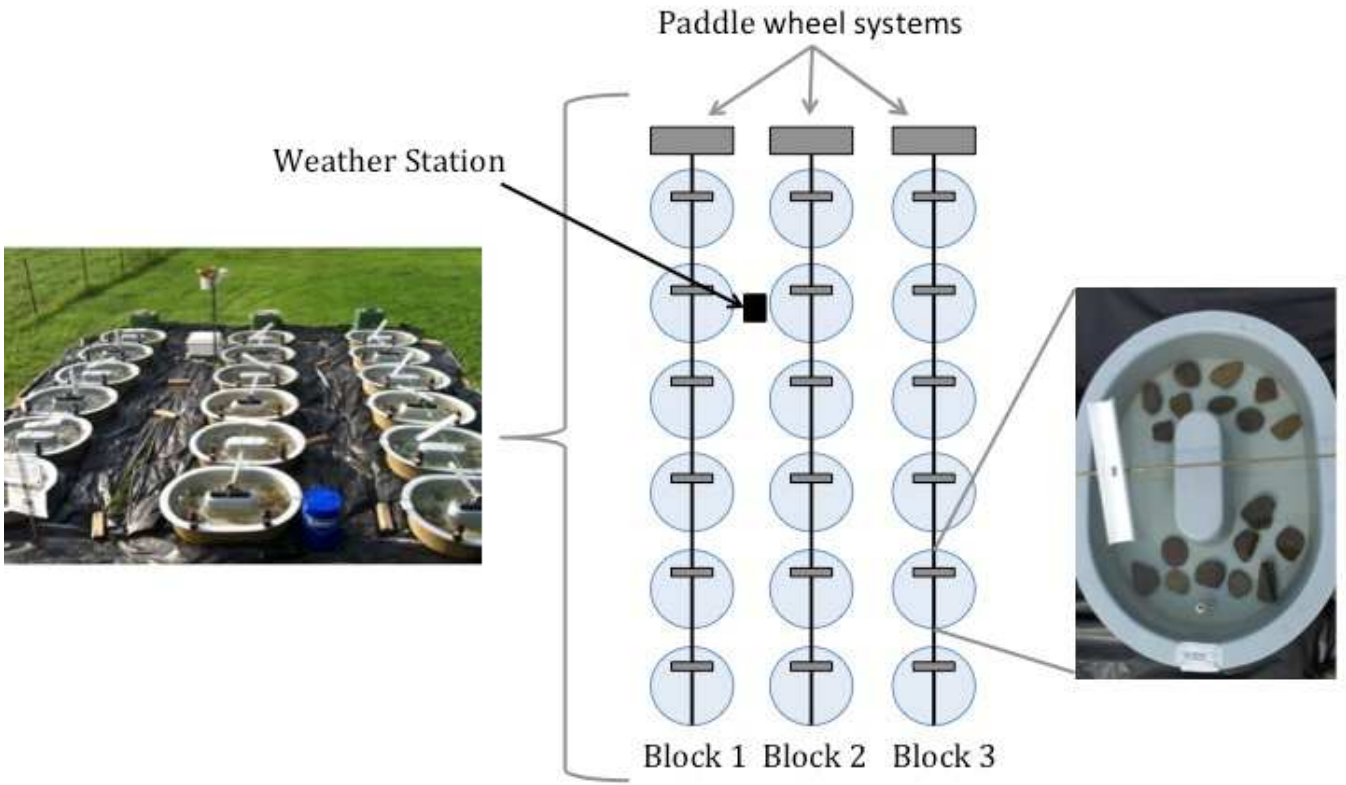

Figure 3.1

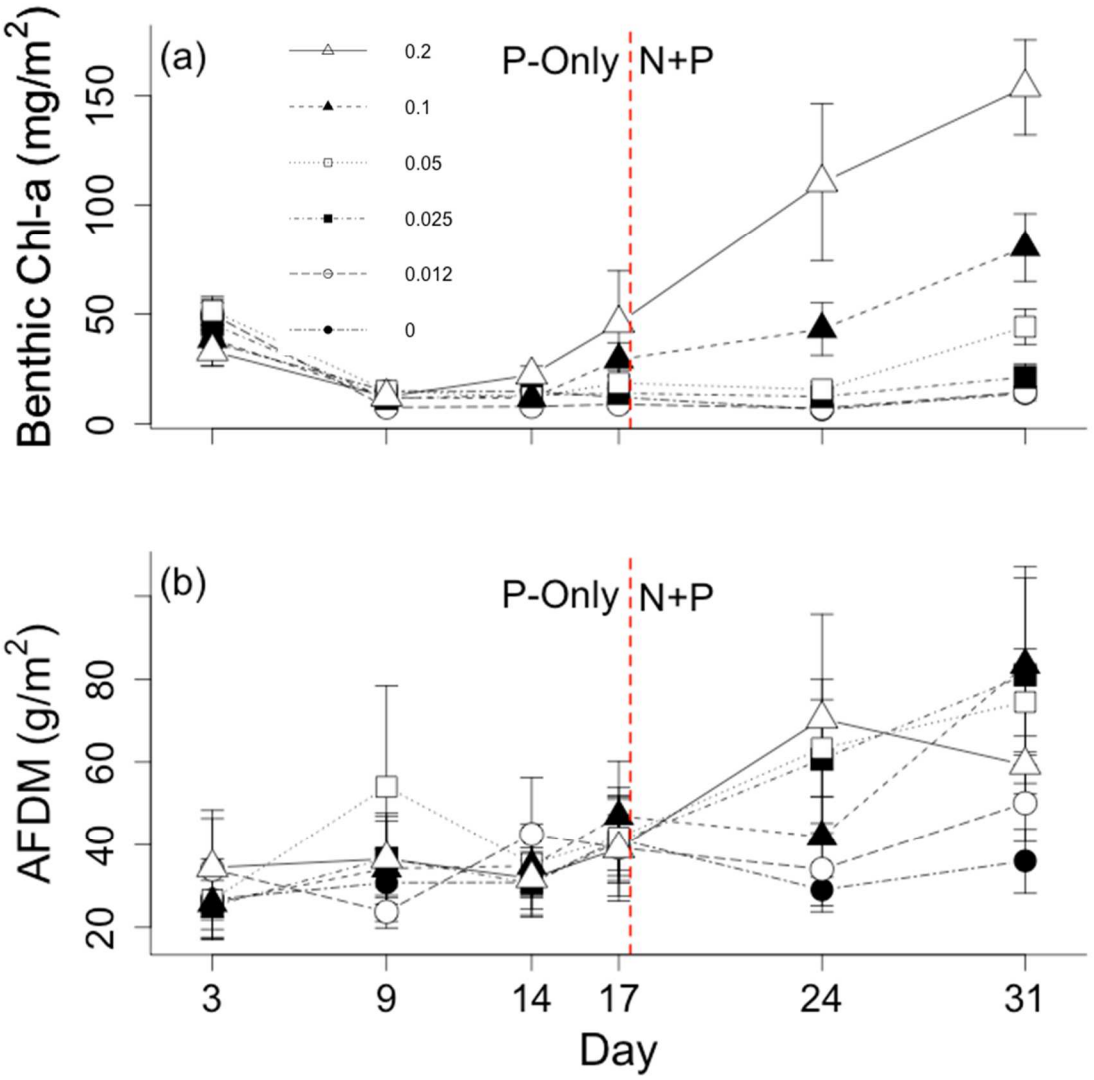
(a)
P-Only
N+P
0.2
0.1
0.05
0.025
0.012
0
Benthic Chl-a (mg/m2)
0
50
100
150
(b)
P-Only
N+P
AFDM (g/m2)
20
40
60
80
3
9
14
17
24
31
Day

