ABSTRACT

Nutrient Enrichment Effects on Stream Periphyton Stoichiometry, Algal and Fish Assemblage Structure, and Grazing Fish-Periphyton Interactions

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Anthropogenic inputs of nitrogen (N) and phosphorus (P) create novel environmental conditions that alter biological organization and ecosystem functioning in freshwaters. My research objectives were: 1) identify levels of nutrient enrichment thresholds for stream periphyton nutrient ratios, individual algal and fish species abundances, and overall assemblage composition; 2) experimentally investigate interactions between P enrichment, an important grazing fish, and periphyton biomass and elemental composition; and 3) examine the influence of nitrogen enrichment on fish-mediated nutrient recycling effects on downstream ecosystem structure and function. Analyses of field collected data indicated that sharp declines in periphyton elemental composition, a synchronous decline in several algal species, and sharp declines in at least two benthic specialist fishes (*Etheostoma spectabile* and *Campostoma anomalum*) occurred when surface-water TP exceeded approximately 20 $\mu g\ L^{-1}$ and when TN was greater than 550 $\mu g\ L^{-1}$. A stream mesocosm experiment demonstrated that grazing by *C. amomalum* decreased benthic C:Chlorophyll *a* ratios, disrupted the continuous deposition

of detritus and inorganic sediment, and as a result, increased periphyton P and N content. Variation in grazer effects on periphyton C:P and C:N ratios across the P enrichment treatments resulted in N:P ratios that were greater on grazed substrates in control and low P streams but grazer effects diminished with increasing P enrichment. A second experiment that measured nutrient recycling effects showed that fish-mediated nutrient recycling decreased periphyton C:N and increased C:P and N:P longitudinally in N enriched streams. Additionally, chlorophyll *a* increased significantly downstream of cages with fish. Comparisons of bacterial biomass production (BBP) and photosynthesis (PS) rates downstream of enclosures indicated that aerial BBP and PS rates and coupling of algal and bacterial production (COV_{BBP-PS}) were all higher downstream of enclosures with fish in high N:P streams. The combined results of these three studies suggest that there are serious biodiversity declines at low levels of nutrient enrichment, and loss of grazing fish species may have important consequences for stream ecosystem structure and function, as grazing fish plays an important role in modulating interactions between surface-water nutrients and benthic resources in streams.

Nutrient Enrichment Effects on Stream Periphyton Stoichiometry, Algal and Fish Assemblage Structure, and Grazing Fish-Periphyton Interactions

by

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A Dissertation

Approved by the Department of Biology

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Robert D. Doyle, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

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December 2011

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ACKNOWLEDGMENTS

I thank my committee: Ryan King, Robert Doyle, Steve Dworkin, Owen Lind, and Joseph White for their help and encouragement in this journey. I would also like to thank Allison Pease and Kirk Winemiller at Texas A&M University, Thad Scott at University of Arkansas, and Jeff Back, Charles Stanley and Ted Valenti at Baylor University for collaborating on various projects during my time at Baylor. I would like to thank the fish crew; Charles Stanley, Justin Grimm, Emily Hooser, and Brianna Kirchner, as well as, Becky Shaftel, Julie Baldizar, Carla Carr, Jeff Back, and Jeff Mink for help in the field. I would especially like to thank the two Jeffs for "keeping it real" in the field. Finally, I would like to thank my wife, Carla, her parents, Carlton and Linda Carr, and my parents Mark and Evie Taylor for all their love and support.

This dissertation was supported by a grant from the U.S. Environmental Protection Agency's Science to Achieve Results (STAR) Graduate Research Fellowship program. Although the research described in this document has been funded in part by the U.S. Environmental Protection Agency's STAR program through grant (FP-91694301-1), it has not been subjected to any EPA review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Additional funding was provided by the Glasscock Fund for Excellence in Environmental Science, the Jack G. and Norma Jean Folmar Research Fund, and a Bob Gardner Memorial grant. The Center for Reservoir and Aquatic Systems Research

provided laboratory and travel support. Additional travel to conferences was supported by the Baylor University Graduate School.

DEDICATION

To my wife Carla and son Carson

Every day you remind me what is important

CHAPTER ONE

Introduction

General Overview

Human activities have become a principal driver of change in earth system processes, leading some researchers to suggest that we have entered a new geologic era, the anthropocene (Crutzen 2002; Meybeck 2003; Steffen et al. 2011). This phenomenon is shifting the physical and chemical properties of many modern ecosystems outside ranges of variability experienced by species across evolutionary time scales (Hobbs et al. 2006). As a result, independent shifts in species' distributions through several inter- and intra-specific mechanisms are leading to unprecedented changes in species composition (Hobbs et al. 2006; Williams and Jackson 2007; Stralberg et al. 2009). Unexpected consequences may arise with the development of novel communities including alteration of ecosystem properties (Hooper et al. 2005), which can lead to regime shifts, and ultimately make ecosystems unresponsive to habitat restoration efforts (Stralberg et al. 2009; Palmer et al. 2010; Clements et al. 2010). Therefore, protecting biodiversity requires identifying critical ranges of variation for key ecological attributes that change natural communities within ecosystems when exceeded (Parrish et al. 2003).

A classic example of a novel environmental gradient driven by human activities is the eutrophication of freshwater ecosystems. Humans have significantly altered the input of nitrogen (N) and phosphorus (P) to freshwater systems, and the resulting eutrophication is a major obstacle in protecting freshwater and coastal marine ecosystems

(Carpenter et al. 1998; Smith et al. 2006). Natural inputs of inorganic N to freshwater systems are derived largely from surrounding geology and vegetation (Dodds 1997), as well as within-stream fixation of atmospheric N_2 to NH_4^+ by bacteria and cyanobacteria. Whereas inorganic P naturally enters freshwater systems through the weathering of phosphate-bearing rocks which are patchily distributed (Notholt et al. 2005). As a result, most freshwater species assemblages have evolved in low nutrient environments. Anthropogenic activities including burning fossil fuels, planting N-fixing crops, production of fertilizer derived from the Häber process (N) and mining of phosphates (P), and the disposal of wastewater from a growing human population and the animals that feed them, have significantly altered the amount and distribution of limiting nutrients across the globe (Vitousek 1997; Carpenter and Bennett 2011). Relatively small increments of anthropogenic nutrients can create novel environmental conditions for aquatic organisms that have evolved under low nutrient conditions, often inducing changes in biological species composition and associated ecosystem processes.

Response of Stream Assemblages to Nutrient Enrichment

Multimetric biological indices were developed over broad scale gradients of general human influence for assessing the ecological health of aquatic ecosystems (Karr and Chu 1997). Unfortunately, these assessments were not developed to characterize the effects of specific stressors on biological endpoints (Norton et al. 2000) and have limited utility in directly supporting the development of numerical water quality criteria for specific stressors, such as excess nutrients. Additionally, many reported linear responses to nutrient enrichment based on aggregate measures (species richness, multi-metric biotic indices, ordination axes) are likely artifacts of *a priori* assignments of taxa tolerance

values or combining abundances of coarse taxonomic, functional, and habitat classes without distinguishing the direction, location, and magnitude of responses (King and Baker 2010). Aggregate measures of assemblage structure that combine the distinctly different responses of declining and increasing species may result in linear or wedge-shaped responses to environmental gradients, and underestimate negative effects of nutrient enrichment on stream assemblages (King and Baker 2010, 2011). The analysis of raw species abundance data with appropriate data analysis techniques allows the "species to do the talking" and potentially provides a clearer, more robust response to environmental gradients, such as nutrient enrichment.

While data analysis techniques may influence response shapes of species assemblages to nutrient enrichment, differences in the relationships between organisms and nutrients may influence responses as well. For example, periphyton species assemblages should show strong relationships with surface-water nutrient concentrations and exhibit threshold responses to low levels of enrichment because evolutionary constraints imposed on algae species in low nutrient environments are relaxed. In contrast, nutrient enrichment influences consumer assemblages indirectly, through altered consumer-resource stoichiometric relationships (Cross et al. 2006; Evans-White et al. 2009), macrophyte habitat structure (Mittelbach 1984), and production / respiration dynamics (Miltner and Rankin 1998) associated with changes in primary producer species composition, biomass, and nutrient content. Each of these factors can influence resource availability and habitat suitability for higher consumers such as fish but may be over-shadowed by other factors experienced by streams. Prairie stream fishes have evolved behavioral and physiological mechanisms as well as life history traits in response

to variable and sometimes harsh conditions associated with dynamic hydrology (Matthews 1987; Matthews 1988; Labbe and Fausch 2000; Matthews and Marsh-Matthews 2003). This ability to cope with highly variable natural conditions may confer a degree of resilience to changes in habitat quality associated with nutrient enrichment. For this reason, it is useful to examine both producer and consumer assemblage responses to nutrient enrichment because these groups represent direct (species optima for growth) and indirect (ecosystem changes associated with enrichment) mechanisms involved in relationships between species assemblage composition and nutrient enrichment.

Interactions between Nutrient Enrichment and Grazer Dynamics on Benthic Resources

Shifts in assemblage structure in response to direct or indirect impacts of nutrient enrichment may result in declines of functionally important species, such as grazing fish. Loss of grazing fish in streams will likely have ecosystem consequences as they can exert considerable influence on structural and functional components of benthic environments (Power 1984; Power et al. 1985; Grimm 1988; Power 1990; Gelwick and Matthews 1992; Flecker and Taylor 2004; Taylor et al. 2006; Bertrand and Gido 2007) and may mitigate or amplify the response of stream periphyton to nutrient enrichment (Stewart 1987; Flecker et al. 2002; Kohler et al. 2011). For example, the effect of grazing is often greater than nutrient enrichment effects on periphyton biomass (Hillebrand 2002), but grazing fish may also influence the elemental composition of attached periphyton via direct consumptive effects and indirect bioturbation or nutrient recycling effects (Taylor et al. 2006; Knoll et al. 2009; McIntyre and Flecker 2010, Kohler et al. 2011). The influence of these two factors on periphyton elemental composition likely varies

depending on other factors such as nutrient content of grazers and their food, grazer biomass, and water column nutrients (Hillebrand et al. 2008).

Ecological stoichiometry (ES) is the study of the balance of multiple chemical substances, particularly C, N, and P in ecological interactions and processes (Elser et al. 1996; Sterner and Elser 2002) and provides an excellent framework for understanding interactions between grazing fish, benthic algae, and nutrients in aquatic systems (Hillebrand et al. 2008). In general, grazers influence the elemental composition of periphyton through several pathways (Figure 1) including:

1. Removal of senescent algal cells that reduce the nutrient-poor detrital component of periphyton communities;
2. Removal of biomass which alters uptake by decreasing diffusion barriers and increasing the relative availability of nutrients for remaining algal biomass (Frost et al. 2002; Hillebrand et al. 2008);
3. Removal of biomass can also change the relative abundance of algae taxa with differing nutrient demands or elemental composition (Frost et al. 2002; Liess and Kahlert 2009); and
4. Regulating the ratios of bioavailable nutrients that are incorporated by periphyton communities through differential recycling of limiting nutrients (Evans-White and Lamberti 2006; Hillebrand et al. 2008; Knoll et al. 2009).

In general, the presence of grazers increases the nutrient content of periphyton resulting in lower C:N and C:P ratios and higher N:P ratios (Hillebrand et al. 2008). Despite this, our understanding of how grazing fish influence periphyton nutrient content is limited (but see Knoll et al. 2009) and few studies have addressed

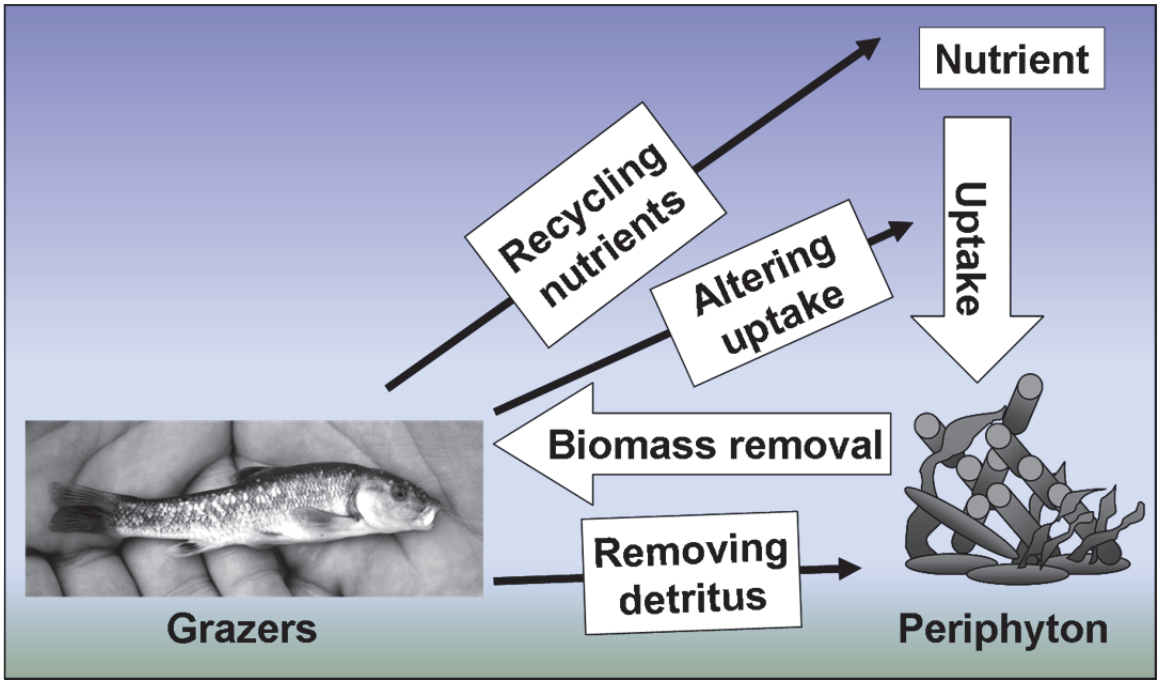

Figure 1. A conceptual model illustrating direct and indirect effects of grazing fish on nutrient stoichiometry. Fish can effect periphyton C:N:P ratios directly through the removal of biomass and indirect mechanisms including removal of detritus, altered nutrient uptake, and nutrient recycling (based on Hillebrand et al. 2008).

interactions between nutrient enrichment, grazing fish and periphyton stoichiometry in stream ecosystems (Kohler et al. 2011).

Nutrient Enrichment, Grazer Dynamics, and Downstream Nutrient Export

Pathways by which grazers influence elemental composition of periphyton have implications for nutrient cycles in streams. Due to the unidirectional flow of water across benthic habitats in streams, nutrients may become depleted along longitudinal gradients due to nutrient uptake by benthic autotrophs and heterotrophs (Mulholland et al. 1995). Consumption of benthic resources and remineralization of nutrients by aquatic consumers facilitate downstream nutrient export (Figure 1). The balancing of these two processes, uptake by the microbial community and remineralization by consumers, results in

nutrient atoms spiraling between biological pools and water column pools as they move downstream. The length of these nutrient spirals depends on stream velocity as well as uptake and remineralization rates which are governed by the nutrient requirements of stream biota (Newbold et al. 1982; Small et al. 2009).

The role of fish in nutrient cycling can constitute an important biogeochemical flux of limiting nutrients and maintain primary production in stream ecosystems (Grimm 1988; Vanni et al. 2002; McIntyre et al. 2008). Nutrient enrichment potentially influences this process by altering consumer-resource stoichiometric relationships (Cross et al. 2006; Evans-White et al. 2009), which may lead to differential excretion of macronutrients (N and P) by consumers, and alter the export of limiting and non-limiting nutrients to downstream habitats (Small et al. 2009). Changes in downstream nutrient availability, associated with fish-mediated nutrient recycling, has the potential to alter periphyton structure (biomass, elemental composition) and function (autotrophic and heterotrophic productivity, internal nutrient cycling) in downstream benthic habitats.

Problem Statement

Given the paucity of information available for species assemblage responses to nutrient enrichment in streams, and the reliance on multi-metric indices by the majority of existing studies, levels of nutrient enrichment at which significant changes in individual species abundance and stream assemblage structure occurs are not well understood and likely underestimated. Additionally, while there is growing evidence that fish influence interactions between water-column nutrients and benthic resources in streams, our understanding of how nutrient enrichment influences these interactions is limited. Understanding how nutrient subsidies alter species distributions, stream

assemblage structure, and interactions between consumers and producers in streams is critical for the development of scientifically defensible, numerical nutrient criteria that is protective of natural stream assemblages and associated ecological processes.

Study Objectives

The following objectives are proposed. 1) Characterize water column nutrients and their influence on periphyton nutrient content, and quantify and compare shifts in individual species and assemblage composition for periphyton and fish across 38 streams in central Texas, USA. 2) Experimentally investigate how grazing by central stonerollers and P availability at three ecologically relevant concentrations influence periphyton biomass and nutrient content in pools of twelve flow-through stream mesocosms. 3) Experimentally investigate the role of fish-mediated nutrient recycling under two different N:P regimes in downstream export of N and P and its influence on downstream ecosystem structure and function by quantifying longitudinal changes in periphyton biomass, stoichiometry, and coupling between algal and bacterial production in twelve flow-through stream mesocosms with or without grazing fish.

Summary of Chapter Contents

This document is divided into 5 chapters, including the current chapter with introductory and background information. Chapter 2 describes a large-scale observational study designed to identify levels of nutrient enrichment that cause abrupt changes in periphyton nutrient content, identify and separate the response of species that decline or increase in response to enrichment, and use this information to identify species and assemblage level thresholds based on both periphyton and fish. Chapter 3 provides

details of a 28 day experiment conducted in 12 large stream mesocosms designed to determine how central stonerollers (*Campostoma anomulum*) influence periphyton biomass and elemental composition under three different P concentrations representing below, at, and above thresholds for P enrichment identified in Chapter 2. Chapter 4 describes a second 42 day mesocosm experiment designed to quantify fish-mediated nutrient recycling by central stonerollers on downstream periphyton structure and function, including periphyton biomass, elemental composition, and coupling of algal and microbial production, in streams above and below thresholds identified for N enrichment in Chapter 2. This document is concluded with chapter 5, which provides a synthesis of the three studies (chapters 2-4), discusses some of the general themes and interconnections between them, and makes recommendations for future avenues of research.

CHAPTER TWO

Stream Biodiversity Thresholds in Response to Low-level Nutrient Enrichment Have Important Implications for Numerical Criteria Development

Introduction

Humans have significantly altered the input of nitrogen (N) and phosphorus (P) to freshwater systems, and the resulting eutrophication is a major obstacle in protecting freshwater and coastal marine ecosystems (Carpenter et al. 1998; Smith et al. 2006). In response, regulatory authorities such as the U.S. Environmental Protection Agency (US EPA) and European Union Water Framework Directive have charged water quality managers with developing defensible, numerical criteria for nutrients that include endpoints that reflect physical, chemical, and biological integrity of aquatic ecosystems (US EPA 1998; Hering et al. 2010). Despite this, development of numerical nutrient criteria has progressed slowly, largely due to insufficient data on nutrients and biological endpoints, and inadequate statistical tools for quantifying levels of enrichment likely to cause biological impairments.

Natural inputs of inorganic N and P to freshwater systems are dependent on watershed geology and vegetation. Nitrogen requirements of freshwater organisms may be augmented by fixation of atmospheric N_2, whereas P is largely derived from the weathering of phosphate-bearing rocks. However, major phosphate deposits are patchily distributed (Notholt et al. 2005), and as a result, most freshwater species assemblages have evolved in low-P environments. For example, periphyton communities dominated by species with low P requirements are common in regions where biogeochemistry is

driven by high Ca and low P availability (Noe et al. 2001). Relatively small increments of anthropogenic P can create novel environmental conditions for algae species that have evolved under low nutrient conditions, and often induces changes in species composition at relatively low-levels of enrichment (Gaiser et al. 2005; Richardson et al. 2007). Shifts in primary producer species composition and biomass in response to P enrichment also can have substantial negative effects on ecosystem processes that influence higher trophic levels (Carpenter et al. 1998; Smith et al. 2006; Miltner and Rankin 1998).

Changes in relative and absolute abundances of periphyton associated with nutrient enrichment influences consumer assemblages through altered consumer-resource stoichiometric relationships (Cross et al. 2006; Evans-White et al. 2009), macrophyte habitat structure (Mittelbach 1984), and production/respiration dynamics (Miltner and Rankin 1998). All of these factors can influence resource availability and habitat suitability for higher consumers such as fish. In streams within the Great Plains region of North America and other semi-arid regions of the world, high flow variability with extended periods of extreme low flows is normal (Poff and Ward 1989; Bernardo and Alves 1999; Dodds et al. 2004). Fishes of prairie streams have evolved behavioral and physiological mechanisms as well as life history traits in response to variable and sometimes harsh conditions associated with dynamic hydrology (Matthews 1987; Matthews 1988; Labbe and Fausch 2000; Matthews and Marsh-Matthews 2003). This ability to cope with highly variable natural conditions may confer a degree of resilience in the face of anthropogenic impacts.

Species-specific responses to nutrient enrichment through direct (species optima for growth) or indirect (ecosystem changes associated with enrichment) mechanisms

likely culminate in shifts in species assemblage composition involving both producers and consumers. Quantifying these patterns will promote understanding of how nutrient subsidies alter stream ecosystems. In regions of the world that experience seasonal low-flow periods, understanding how nutrient subsidies influence biotic communities is particularly germane. In some catchments, wastewater discharges now account for > 90% of instream flow during low-flow periods (Brooks et al. 2006). Southern regions of North America are expected to experience reduced magnitude, frequency, and duration of stream flows due to climate change (Sun et al. 2008). This emerging threat, combined with increased water consumption and nutrient enrichment associated with rising human population growth will have significant impacts on freshwater biodiversity (Dudgeon 2010; Palmer et al. 2009).

We quantified the responses of periphyton and fish species assemblages across 38 streams spanning a steep gradient of N and P in central Texas, USA. We hypothesized that periphyton nutrient content and species assemblages would show strong relationships with surface-water nutrient concentrations and exhibit threshold responses to low levels of enrichment because evolutionary constraints imposed on algae species in low nutrient environments are relaxed which then favors shifts to novel assemblage compositions. In contrast, we hypothesized that indirect effects of nutrient enrichment on fish species distributions would be weaker and more difficult to detect because of the harsh physicochemical conditions associated with highly variable flows in streams of the Southern Great Plains region.

Materials and Methods

Study Area and Field Sampling

We studied 38 wadeable streams located in the Cross Timbers ecoregion and Brazos and Trinity River basins in Texas (Figure 2). Hydrology within this portion of the Southern Great Plains is highly variable due to seasonal precipitation patterns that include long, hot summers (Matthews et al. 2005). Study sites were selected to provide broad geographic coverage and a range of landscape features that drive nutrient enrichment (pasture and wastewater treatment plant [WWTP] discharge) resulting in a wide range of stream nutrient conditions (Figure 2, Table 1). At each sample site, we collected data on water chemistry, instream and riparian habitat variables, periphyton nutrient content and species composition, and fish relative abundance following Texas Commission on Environmental Quality protocols (Texas Commission on Environmental Quality (TCEQ) 2005) and protocols outlined in King et al (2009).

Environmental Variables

We selected a suite of environmental variables that were hypothesized to be associated with changes in species composition related to nutrient enrichment gradients. We considered five classes of variables: (a) catchment physiography, (b) land cover, (c) instream and riparian habitat, (d) water chemistry and (e) periphyton biomass and tissue chemistry. We initially included a wide range of environmental variables, but narrowed these down to a few non-redundant variables within each broad category using scatterplot matrices and associated correlation coefficients combined with analysis of variance

inflation factors for variables significantly correlated with periphyton or fish assemblage composition (Table 1) (see Data analysis section for more detail).

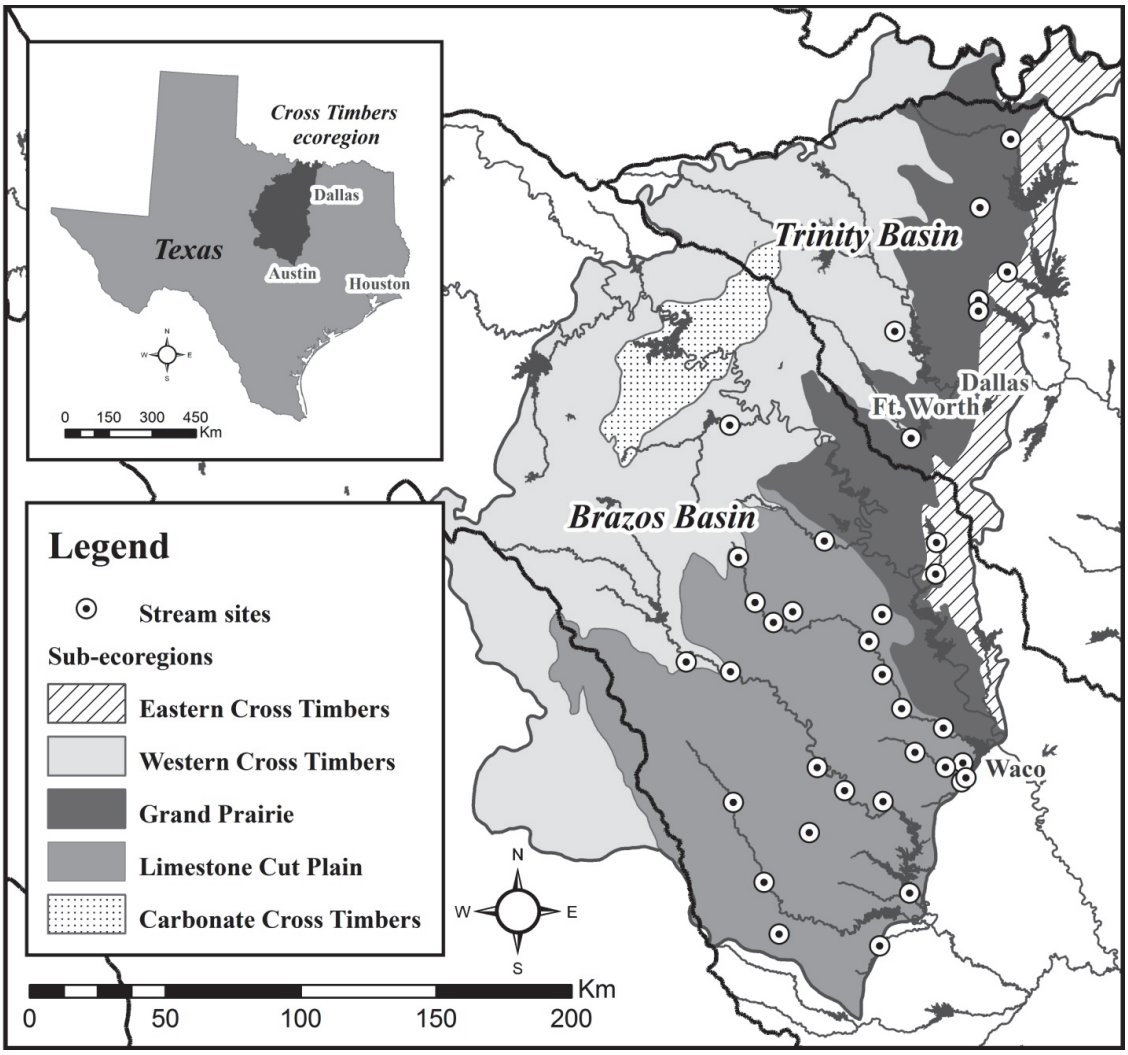

Figure 2. Locations of the 38 study sites across the two river basins within the Cross Timbers ecoregion.

Twenty-three landscape-scale variables describing spatial relationships (coordinates), physical characteristics and topography, land cover, and distribution of disturbance points (outfalls and dams) were calculated for each site within the ecoregion

using ArcGIS v. 9.2 (Pease et al. *in press*). We measured forty-one local habitat variables representing geomorphology, discharge, substrate composition, cover, bank

Table 1. Selected physical characteristics, water chemistry and benthic nutrient content for all 38 sampling sites used in the final analyses. Surface water TN and periphytpn C:N are listed for reference despite being highly correlated with phosphorus variables.

Variable	Min	Max	Mean	Median
Latitude DS	30.9	33.6	32.00	31.88
Watershed area km^2	68	6112	845	404
WWTP km^2	0	0.12	0.01	0
% Shrub	0	56.6	12.6	4.9
% Pasture	0.1	18.6	5.6	3.3
Discharge	0	24.6	3.5	1.3
Mud/silt	0	48.3	8.4	2.2
Bank Slope	17.9	64.3	34.3	34.1
Specific Conductivity	184	1225	646	604
Cl	7	140	41	24
TSS	1	105	14	7
Surface Water TP	7	2380	296	29
Surface Water TN	127	15860	1635	463
Periphyton C:P	78	704	273	192
Periphyton C:N	9	25	18	16

condition, canopy cover and other instream and riparian characteristics following TCEQ protocols (TCEQ 2005; Pease et al. *in press*).

Surface water nutrient sampling consisted of triplicate surface-water instantaneous grab samples. Surface-water grab samples for total phosphorus (TP), total nitrogen (TN) were analyzed using the molybdate and cadmium reduction method, respectively, following persulfate digestion (APHA 1998). Total alkalinity, chloride (Cl), total suspended solids, volatile suspended solids, sulfate, total dissolved solids (TSS), and fluoride were sampled and analyzed in accordance with TCEQ Surface Water Quality Monitoring Procedures (TCEQ 2003). We also collected reach-scale composite samples of epilithic periphyton for analysis of nutrient content and species composition by removing periphyton from surface of at least 25 rocks for a composite periphyton slurry

following methods outlined in (King et al. 2009). All samples were stored in Nalgene dark bottles and transported on ice (4ºC) to the lab within 24 hours.

Periphyton samples were homogenized, subsampled, and filtered onto preweighed Whatman GF/F (pore size = 0.7 μm) filters for quantification of chlorophyll a, dry mass, and AFDM following (Steinman et al. 2006). Additional subsamples were dried at 60°C for 48 hours and pulverized into a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc.) for analysis of nutrient content. We measured C and N content of periphyton using a ThermoQuest Flash EA™ 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) following fuming with HCl to drive off inorganic carbonates (Hill and Middleton 2006). Periphyton P content was analyzed using the molybdate method following a 1-hour digestion in 15 mL of distilled water with 1.8 mL of a mixture of peroxodisulphate (30 g L-1 K2S2O8), boric acid (50 g L-1 H3BO3) and sodium hydroxide (15 g L-1 NaOH) at 121°C (Faerovig and Hessen 2003). Soil (Thermo Finnigan 1.99% C) and peach leaf (SRM 1547, 0.137% P, 0.298% N) standards were analyzed to assure C, N, and P recoveries met QA/QC standards (± 10%) for each sample run.

Species Assemblage Composition

Additional aliquots of homogenized periphyton samples were preserved for species identifications in accordance with taxonomic methods for soft algae and diatoms (TCEQ 2005). One soft algae and one diatom sample were sorted and identified per stream with at least 500 diatom and 300 soft algae cells identified per respective sample (TCEQ, 2005). We quantified fish assemblage composition by sampling all obvious habitat components (e.g., open pools or runs, undercut banks, brush piles, rocks, riffles)

within each survey reach with a backpack electrofisher (Smith-Root Model LR-24) and seine net (4.6 m x 1.8 m, or 1.8 m x 1.8 m). Crews of 3-4 people electrofished each study reach in a single upstream pass with a minimum effort of 900 seconds. The reach was then sampled with a seine net with a minimum of six 10-m hauls that had covered all habitat components within the study reach. If the sixth haul yielded species not previously collected, additional seine hauls were made until no additional species were captured. Fish specimens were identified, counted, and either released unharmed into the habitat or euthanized by ice-bath immersion then preserved in 10% buffered formalin for later identification according to Hubbs et al. (1991) and Thomas et al. (2007). Numerical abundance of each algae and fish species was recorded for each study reach for analyses of species assemblage structure.

Data Analysis

We used a combination of non-metric multidimensional scaling (MDS) ordinations, generalized additive models (GAM), non-parametric change-point analysis (nCPA), and Threshold Indicator Taxa ANalysis (TITAN), to analyze assemblage composition, periphyton nutrient ratios, and individual species responses to surface water nutrients and other catchment and reach-scale variables hypothesized to be important drivers of stream communities in this region. Ordinations were used to extract and visualize gradients in community composition and identify the strongest catchment and reach-scale environmental correlates of algae and fish species composition. GAMs were used in combination with nCPA to contrast the response of periphyton stoichiometry to surface water TN and TP to enhance interpretations of algal species responses to surface-water nutrients. TITAN was used subsequently to identify individual species that

significantly declined or increased with nutrient enrichment variables and estimate the degree of synchrony in their responses as an indication of assemblage-level thresholds. GAMs were also used to validate and develop predictive relationships between the response of individual fish species identified using TITAN and nutrient enrichment variables and respective sources.

Ordination and environmental vector fitting. We conducted MDS ordinations (Minchin 1987; Clarke 1993) on log10(x)-transformed abundances of periphyton and fish species using Bray-Curtis dissimilarity (BCD) as the distance measure in the vegan package in R 2.11.2 (Oksanen et al. 2011, R core development team 2010). We used the function 'envfit' to examine linear correlations between assemblages (MDS axes) and environmental factors associated with nutrient enrichment, and assessed significance of the fitted environmental vectors using a permutation procedure (1000 permutations) (Oksanen et al. 2010). Ordination plots were rotated to have the strongest correlation with the TP vector on axis 1. Additionally, we overlaid bubble plots depicting TP concentrations within site ordination plots to look for nonlinearity in observed relationships with nutrient enrichment. All significant environmental vectors were assessed for collinearity within the broad categories of catchment physiography, land cover, stream reach, water chemistry and periphyton stoichiometry using scatterplot matrices and associated correlation coefficients combined with analysis of variance inflation factors. Environmental variables were removed if they had strong correlations ($r > 0.7$) with other factors and increased variance inflation factors within groups of variables to greater than 3 (Zuur et al. 2009). Only vectors for the remaining variables were plotted in MDS plots and used in subsequent analyses. The exception was TN,

periphyton C:N ratios, and Cl, which we included despite strong relationships with surface water TP and periphyton C:P ratios because we wanted to contrast assemblage responses to both N and P because both nutrients are fundamental to stream productivity whereas Cl provides a conservative tracer of the relative contribution of wastewater discharges to stream flow. Sites with no WWTP had TP < 50 μg L^{-1} and Cl < 40 μg L^{-1}, whereas sites with the highest permitted WWTP discharges also had the highest levels of TP and Cl. (Figure 3a). Discharge at sites with higher TP and Cl concentrations was linearly related to WWTP permitted discharge after accounting for catchment size (Figure 3b). Similar results were observed for TN as the two nutrients were highly correlated across our study sites.

We also estimated differences in assemblage structure between sites among the Brazos and Trinity basins to assess whether basins were confounding relationships between species and environmental variables. Using basins as a grouping variable, we assessed the dispersion of assemblage composition within each group within ordination space using permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP) with the functions 'adonis' and 'betadisper' (Oksanen et al. 2010). We also assessed relationships between latitude and tested for basin differences in environmental variables that were associated with assemblage structure using Spearman rank correlation analysis and Wilcoxon rank-sum tests.

Generalized additive modeling (GAM). We used GAM to fit responses of periphyton nutrient ratios to surface water nutrients. GAMs were performed using the

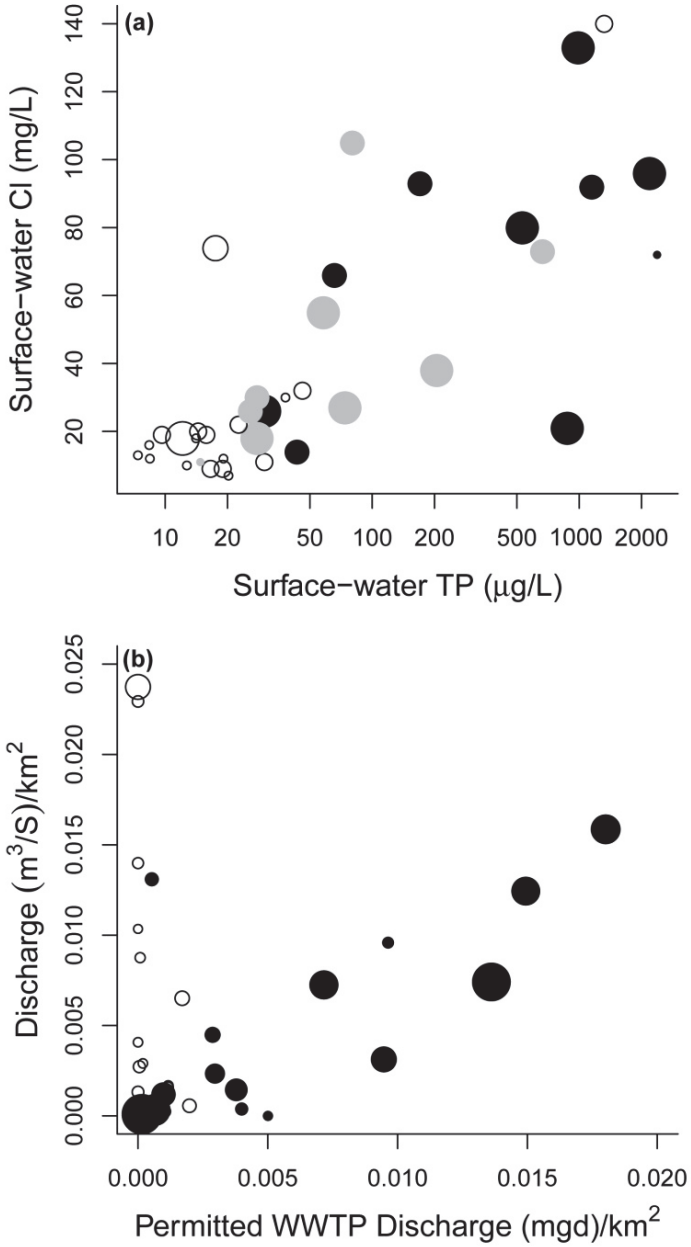

Figure 3. Relationship between surface water TP (µg L^{-1}) and Cl (mg L^{-1}) (a). Sizes of circles represent 25th, 50th and 75th quantiles of observed pasture. Open circles = < 50th, grey circles = 50th-75th, and black circles = > 75th percentile for WWTP influence (mgd/watershed km^2). Panel b represents relationship between permitted WWTP discharge in million gallons per day (mgd) and measured stream discharge (m^3 S^{-1}). Both measures are divided by watershed area (km^2) to remove stream size effects. Size of circles represents surface water Cl (mg L^{-1}) and open circles are sites with surface water TP below thresholds identified for declining fish species. One site, that had exceptionally high watershed size adjusted WWTP discharge (0.1226) and corresponding stream discharge (0.0895) is not plotted but fit the relationship for effluent-dominated streams.

mgcv package in R 2.11.2 (Wood 2006). This package uses splines for smoothers which are formed by dividing the gradient for the explanatory variable into intervals and fitting a cubic polynomial equation to each interval. Periphyton nutrient content data were positively skewed so we used the gamma distribution to model fits with surface-water nutrients. Generalized cross-validation was used to determine the optimal amount of smoothing, and segments were merged together to form smoothers with the effective degrees of freedom (EDF) representing the amount of smoothing and values >1 indicating an increasingly nonlinear relationship (Zuur et al. 2009). In datasets with small sample sizes, models selected by cross validation may be over-fit. To avoid this problem, an upper limit was set for the degrees of freedom (k – 1) for the individual smoother in each model by limiting the number of dimensions (k) to 4. *P*-values obtained from GAM for smoothing splines are approximate, and we therefore used *P*-values <0.001 to identify significance (Zuur et al. 2009). We presented fitted responses for models for each nutrient ratio response graphically with 95% confidence limits.

Non-parametric changepoint analysis. We used non-parametric changepoint analysis (nCPA) (King and Richardson 2003; Qian et al. 2003) to complement the GAM analysis of periphyton nutrient ratios in response to TN and TP by estimating the level of TN or TP that resulted in the greatest change in C:N, C:P, and N:P ratios. Non-parametric changepoint analysis identifies values along an environmental gradient (TN, TP) that separates the response data (periphyton nutrient ratios) into the two groups having the greatest difference in means and/or variances. This can also be thought of as the degree of within-group variance relative to the between group variance. Uncertainty around an observed change point was estimated using a bootstrap simulation (n = 1000)

that resamples (with replacement) the original dataset, and recalculates the change point for each simulation. We used the 5th and 95th percentiles of the distribution of change points from the bootstrap simulation as lower and upper confidence limits. Increasingly narrow confidence limits indicate greater certainty about the location and magnitude of a change point, whereas wide confidence limits imply a weaker, more gradual change point or zone of change.

Threshold indicator taxa analysis (TITAN). We further analyzed taxa responses to each of the important environmental variables identified in the MDS analysis using TITAN (Baker and King 2010). We hypothesized pasture to be the dominant land-cover variable associated with nutrient enrichment. Cl was correlated with nutrient enrichment due to cumulative WWTP discharge (Figure 3). We therefore limited our examination of species- and assemblage-level thresholds to measures of enrichment (surface water TP, TN, and periphyton C:P and C:N ratios), and potential sources (pasture and Cl as a surrogate for WWTP). We used TITAN to identify levels of each environmental variable that corresponded to the greatest change in the frequency and abundance of individual taxa and to assess the relative degree of synchrony in the decline or increase of responsive taxa as an indicator of assemblage-level thresholds.

TITAN is a threshold detection method that combines deviance reduction techniques with indicator species analysis. Association is measured by taxon abundances weighted by their occurrence in each partition (Dufrene and Legendre 1997) and standardized as z-scores to facilitate cross-taxon comparison via permutation of samples along the predictor gradient. TITAN distinguishes declining and increasing taxa, and tracks the cumulative responses of each component within the assemblage.

Bootstrapping is used to identify reliable threshold indicator taxa and the uncertainty around the location of taxon and assemblage change points. TITAN can be used to identify individual species that exhibit threshold responses as well as provide evidence for an assemblage threshold through synchronous changes in abundance of many taxa within a narrow range of predictor values. Prior to performing TITAN, we $\log_{10}(x)$-transformed abundances of each of 148 periphyton and 31 fish taxa (taxa occurring at less than 3 sites were removed from analysis). Individual taxa were deemed significant threshold indicators when reliability and purity were both > 0.95. We interpreted assemblage-level responses as thresholds when multiple taxa declined or increase within a narrow region along an environmental gradient, leading to a sharp peak and narrow confidence limits (95%) around the the sum of individual taxa responses (z scores). TITAN was run with the TITAN package in R.2.11.2 (Baker and King 2010).

We validated TITAN results for fish by using GAMs to model responses of significant declining and increasing fish species identified by TITAN to the strongest nutrient correlates of community structure to better characterize the shape of the stressor-response relationship. We performed GAMs only on fish species because many algal species were significant threshold indicators and scatterplots revealed patterns that were distinctly nonlinear, thus fitting individual GAM models to each of them was impractical, if not gratuitous. We performed GAMs as stated previously except we used untransformed species abundance with the negative binomial distribution for all fits because our fish abundance data contained zeros and was overdispersed (Zuur et al. 2009). We also created GAM models with separate smoothers for each river basin when median values for predictor variables were significantly different between basins. We

assessed effects of incorporating separate basin smoothers on model fit using Akaike's Information Criterion corrected for small sample size (AICc). We compared base models with and without separate smoothers and selected the more complex model if Δ AICc ≥ 3 (Burnham and Anderson 1998). We presented fitted responses for models that explained the most variation for each species graphically with 95% confidence limits.

Results

Environmental Variables and Assemblage Structure

Ordination of periphyton assemblage composition sorted streams along two major axes that explained 79% of the original distances in n-dimensional space (2-D stress = 17.7). The first axis represented a gradient primarily defined by nutrient enrichment variables, whereas the second represented a shorter gradient associated with stream discharge (Figure 4a). Variation in periphyton assemblage composition was most strongly linked to measures of nutrient enrichment defined by TP, TN, periphyton C:P and C:N ratios, and Cl. Bubble plots of TP overlaid on the MDS plot help validate the statistical relationship between TP and periphyton, illustrating that sites with higher TP dramatically increased along the gradient identified by nutrient enrichment vectors (Figure 4a). Catchment pasture and accompanying environmental stressors (mud/silt, TSS) were closely associated with the nutrient enrichment gradient along axis 1, and WWTP density was associated with both nutrient enrichment as well as discharge on axis 2 (Figure 4a).

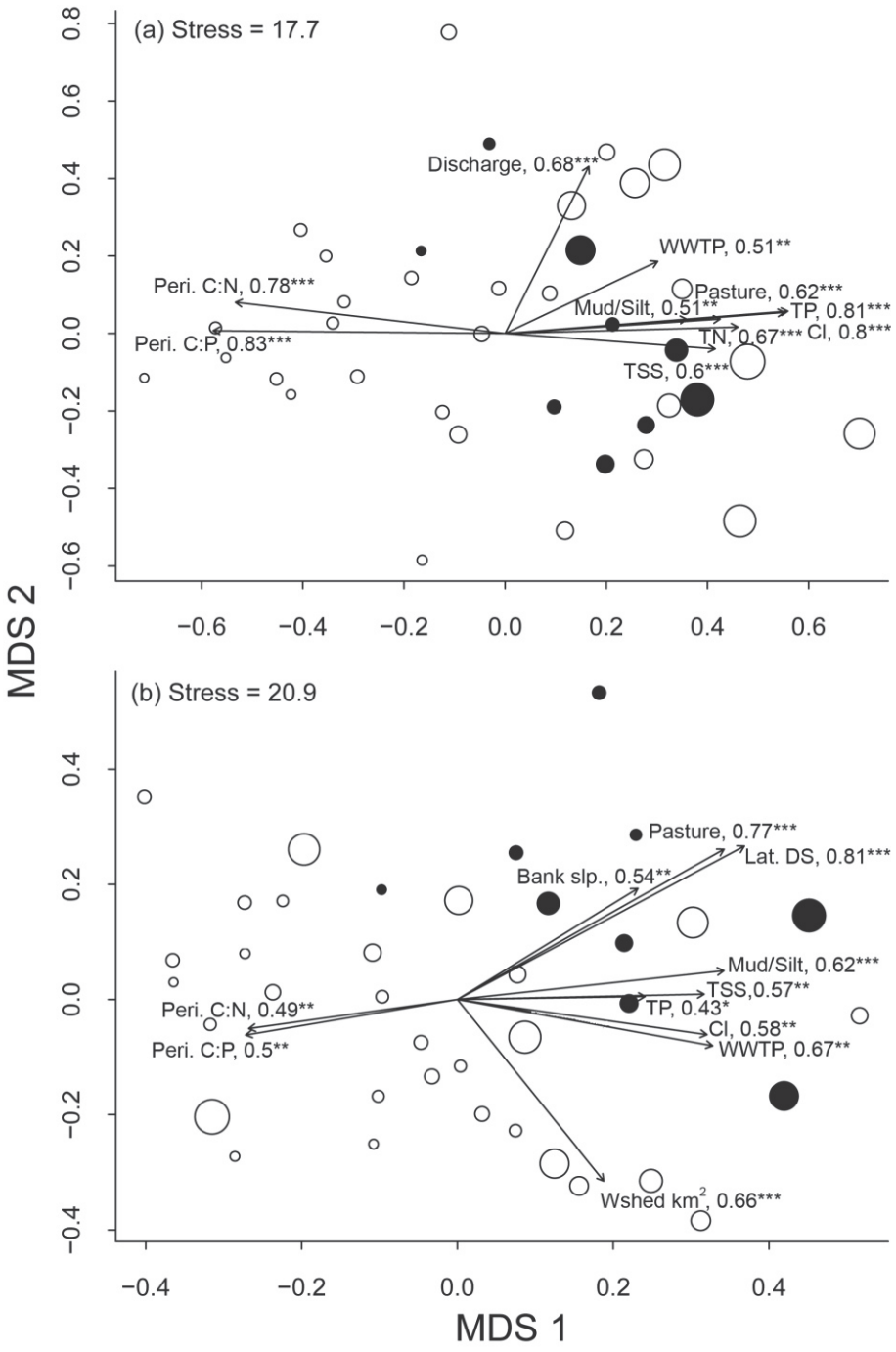

Figure 4. Nonmetric multidimensional scaling (NMS) ordination of individual sites using periphyton (a) and fish (b) assemblage compostion. Symbol shading indicate membership among the Brazos (open) and Trinity (filled) river basins. Environmental vectors show the direction and magnitude of significant correlations between environmental factors and assemblage composition within the ordination space and associated correlation coefficients (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Ordinations are rotated on axis 1 to surface water TP (µg L^{-1}) which is represented by size of circles.