Figure 3.2

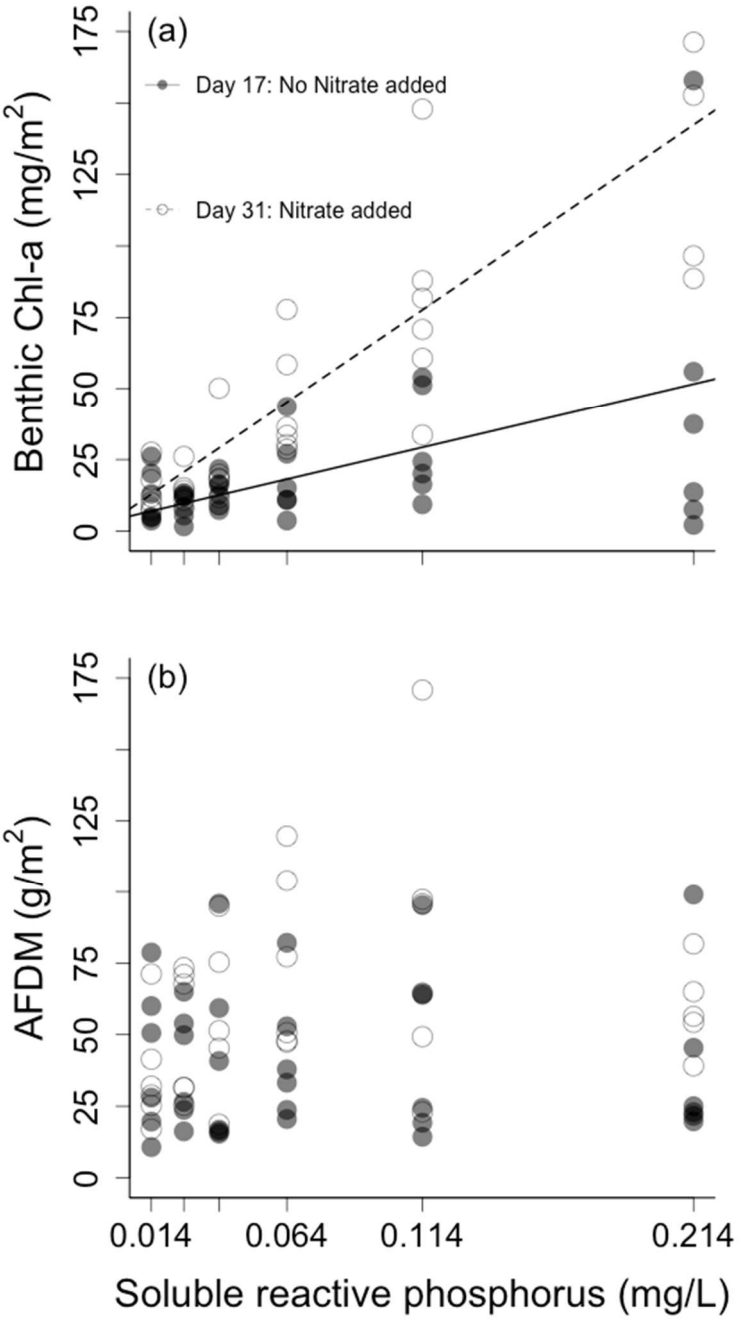
(a)
Day 17: No Nitrate added
Day 31: Nitrate added
Benthic Chl-a (mg/m2)
0
25
50
75
125
175
(b)
AFDM (g/m2)
0
25
50
75
125
175
0.014
0.064
0.114
0.214
Soluble reactive phosphorus (mg/L)