Fish assemblage composition sorted sites along two major axes that explained 73 % of the original distances in n-dimensional space (2-D stress = 20.9). In contrast to patterns in periphyton assemblages, composition of fish species assemblages was moststrongly linked to latitudinal gradients on axis 1 and size gradients on axis 2 (Figure 4b). Percent pasture in the catchment and bank slope had significant associations with fish assemblage structure along the same trajectory as latitudinal gradients (Figure 4b). Fish assemblage structure also was related to measures of nutrient enrichment on axis 1, with periphyton C:P and C:N ratios decreasing and TP increasing in conjunction with increased mud/silt substrates, TSS, WWTP density and higher Cl. The latter two were associated with fish assemblage structure on a very similar trajectory. However, relationships between fish species composition and nutrient enrichment were weaker than those observed with periphyton assemblage structure (Figure 4). While most sites with high TP sorted to the right of the plot in conjunction with the TP environmental vector, two sites with high TP and WWTP influence had fish species composition similar to sites with low pasture, low mud/silt substrates and nutrients (Figure 4b). Latitudinal gradients in fish species composition among river basins were driven by clear separation of fish assemblages in ordination space based on river basin (PERMANOVA, $F_{1, 36} = 9.51$, $P = 0.009$) (Figure 4b). Variability in fish species composition within the Brazos versus Trinity basin was not significantly different (PERMDIST, $F_{1,36} = 0.91$, $P = 0.36$). However, latitude was correlated with catchment % pasture (Spearman rank correlation, $r = 0.71$, $P < 0.001$) and this pattern was driven by higher median values in the Trinity Basin (Wilcoxon rank-sum test, $W = 251$, $n_1 = 9$, $n_2 = 26$, $P < 0.001$).

Periphyton Nutrient Content Thresholds

Surface-water TP and TN were highly correlated across our study sites (Pearson's product-moment correlation, r = 0.86, $P < 0.001$). Periphyton nutrient ratios declined sharply (EDF = 1-1.6) with low levels of nutrient enrichment, but in every case had a much stronger relationship with surface-water TP than TN (Figure 5). There was a significant change point in periphyton C:P ratios at surface-water TP concentrations > 18.2 (17.4-55.9) μg L^{-1}, after which we observed C:P ratios below ~ 200 across the remainder of the TP gradient (Figure 5a). There was a similar shift from sites with high to low periphyton C:P around TN concentrations of 266 μg L^{-1} but the confidence in this change-point was low, the predictive GAM relationship explained substantially less deviance in the data, and the scatterplot indicated that one site with high surface-water TN (1783.3 μg L^{-1}) but low TP (16.6 μg L^{-1}) concentrations had high C:P ratios (Figure 5b). We observed similar patterns between surface-water nutrients and periphyton C:N (Figures 5c-d) and N:P (Figures 5e-f). Shifts in nutrient ratios occurred at similar areas observed for C:P along both nutrient gradients (TP:10-60 μg L^{-1}; TN: 236-2285 μg L^{-1}), predictions based on surface-water TP always explained more deviance, and periphyton nutrient content for one site with high surface-water TN but low TP was not well represented by GAM or nCPA models when TN was the predictor (Figures 4d, f).

Species and Assemblage Thresholds

TITAN, periphyton. Multiple algal species declined sharply in response to surface water TP, TN, periphyton nutrient ratios, pasture land cover, and Cl (Table 2). We identified 26 taxa that declined in response to at least one of the environmental variables

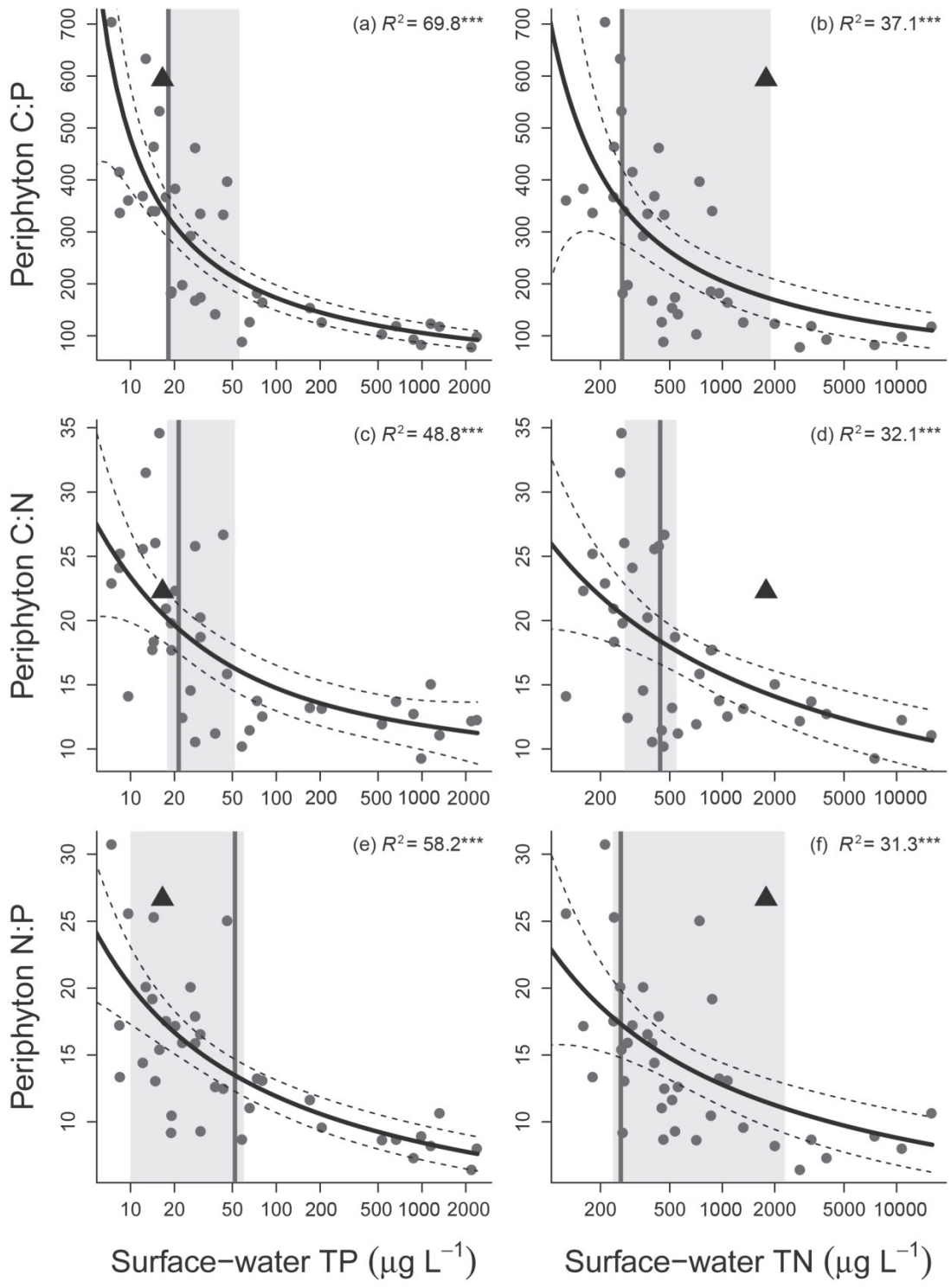

Figure 5. GAM smoothers for periphyton C:P (a-b), C:N (c-d), and N:P (e-f) in response to surface-water TP and TN. Solid lines represent predicted ratios with 90% CI (dashed lines). Vertical grey line indicates nCPA changepoint with light grey area representing 90% CI. R^2 = null deviance – residual deviance / null deviance and $P < 0.001$ is indicated by ***.

related to nutrients. Surface water TP had the highest number of declining taxa among the environmental variables. Sixteen of 18 negative (*z*-) indicator taxa declined sharply in response to TP between 16.2 to 27.8 μg L^{-1} (Figure 6a, Table 2). This synchronous decline in many taxa resulted in a sharp peak in the sum of the individual taxa z scores (sum (*z*-)) and provided evidence for an assemblage threshold at ~ 21 (13.4-28.9) μg L^{-1} (Table 2). In contrast, algal species responses to TN were weaker than TP (Figure 6b, Table 2). Only seven of the 18 species that declined in response to TP also declined in response to surface-water TN (Table 2). The greatest degree of change in algal species composition in response to TN was observed at 280.2 μg L^{-1} (249.7–462.5) μg L^{-1} (Figure 6b, Table 2).

Most of the taxa that declined with increasing TP or TN also responded negatively to periphyton nutrient ratios (Figures 6c-d, Table 2). However, in contrast to the single change point observed in response to TP and TN, two distinct change points were evident in the response to periphyton C:P (Figure 6c). Several taxa declined synchronously between 312 and 406 C:P, followed by a second decline between 171 and 178 C:P (Figure 6c). A very similar suite of taxa declined in response to periphyton C:N ratios between 12.9 and 25.4, % pasture (0.9-3.8%), and Cl (17-28.5 mg L^{-1}) (Figure 6d, Table 2). Following the decline of multiple algal species, many others sharply increased in response to catchment and reach scale indicators of nutrient enrichment (Figure 6, Table 2). However, positive indicator taxa were generally less synchronous in the location of increase and less abrupt in their individual response to these variables (Figure 6, Table 2). The largest number of algal species increased in response to TP and periphyton C:N ratios. Eighteen species increased sharply between 14.3 and 125.1 μg L^{-1}

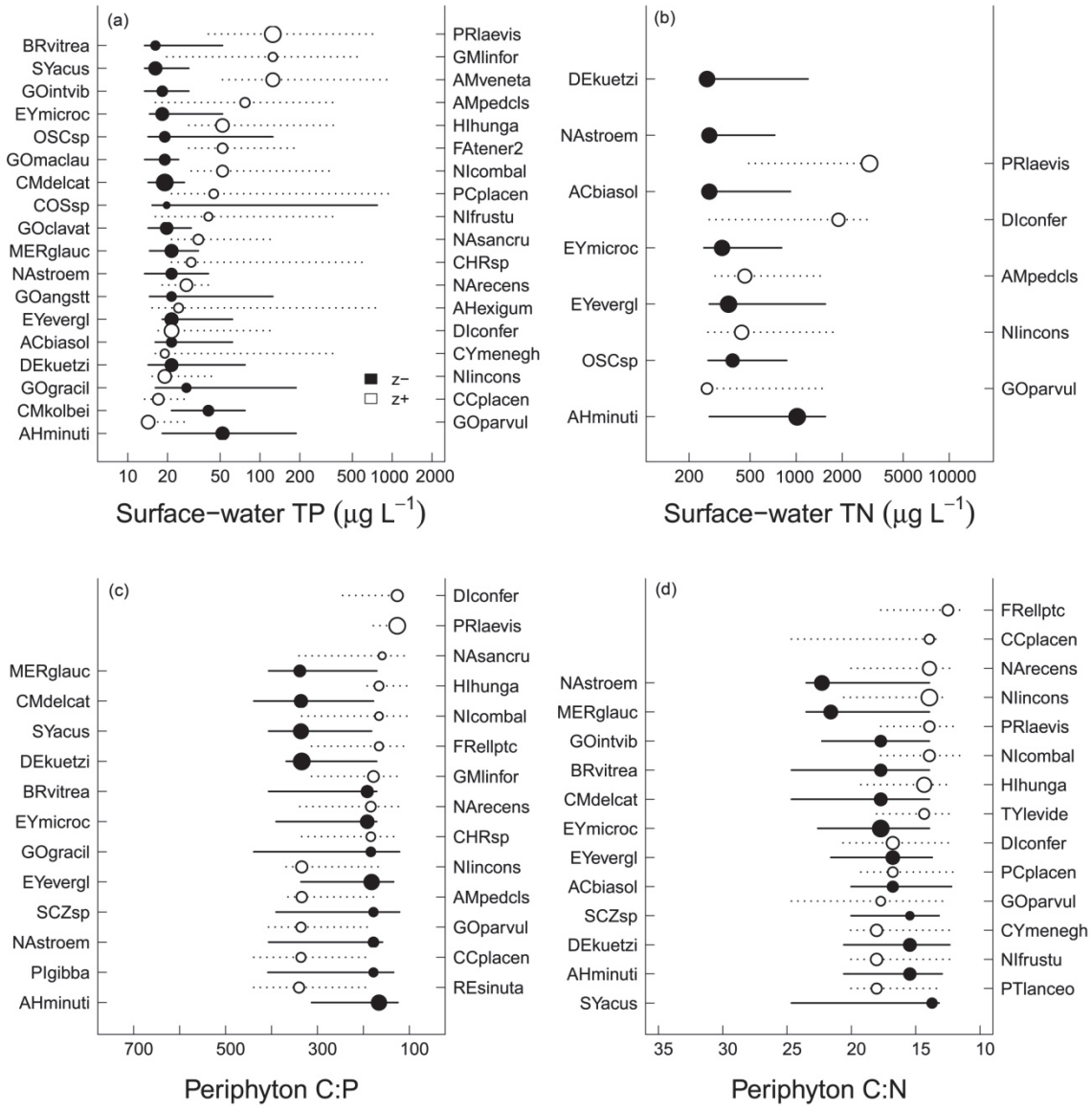

Figure 6. Significant periphyton indicator taxa identified in Threshold Indicator Taxa Analysis (TITAN) across surface water TP (µg L^{-1})(a), surface water TN (µg L^{-1}) (b), periphyton C:P (c), and periphyton C:N (d) gradients spanning 38 sample sites. Significant (purity ≥ 0.95, reliability ≥ 0.90, $p \leq 0.05$) indicator taxa are plotted in increasing order with respect to their observed change point. Solid symbols correspond to negative (z-) indicator taxa, whereas open symbols correspond to positive (z+) indicator taxa. Symbols are sized in proportion to magnitude of the response (z scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1000 bootstrap replicates

Table 2. Declining (*z*-) and inceasing (*z*+) assemblage and taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) of periphyton species composition in response to surface-water total nitrogen and phosphorus (TN and TP, µg L^{-1}), periphyton C:P and C:N (molar ratio), % pasture (catchment), and chloride (Cl, mg L^{-1}) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*) and upper (*95%*) values in parentheses that correspond to change point quantiles of 1000 bootstrap replicates.

	Sp. code	TP	TN	Periphyton C:P	Periphyton C:N	% Pasture	Chloride
Assemblage threshold (*z*-)		21.4 (13.4-28.9)	280.2 (249.7-462.5)	191.6 (110.5-334.1)	16.8 (25.4-13.7)	2.1 (0.9-3.8)	20.5 (17.0-28.5)
Declining species (*z*-)							
Achnanthes biassolettiana	ACbiasol	21.4 (16.1-61.9)	271.0 (249.7-918.2)	-	16.8 (21.6-13.2)	-	20.5 (13.5-31.0)
Achnanthidium minutissimum	AHminuti	52.1 (18.2-187.5)	1016 (271.0-1551.7)	165.9 (121.2-376.0)	15.5 (20.6-12.9)	2.6 (1.2-7.1)	35.0 (18.0-69.0)
Brachyseira vitrea	BRvitrea	16.2 (13.4-52.1)	-	245.1 (178.0-438.6)	17.7 (24.7-13.9)	-	26.0 (17.0-30.0)
Cymbella affinis	CMaffins	-	-	-	-	-	15.0 (11.5-30.0)
C. delicatula	CMdelcat	19.1 (14.3-26.8)	-	335.8 (182.0-462.9)	17.7 (24.7-13.9)	-	19.5 (12.5-4.2)
C. kolbei	CMkolbei	40.7 (21.4-77.0)	-	-	-	3.1 (1.5-7.1)	11.5 (11.5-46.5)
C. laevis	CMlaevis	-	-	-	-	-	13.5 (11.5-20.5)
Cosmarium sp.	COSsp	19.6 (15.3-770.3)	-	-	-	-	-
Encyonema silesiacum	ECsilesi	-	-	-	-	-	18.0 (12.5-72.6)
Encyonopsis evergladianum	EYevergl	21.4 (18.2-61.9)	362.0 (271.0-1551.7)	178.0 (134.0-335.8)	16.8 (21.6-13.7)	1.5 (0.9-7.8)	26.0 (13.5-31.0)
E. microcephala	EYmicroc	18.2 (14.6-52.1)	328.2 (249.7-803.6)	312.8 (178.0-406.2)	17.7 (22.6-13.9)	1.5 (0.9-4.5)	24.0 (17.0-35.0)
Fragilaria capucina	FRcapuci	-	-	-	-	-	26.5 (12.5-46.5)
Gloethece sp.	GLHsp	-	-	-	-	3.1 (1.6-12.3)	-
Gomphonema angustatum	GOangstt	21.4 (14.6-125.1)	-	-	-	-	12.5 (11.5-35.0)
G. clavatum	GOclavat	19.7 (14.3-30.2)	-	-	-	2.6 (0.9-3.8)	18.5 (12.5-30.0)
G. gracile	GOgracil	27.8 (16.2-187.5)	-	183.7 (124.7-438.6)	-	4.5 (1.5-8.6)	-
G. intricatum var. *vibrio*	GOintvib	18.2 (13.4-28.9)	-	-	17.7 (22.3-13.9)	-	-
G. maclaughlinii	GOmacla	19.1 (13.4-24.2)	-	-	-	-	-
Merismopedia glauca	MERglau	21.4 (14.6-34.2)	-	335.8 (165.9-406.2)	21.6 (23.5-13.9)	2.6 (0.9-8.4)	20.5 (18.0-28.5)
Sellaphora stroemii	NAstroem	21.4 (13.4-40.7)	271.0 (249.7-725.0)	178.0 (159.0-438.6)	22.3 (23.5-13.9)	-	20.5 (13.5-28.5)
Pinnularia gibba	PIgibba	-	-	312.8 (178.0-406.2)	-	-	-

Table 2. Continued.

	Sp. code	TP	TN	Periphyton C:P	Periphyton C:N	% Pasture	Chloride
Schizothrix sp.	SCZsp	-	-	178.0 (121.2-406.2)	15.4 (20.0-13.2)	-	-
Oscillatoria sp.	OSCsp	19.1 (14.3-125.1)	384.7 (266.0-867.8)	-	-	-	-
Synedra acus	SYacus	16.2 (13.4-28.9)	-	335.8 (178.0-406.2)	13.7 (24.7-13.2)	1.2 (0.9-5.8)	-
S. ulna	SYulna	-	-	-	-	-	86.0 (18.5-92.5)
Assemblage threshold (*z*+)		40.7 (26.8-778.4)	440.8 (284.7-3016.7)	335.8 (170.9-438.5)	16.8 (25.4-13.7)	7.1 (3.3-12.1)	26.0 (21.5-92.5)
Increasing species (*z*+)							
Achnanthes lanceolata	PTlanceo	-	-	-	18.0 (20.0-12.9)	-	-
Achnanthidium exiguum	AHminuti	24.2 (15.3-770.3)	-	-	-	-	21.5 (17.9-73.5)
Amphora pediculus	AMpedcis	77.0 (16.2-368.3)	462.5 (295.7-1568.7)	334.1 (165.9-363.9)	-	4.5 (1.5-12.1)	69.0 (12.5-86.0)
Amphora sabiniana	AMsabina	-	-	-	-	-	19.0 (15.0-47.2)
A. veneta	AMveneta	125.1(52.1-932.2)	-	-	-	-	69.0 (31.0-92.5)
Cocconeis placentula	CCplacen	17.0 (13.4-26.8)	-	335.8 (183.7-438.6)	13.9 (24.7-12.9)	3.3 (7.8)	-
Characium sp.	CHRsp	30.2 (21.4-598.3)	-	191.5 (124.7-334.1)	-	-	26.0 (19.0-69.0)
Cyclotella meneghiniana	CYmemegh	19.1 (16.2-368.3)	-	-	18.0 (20.1-12.2)	-	-
Diadesms confervacea	DIconfer	21.4 (16.9-125.1)	1891.7 (271.0-3016.7)	159.0 (121.2-313.9)	16.8 (20.6-12.2)	2.6 (1.4-12.1)	21.5 (18.0-60.5)
Fallatia tenera	FAtener2	52.1 (28.9-187.5)	-	-	-	-	-
Fragilaria elliptica	Frellpct	-	-	159.0 (110.5-312.8)	12.5 (17.7-11.3)	-	46.5 (24.0-86.0)
Gomphomena parvulum	GOparvul	14.3 (13.4-28.9)	261.8 (249.7-1551.7)	335.8 (183.7-406.2)	17.7 (24.7-12.9)	-	-
Gomphosphenia lingulatiformis	GMlinfor	125.1 (19.7-598.3)	-	178.0 (118.4-312.8)	-	8.4 (4.5-12.3)	-
Hippodonta hungarica	HIhunga	52.1 (28.9-368.3)	-	165.9 (110.5-245.1)	14.3 (19.3-12.5)	7.1 (1.5-12.1)	92.5 (18.0-92.5)
Navicula recens	NArecens	27.8 (18.2-44.7)	-	183.7 (118.4-338.2)	13.9 (20.0-12.2)	10.9 (4.5-12.3)	20.5 (18.0-77.0)
N. sanctaecrucis	NAsancru	34.2 (21.4-125.1)	-	335.8 (110.5-340.0)	-	3.8 (2.6-11.5)	-
Nitzschia angustatula	NIangtu	-	-	-	-	11.5 (5.8-12.3)	-
N. angustata	NIangust	-	-	-	-	4.5 (2.6-12.3)	-
N. compressa var. *balatonis*	NIcombal	52.1 (30.2-368.3)	-	191.5 (110.5-312.8)	13.9 (17.7-11.3)	7.8 (3.8-11.5)	26.0 (21.5-92.5)
N. frustulum	NIfrustu	40.7 (16.2-368.3)	-	-	18.0 (20.0-12.1)	-	-

Table 2. Continued

	Sp. code	TP	TN	Periphyton C:P	Periphyton C:N	% Pasture	Chloride
N. inconspicua	NIincons	19.1 (15.3-44.7)	440.8 (266.0-1891.7)	334.1 (158.4-368.0)	13.9 (20.6-12.5)	6.9 (1.5-12.1)	20.5 (13.5-86.0)
N. levidensis	TYlevide	-	-	-	14.3 (18.0-12.5)	-	-
N. solita	NIsolit	-	-	-	-	-	-
Placoneis placentula	PCplacen	44.7 (21.4-932.2)	-	-	16.8 (19.3-12.1)	-	30.0 (19.5-86.0)
Pleurosira laevis	PRlaevis	125.1 (40.7-770.3)	3016.7 (490.7-3016.7)	126.2 (118.0-178.0)	13.9 (17.7-11.7)	-	69.0 (26.0-77.0)
Reimeria sinuate	REsinuta	-	-	338.2 (245.1-438.6)	-	-	-
Terpsinoe musica	TEmusica	-	-	-	-	9.8 (7.1-12.3)	-
Tryblionella apiculata	TYapicul	-	-	-	-	6.9 (3.3-12.3)	31.0 (18.5-92.5)

TP, whereas only five taxa increased in response to TN between 261.8 and 3016.7 μg L^{-1} (Figure 6a-b). Most of the taxa that increased in response to TP also responded positively to other catchment and reach scale nutrient indicators, with 14 taxa increasing between 159 and 340 C:P, 12.4 and 18 C:N, 13 taxa between 2.6 and 11.5 % pasture, 14 taxa between 19 and 92.5 mg L^{-1} Cl (Figure 6c-d, Table 2).

TITAN, fish. Eleven species declined or increased in response to at least one environmental gradient. Three species declined in response to TP with *Campostoma anomalum* (Rafinesque) and *Etheostoma spectabile* (Agassiz) declining sharply at concentrations > 27.8 μg L^{-1} and 19.7 μg L^{-1}, respectively (Figure 7a). A sum (*z*-) change point representing an assemblage threshold was observed at surface water TP, 27.7 μg L^{-1} (16.2-187.5) (Table 3). Only *E. spectabile* was a significant declining species in response to surface water TN (Figure 7b). Three species, including *C. anomalum*, *E. spectabile*, *and Lepomis macrochirus* (Rafinesque) had significant declining (*z*-) change points at periphyton C:P between 165.9 and 170.9 (Figure 7c) with an assemblage threshold for declining species between 118.4 and 334.1 (Table 3). Four species including *C. anomalum* and *E. spectabile* had significant declining change points in response to periphyton C:N ratios and TITAN identified a sum (*z*-) assemblage change-point between 18.5 and 11.7 (Figure 7d). *Campostoma anomalum* and *E. spectabile* both declined sharply when pasture was > 4.5% with two additional species declining at higher pasture cover, but sum (*z*-) values did not indicate a strong negative assemblage threshold for pasture (Table 3). Three species, including *C. anomalum* and *E. spectabile* declined sharply in response to surface water Cl > 18.0 and 30.0 mg L^{-1} and cumulative declines in

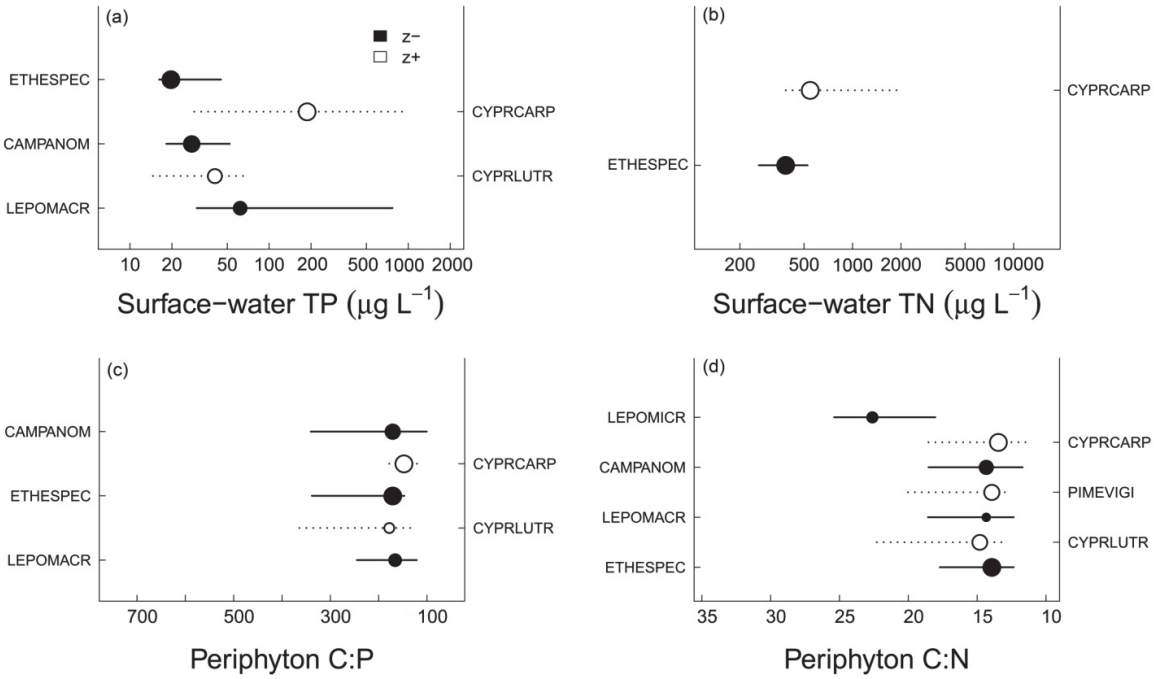

Figure 7. Significant fish indicator taxa identified in Threshold Indicator Taxa Analysis (TITAN) across surface water TP (µg L^{-1}) (a), surface water TN (µg L^{-1}) (b), periphyton C:P (c), and periphyton C:N (d) gradients spanning 38 sample sites. Significant (purity ≥ 0.95, reliability ≥ 0.90, $p \leq 0.05$) indicator taxa are plotted in increasing order with respect to their observed change point. Solid symbols correspond to negative (z-) indicator taxa, whereas open symbols correspond to positive (z+) indicator taxa. Symbols are sized in proportion to magnitude of the response (z scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1000 bootstrap replicates.

negative (*z*-) fish indicator taxa suggested an assemblage change point of 18.0 mg L^{-1} (12.5-73.7) (Table 3).

Cyprinella lutrensis (Baird and Giraard) and *Cyprinus carpio* (Linneaus) were consistently identified as increasing (z+) taxa for most of the reach and landscape scale nutrient enrichment factors (Figure 7, Table 3). *Cyprinella lutrensis* (Baird and Giraard) increased sharply in response to higher TP, lower C:P and C:N ratios, increased pasture, and increasing Cl concentrations (Figure 7a, c-d, Table 3). Likewise, *Cyprinus carpio* (Linneaus) abundance increased at high TP and TN concentrations, low C:P and C:N

Table 3. Declining (*z*-) and increasing (*z*+) assemblage and taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) of fish species composition in response to surface-water total phosphorus (TP, µg L^{-1}), periphyton C:P and C:N (molar), % pasture, and chloride (Cl, mg L^{-1}) in central Texas streams. The observed change point is shown for each taxon with lower (*5%*) and upper (*95%*) values in parentheses that correspond to change point quantiles of 1000 bootstrap replicates.

	Sp. code	TP	TN	Periphyton C:P	Periphyton C:N	% Pasture	Chloride
Assemblage threshold (*z*-)		27.8 (16.2-187.5)	420.2 (261.8-525.8)	165.95 (118.4-334.1)	14.3 (25.4-12.9)	8.6 (2.6-11.5)	18.0 (12.5-73.7)
Species (*z*-)							
Campostoma anomalum	CAMPANOM	27.8 (18.2-52.1)	-	170.9 (124.7-340.0)	14.3 (18.5-11.7)	4.5 (1.5-12.3)	18.0 (12.5-77.0)
Cyprinella venusta	CYPRVENU	-	-	-	-	12.3 (2.6-12.3)	-
Etheostoma spectabile	ETHESPEC	19.7 (16.2-45.1)	384.7 (261.8-525.8)	170.9 (134.0-340.0)	13.9 (17.7-12.3)	4.5 (2.4-8.8)	30.0 (13.5-72.5)
Ictalurus punctatus	ICTAPUNC	-	-	-	-	10.9 (5.8-12.3)	-
Lepomis macrochirus	LEPOMACR	61.9 (30.2-770.3)	-	165.9 (121.2-245.1)	14.3 (18.6-12.3)	-	92.5 (18.9-92.5)
Lepomis michrolophus	LEPOMICR	-	-	-	22.6 (25.4-18.0)	-	-
Assemblage threshold (*z*+)		30.2 (19.0-77.0)	384.7 (271.0-3016.7)	170.9 (126.2-376.0)	13.9 (18.5-11.7)	3.26 (2.4-10.9)	30.0 (12.0-35.6)
Species (*z*+)							
Cyprinus carpio	CYPRCARP	187.5 (28.9-932.2)	546.2 (384.7-1891.7)	126.2 (110.5-178.0)	13.5 (18.5-11.3)	-	46.5 (26.5-77.0)
Cyprinella lutrensis	CYPRLUTR	40.7 (14.6-69.8)	-	170.9 (126.2-439.8)	14.8 (22.3-13.2)	2.6 (0.9-4.5)	13.5 (11.5-18.5)
Dorosoma cepidianum	DOROCEPI	-	-	-	-	8.8 (2.8-12.1)	-
Lythrurus umbratilis	LYTHUMBR	-	-	-	-	10.9 (3.3-12.3)	-
Pimephales vigilax	PIMEVIGA	-	-	-	13.9 (20.0-12.6)	3.1 (0.9-7.1)	13.5 (6.5-19.6)

ratios, and Cl concentrations indicative of WWTP influence (Figures 3 and 7a-d, Table 3). *Pimephales vigilax* (Baird and Girard) had threshold responses similar to *C. lutrensis* for pasture and Cl (Table 3)

GAM, fish. Generalized additive models confirmed that *C. anomalum* and *E. spectabile*, the two dominant declining species identified by TITAN, declined in a nonlinear fashion in response to surface water TP and pasture (Table 4). Pasture was the strongest single predictor of *C. anomalum* abundance which decreased in a non-linear fashion with pasture, but also decreased in response to increasing Cl as well as TP values above the threshold (27.8 μg L^{-1}) identified by TITAN (Figure 8a). TP was the strongest single predictor for *E. spectabile* abundance but a pasture model that incorporated separate predictive relationships for each basin explained more deviance. This model identified a strong, nonlinear relationship between *E. spectabile* and pasture within the Brazos river basin only (Table 4). *Etheostoma spectabile* abundance decreased at low concentrations of surface-water TP, and was at or near 0 for the majority of the remaining nutrient gradient with the exception of two sites that had exceptionally high TP and Cl concentrations indicative of WWTP subsidized stream flow (Figure 8b). Nonlinear relationships between several environmental variables and the dominant increasing species from the TITAN analysis were confirmed with GAMs (Table 4). All nutrient variables were significant nonlinear predictors of *C. lutrensis* abundance; among all of the variables examined, pasture explained the most variation in abundance (Table 4). *Cyprinella lutrensis* abundance increased with % pasture, but was exceptionally high when % pasture exceeded 5 %, TP was above the TITAN threshold (40.7 μg L^{-1}), and Cl concentrations indicated WWTP influence (Figure 8c). *Cyprinus carpio* abundance

Table 4. Results of GAM fits between environmental variables and dominant fish indicator taxa identified in TITAN anlaysis. Models are based on estimated smothers for the whole dataset and separate smoothers for each river basin when median predictor values (Pasture) were significantly different between basins.

			Separate smoothers[b]	
Predictor	R^{2a}	EDF[c]	R^{2a}	EDF[c]
Campostoma anomalum				
TP	26.0	**2.9**	NA	NA
TN	12.9	**1.2**	NA	NA
Periphyton C:P	NC	NC	NA	NA
Periphyton C:N	17.7	**2.3**	NA	NA
Pasture	32.3*	**1.0**	34.7	**1.7**/**1.0**
Cl	31.6	**2.9**	NA	NA
Etheostoma spectabile				
TP	45.7	**2.9**	NA	NA
TN	NC	NC	NA	NA
Periphyton C:P	NC	NC	NA	NA
Periphyton C:N	30.9	**2.2**	NA	NA
Pasture	35.8	**1.0**	51.6*	**1.7**/1.0
Cl	NC	NC	NA	NA
Cyprinella lutrensis				
TP	29.4	**2.5**	NA	NA
TN	NC	NC	NA	NA
Periphyton C:P	18.6*	**1.0**	NA	NA
Periphyton C:N	21.9	**2.0**	NA	NA
Pasture	34.6*	**2.4**	35.0	**1.8**/1.0
Cl	21.0	**2.9**	NA	NA
Cyprinus carpio				
TP	36.4	**1.0**	NA	NA
TN	NC	NC	NA	NA
Periphyton C:P	52.2*	**2.7**	NA	NA
Periphyton C:N	37.0	**1.0**	NA	NA
Pasture	13.8*	**1.0**	13.9	1.0/1.0
Cl	27.8	**1.6**	NA	NA

[a] R^2 = Explained deviance = null deviance – residual deviance/null deviance (Zurr et al. 2009).
[b] Separate smoothers extimated for Brazos and Trinity river basins.
[c] Effective degrees of freedom (EDF) in bold signify significant smoothers ($P < 0.001$) with EDF for separate smoothers read as Brazos/Trinity.
*indicates best fit model given the data based on Δ AICc > 3.
NC = model did not converge.
NA = not applicable.

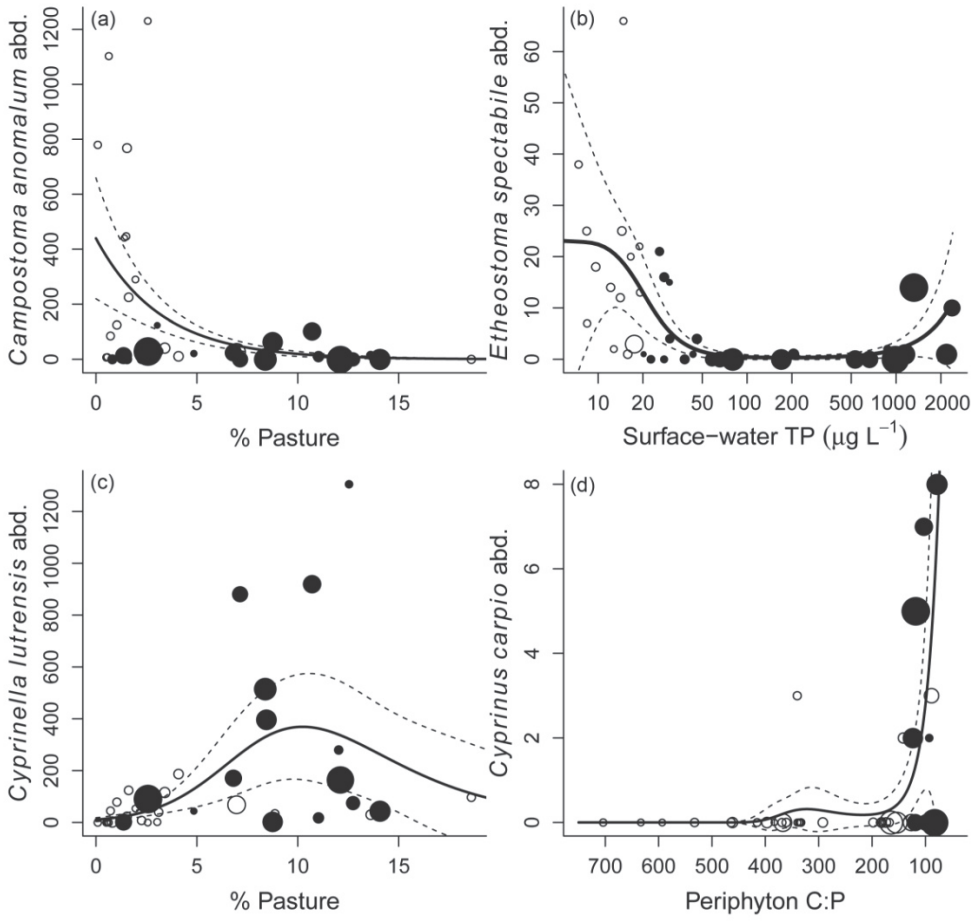

Figure 8. GAM smoothers for *Campostoma anomalum* (a), *Etheostoma spectabile* (b), *Cyprinella lutrensis* (c) and *Cyprinus carpio* (d) abundance with nutrient enrichment factors. Open symbols represent sites below surface water TP (µg L^{-1}) species level threshold identified by TITAN. Size of dots in all plots represents the distribution of Cl (mg L^{-1}) concentrations among sites.

demonstrated a clear nonlinear threshold response to periphyton C:P that explained considerably more variance (52.2) than any other variable (Table 4; Figure 8d). Most sites with high *C. carpio* abundance also had TP values above the TITAN threshold (187.5 µg L^{-1}) for *C. carpio* as well as Cl levels indicative of strong WWTP influence (Figure 8d).

Discussion

Low levels of nutrient enrichment, particularly P, corresponded with significant shifts in periphyton nutrient content and sharp, synchronous declines in many algae species as well as species representing unique functional groups within the stream fish assemblage (e.g. periphyton grazers; riffle-dwelling benthic invertivores). Previous studies have reported changes in periphyton assemblages based on biotic indices at relatively low levels of P enrichment (Gaiser et al. 2005; Richardson et al. 2007; Stevenson et al. 2008a). Our study provides new insights into periphyton assemblage responses to nutrient enrichment by demonstrating that changes in species composition are a function of synchronous declines in many taxa with small increases in P (13.4 - 28.95 μg L^{-1}) along with more gradual increases in tolerant taxa over a broader gradient of P enrichment (26.8 - 778.4 μg L^{-1}). Similarly, while overall fish assemblage structure showed a weak response to P enrichment in a manner similar to results from previous studies (Miltner and Rankin 1998; Wang et al. 2007; Johnson et al. 2006; Johnson and Hering 2009), our approach identified declines in abundance of two functionally important species (*C. anomalum*, *E. spectabile*) at similarly low levels of P enrichment and its hypothesized sources (pasture cover, WWTP effluent). Significant declines in functionally important species, coupled with increasing abundances of the tolerant minnow, *C. lutrensis*, and non-native *C. carpio* across our P enrichment gradient, suggests that P inputs and/or associated landscape drivers can have stronger effects within fish assemblages than previously thought.

Nitrogen covaried with P across our study sites and in every case of elevated P, N was also elevated above background. Our analysis of assemblage changes in response to

nutrient enrichment provided evidence that covariation in N and P enrichment occurred across our sites. Almost all of the species that had significant negative change-points in response to N enrichment were a subset of species that also declined in response to P enrichment. Additionally, patterns in the response of periphyton nutrient content to N enrichment were always weaker than in response to P enrichment. Higher uncertainty in periphyton nutrient content models based on surface-water TN was driven primarily by the fact that some streams had high N but low P because of different sources (rowcrop), and under those conditions streams did not respond to N enrichment. The sharp decline in periphyton C:N and N:P with P enrichment demonstrates that N is important in central TX streams but whether there would have been a strong response to P alone under very low N is unlikely. The fact is that virtually all sources of P are also sources of N, whereas not all sources of N are sources of P. Thus, managing sources of P should also include best management practices for N reduction.

Anthropogenic P enrichment creates new environmental conditions in stream ecosystems to which aquatic assemblages respond. Previous studies have reported linear responses (Miltner and Rankin 1998; Johnson and Hering 2009; Pan et al. 2000; King et al. 2004; King and Richardson 2007; Justus et al. 2010), threshold responses (Richardson et al. 2007; Stevenson et al. 2008a; King and Richardson 2003; Evans-White et al. 2009), or subsidy-stress relationships (King and Richardson 2007) between aggregate measures of aquatic assemblage structure (ordination axes, metrics) and increasing P enrichment. Linear or subsidy-stress responses to P enrichment based on aggregate measures (species richness, multi-metric biotic indices, ordination axes) can be artifacts of a priori assignments of taxa tolerance values or combining abundances of coarse taxonomic,

functional, and habitat classes without distinguishing response direction, location, and magnitude (King and Baker 2010). For example, King et al. (2011) demonstrated synchronous declines of numerous but less frequent macroinvertebrate taxa and poorly defined change points and gradual increases in tolerant taxa in response to urban land cover in catchments. Aggregate measures of assemblage structure that combine these distinctly different responses may result in linear or wedge-shaped responses to environmental gradients, and may subsequently underestimate negative effects (King and Baker 2010, 2011). Likewise, subsidy-stress relationships (Odum et al. 1979) based on aggregate measures of assemblage structure may be an artifact of overlapping responses between increasing and declining species.

Recent studies have observed threshold changes in periphyton assemblage structure (based on aggregated diatom biotic indices) at low levels of phosphorus enrichment in streams (Justus et al. 2010; Stevenson et al. 2008a; Wang et al. 2009). Similarity in threshold responses between our results based on TITAN and diatom biotic indices used by others is likely due to concerted efforts toward identifying declining and increasing diatom species in response to P enrichment (Potapova and Charles 2007). Several of the taxa that declined with P enrichment in our study have been shown to have optima at relatively low levels of TP (Justus et al. 2010; Stevenson et al. 2008; Wang et al. 2009). Our declining species list corresponded particularly well with the low-P indicator taxa identified by (Potapova and Charles 2007) for streams in the United States. In every case, declining species identified in our study were previously identified as significant indicator species of low-P conditions. Species we identified as increasing in response to P enrichment also agreed well with high-P indicator taxa identified by

Potapova and Charles (2007) and those used by others in diatom indices that measure tolerant species response to nutrient enrichment (Stevenson et al. 2008a; Stevenson et al. 2008b). All declining and increasing species along the N enrichment gradient were a subset of P enrichment responders and were likely responding to P enrichment.

Negative (*z*-) assemblage thresholds for the fish dataset were similar to the periphyton TP thresholds (27.4 μg L^{-1}) but with much higher confidence limits. Despite weak correlation between P enrichment and fish assemblage structure and low confidence in fish assemblage thresholds, TITAN identified species that had negative and positive threshold responses to increasing phosphorus. In particular, two benthic species, *E. spectabile* and *C. anomalum*, showed negative declines at low levels of P enrichment and its inferred sources (pasture, effluent). These two species also declined in response to N enrichment, which covaried with P, but had high abundances in streams with high N but low P enrichment. The lack of many significant declining species precludes identifying reliable fish community thresholds for P enrichment. However, quantifying sharp declines in *E. spectabile* and *C. anomalum* at low levels of enrichment is important, because these species represent unique functional groups within fish assemblages of central Texas streams. Few darter species occur synoptically in central Texas streams, and a single *Etheostoma* species often is the only representative of the riffle-dwelling benthic-invertivore guild at the local habitat scale. Trophic cascades associated with loss of benthic invertivores could limit periphyton biomass response to P enrichment and negate potential positive response of *C. anomalum*, a periphyton grazer, to P enrichment (Hargrave et al. 2006). *Campostoma* consume benthic algae, detritus and smaller fractions of associated invertebrates (Evans-White et al. 2001) and can influence

periphyton composition and structure, overall organic matter dynamics, and sediment accumulation in streams (Matthews 1998). Elimination of this functional group likely would impact stream ecosystem processes and structure.

What Landscape Factors Influence P Enrichment-Assemblage Structure Relationships?

This study identified associations between species assemblages, nutrient enrichment and putative nonpoint (pasture) and point (WWTP) nutrient sources within our study region. Low levels of nutrient enrichment and effects on stream biota have been correlated with pasture and associated activities including cattle grazing and application of waste from confined animal feeding operations (Justus et al. 2010). Periphyton assemblages seemed to respond directly to nutrient enrichment gradients, whereas fish assemblages showed stronger relationships with pasture than P enrichment or other related variables, suggesting that other pasture-related stressors, such as increased bank erosion and mud/silt substrates, may have contributed to fish responses. Large-scale dairy operations in our study region apply manure as fertilizer, and as a result, land classified as pasture can be a substantial source of P entering streams. This widespread practice also elevates P in shallow, sub-surface groundwater that flows into stream bank sediments during low-flow periods and can result in substantial inputs of sediment-bound P to streams through bank erosion (Thompson and McFarland 2010). Municipal wastewater effluent generally results in increased levels of nutrient enrichment, and in our study WWTP defined the upper limit of the enrichment gradient. Surface and subsurface flows from pasture areas combined with sewage treatment discharges defined the P enrichment gradient in our study region.

Additional spatial factors could influence relationships between assemblage composition and nutrient gradients. Latitude had a strong relationship with fish assemblage composition and was confirmed by a significant basin effect. The major rivers of Texas flow from northwest to southeast and enter the Gulf of Mexico as independent drainage basins. Although many broad-scale zoogeographic patterns can be explained by dispersal limitation across basin divides (Hubbs et al. 1991; Hoeinghaus et al. 2007), composition of central Texas stream fish assemblages tends to be related more strongly to gradients of precipitation and geology that are reflected in ecoregional differences in fish assemblage structure (Pease et al. *in press*). A few species included in our analysis of fish assemblage structure only occur in one basin. However, species that had strong associations with, and were likely driving patterns of species turnover along measured environmental gradients did not exhibit basin affinities. Patterns in land cover can be driven by underlying spatial drivers that may confound ecological relationships (King et al. 2005). Latitudinal gradients and basin differences in fish assemblage structure were potentially related to associated gradients and basin differences in pasture land cover. The separation of our sites by basin corresponds with a subecoregional boundary between the northern Grand Prairie and southern Limestone Cut Plain portions of the Cross Timbers ecoregion (Figure 2). Geological conditions and soil characteristics of the Grand Prairie region support vegetation more similar to the adjacent Northern Blackland Prairie which might explain the high proportion of sites with higher pasture land cover within the Trinity Basin (Griffith et al. 2004). Additionally, historical landscape degradation surrounding the Dallas-Ft. Worth Metroplex has altered biological communities in rural streams adjacent to heavy urban areas within the Trinity Basin

(Cuffney et al. 2010). Greater amounts of pasture cover associated with Trinity Basin sites likely increased inter-basin differences in fish assemblage structure within the region in the present study. This was somewhat confirmed by our GAM results for declining species. Models that incorporated separate basin smoothers had higher EDFs that were always significant for the Brazos basin, likely due to the longer % pasture gradient observed across our study sites in the Brazos (range = 0.09-12.11%) verses Trinity (range = 6.97=18.6%) basins. Fish assemblages are also influenced by catchment size and its covarying factors including stream order, elevation, width and discharge (Matthews and Robison 1998; Angermeier and Winston, 1999). We controlled for catchment size by including sites within a range of watershed sizes and a range of nutrient profiles uncorrelated with catchment size. Our results indicated that fish assemblage structure was correlated with catchment size but along an orthogonal axis to the dominant species gradient strongly associated with nutrient enrichment variables.