Figure 3.3

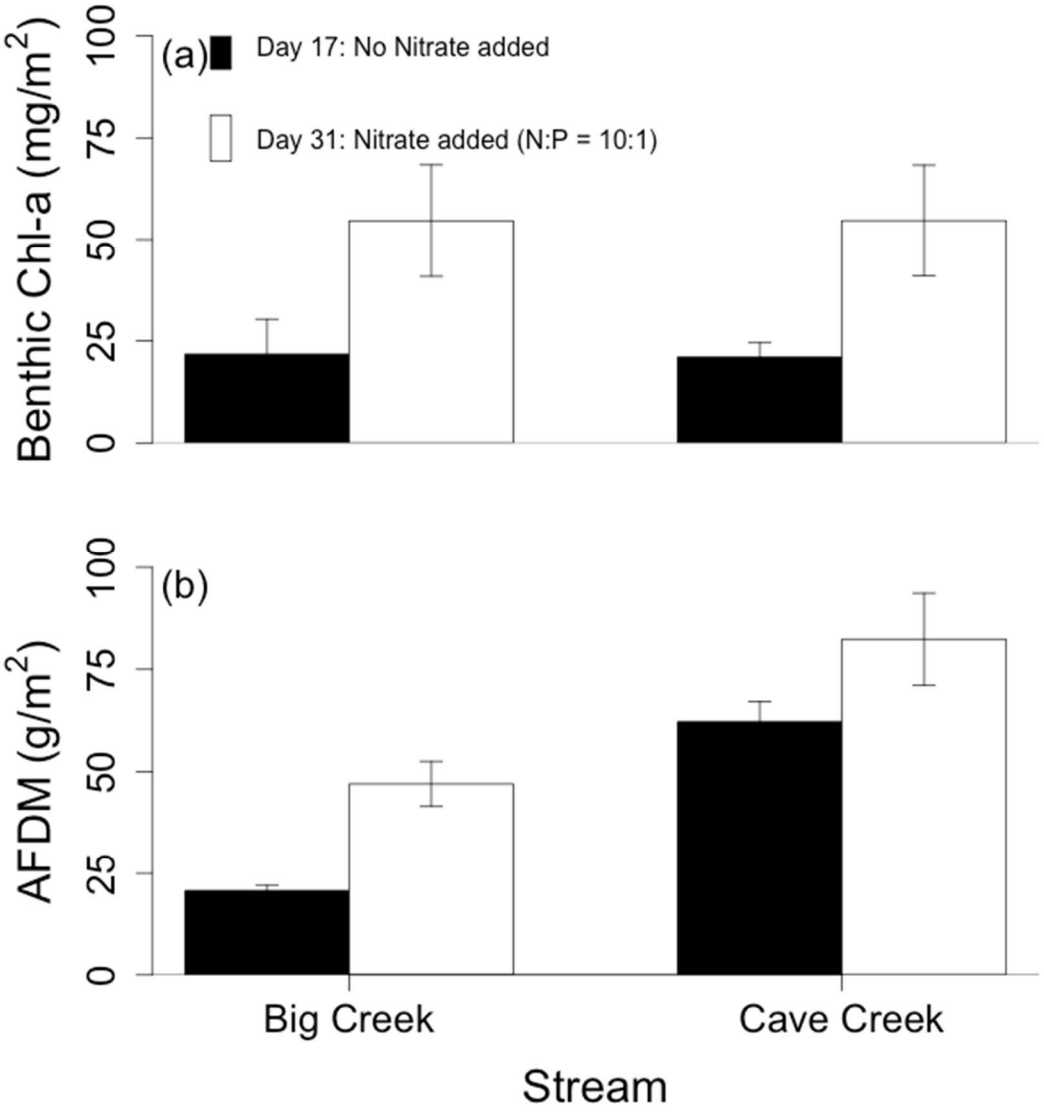
(a)
Day 17: No Nitrate added
Day 31: Nitrate added (N:P = 10:1)
Benthic Chl-a (mg/m2)
0
25
50
75
100
(b)
AFDM (g/m2)
0
25
50
75
100
Big Creek
Cave Creek
Stream