Do Periphyton and Fish Assemblages Respond Differently to P Enrichment?

Southern Great Plains streams have dynamic flow regimes characterized by flood events and extended periods of low flows (Poff and Ward 1989; Bernardo and Alves 1999; Dodds et al. 2004). The structure of both periphyton and fish species assemblages is limited by hydrologic disturbance regimes. After high-flow scour events and stream dewatering, benthic algae must recolonize the surface of stream substrates. The interaction between scour events and available nutrients determines biomass accrual and structuring of algae species assemblages (Riseng et al. 2004; Stevenson et al. 2006). Thresholds for algae associated with low P probably represent levels that exceed maximum growth rate potential and define where along P enrichment gradients species

are no longer competitive. For example, (Manoylov and Stevenson 2006) experimentally demonstrated that *Achnanthidium minutissimum* (Kützing), a species that declined at low levels of P in our study, have high growth rates in low-P conditions, but are not competitive with other taxa in high-P environments. A species that responded positively to P enrichment in our study, *Gomphonema parvulum* (Kützing), is a stalked diatom that is a superior competitor for light and space in high-density algal biofilms. Transplant studies have demonstrated that this species declines when transferred from high- to low-P streams while species including *A. minutissimum* and *Achnanthes biassolettiana* (Lange-Bertalot), both species identified as low P taxa by the current study, increased (Rimet et al. 2009). As nutrient concentrations increase, new algae assemblages develop based on interactions between differences in optimal growth rates, competition for substrate during recolonization, and structural requirements for establishment of particular growth forms (Larned 2010).

Fish assemblage structure in Great Plains streams is strongly influenced by abiotic factors, including physical disturbances such as flood and drought (Matthews and Marsh-Matthews 2003; Fausch et al. 2002). Consequently, fish assemblages tend to be dominated by physiologically tolerant species capable of surviving stressful conditions, seeking refugia, and/or rapidly recolonizing habitats following extirpation (Matthews 1987; Labbe and Fausch 2000; Fausch and Bramblett 1991; Ostrand and Wilde 2002). Seasonal low-flow periods and increasing P enrichment interact to increase the magnitude of diel fluctuations in dissolved oxygen and pH across our study sites (Valenti et al. 2011). Large dissolved oxygen fluctuations in enriched streams could exceed the physiological limits of *E. spectabile* and *C. anomalum* within isolated pools during low-

flow conditions (Miltner and Rankin 1998; Miltner 2010). Usable habitat would be severely restricted because these small fishes may be forced to seek refuge from harsh conditions in dewatered riffles by entering pools where piscivorous fish reside (Power and Matthews 1983; Power et al. 1985).

We observed higher *E. spectabile* abundances indicative of low P streams at a few sites with TP concentrations greater than species thresholds identified by TITAN (Figure 6b). This inconsistency might have been due to higher and more stable low flows related to sewage treatment discharges and/or network connectivity to higher quality habitat for sites with high WWTP influence. Discharge was not a significant environmental factor associated with fish assemblages or individual species distributions. Identifying such associations may have been prevented by a lack of continuous discharge measures in a region characterized by high flow variability. However, effluent discharge can make up a considerable portion of stream baseflows during dry periods, and can introduce effluent-related chemical stressors (Brooks et al. 2006). In our study region, variability in stream discharge of effluent-dominated streams appears to be related to permitted discharge rates (Figure 3b). One of our study sites with exceptionally high TP and higher *E. spectabile* abundance had the highest cumulative WWTP permitted discharge rate as well as the highest recorded discharge among our study sites. The other site was close to a WWTP discharge point, above which, the stream did not receive effluent. Streams with elevated discharge due to WWTPs might serve as refugia for sensitive species during dewatering of more natural stream segments. However, position within the drainage network and its influence on dispersal might influence relationships between P enrichment, potential flow

refugia, and recolonization of sensitive species from less impacted habitats (Osborne and Wiley 1992; Hitt and Angermeier 2008).

Cyprinella lutrensis abundance was greater in streams with high % pasture, total phosphorus and chloride concentrations. *Cyprinella lutrensis* is a generalist that can occupy a variety of habitats (Matthews 1985), have high and continuous reproductive effort (Marsh-Matthews et al. 2002; Herrington and DeVries 2008), and can outcompete other native minnows under certain conditions, such as high turbidity (Douglas et al. 1994). These factors have made *C. lutrensis* successful invaders of several Western and Southeastern basins (Schade and Bonar 2005; Walters et al. 2008), as well as disturbed systems within their native range (Matthews 1985; Wilde and Ostrand; 1999). *Cyprinus carpio* numbers increased at high levels of nutrient enrichment and chloride levels, but did not show strong relationships with % pasture, which suggests that WWTP affected fish abundance. This is probably because *C. carpio* are tolerant to organic pollution, and effluent discharges can even enhance their growth and condition (Smolders et al. 2004).

What are the Implications for Nutrient Management in Streams?

This study presents multiple lines of evidence that support the development of nutrient criteria at low levels of P (~ 20 μg L^{-1}) to protect natural periphyton and fish assemblages in our study region. First, all measures of periphyton nutrient content showed significant shifts indicative of a change in nutrient status between surface-water TP concentrations of 10 and 60 μg L^{-1} (90% CI) with change-points occurring at 18, 21, and 52 μg L^{-1} for periphyton C:P, C:N, and N:P ratios, respectively. Second, there was a cumulative decline of sensitive species within periphyton assemblages around 21.4 μg L^{-1} (13.4-28.9). Third, we observed significant declines in two functionally important

benthic fish species (*E. spectabile*, *C. anomalum*) at similar levels of P enrichment, 19.7 μg L^{-L} (16.2-45.1) and 27.8 μg L^{-L} (18.2-52.1), respectively. These findings combined with more gradual increases in weedy algae species, as well as tolerant minnows and invasive carp at higher concentrations of TP (~30-200 μg L^{-L}) support our assertion that major shifts in assemblage structure occur at low levels of P enrichment. The threshold approach and validation through predictive modeling employed in the current study should provide water quality managers with a framework for setting numerical criteria for P that is protective of natural stream assemblages in our study region (King and Richardson 2003; Soranno et al. 2008). Regulatory agencies should consider similar approaches when developing numerical criteria for nutrients in streams from other regions.

Managing sources of P should also include best management practices for N reduction. Nitrogen covaried with P across our study region and our analyses provides evidence that P was the limiting nutrient that stream assemblages were responding to. Despite this, our analyses suggests that major shifts in periphyton C:N occur at TN concentrations of 440 μg L^{-1} (275.2-546.2) which corresponds well with declining periphyton taxa thresholds of 231.8 μg L^{-1} (236.2-228.5), and where increasing periphyton species begin to dominate, 440.8 μg L^{-1} (384.7-3016.7). Additionally, *Etheostoma spectabile* declined and invasive *C. carpio* increased at TN concentrations of 384.7 μg L^{-1} (271.0-803.6) and 546.2 μg L^{-1} (384.7-1891.7), respectively. These results cumulatively suggest that major shifts in both periphyton nutrient content and stream assemblages occur at TN concentrations as low as ~240 μg L^{-1} and are highly likely at concentrations ~550 μg L^{-1}. Criteria based on these numbers should also prove useful in

managing nutrient enrichment in streams, with the caveat that organisms in our study likely only respond to N when P is not limiting. Managers should also consider the influence of N enrichment in streams on receiving water bodies with different nutrient limitation status when developing numerical criteria, as N is often the nutrient that impacts estuaries and associated coastal ecosystems (Smith et al. 2006).

Findings from the current study also point to the need for further research that will reveal mechanisms that account for relationships between P enrichment, its sources (pasture, WWTP) and local periphyton and fish assemblages in streams. For example, overall patterns in our data suggested declines in benthic fish species at low levels of P enrichment. Yet, *E. spectabile* and *C. anomalum* did inhabit certain streams with high levels of permitted WWTP discharges, presumably due to flow enhancement. Experimental studies that examine fish response to P enrichment under different flow conditions are needed to better understand this interaction, and we caution water resource managers when considering the potential for streams with high WWTP inputs to function as flow refugia. First, source-sink dynamics between effluent-dominated streams and their tributaries are not well understood, and if tributaries contain high quality habitats, then streams receiving effluent might serve as population sinks during low-flow periods (Waits et al. 2008). Second, P enrichment and flow stability in streams receiving high amounts of effluent, increase abundance of tolerant native minnows as well as invasive species. Greater diversity of native minnows, despite being tolerant, combined with presence of more sensitive species, increases richness and biotic index scores used by regulatory agencies to evaluate water quality in the region. These indices, in their current state, are not adequate for measuring nutrient impacts. Finally, continued population

growth and climate change (hotter, drier summers) in Texas will put additional stress on water resources. Streams with discharge that is currently maintained by effluent during the dry season will likely become low-flow environments with high P loads as water becomes scarcer in the southwestern United States, which may have consequences for both sensitive and tolerant species.

CHAPTER THREE

Response of Periphyton Stoichiometry to Phosphorus Enrichment is Influenced by the Grazing Minnow *Campostoma anomalum* in Stream Mesocosms

This chapter published as: Taylor JM, Back, JA, King RS. *Accepted with minor revisions.* Response of periphyton stoichiometry to phosphorus enrichment is influenced by the grazing minnow *Campostoma anomalum* in stream mesocosms. Freshwater Science.

Introduction

Freshwater benthic habitats are important sites of uptake, transformation and recycling of essential elements (carbon (C), nitrogen (N), and phosphorus (P)) and often form the basis for diverse and productive food webs (Cross et al. 2006). Periphyton form a complex community of autotrophic and heterotrophic organisms intertwined with dead organic matter on benthic surfaces (Frost et al. 2005; Hillebrand et al. 2008). The structural and functional composition of periphyton is influenced by many factors including disturbance, limiting abiotic factors such as nutrients and sediment, competitive interactions and herbivory (Larned 2010).

Anthropogenic nutrient loading is a widespread problem and the response of periphyton to nutrient subsidies can create a wide range of undesirable changes in freshwater ecosystems (Carpenter et al. 1998; Smith et al. 2006). Grazing fish can exert considerable influence on structural and functional components of benthic environments (Power 1984; Power et al. 1985; Grimm 1988; Power 1990; Gelwick and Matthews 1992; Flecker and Taylor 2004; Taylor et al. 2006; Bertrand and Gido 2007) and may mitigate or amplify the response of stream periphyton to nutrient enrichment (Stewart 1987; Flecker et al. 2002; Kohler et al. 2011). For example, the effect of grazing is often

greater than nutrient enrichment effects on periphyton biomass (Hillebrand 2002). While benthic feeding fish may influence the elemental composition of attached periphyton via direct consumptive effects and indirect bioturbation or nutrient recycling effects (Taylor et al. 2006; Knoll et al. 2009; McIntyre and Flecker 2010, Kohler et al. 2011), the influence of these two factors on periphyton elemental composition likely varies depending on other factors such as nutrient content of grazers and their food, grazer biomass, and water column nutrients (Hillebrand et al. 2008).

Ecological stoichiometry (ES) is the study of the balance of multiple chemical substances, particularly C, N, and P in ecological interactions and processes (Elser et al. 1996; Sterner and Elser 2002). ES has made considerable contributions to understanding interactions between grazers, benthic algae, and nutrients in aquatic systems (Hillebrand et al. 2008). In general, grazers influence the stoichiometry of C, N, and P in periphyton through several pathways. For example, grazers remove senescent algal cells that reduce the nutrient-poor detrital component of periphyton communities. Removal of both senescent and living periphyton matter may alter nutrient uptake rates by decreasing diffusion barriers and increasing the relative availability of nutrients for remaining algal biomass (Frost et al. 2002; Hillebrand et al. 2008). Herbivores can also influence periphyton nutrient content by changing the relative abundance of taxa with differing nutrient demands or elemental composition (Frost et al. 2002; Liess and Kahlert 2009). Additionally, nutrient recycling by grazers can regulate the ratios of bioavailable nutrients that are incorporated by periphyton communities (Evans-White and Lamberti 2006; Hillebrand et al. 2008; Knoll et al. 2009). In general, the presence of grazers increases the nutrient content of periphyton resulting in lower C:N and C:P ratios and

higher N:P ratios (Hillebrand et al. 2008). Despite this, our understanding of how grazing fish influence periphyton nutrient content is limited (but see Knoll et al. 2009) and few studies have addressed interactions between nutrient enrichment, grazing fish and periphyton stoichiometry in stream ecosystems (Kohler et al. 2011).

There is a paucity of grazing fish species in North America and this functional role is often occupied primarily by the central stoneroller (*Campostoma anomalum* Rafinesque) in temperate streams of eastern and midwestern North America (Matthews 1998). Central stonerollers consume large amounts of algae, detritus and animal matter (Evans-White et al. 2001; Evans-White et al. 2003) which influences periphyton composition and structure, overall organic matter dynamics, and sediment accumulation on grazed substrates (Matthews 1998). Field data from central Texas streams suggest that central stoneroller abundance declines in response to sources of nutrients (pasture and effluent discharges) but can exist in enriched systems associated with effluent discharges that maintain summer baseflows (Pease et al. *in press*). Thus, our understanding of how this functionally important species interacts with benthic environments across P enrichment gradients is fundamental to understanding P enrichment effects on stream ecosystems.

The goal of our study was to investigate how grazing by central stonerollers and P availability at three ecologically relevant concentrations influence periphyton biomass and nutrient content in pools of twelve flow-through stream mesocosms. Due to the flow-through design of our mesocosms, physical characteristics of the source water habitat, and the depositional nature of our experimental habitat (pools), sediment deposition was observed early on during the experiment and provided a potential

interacting environmental factor to our study design. Shifts in the relative amounts of nutrient poor sediment and detritus in response to fish grazing and changes in nutrient content of individual periphyton components in response to P enrichment both potentially influence periphyton nutrient content. Therefore we hypothesized that: 1) grazing fish would remove detritus and sediment from benthic surfaces resulting in a higher proportion of algae in periphyton biomass; 2) this process would result in higher nutrient content of periphyton; and 3) nutrient content and grazer influence on nutrient content would vary with P enrichment.

Materials and Methods

Experimental Design

We conducted this study in shallow (40 cm depth) pool sections of twelve outdoor flow-through stream mesocosms located at the Baylor Experimental Aquatic Research (BEAR) facility in McLennan Co., TX. Each BEAR stream receives 180 L min^{-1} of relatively low-nutrient surface water from an 80 hectare wetland fed by the North Bosque River (Figure 9 A). Water flows through each stream once before returning to the head of the wetland. Streams are stratified into 0.61 m wide × 18.3 m long riffles (upper), glides (middle) and 1.7 m^2 pools (lower) (Figure 9 B-C). We filled each pool with a 10 cm layer of local limestone gravel and cobble and established grazed and ungrazed areas using fish exclusion cages. Fish exclusion cages consisted of two (0.29 m^2) compartments, one that was completely enclosed and one that was enclosed on all but one side with 6mm polypropylene mesh (Industrial Netting, Minneapolis, MN, USA) to create similar light environments for grazed and ungrazed substrates (Figure 9 C). Pools

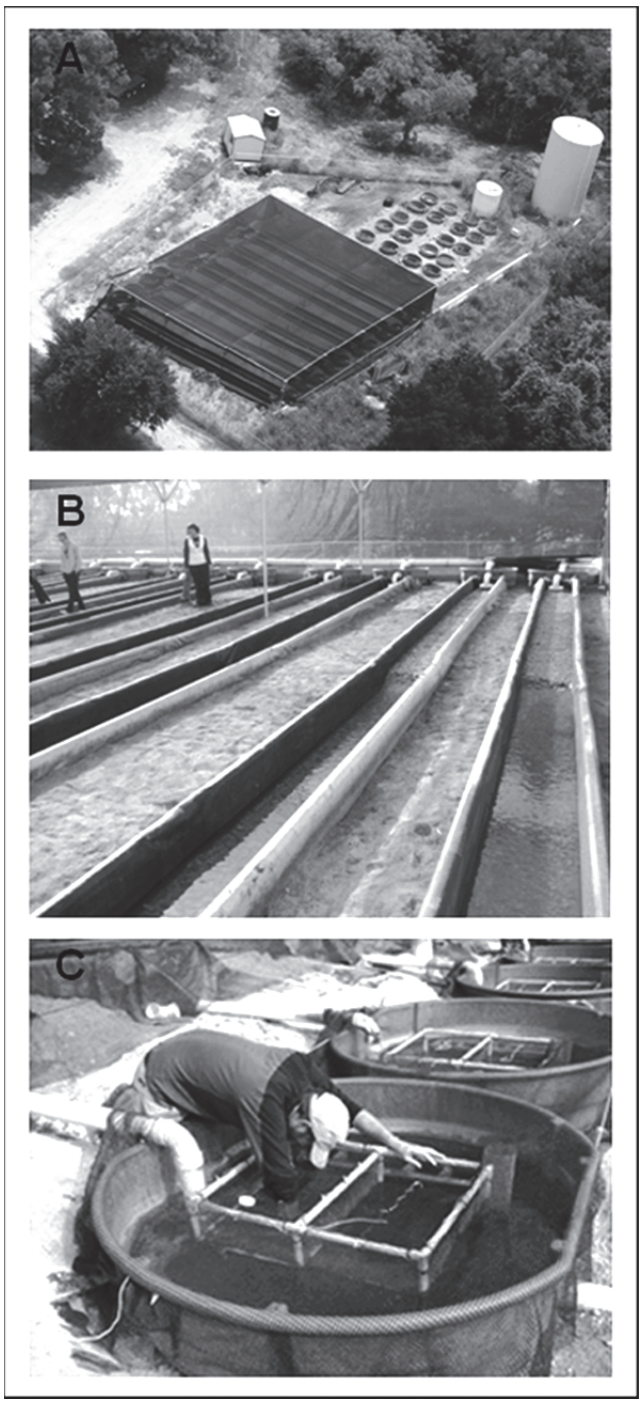

Figure 9. Baylor Experimental Aquatic Research (BEAR) mesocosm facility located at the Lake Waco Wetlands in McClennan Co., TX (A). Twelve stream mesocosms receive water from an 80 hectare wetland which then flows through riffle/run sections (B) before emptying into pools and returning to the wetland via a standpipe (C).

were covered by 30% shade cloth to approximate riparian canopies and ambient photosynthetically active radiation of lower order streams (1,000–1,200 μE m^{-2} s^{-1}; R.S. King unpublished data), reduce variability of sunlight across experimental units, and prevent loss of fish due to jumping or bird predation. Twenty-four evenly spaced cobbles were placed in each section and streams were allowed to run without nutrient additions or fish from 31-January to 10-Mar-2008 to allow periphyton to grow at low nutrient concentrations. Streams were seeded with cobble and associated periphyton, organic matter, and macroinvertebrates on 01-Feb and 15-Feb-2008 from Neils Cr., TX (31.6995°N, 97.5309°W; PO_4-P = 7.5 ± 1.1 μg L^{-1}) and the North Bosque R., TX (31.9769°N, 98.0397°W; PO_4-P = 79.4 ± 38.1 μg L^{-1}). Neils Cr. and the North Bosque R. were selected to introduce taxa from streams spanning a similar range of PO_4-P as the experimental treatments.

Experimental dosing of phosphorus was initiated on 11-Mar-2008. We used peristaltic pumps calibrated to deliver solutions of dibasic sodium phosphate (NaH_2PO_4) from 50-L carboys to mixing tanks before discharging water at low (20 μg L^{-1}) and high (100 μg L^{-1}) PO_4-P concentrations into mesocosms. Control streams were not dosed and received background water from the wetland (8 μg L^{-1}) PO_4-P. We chose these concentrations because our field studies in central Texas streams have identified consistent nonlinear changes in periphyton taxonomic composition, biomass, and nutrient content at concentrations ~ 20 μg L^{-1} PO_4-P (Chapter Two). Each P treatment was replicated in four streams.

We collected central stonerollers from Harris Cr., McLennan Co., TX (31.4596 °N, 97.2925 °W; PO_4-P = 9.7 ± 1.8 μg L^{-1}) and stocked each stream to represent size

structure and densities comparable to those observed in natural streams (9 fish m^{-2}; mean biomass m^{-2} = 70.3 ± 2.22 g). Treatments were arranged in a split-plot design, with pools stratified across the three phosphorus treatments as whole plots and grazer treatments within pools as split-plots. The study ran for 28 days and was concluded on 08-April-2008.

Data Collection

Water samples were collected from the outflow of each stream one month and again one week before the experiment was initiated to determine ambient dissolved nutrient concentrations. During the study triplicate samples were collected from each stream twice weekly and analyzed for PO_4-P, NO_2-N + NO_3-N and NH_3-N on a Lachat Quickchem 8500 autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). On days 0, 14 and 28 we collected composite periphyton samples from each sample unit by scraping periphyton from the upper surface of six randomly selected rocks. Periphyton was homogenized, subsampled, and filtered onto preweighed Whatman GF/F (pore size = 0.7 μm) filters for quantification of chlorophyll *a*, dry mass, and AFDM following (Steinman et al. 2006). Additional subsamples were dried at 60°C for 48 hours and pulverized into a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc.) for analysis of nutrient content. We measured nutrient content as % C, N and P. We estimated C and N content of periphyton using a ThermoQuest Flash EA™ 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) following fuming with HCl to drive off inorganic carbonates (Hill and Middleton 2006). Periphyton P content was analyzed with a Lachat QuikChem 8500 flow-injection autoanalyzer using the molybdate colorimetric method following a 1-hour digestion in 15

mL of distilled water with 1.8mL of a mixture of peroxodisulphate (30 g L^{-1} $K_2S_2O_8$), boric acid (50 g L^{-1} H_3BO_3) and sodium hydroxide (15 g L^{-1} NaOH) at 121 °C (Faerovig and Hessen 2003). Soil (Thermo Finnigan 1.99 % C) and peach leaf (SRM 1547, 0.137 % P, 0.298 % N) standards were analyzed to assure C, N, and P recoveries met QA/QC standards (± 10%) for each sample run.

Data Analysis

We tested for differences in periphyton AFDM, chlorophyll *a*, C:chlorophyll *a*, inorganic sediment, % C, % N, % P, C:P, C:N and N:P among different P enrichment (P), grazing (Graz) and sampling date (Date) treatments using linear mixed models (LMM) with the package nlme (Pinheiro and Bates 2000) in the freely available R statistical programming language (R Core Develoment Team 2011). Linear mixed models are useful in analyzing split-plot or repeated measures designs by allowing random effects to specify a grouping structure for the data whereas fixed effects specify the treatments of interest (Pinheiro and Bates 2000). We specified stream as a random effect (intercept only) to account for non-independence of grazed and un-grazed samples (split-plot) and multiple dates collected from the same stream. Fixed factors included P enrichment (low, med, high), grazing (grazed, un-grazed), and date (day 14, day 28). The restricted maximum likelihood (REML) criterion was used to fit all models. We assessed assumptions of LMM visually with normality plots (qqnorm) and standardized residual plots across treatments (Pinheiro and Bates 2000). In cases where error variances differed across stratums of treatments we incorporated this heterogeneity into our model by modeling variance separately among treatments using the VarIdent command (Pinheiro and Bates 2000). We assessed effects of incorporating heterogeneity of error

variances on model fit using Akaike's Information Criterion (AIC). We compared base models with and without variance structure and selected the more complex model if Δ AIC ≥ 3 (Burnham and Anderson 1998). In some cases log-transformation of data was necessary to meet assumptions of normality and heterogeneous error variances (Zuur et al. 2009). Differences in periphyton measures between levels of fixed factors and across interactions were assessed when factors were significant. We built contrast matrices from our models and used these to conduct Tukey HSD multiple comparsions of means with the glht function of the multcomp package (Bretz et al. 2011) in R ver. 2.11.1 (R Development Core Team 2011). We interpreted p-values less than 0.05 as significant patterns.

Results

Water Column Nutrients

Experimental PO_4-P manipulations yielded mean concentrations that were very close to nominal treatment concentrations of 8, 20, and 100 μg L^{-1} PO_4-P for the control, low and high treatments (Table 5). Dissolved inorganic nitrogen was similar across the three treatments (Table 5). Molar N:P ratios reflected the P enrichment gradient with N:P ratios decreasing across the P enrichment treatments (Table 5).

Table 5. Mean ± SE dissolved P and N concentrations in μg L^{-1} across the P enrichment treatments during the 28 days of PO4-P dosing. Dissolved nutrients (PO4-P, DIN) were sampled in triplicate for each stream, twice weekly during the study period (11 March – 7 April 2008; n = 9 sampling events).

Treatment	PO_4-P	DIN	N:P (molar)
Control	8.59 ± 0.33	306.39 ± 12.71	83.09 ± 5.15
Low	19.52 ± 0.91	303.01 ± 12.72	36.57 ± 2.06
High	108.36 ± 3.89	304.39 ± 12.67	6.35 ± 0.32

Biomass and Sediments

Total biomass (AFDM), chlorophyll *a*, C:chlorophyll *a*, and inorganic sediments did not vary with P enrichment. The influence of grazing fish on these measures varied with time (Table 6; Figure 10 A-D). Grazing fish reduced total biomass (AFDM mg cm^{-2}) on day 14 (G = 1.04 ± 0.16, UG = 2.01 ± 0.41) but there was no difference between grazed and ungrazed treatments on day 28 (Table 6-7; Figure 10 A). Chlorophyll *a* (μg cm^{-2}) was not influenced by grazing fish on day 14 ($p > 0.05$). However, by day 28 there was less chlorophyll *a* in ungrazed compared to grazed treatments (G = 4.79 ± 0.64, UG = 2.01 ± 0.27; Table 6-7; Figure 2 B). Overall, C:chlorophyll *a* ratios were lower in the presence of grazing fish (G = 85.85 ± 13.71, UG = 225.92 ± 24.99) indicating that fish decreased the detritus component of periphyton mats. However, increases in AFDM (Figure 10 A) in grazed treatments and decreases in chlorophyll *a* in ungrazed treatments (Figure 10 B) resulted in C:chlorophyll *a* ratios increasing by 60% and 48% respectively for each grazer treatment on day 28 (Table 6; Figure 10 C). Grazing fish removed inorganic sediments (mg cm^{-2}) from benthic substrates on day 14 (G = 1.01 ± 0.12, UG = 4.71 ± 0.37) but by day 28, overall sediment levels were similar in grazed and ungrazed treatments (G = 3.22 ± 0.58, UG = 4.29 ± 0.40) and not significantly different (Table 6-7; Fig. 10 D).

Elemental Composition

Overall, periphyton % P was significantly greater in high P (0.17 ± 0.02 %) than in control (0.08 ± 0.01 %) and low P (0.09 ± 0.01 %) streams (Table 6-7, Fig.11 a). Fish grazing increased periphyton % P by 40 % on average but there was a significant interaction with P enrichment and date (Table 6). Fish grazing increased periphyton % P

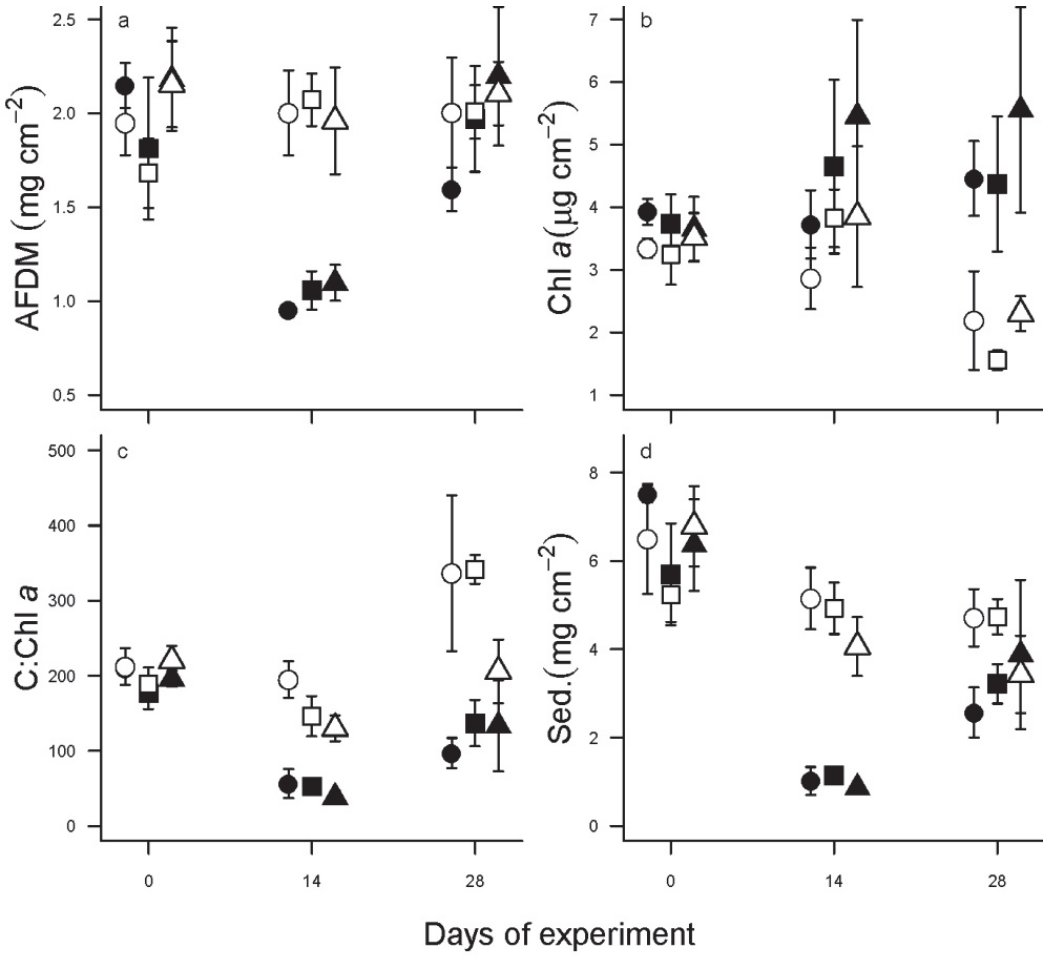

Figure 10. Mean (± SE) periphyton ash free dry mass (AFDM) (A), chlorophyll (Chl) *a* (B), C:chl *a* ratios (C), and inorganic sediment (Sed.) (D) in different grazer treatments across a P enrichment gradient on three dates. Shapes represent P enrichment levels (circles = control, squares = low, triangles = high) and shading represent grazer treatments (closed = grazed, open = ungrazed).

across all P treatments (Table 7) but appeared to have greater effects in high P treatments (Fig. 11 a). Mean comparison tests showed that grazing increased periphyton % P on both day 14 (G = 0.17 ± 0.03, UG = 0.09 ± 0.01 %) and day 28 (low G = 0.12 ± 0.02, UG = 0.08 ± 0.01 %) and the interaction was more related to differences between dates for grazed substrates (Table 7). Mean periphyton % N did not vary between control (0.82 ± 0.07) and low P streams (0.80 ± 0.06) but was significantly higher in high P (0.98 ± 0.1)

Table 6. Mixed-model analysis of variance *F* statistics for periphyton response variables in experimental streams. Degrees of freedom are indicated by subscripts. Bold denotes significant difference. Italics denote marginally significant difference ($p < 0.1$).

Source of variation	*F*	*P*	*F*	*P*
	Ash free dry mass		Chlorophyll *a*	
$P_{2, 9}$	1.02	0.398	0.73	0.507
Grazer (G) $_{1, 27}$	34.06	**<0.001**	16.64	**<0.001**
Date (D) $_{1, 27}$	23.48	**<0.001**	3.81	*0.061*
P × G $_{1, 27}$	0.74	0.485	0.03	0.974
P × D $_{2, 27}$	0.35	0.709	0.57	0.572
G × D $_{1, 27}$	20.66	**<0.001**	5.48	**0.027**
P × G × D $_{2, 27}$	0.08	0.922	0.20	0.818
	C:chl *a*		Inorganic sed	
$P_{2, 9}$	1.79	0.220	0.40	0.681
Grazer (G) $_{1, 27}$	60.42	**<0.001**	33.55	**<0.001**
Date (D) $_{1, 27}$	27.95	**<0.001**	4.73	**0.039**
P × G $_{1, 27}$	0.51	0.608	1.62	0.216
P × D $_{2, 27}$	0.69	0.509	0.20	0.818
G × D $_{1, 27}$	1.26	0.271	10.18	**0.004**
P × G × D $_{2, 27}$	0.44	0.649	0.39	0.683
	% Phosphorus		C:P ratio	
$P_{2, 9}$	66.37	**<0.001**	97.21	**<0.001**
Grazer (G) $_{1, 27}$	59.05	**<0.001**	5.25	**0.030**
Date (D) $_{1, 27}$	9.67	**0.004**	23.04	**<0.001**
P × G $_{1, 27}$	4.64	**0.019**	5.68	**0.009**
P × D $_{2, 27}$	1.42	0.259	0.39	0.679
G × D $_{1, 27}$	6.339	**0.018**	2.79	0.106
P × G × D $_{2, 27}$	0.29	0.7546	0.21	0.809
	% Nitrogen		C:N ratio	
$P_{2, 9}$	5.35	**0.029**	10.37	**<0.005**
Grazer (G) $_{1, 27}$	178.92	**<0.001**	91.81	**<0.001**
Date (D) $_{1, 27}$	5.96	**0.022**	16.10	**0.004**
P × G $_{1, 27}$	2.98	*0.068*	6.85	**0.004**
P × D $_{2, 27}$	0.19	0.831	0.86	0.433
G × D $_{1, 27}$	10.75	**0.003**	12.35	**0.002**
P × G × D $_{2, 27}$	0.036	0.965	0.06	0.944
	% Carbon		N:P ratio	
$P_{2, 9}$	0.71	0.516	41.03	**<0.001**
Grazer (G) $_{1, 27}$	270.73	**<0.001**	19.83	**<0.001**
Date (D) $_{1, 27}$	0.07	0.790	50.14	**<0.001**
P × G $_{1, 27}$	0.07	0.934	3.33	*0.051*
P × D $_{2, 27}$	0.13	0.877	0.06	0.940
G × D $_{1, 27}$	5.85	**0.023**	0.13	0.722
P × G × D $_{2, 27}$	0.36	0.363	0.20	0.823

Table 7. Summary of multiple comparison results for significant P enrichment and grazer interaction effects identified in mixed-model analysis of variance. NS denotes where response variable was not significant for P enrichment or interaction in the mixed-model analysis of variance (Table 2). Bold denotes significant difference ($p < 0.05$). Italics denote marginally significant difference ($p < 0.1$).

Response	P enrichment[a]		P enrichment × Grazer[b]		Grazer × Date[c]	
AFDM	NS	-	NS	-	**D 14** D 28	***p* < 0.001** *p* = 0.999
Chlorophyll *a*	NS	-	NS	-	D 14 **D 28**	*p* = 0.536 ***p* = 0.044**
Inorganic sed	NS	-	NS	-	**D 14** D 28	***p* = 0.006** *p* = 0.783
% Phosphorus	C = L **C < H** **L < H**	*p* = 0.201 ***p* < 0.001** ***p* < 0.001**	**C** **L** **H**	***p* = 0.005** ***p* = 0.007** ***p* = 0.001**	**G** UG	***p* < 0.001** *p* = 0.712
% Nitrogen	C = L **C < H** **L < H**	*p* = 0.997 ***p* = 0.009** ***p* = 0.012**	NS	-	G *UG*	*p* = 0.678 *p = 0.059*
% Carbon	NS	-	NS	-	**D 14** **D 28**	***p* < 0.001** ***p* < 0.001**
C:P ratio	C = L **C > H** **L > H**	*p* = 0.119 ***p* < 0.001** ***p* < 0.001**	C L **H**	*p* > 0.988 *p* = 0.899 ***p* = 0.002**	NS	-
C:N ratio	NS	-	G: C = L **G: C > H** **G: L > H** UG: C = L UG: C = H UG: L = H	*p* = 1.000 ***p* < 0.001** ***p* = 0.030** *p* = 1.000 *p* = 0.952 *p* = 0.714	G **UG**	*p* = 0.965 ***p* = 0.006**
N:P ratio	**C > L** **C > H** **L > H**	***p* = 0.017** ***p* < 0.001** ***p* < 0.001**	**C** *L* H	***p* = 0.023** *p =0.097* *p* = 0.995	NS	-

[a] C = control, L = low phosphorus, H = high phosphorus
[b] Reported values for C, L, and H represent grazer differences within each P enrichment level
[c] Reported values for D 14 and D 28 represent grazer differences on each date. G and UG represent date differences within each grazer treatment

streams (Table 7). This pattern was only observed in grazed treatments despite no significant P × G interaction (Table 6, Fig. 11 b). Grazing increased periphyton % N on both dates but there was a significant interaction with date (Table 6). Percent N increased

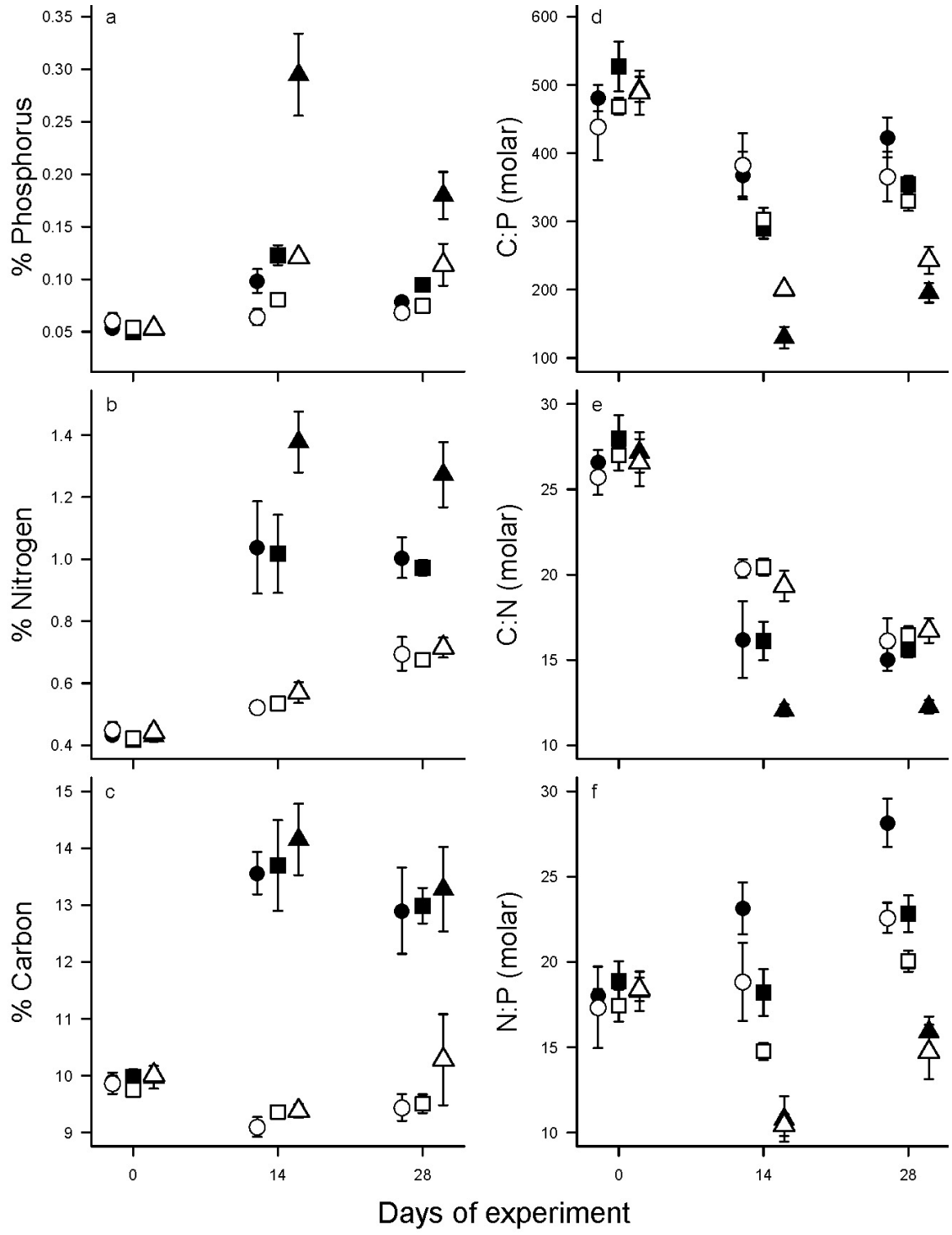

Fig. 11. Mean (± SE) periphyton % P (A), % N (B), % C (C), C:P (D), C:N (E) and N:P (F) in different grazer treatments across a P gradient on three dates. Shapes represent P enrichment levels (circles = control, squares = low, triangles = high) and shading represent grazer treatments (closed = grazed, open = ungrazed).

between the two dates in un-grazed treatments (Table 7; Fig. 11 b). Periphyton % C did not respond to P enrichment. Grazed treatments had significantly higher periphyton % C but these effects decreased slightly between sampling dates (Table 6; Fig. 11 c).

Nutrient Ratios

Nutrient ratios showed a variety of responses to P enrichment and grazing fish (Fig. 11 D-F). Average C:P ratios were lower in high P (192.19 ± 12.35) than control (384.91 ± 17.72) and low P (319.21 ± 9.2) streams (Table 6-7; Fig. 11 D). Fish grazing significantly decreased C:P ratios in high P streams (G = 162.67 ± 15.89, UG = 221.73 ± 12.25) but did not influence C:P ratios in control and low P streams (Table 6-7, Fig. 11 D). C:P ratios increased with time across all treatments (Day 14 =278.81 ± 20.9, Day 28 = 318.74 ± 18.02; Table 6). P enrichment influenced periphyton C:N ratios but only in grazed streams (Table 6, Fig. 11 E). On grazed substrates, C:N ratios were significantly lower in high P (12.14 ± 0.24) than control (15.61 ± 1.12) and low P (15.85 ± 0.56) streams (Table 7). Mean C:N ratios were lower on day 28 but this was primarily due to decreased C:N ratios on ungrazed substrates (Day 14 = 20.04 ± 0.38, Day 28 = 16.44 ± 0.48; Table 7; Fig. 11 F). Periphyton N:P ratios decreased significantly with each level of P enrichment (control = 23.19 ± 1.12, low P = 18.96 ± 0.87, high P = 12.97 ± 0.81; Table 6-7; Fig. 11 F). Grazing increased N:P ratios significantly in control (G = 25.65 ± 1.35, UG = 20.71 ± 1.34) and marginally in low P (G = 20.52 ± 1.19, UG = 17.4 ± 1.06) streams but had no effect in high P streams (G = 13.36 ± 1.22, UG = 12.58 ± 2.63; Table 7; Fig. 11 F). Overall, mean periphyton N:P ratios increased between the two sampling dates (Day 14 = 16.04 ± 1.07, Day 28 = 20.71 ± 1.03; Table 6).

Discussion

We investigated how P enrichment and presence/absence of grazing fish affect periphyton biomass and elemental composition of periphyton in stream mesocosms. The central finding of our study is that grazing by central stonerollers maintained a higher proportion of algae in the periphyton matrix and as a result, the response of periphyton stoichiometry to experimental P enrichment was stronger on grazed substrates. C:Chl *a* ratios were ≤ 100 in conjunction with higher periphyton P and N content on grazed substrates, evidence for our first two hypotheses; fish increased relative proportions of nutrients by removing nutrient poor detritus and sediment from benthic surfaces and maintaining a relatively high proportion of algae in the periphyton matrix (Frost et al. 2002; Hillebrand et al. 2008). However, C:P ratios generally decline with increased algal cellular C (Frost et al. 2005) and we observed no grazing fish effects on C:P ratios in control and low P streams despite lower C:Chl *a* ratios. Carbon:Chl *a* ratios also increase when exposed to more light (Geider 1987) and increased light may have stimulated photosynthesis and associated nutrient demand. However, at high P enrichment levels C:P ratios were significantly lower on grazed surfaces, presumably because algal cells can incorporate excess P not utilized for growth (Borchardt 1996). Incorporation of N into the periphyton matrix was linked to both grazing and P enrichment. C:N ratios were lower and only responded to P enrichment on grazed surfaces. Positive grazer effects were observed on periphyton N:P ratios in low P streams but diminished with P enrichment. Differential responses of periphyton nutrient ratios to grazing fish across P treatments provide evidence for our third hypothesis; P enrichment influences grazer induced changes in periphyton stoichiometry. These results contribute to the growing

body of literature that suggest organisms can modify resource quality under limiting abiotic conditions (nutrients, sediments) but also present new evidence that P enrichment may alter these interactions in benthic habitats.

Previous studies on grazing fish have found that when grazing fish interact with physical factors that limit food resources such as sediment and detritus, the net effects of grazing can enhance autochthonous resources (Power 1990). Lower detritus and sediments in grazed treatments on day 14 and lower chlorophyll *a* in un-grazed treatments on day 28 had an overall net effect of grazing fish maintaining significantly lower C:Chl. *a* ratios. Despite increased AFDM and sediments on day 28, grazed substrates had C:Chl. *a* ratios below or near 100, indicative of relatively high algal cellular content in natural organic matter (Geider, 1987). Decreased C:Chl *a* ratios on grazed surfaces may be the result of increased light (Geider 1987) or decreased detrital material within the periphyton matrix (Frost et al. 2002). While these results partially support the findings of Power (1990) they suggest that the interplay between grazing fish, sediment and autochthonous resources may be more complex in our experimental system and questions the overall importance of sediment removal by central stonerollers in natural streams.

Initial removal of sediments by grazing fish early in the study likely facilitated the establishment of *Cladophora glomerata* which was observed as a close cropped layer on grazed substrates in pools on day 28. *Cladophora* generally prefers solid substrates (Dodds and Gudder 1992) and was confined to the vertical edges of some rocks in the un-grazed treatments due to high sediment loads and never became well established (Figure

12). Lack of grazing effects on inorganic sediments coupled with no change in chlorophyll *a* on grazed substrates but decreased chlorophyll *a* on un-grazed substrates suggest that filaments of *Cladophora* on grazed substrates were long enough to maintain chlorophyll *a* cells above a sediment layer that accumulated within the macroalgal layer on day 28. Increased sedimentation on grazed substrates over time may have also been an artifact of our mesocosm pool design. It is plausible that a portion of sediment re-suspended through grazing activity resettled within pools and was not exported out of the

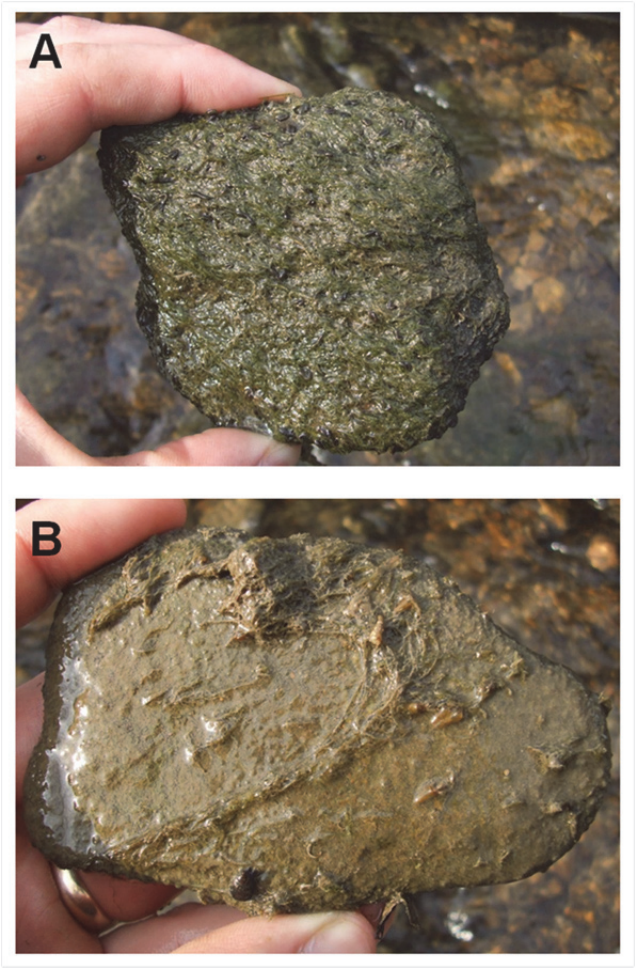

Figure 12. *Cladophora glomerata* growth on grazed (A) and un-grazed (B) substrates on day 28 of the study.

system through a standpipe which pulled wate from the top of the water column (Figure 9). Over time total sediment within the pool may have reached a critical threshold at which central stonerollers could no longer prevent sediment accumulation. This suggests that central stonerollers do influence sediment distribution on benthic substrates in streams with relatively low sediment inputs but may be unable to process heavy sediment loads indicative of heavily disturbed habitats. Power (1990) observed that the grazing fish *Ancistrus* can reduce moderate sediment accumulation in tropical streams but high sediment loads discouraged small fish from feeding on benthic surfaces. Sediment cover is an important factor structuring fish communities in central Texas streams where we have observed declining central stonerollers abundance within communities experiencing increased sediment loads (Pease et al. *in press*).