Figure 3.4

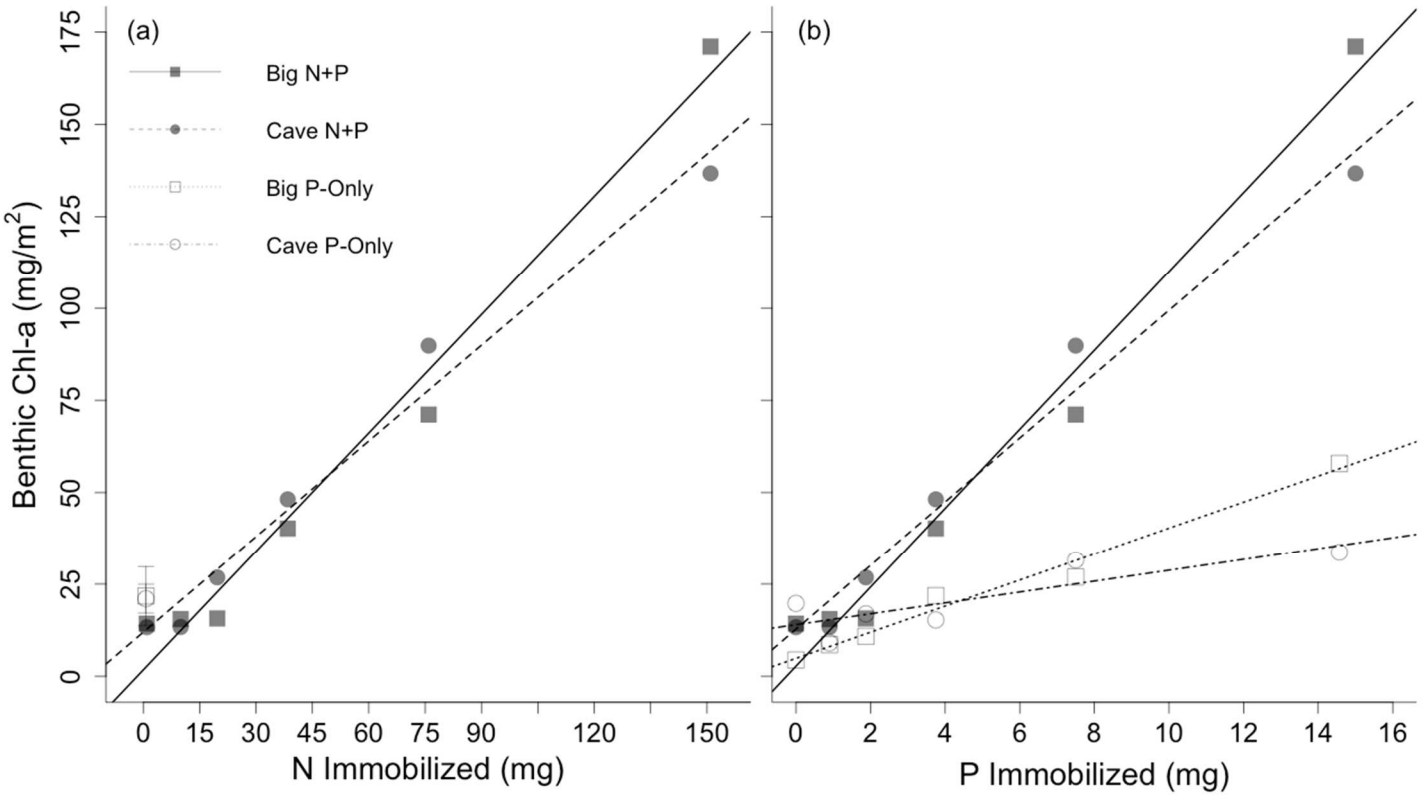
(a)
(b)
Big N+P
Cave N+P
Big P-Only
Cave P-Only
Benthic Chl-a (mg/m^2)
0
25
50
75
100
125
150
175
0
15
30
45
60
75
90
120
150
N Immobilized (mg)
0
2
4
6
8
10
12
14
16
P Immobilized (mg)