We did not observe a clear P enrichment effect on algal biomass in either the grazed or un-grazed treatments. Other studies of streams vary in the extent to which periphyton respond to nutrient enrichment under different grazer treatments. Stewart (1987) compared the effects of stonerollers in unenriched and fertilized pools. While primary productivity increased with P enrichment, central stonerollers limited standing stocks of periphyton regardless of nutrient enrichment. Similarly, Flecker et al. (2002) found that periphyton biomass was limited by both grazing fish and limiting nutrients but fish effects on algal biomass were stronger. While periphyton biomass is controlled by top-down and bottom-up mechanisms, top-down control is often greater because nutrient enrichment represents a relative relief from limitation while grazer exclusion represents a categorical removal of grazing pressure (Hillebrand 2002). In our study, grazer exclusion replaced one limiting factor (fish) with a stronger limiting factor (sediment accumulation)

that resulted in lower algal biomass and no response to P enrichment. Most experimental studies examining top-down verses bottom-up effects on periphyton biomass have been conducted in closed mesocosm systems (aquariums or recirculating streams) or in natural systems during low flow periods and, as a result, potentially underestimated the important influence of grazer-sediment interactions on periphyton biomass response to nutrient enrichment.

Grazing fish can influence algal C through unselective grazing which removes both detritus and algal cells, of which only the nutrient-rich algal portion regenerates (Frost et al. 2002; Hillebrand et al. 2008). There was clear evidence that grazing fish increased the relative amount of organic C by removing inorganic C components and increasing the relative proportion of algal cells in periphyton. Increased proportions of algal cellular C has been linked to higher nutrient content in periphyton (Frost et al. 2005) and was likely the mechanism by which fish grazing increased periphyton % P and N and the response of periphyton % P to P enrichment. These results, combined with the fact that C:Chl. *a* ratios did not respond to P enrichment, suggest that increased algal content on grazed substrates influenced uptake and storage of excess P within the periphyton matrix. Autotrophs have the ability to assimilate P even when it is not needed for growth (Borchardt 1996) and as the proportion of algal C increases in periphyton, algal nutrient content has a greater influence on periphyton nutrient content (Frost et al. 2005). Stronger responses of grazed substrates to nutrient enrichment in our study are likely the result of higher relative amounts of algal cells which permitted more luxury uptake and storage of P within the periphyton matrix at high P enrichment levels. At high P

enrichment levels, excess P on grazed substrates likely influenced incorporation of N into the periphyton matrix as a result of potential shifts towards N limitation.

In general, grazers increase incorporation of limiting nutrients into periphyton (Hillebrand et al. 2008). The lack of grazing effects on C:P ratios in our control and low P streams may be due to higher proportions of organic matter with a proportionally similar amount of P on grazed substrates. However there was no difference in periphyton P between control and low streams and the fact that both % C and chlorophyll *a* were significantly higher in grazed treatments, an indication that autotrophs were photosynthesizing more (Sterner and Elser 2002). High levels of bicarbonate in central Texas streams due to their underlying lithology (Dworkin 2003) combined with dissolved CO_2 from turbulent mixing suggests that elemental composition of new autotrophic production in our system must be largely driven by the relative supply of light, N and/or P (Frost et al. 2002). Our study was not explicitly designed to test how interactions between light limitation and P limitation influence periphyton nutrient content, but grazing fish presumably increased light: nutrient supply ratios on grazed substrates by removal of sediments early in the study. Benthic grazers and detritivores play a key role in removing sediments from benthic surfaces which can increase primary production (Power 1984; Power 1990; Flecker 1992; Flecker 1996; Taylor et al. 2006; Winemiller et al. 2006). Additionally, grazing may stimulate biomass-specific primary production directly by stimulating an early successional periphyton community with faster growing organisms and less over-story algae (Stewart 1987; Gelwick and Matthews 1992). Grazer-induced changes in primary productivity can have consequences for nutrient demand in benthic habitats (Flecker et al. 2002). Higher photosynthesis rates due to

increased light and/or high turnover rates associated with grazing potentially induced P-limitation at concentrations below 20 μg L^{-1} PO_4-P which would explain the lack of response of periphyton C:P ratios to grazing in control and low P streams. In highly enriched streams, C:P values for grazed periphyton were lower than un-grazed periphyton, evidence that P availability exceeds grazer-mediated demand, and luxury uptake of P occurred within the periphyton community. Luxury uptake of P not only lowered C:P ratios but potentially stimulated N demand and incorporation in the periphyton matrix, resulting in greater differences in C:N ratios. Further studies that directly measure C fixation (^{14}C-HCO_3^- uptake), P and N uptake, and separate indirect (sediment removal) and direct effects of grazing fish are needed to test a grazer-induced, sediment-driven light: nutrient hypothesis (Sterner et al. 1997).

In general, grazers reduced C:P and C:N ratios but tend to increase N content more so than P content resulting in higher N:P ratios in the presence of grazing (Hillebrand et al. 2008). The sign and strength of grazer effects on periphyton nutrient ratios is dependent on factors such as the nutrient content of grazers and their food, grazer biomass, the amount of biomass removal, and water column nutrients (Hillebrand et al. 2008). As all of our streams contained similar biomass of central stonerollers and grazers had similar effects on biomass across the P enrichment treatments, our results suggest that phosphorus enrichment and its influence on consumer-resource stoichiometric mismatches had the greatest influence on fish grazing effects on periphyton nutrient content in our streams. However, the role of sediment interactions in this relationship remains unclear and similar studies that remove the influence of sediment on

relationships between fish grazing, nutrient enrichment and periphyton stoichiometry are needed.

Our results provide strong evidence for the role of grazer-induced increased algal content within the periphyton matrix in increasing % C, P and N but we cannot completely rule out other mechanisms that may have contributed to the response of periphyton elemental composition to grazing fish and P enrichment. Grazers also reduce diffusion barriers between algal cells and available nutrients by removing over-story algae, organic matter and inorganic sediments (Frost et al. 2002; Hillebrand et al. 2008). While we did not measure nutrient uptake directly, changes in periphyton P content mirrored changes in AFDM on grazed substrates and suggests that periphyton nutrient content was influenced by grazer-induced mechanisms that increased nutrient uptake in periphyton. Shifts in periphyton species composition can contribute to changing nutrient content in response to both grazing and nutrient enrichment (Frost et al. 2002; Hillebrand et al. 2008; Liess and Kahlert 2009). We did not quantify periphyton community structure across our treatments but there were obvious qualitative differences in *C. glomerata* between grazed and un-grazed treatments by day 28 of our study (Figure 12). Despite these differences, there was no difference in AFDM or chlorophyll *a* across nutrient treatments but P and N content response was greater on grazed substrates. *Cladophora* readily incorporates and utilizes excess P for growth in freshwater systems (Dodds and Gudder 1992) and may have been at least partially responsible for stronger elemental responses of the periphyton community to P enrichment on grazed substrates. Potential changes in the invertebrate community in response to fish grazing may influence whole community consumer-resource stoichiometric relationships. We did not

monitor invertebrate communities during our study. However, fish are the most nutrient-rich organisms in streams and serve as the dominant pool of nitrogen and phosphorus in stream ecosystems when abundance is high (McIntyre and Flecker 2010). The relatively high biomass of fish (~70 g m^{-2}) in our experimental units suggests their influence on periphyton stoichiometry would have overridden any influence of changing macroinvertebrate abundances. Finally, regeneration of nutrients by fish excretion or egestion (Evans-White and Lamberti 2006) can also contribute to changes in periphyton nutrient content but our study was not designed to separate this pathway from consumptive effects. However the influence of fish excretion on nutrient dynamics in streams appears to be more related to species identity and abundance rather than resource stoichiometry (Vanni et al. 2002; McIntyre et al. 2008; Knoll et al. 2009; McManamay et al. 2011; Wilson and Xenopoulos 2011; Kohler et al. 2011) and the relative size of central stonerollers compared to benthic boundary layers and the high flow rates in our experiment likely increased the spatial scale of this important nutrient pathway beyond the extent of our experimental units (Frost et al. 2002). More experiments that separate this important mechanism from direct grazing effects are needed to clearly define the role of this consumer-mediated nutrient pathway in streams (Knoll et al. 2009).

While our findings are limited to experimental mesocosms, the influence of stonerollers on sediment accumulation on benthic surfaces has several potential ecological implications for stream food webs. First, by increasing periphyton algal content, *C. anomalum* increases the palatability of benthic food resources for other grazing organisms (Vaughn et al. 1993). Second, maintaining close-cropped filamentous algae layers on benthic substrates may also influence periphyton stability and nutrient

retention in stream habitats. Stonerollers potentially decrease export of algal biomass by consuming algae before it reaches lengths susceptible to sloughing or scour during storm events. This shift from abiotic to biotic control of algal biomass not only potentially increases algal resource stability (Pringle and Hamazaki 1997) but may also benefit consumers other than grazers by converting coarse algal resources into fine particulate benthic organic matter (Gelwick and Matthews 1992). Third, the influence of grazing on periphyton stoichiometry and its interaction with P enrichment has potential ecological consequences for other species and stream riffle communities as a whole. Low P content in benthic resources creates stoichiometric imbalances in autotroph-herbivore interactions that put food quality limitations on fast growing organisms and constrain food web dynamics in nutrient poor freshwater ecosystems (MacKay and Elser 1998; Elser et al. 2000). Stronger responses of periphyton P content to P enrichment on substrates exposed to *C. anomalum* grazing suggest that stonerollers facilitate the incorporation of excess P into benthic foodwebs and as a consequence, potentially remove stoichiometric constraints on other herbivorous organisms at lower levels of P enrichment. As a result, shifts in community structure related to grazer-induced changes in algal content and its response to P enrichment, may manifest in simple communities of fast-growing organisms with lower trophic diversity.

CHAPTER FOUR

Fish-Mediated Nutrient Recycling and Benthic Microbial Processes: Can Consumers Influence Stream Nutrient Cycling at Multiple Spatial Scales?

Introduction

Consumers play an important role in biogeochemical cycles within ecosystems by controlling availability of limiting resources (Chapin et al. 2000). Consumer influence on nutrient cycling depends on relative biomass within systems as well as differences between resource nutrient content and consumer requirements for growth, maintenance, and reproduction (Elser and Urabe 1999; Sterner and Elser 2002; Vanni 2002; Hillebrand et al. 2008). Thus, species that have unique relationships between diet nutrient content and body elemental composition and/or are numerically abundant may play disproportionately important roles in the recycling, transport or retention of nutrients within stream ecosystems (Evans-White and Lamberti 2006; McIntyre et al. 2007; Vaughn 2010; Small et al. 2011).

Nutrients may become depleted along longitudinal gradients in streams due to nutrient uptake by benthic autotrophs and heterotrophs (Mulholland et al. 1995). Aquatic consumers facilitate downstream nutrient export by consuming benthic resources (periphyton, detritus, lower consumers) and remineralizing nutrients bound up in organic material for downstream uptake (Vanni 2002). The balancing of these two processes, uptake by the microbial community and remineralization by consumers, results in nutrient atoms spiraling between biological pools and water column pools as they move downstream. The length of these nutrient spirals is dependent on stream velocity as well

as uptake and remineralization rates which are governed by the nutrient requirements of stream biota (Newbold et al. 1982; Small et al. 2009). This process can constitute an important biogeochemical flux of limiting nutrients and maintain primary production in stream ecosystems (Grimm 1988; Vanni et al. 2002; McIntyre et al. 2008). However, depending on stoichiometric differences between organisms and their food, differential excretion of macronutrients (N and P) potentially leads to decoupling of limiting and non-limiting nutrient spirals in stream ecosystems (Small et al. 2009).

Fish have high proportions of P-rich bone and scales (Sterner and George 2000; Hendrixson et al. 2007) which can make growth of some species P-limited (Hood et al. 2005). In unbalanced ecosystems (excess N, limited P), fish potentially retain P and increase excretion rates of N to maintain optimal body N:P ratios (Small et al. 2009). Fish species vary widely in their nutrient requirements and as a result, high fish species diversity in tropical streams can compensate for negative effects of some species on P-recycling, resulting in overall high consumer-driven nutrient turnover of limiting nutrients in these ecosystems (McIntyre et al. 2008). In temperate stream ecosystems however, comparably low fish diversity may result in a few numerically abundant taxa having considerable influence on nutrient cycling (Grimm 1988).

While fish-mediated nutrient recycling may influence nutrient spiraling at the stream scale, stoichiometric constraints on cycling of nutrients between producer, consumer and water column pools may also influence smaller scale nutrient cycles within streams. Inorganic nutrients play a central role in the coupling of bacterial and algal production within periphyton matrices (Rier and Stevenson 2001; Rier and Stevenson 2002; Carr et al. 2005). While bacterial production relies on metabolism of algal

extracellular organic carbon (EOC) (Haack and Mcfeters 1982; Murray et al. 1986), recent studies support the hypothesis that algae also rely on bacterial-regenerated nutrients to support benthic production in nutrient-poor aquatic systems (Rier and Stevenson 2002b; Sharma et al. 2005; Scott et al. 2008; Borovec et al. 2010). Internal recycling of nutrients within periphyton maintains biomass in nutrient-deficient streams (Mulholland et al. 1995). There is growing evidence that this important ecosystem function decreases with anthropogenic nutrient subsidies (Scott et al. 2008; Lyon and Ziegler 2009). Thus, changes in nutrient availability initiated by consumer-mediated nutrient recycling may influence internal nutrient cycling and coupling of bacterial-algal production in stream periphyton. However, this is dependent on stoichiometric relationships between limiting nutrients, stream consumers, and their food.

In this study we explore the role of fish-mediated nutrient recycling in downstream export of N and P by quantifying longitudinal changes in periphyton biomass, production, and stoichiometry in twelve flow-through stream mesocosms with or without grazing fish. Our study organism was the central stoneroller (*Campostoma anomalum*; Cyprinidae). Central stonerollers can exert considerable control over biomass and ecological processes in benthic habitats (Gelwick and Matthews 1992; Matthews 1998) and are a model consumer for investigating consumer-mediated nutrient recycling in temperate streams. We established stream mesocsoms with low (N:P = 11) and high (N:P =177) ratios to evaluate the prediction that P-rich consumers will decouple N and P cycles in streams where nutrients are un-balanced (Small et al. 2009). We hypothesized that fish would increase downstream N and P availability compared to fishless streams and maintain periphyton nutrient content and biomass along the stream gradient in low

N:P streams. In contrast, we hypothesized that fish would recycle more N than P and this would have minimal influence on net N availability but decrease P availability in high N:P streams, thus there would be longitudinal declines in periphyton P content and biomass in the presence of fish. To link influences of grazing fish on nutrient cycling in streams with changes in internal nutrient cycling within periphyton mats we also compared algal-bacterial coupling downstream of fish enclosures across our treatments. We hypothesized that increased nutrient availability from fish excretion would decrease algal-bacterial coupling in low N:P streams. In high N:P streams, we hypothesized that fish would increase algal-bacterial coupling in response to decreased P availability downstream of fish enclosures.

Materials and Methods

Experimental Design

Our experiment was designed to contrast directional nutrient cycling effects of central stonerollers on periphyton communities under two different N:P regimes by altering N availability. We conducted the experiment in twelve outdoor flow-through stream mesocosms that receive relatively low-nutrient surface water from an 80 hectare constructed wetland fed by the North Bosque River in McLennan Co., TX (Figure 13A). We set up the mesocosms to simulate normal summer low-flow conditions in the region (Pease et al. *in press*). Water flowed at a rate of 15 L min^{-1} interstitially through a 9 x 0.61 m long riffle before flowing through the 9 x 0.61 m run section and returning to the wetland. Four months before initiation of the experiment, each run was completely covered with a 10 cm layer of gravel and 75 similar sized cobbles were placed

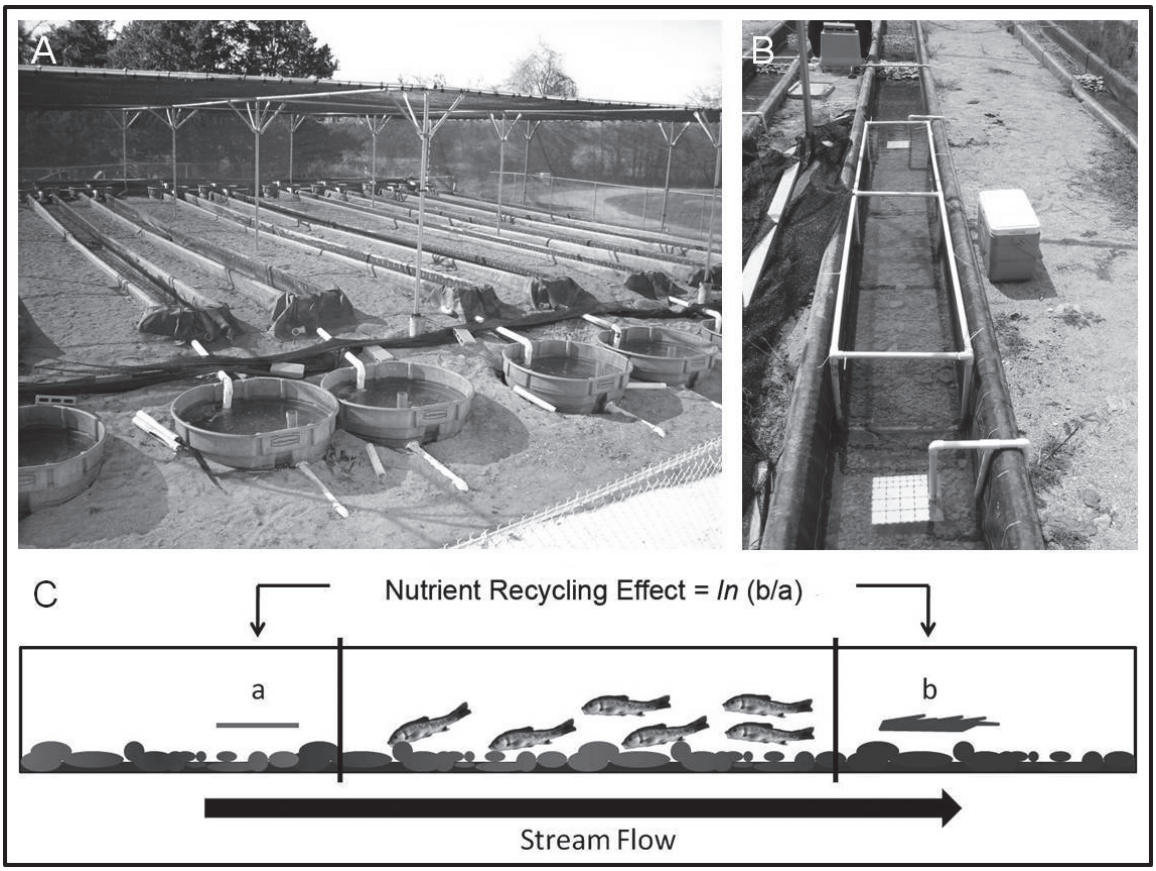

Figure 13. Stream mesocosms at the Baylor Experimental Aquatic Research facility (A), fish enclosure cage and upstream/downstream grazer exclusion platforms in stream run, and stream mesocosm design (C). Note: only six mesocosms had fish.

within each fish enclosure in 15 rows of five rocks each to allow development of natural periphyton and macroinvertebrate communities. During the study, severe nitrogen limitation typical of summer conditions in the source water wetland (Scott et al. 2005) resulted in low background nutrient concentrations of source water (DIN = 54.8 ± 5.2 µg L^{-1}, SRP = 10.5 ± 0.4 µg L^{-1}, N:P = 10.8 ± 0.7). We used peristaltic pumps calibrated to deliver solutions of sodium nitrate (NaH_2NO_3) from 50-L carboys at DIN concentrations of 1000 µg L^{-1} to create an unbalanced nutrient regime (DIN= 775.9 ± 24.8 µg L^{-1}, SRP = 10.5 ± 0.4 µg L^{-1}, N:P = 177.4 ± 8.6) in six of the twelve streams (Figure 14). We placed 3 m long fish enclosure cages (6mm polypropylene mesh (Industrial Netting,

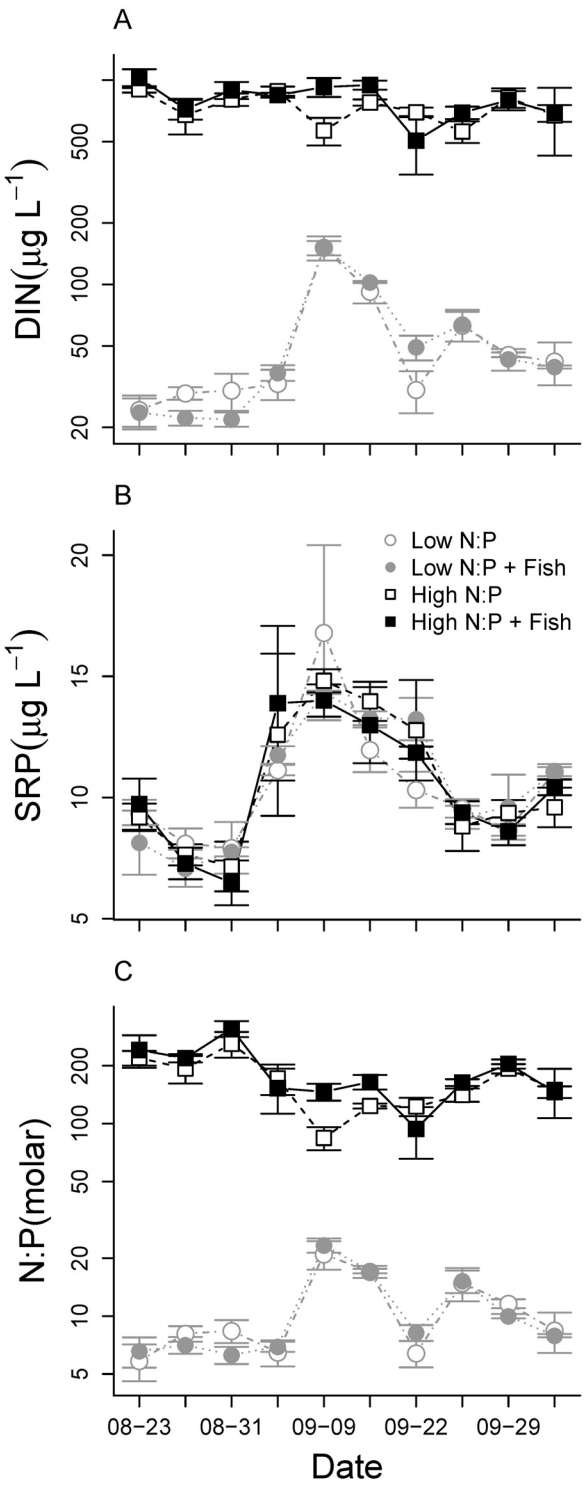

Figure 14. Mean (± SE) surface water dissolved inorganic nitrogen (DIN) (A), soluble reactive phosphorus (SRP) (B), and molar N:P ratios (C) across time in streams representing different N-enrichment and fish treatments.

Minneapolis, MN, USA), 1.83 m^2 surface area) in the center of each stream run that allowed the unidirectional movement of water through the cages from areas upstream to downstream of cages (Figure 13B). Thus, periphyton upstream of cages with fish was not exposed to fish-mediated nutrient recycling, whereas downstream periphyton communities were exposed to potential nutrient-mediated effects of fish (Fig. 13C). Stream runs were covered by 30% shade cloth to approximate riparian canopies and ambient photosynthetically active radiation of regional streams (1,000–1,200 $\mu E\ m^{-2}\ s^{-1}$; R.S. King unpublished data), reduce variability of sunlight across experimental units, and prevent loss of fish due to jumping or bird predation.