Figure 3.5

4. CONCLUSION

Accelerated eutrophication of the world's freshwater resources has elicited concern from the scientific community about potential widespread repercussions of nutrient pollution (Smith et al. 1999). Because of this, the need for scientifically defensible numeric nutrient standards in streams has been endorsed for years but has not yet been fully realized (Dodds and Welch 2000; Evans-White et al. 2013). Nonetheless, there are many opportunities to develop defensible nutrient criteria development through the use of field and experimental studies and protect stream ecosystem services (Stevenson and Sabater 2010). Understanding algal-nutrient relationships and determining comparability of popular data collection protocols are pertinent steps on the journey to acquiring knowledge about how to best pinpoint numerical nutrient thresholds (Aloi 1990; Dodds et al. 1998). Additionally, algal-nutrient investigations focused on smaller spatial scales aid in reducing natural variation in nutrients and algae due to such factors as climate, geology, watershed area, and land use and provide managers with the best tools for understanding the dynamics of individual watersheds (Smucker et al. 2013). The focus of this thesis was to further contribute to the development of numeric nutrient criteria by: (1) comparing two common data collection methods used in periphyton field bioassessments and (2) providing experimental evidence of watershed-specific algal biomass responses across manipulated nitrogen (N) and phosphorus (P) gradients.

Evidence suggests pre-existing datasets collected using the delimiter-reduced and whole-surface periphyton removal techniques may be compiled into larger datasets, particularly when data collected during different seasons are regarded separately. Although repeated measures ANOVA revealed methods were comparable throughout the study, sometimes variability in biomass across seasons was significantly different. Biomass collected during months with cooler

temperatures (i.e. November and February) exhibited the most variability between methods (i.e. heteroscedasticity). Results of the method comparison study support literature advising that confounding factors other than nutrients may greatly influence variability in periphyton biomass and the magnitude of influence is seasonally-dependent (Biggs 1995; Hillebrand 2008; Villeneuve et al. 2010). For example, the importance of seasonal grazing impacts on periphyton biomass in the Ozarks, as suggested by Power et al. (1988), possibly occurred during the study. Knowing the comparability of periphyton bioassessment methods is beneficial to managers attempting to model algal biomass-nutrient relationships with pre-existing datasets. Still, studies with larger sample sizes and within other ecoregions would be advantageous in confirming these results, as would comparison of other data collection methods used in periphyton bioassessment.

Results of the nutrient enrichment experiment provided additional experimental support for the growing body of literature that suggests streams can be co-limited by N and P (Elser et al. 2007; Lange et al. 2011) and confirmed that Ozark stream periphyton productivity are limited by both N and P when confounding factors are controlled. Increased N and P enrichment were experimentally shown to stimulate increases in algal biomass over a 31 day period, suggesting streams in pristine watersheds, like the Buffalo River Basin, may be vulnerable to increased nutrient loading primarily from changing land uses (Stevenson et al. 2008). Unlike similar nutrient manipulation studies, no thresholds in algal biomass were observed throughout the applied nutrient gradient (i.e. 0 to 0.2 mg/L) when stimulated by P alone or N + P (10:1 mass ratio) but this finding is likely due to differences in nutrient saturation between flow through and pulsed nutrient addition study designs (Bothwell 1989; Hill et al. 2009; Rier and Stevenson 2006). The observed algal biomass-nutrient relationships in this study are particularly unsettling because the economy of the Buffalo National River Watershed is primarily supported by tourism,

and reduced economic values have been reported when streams are no longer aesthetically appealing due to increases in algal biomass (algal biomass > 100-150 mg/m^2) (Dodds et al. 2009; Suplee et al. 2009; Welch et al. 1988). More experimental evidence and regional analysis of combined datasets is warranted to improve understanding of the comparability of manipulated algal biomass-nutrient experiments and the response of periphyton to nutrient stimulation under natural conditions with the presence of other biomass-limiting effects (i.e. variable grazing, shading, and flow).

Together, results from this thesis work provide unique insight into using algal biomass responses to nutrient enrichment as a tool for developing numeric nutrient standards in order to better protect aquatic resources, specifically at the watershed and level II ecoregion scales. Results suggest algal biomass can exhibit positive responses to N and P enrichment. However, the presence of confounding factors may limit biomass accrual in natural systems. Additionally, the possibility of combining periphyton biomass datasets collected at relatively small spatial scales using different bioassessment methods supports increased collaboration between different government agencies, universities, and other interest groups. Future studies directed toward understanding the dynamic influence confounding factors may have on periphyton biomass are needed, and the extent that experimentally derived algal-nutrient datasets represent natural responses remains unknown.

4.1 References

Aloi, J. (1990). A critical review of recent freshwater periphyton field methods. *Canadian Journal of Fisheries and Aquatic Sciences*, *47*, 656–670.

Biggs, B. J. F. (1995). The contribution of flood disturbance, catchment geology and land use to the habitat template of periphton in stream ecosystems. *Freshwater Biology*, *33*(3), 419–438.

Bothwell, M. L. (1989). Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*, 1293–1301.

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., et al. (2009). Eutrophication of U. S. freshwaters: analysis of potential economic damages. *Environmental Science & Technology*, *43*(1), 12–19.

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

Dodds, W. K., & Welch, E. B. (2000). Establishing nutrient criteria in streams. *Journal of North American Benthological Society*, *19*(1), 186–196.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., et al. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135–1142. doi:10.1111/j.1461-0248.2007.01113.x

Evans-White, M. A., Haggard, B. E., & Scott, J. T. (2013). A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality*, *42*(4), 1002–1014. doi:10.2134/jeq2012.0491

Hill, W. R., Fanta, S. E., & Roberts, B. J. (2009). Quantifying phosphorus and light effects in stream algae. *Limnology and Oceanography*, *54*(1), 368–380.

Hillebrand, H. (2008). Grazing regulates the spatial variability of periphyton biomass. *Ecological Society of America*, *89*(1), 165–173.

Lange, K., Liess, A., Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2011). Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, *56*(2), 264–278. doi:10.1111/j.1365-2427.2010.02492.x

Power, M. E., Stewart, A. J., & Matthews, W. J. (1988). Grazer control of algae in an Ozark

mountain stream: effects of short-term exclusion. *Ecology*, *69*(6), 1894–1898. doi:10.2307/1941166

Rier, S. T., & Stevenson, R. J. (2006). Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. *Hydrobiologia*, *561*(1), 131–147. doi:10.1007/s10750-005-1610-6

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, *100*, 179–196. doi:10.1016/S0269-7491(99)00091-3

Smucker, N. J., Becker, M., Detenbeck, N. E., & Morrison, A. C. (2013). Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. *Ecological Indicators*, *32*, 51–61. doi:10.1016/j.ecolind.2013.03.018

Stevenson, R. J., Hill, B. H., Herlihy, A. T., Yuan, L. L., & Norton, S. B. (2008). Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of the North American Benthological Society*, *27*(3), 783–799. doi:10.1899/07-077.1

Stevenson, R. J., & Sabater, S. (2010). Understanding effects of global change on river ecosystems: science to support policy in a changing world. *Hydrobiologia*, *657*, 3–18. doi:10.1007/s10750-010-0392-7

Suplee, M. W., Watson, V., Teply, M., & McKee, H. (2009). How green is too green? public opinion of what constitutes undesirable algae levels in streams. *Journal of the American Water Resources Association*, *45*(1), 123–140. doi:10.1111/j.1752-1688.2008.00265.x

Villeneuve, A., Montuelle, B., & Bouchez, A. (2010). Influence of slight differences in environmental conditions (light, hydrodynamics) on the structure and function of periphyton. *Aquatic Sciences*, *72*(1), 33–44. doi:10.1007/s00027-009-0108-0

Welch, E. B., Jacoby, J. M., Horner, R. R., & Seeley, M. R. (1988). Nuisance biomass levels of periphytic algae in streams. *Hydrobiologia*, *157*, 161–168.