At the onset of the experiment we suspended grazer exclusion platforms upstream and downstream of each fish enclosure with twelve 5.08 cm and sixteen 2.54 cm unglazed ceramic tiles to quantify effects of nutrient excretion occurring within cages on periphyton biomass, stoichiometry and microbial production. We collected central stonerollers from Salado Cr., TX (31.4596 °N, 97.2925 °W) and stocked 3 streams within each nutrient treatment to represent size structure from a local central Texas stream and densities at which fish exhibited natural shoaling and grazing behavior (15 fish m^{-2}). Treatments included 3 whole stream replicates of each fish treatment (fish/no fish) within each nutrient regime (N:P = 11 and 177). The study ran for 42 days and was concluded on 30-September-2009.

Data Collection

Dissolved nutrients were collected from the center of each stream weekly for PO_4-P, $NO_2N + NO_3N$ and NH_3N and analyzed using standard methods (APHA 1998) on a Lachat Quickchem 8500 autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). On

days 0, 14, 28, and 42 we monitored biomass and stoichiometry of food resources by collecting composite periphyton samples from six randomly selected cobbles. We collected periphyton samples from 12 tiles upstream and downstream of each fish enclosure on day 42 to quantify fish-mediated nutrient recycling effects. Periphyton was scraped off substrates, homogenized, subsampled, and filtered onto preweighed Whatman GF/F (pore size = 0.7 μm) filters for quantification of chlorophyll *a*, dry mass, and AFDM following (Steinman et al. 2006). Additional periphyton slurry subsamples were transferred to 50 mL centrifuge tubes and separated from inorganic material by centrifugation in colloidal Si using the method of (Hamilton et al. 2005). The organic fraction was then dried at 60°C for 48 hours and pulverized into a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc.) for analysis of nutrient content. We measured nutrient content as % C, N and P. We estimated C and N content of periphyton using a ThermoQuest Flash EA™ 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts). Phosphorus content was analyzed with a Lachat QuikChem 8500 flow-injection autoanalyzer using the molybdate colorimetric method following a 1-hour digestion in 15mL of distilled water with 1.8mL of a mixture of peroxodisulphate (30 g L^{-1} $K_2S_2O_8$), boric acid (50 g L^{-1} H_3BO_3) and sodium hydroxide (15 g L^{-1} NaOH) at 121 °C (Faerovig and Hessen 2003). Soil (Thermo Finnigan 1.99 % C) and peach leaf (SRM 1547, 0.137 % P, 0.298 % N) standards were analyzed to assure C, N, and P recoveries met QA/QC standards (± 10%) for each sample run.

We measured fish body stoichiometry and excretion rates for eight individuals from each stream on day 42. Fish were collected by herding individuals into a dipnet and immediately placing fish into a covered bucket for 15 min to recover from shock. We

placed single fish (69.7 ± 2.1 mm, std. length) in 1 L bottles filled with 800 mL of filtered low N:P stream water and placed bottles in stream during incubation. A background nutrient sample was collected prior to adding fish and at 15, 30, 60, 90 and 120 minutes to look for signs of stress or starvation-induced excretion rates (Whiles et al. 2009). Water samples were analyzed for PO_4-P and NH_3N following methods above. Mass specific excretion rates for each time period were calculated as the change in concentration per unit time divided by the wet mass of the fish. Fish were measured, weighed and frozen for subsequent tissue analysis. We dried individual fish at 60 ºC after removing intestines, then ground samples to a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc.) followed by mortar and pestal. Fish tissue C, N, and P content were measured using the same methods described for periphyton.

On day 42 we measured autotrophic and heterotrophic microbial production simultaneously on individual tiles downstream of fish enclosures using ^{14}C-HCO_3^- uptake and ^3H-L-leucine incorporation into protein, respectively (Neely and Wetzel 1995; Scott et al. 2008). In each stream, tiles were incubated in 60 mL wide-mouth jars fitted with open-top caps lined with silicon septa and filled with filtered (0.2 µm) stream water. Three jars were left unwrapped for light incubation, and 1 was wrapped in aluminum foil for dark incubation. An additional killed control jar was incubated in light. We injected each jar with 25 µL of ^{14}C-labeled $NaH^{14}CO_3$ solution (45 µCi mL^{-1}) and placed jars face down with periphyton facing up in the stream at a depth of 10 cm. After 1.5 h, 25 µL of ^3H-labeled L-leucine (75 µCi mL^{-1}) was injected into each jar. Incubations continued for an additional 30 minutes. Incubations were stopped by adding buffered formalin to a final

concentration of 4% after which, samples were returned to the laboratory and stored at 4°C until processing.

Laboratory processing of radio-labeled periphyton samples followed (Scott et al. 2008). Briefly, site water in incubation jars was replaced with 5% TCA, and placed on ice for 1 h. After chilling, the TCA was removed and we removed periphyton from tiles with a toothbrush, rinsed sample into a slurry with 5% TCA, and diluted to a known volume (30–50 mL) with 5% TCA. Samples were then homogenized with a vortex mixer and a subsample was filtered onto a precombusted and weighed glass-fiber filter (GF/F; pore size = 0.7 μm) for determination of AFDM. A 2nd subsample was filtered onto a 0.2 μm pore size polycarbonate filter, washed twice with 5% TCA, once with 80% ethanol, and once with deionized (DI) water, placed in scintillation vials with 5 mL of alkaline solution (0.5 mol/L NaOH, 25 mmol/L ethylenediamine-tetra-acetic acid [EDTA], and 0.1% sodium dodecylsulfate) and agitated for 1 h at 85 ºC to dissolve material attached to polycarbonate filters (Buesing and Gessner 2003). We radioassayed a 2.5 mL aliquot of this solution for ^{14}C and ^{3}H activity on a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Fullerton, California). Activities of both isotopes were corrected for quench with external standards and then converted from radioactivity estimates to HCO_3^- and leucine uptake rates based on the specific activity of each isotope used in incubations. Autotrophic and heteroptrophic production were normalized to the surface area of tiles and AFDM and express as μg C mg^{-1} cm^{-2} h^{-1} and μg C mg^{-1} AFDM h^{-1}, respectively.

Data Analysis

We tested for differences in fish stoichiometry and nutrient excretion rates, as well as changes in periphyton C:N, C:P, N:P, and chlorophyll *a* within fish enclosures across time among treatments using linear mixed models (LMM) (Pinheiro and Bates 2000). We specified stream as a random effect (intercept only) to account for non-independence of nested samples or multiple dates collected from the same stream. Fixed factors included stream N:P ratio for fish stoichiometry and excretion data, and stream N:P ratio, fish, and date for fish enclosure periphyton data. The restricted maximum likelihood (REML) criterion was used to fit all models. We assessed assumptions of LMM visually with normality plots (qqnorm) and standardized residual plots across treatments (Pinheiro and Bates 2000). In cases where error variances differed across stratums of treatments we incorporated this heterogeneity into our model by modeling variance separately among treatments using the VarIdent command (Pinheiro and Bates 2000). In some cases we $\log_{10}(x)$-transformed data to meet normality assumptions (Zuur et al. 2009). We also checked for temporal autocorrelation by running the repeated measures model with and without temporal covariance structures. We assessed effects of incorporating heterogeneity of error variances and temporal autocorrelation on model fits using Akaike's Information Criterion corrected for small sample size (AICc) (Burnham and Anderson 1998). We compared base models with and without variance structure and selected the more complex model if Δ AICc ≥ 3 (Burnham and Anderson 1998).

To integrate temporal effects of nutrient cycling within streams independent of differences between streams we assessed longitudinal changes in periphyton nutrient content and biomass by comparing the response variable on tiles downstream of cages to

upstream of cages using log effect ratios, $\ln(R_{downstream}/R_{upstream})$ where R is the response variable (C:N, C:P, N:P or chl. *a*) (Osenberg et al. 1997). We used generalized least squares models (GLS) with the REML criterion to compare effect ratios between fish treatments within each nutrient regime. We assessed assumptions of GLS as in LMM models and incorporated heterogeneity of error variances when it differed among treatments (Pinheiro and Bates 2000). In some cases we $\log_{10}(x)$-transformed data to meet normality assumptions (Zuur et al. 2009).

We calculated the amount of BBP stimulated by PS on each tile by subtracting the BBP rate measured in dark incubations from light incubations (BBP_L-BBP_D). We determined if PS stimulated BBP by assessing the relationship between mean BBP_L-BBP_D and mean PS across all streams using model II major-axis regression because our predictor (mean PS) and response (mean BBP_L-BBP_D) were random estimates with error (Legendre and Legendre 1998). We quantified PS-BBP_L coupling as the covariance value between PS and BBP_L within each stream which is calculated as:

$$\mathrm{COV}_{\mathrm{PS-BBP}} = \sum_{i=1}^{n} \frac{(\mathrm{PS}_i - \mathrm{PS})(\mathrm{BBP}_{\mathrm{L}i} - \mathrm{BBP}_{\mathrm{L}})}{n},$$

where *i* is the incubation replicate (Scott et al. 2008). We assessed the influence of fish nutrient recycling on algal-bacterial coupling rates ($COV_{PS\text{-}BPP}$) within each nutrient treatment using GLS models and associated methods described in the previous paragraph. We also used ordinary least squares (OLS) regression to examine relationships between $COV_{PS\text{-}BPP}$ and nutrient and biomass variables that responded to our experimental manipulation.

We performed all statistical analyses using the R statistical programming language (R Core Development Team 2011). We used the nlme package (Pinheiro and Bates 2000) to run LMM and GLS, the lmodel2 package (Legendre 2008) for major axis regression, and the "lm" function for OLS regression. Following previous mesocosm studies using large experimental units (Threlkeld and Drenner 1987; Gelwick and Matthews 1992), we set alpha = 0.10 to test for significant effects between our treatments in LMM and GLS while balancing Type I and Type II errors.

Results

Fish Body Stoichiometry and Excretion Rates

There was no difference in fish whole body nutrient content between low and high N:P streams (Table 8). Mass specific excretion rates across time were initially highly variable but were fairly constant from 60 to 120 min, indicating that longer incubations provided more reliable estimates of excretion rates. There were no differences in mean fish excretion rates for N or P based on 60 min incubations between low and high N:P streams (Table 8). N:P ratios of fish excretion were variable within both treatments and there was no difference between treatments (Table 8).

Periphyton Stoichiometry and Biomass within Fish Enclosures

Periphyton C:N ratios responded negatively to N enrichment over time. Periphyton C:N from high N:P streams were significantly different from low N:P streams by day 42 (Figure 15A, Table 9). Periphyton exposed to fish grazing always had lower C:N ratios in high N:P streams (Figure 15A, Table 9). Periphyton C:P ratios increased during the study, but primarily in low N:P streams (Figure 15B, Table 9). Periphyton

Table 8. Mean ± 1 SE of fish whole body nutrient content from low and high N:P streams and t test (df = 4) results for differences between treatment effects on stoichiometric ratios. t values and associated p values are based on a linear mixed effects model (LME) that incorporates nested fish within each stream. Different variances for each stream were incorporated into the N:P body stoichiometry model, and all excretion stoichiometry models.

	Surface water N:P ~ 11	Surface water N:P ~ 177	*t*	*P*
Body stoichiometry				
C:N	4.216 ± 0.046	4.258 ± 0.080	0.45	0.676
C:P	77.353 ± 2.555	82.333 ± 4.810	0.91	0.413
N:P	18.322 ± 0.805	19.284 ± 0.780	0.98	0.384
Excretion stoichiometry				
N (μmol g^{-1} h^{-1})	1.200 ± 0.081	1.354 ± 0.107	0.70	0.521
P (μmol g^{-1} h^{-1})	0.146 ± 0.030	0.159 ± 0.034	0.69	0.527
N:P	19.908 ± 8.960	29.299 ± 15.580	0.45	0.679

N:P ratios increased with time during the study but did not differ among N-enrichment or fish treatments (Figure 15C, Table 9). High N:P streams had more chlorophyll *a* than low N:P streams by day 42 (Figure 15D, Table 9). Chlorophyll *a* was lower in streams with fish on day 28 but fish effects were only observed in low N:P streams by day 42 (Figure 15D, Table 9).

Nutrient Cycling Effects

Fish effects on periphyton stoichiometry and algal biomass differed between the nutrient treatments. In low N:P streams, periphyton C:N, C:P, or N:P ratios did not change longitudinally in streams with or without fish (Fig. 16A-C, Table 10). Likewise, benthic chl. *a* did not change longitudinally in streams with or without fish (Fig. 16D, Table 10). Conversely, there were longitudinal changes in periphyton C:N, C:P, and N:P ratios in high N:P streams with and without fish (Fig. 16A-C). However, declines in periphyton C:N were significantly greater in streams with fish than without fish, whereas C:P and N:P ratios also trended higher in stream with fish (Fig. 16A-C, Table 10). Further, longitudinal chl. *a* increased significantly in high N:P streams with fish but did not increase in fishless streams (Fig. 16D).

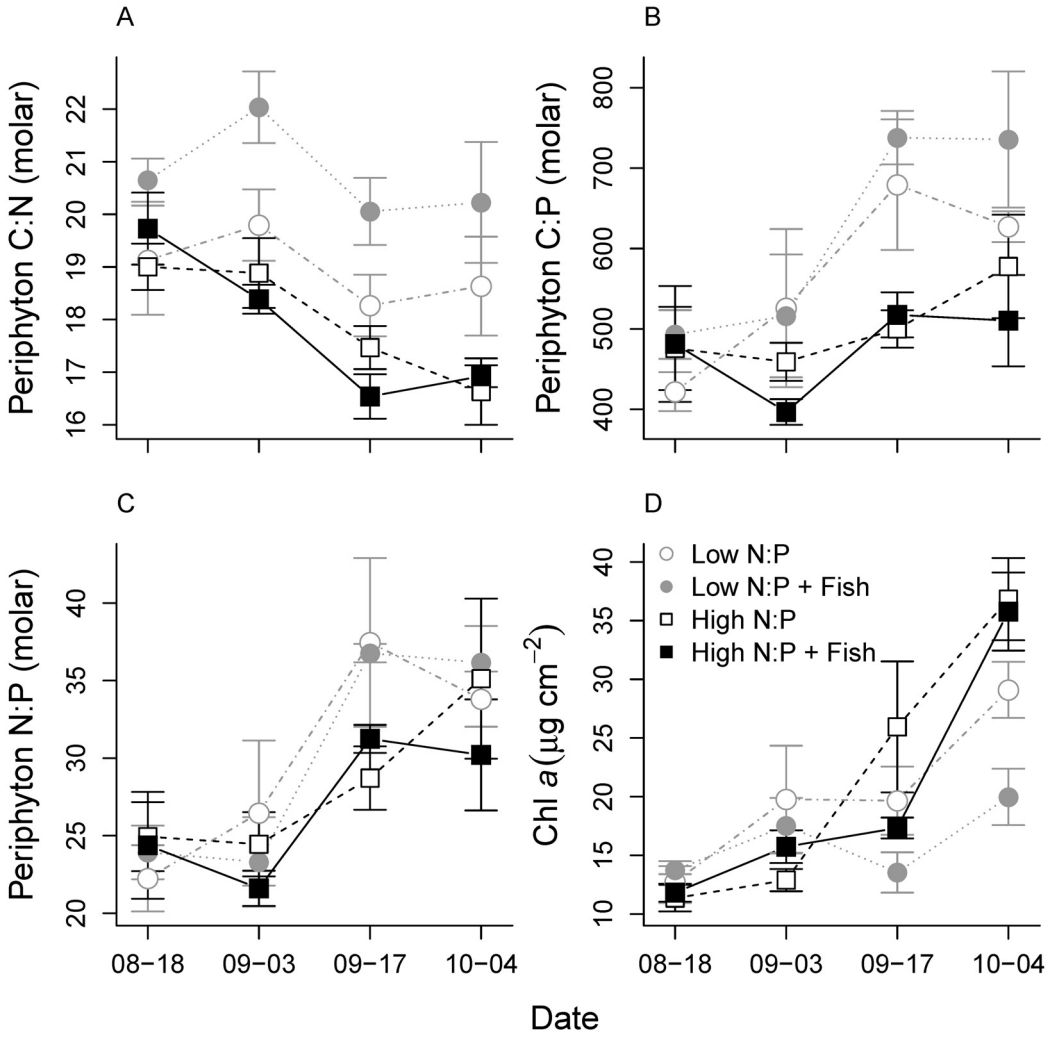

Figure 15. Mean (± SE) periphyton C:N (A), C:P (B), N:P (C), and chl. *a* (D) across time in streams representing different N-enrichment and fish treatments.

Downstream Periphyton Production

BBP_L rates were always greater than BBP_D rates and mean BBP_L - BBP_D increased significantly with mean PS across all streams (Figure 17). There were no differences in areal or biomass-corrected BBP and PS rates downstream of cages with and without fish in low N:P streams (Figure 18A-D, Table11). Areal BBP and PS rates were significantly greater in high N:P streams with fish (Figure 18A and C, Table 11).

Table 9. Effects of nitrogen enrichment and fish grazing on measures of periphyton nutrient content and biomass. *F* values and associated *p* values are based on a linear mixed effects model (LME) that incorporates repeated measures within each stream. Different variances for nitrogen, fish, and date were incorporated into C:P, N:P and Chl. *a* models respectively.

Source of variation	*F*	*P*
	C:N ratio	
Nitrogen $_{1, 8}$	28.26	**<0.001**
Fish $_{1, 8}$	5.51	**0.047**
Date $_{1, 32}$	10.56	**0.003**
Nitrogen × Fish $_{1, 8}$	6.94	**0.030**
Nitrogen × Date $_{1, 32}$	4.33	**0.046**
Fish × Date $_{1, 32}$	0.04	0.837
Nitrogen × Fish × Date $_{2, 27}$	0.06	0.813
	C:P ratio	
Nitrogen $_{1, 8}$	11.42	**0.002**
Fish $_{1, 8}$	0.00	0.987
Date $_{1, 32}$	9.66	**0.004**
Nitrogen × Fish $_{1, 8}$	1.91	0.175
Nitrogen × Date $_{1, 32}$	3.71	*0.061*
Fish × Date $_{1, 32}$	0.17	0.685
Nitrogen × Fish × Date $_{2, 27}$	0.41	0.525
	N:P ratio	
Nitrogen $_{1, 8}$	3.39	0.103
Fish $_{1, 8}$	0.28	0.609
Date $_{1, 32}$	16.63	**<0.001**
Nitrogen × Fish $_{1, 8}$	0.32	0.588
Nitrogen × Date $_{1, 32}$	0.66	0.424
Fish × Date $_{1, 32}$	0.07	0.771
Nitrogen × Fish × Date $_{2, 27}$	0.22	0.644
	Chl. *a*	
Nitrogen $_{1, 8}$	1.42	0.268
Fish $_{1, 8}$	0.23	0.646
Date $_{1, 32}$	192.27	**<0.001**
Nitrogen × Fish $_{1, 8}$	0.03	0.879
Nitrogen × Date $_{1, 32}$	24.05	**<0.001**
Fish × Date $_{1, 32}$	7.52	**0.009**
Nitrogen × Fish × Date $_{2, 27}$	2.40	0.131

However, differences in downstream biomass-corrected BBP and PS rates between fish treatments in high N:P streams were not different (Figure 18F, Table 11). In low N:P streams, areal and biomass-corrected coupling rates ($COV_{PS\text{-}BPP}$) were more variable in streams with fish but were not significantly different from fishless streams (Figure 18E-F, Table 11). Both areal and biomass-corrected coupling rates ($COV_{PS\text{-}BPP}$) were

significantly greater in streams with fish than those without fish in high N:P streams (Figure 18E-F, Table 11). Both chl. *a* and algal-bacterial coupling rates (COV_{PS-BPP}) declined with periphyton C:N content (Figure 19A-B), resulting in a positive relationship between algal-bacterial coupling and algal biomass (Figure 19C).

Discussion

Role of Central Stonerollers in Decoupling of N and P Cycling in Stream Ecosystems

Longitudinal changes in periphyton stoichiometry indicate that central stonerollers differentially excrete N and P and decouple spiraling of these two important macronutrients when N:P ratios are high in streams. These results suggest that the relative importance of grazing fish in stream nutrient spiraling varies with nutrient availability and supports predictions from simulation models; that N enrichment can influence P availability by increasing the differential excretion of N and P by consumers (Small et al. 2009). In low N:P streams, periphyton stoichiometry and algal biomass did not change along the stream continuum in streams with or without fish, suggesting that fish nutrient recycling was not an important factor in enhancing downstream nutrient availability. In contrast, periphyton C:N ratios decreased, C:P and N:P ratios trended higher, and chl. *a* increased longitudinally in streams with fish when background DIN was 1000 μg L^{-1}. Dissolved P was relatively low (10.5 μg L^{-1}) across all streams, but N-enriched periphyton in high N:P streams likely resulted in central stonerollers excreting more N and less P to maintain optimal N:P ratios for growth and maintenance. An alternative explanation for longitudinal changes in periphyton C:N ratios is fish excretion enhanced downsteam uptake of DIN by consuming periphyton with higher N content due

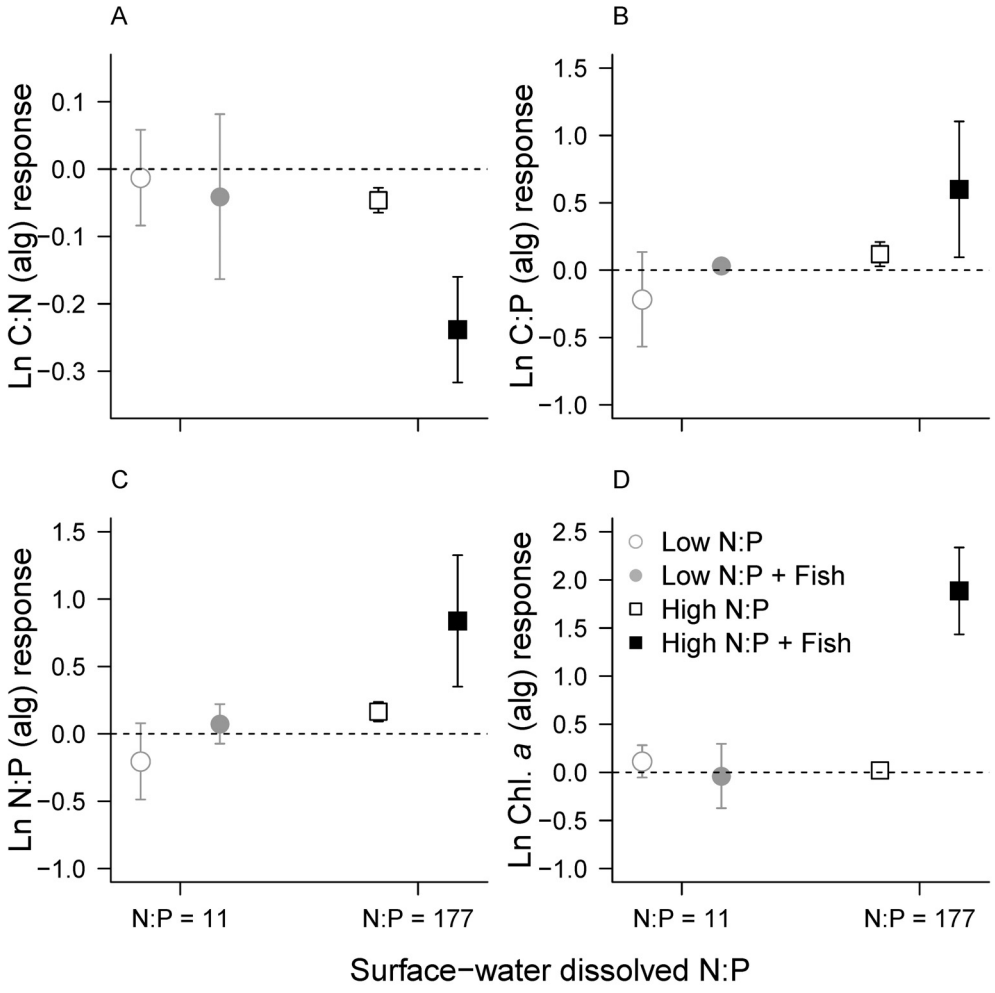

Figure 16. Mean (± SE) *ln* effect ratios for periphyton C:N (A), C:P (B), N:P (C), and chl. *a* (D) in streams representing different N-enrichment and fish treatments.

to NO_3 uptake and excreting N as NH_4^+, a form that many algae selectively use (Kemp and Dodds 2002). However, trends in periphyton C:P and N:P data combined with significant changes in C:N ratios provide stronger support for our first explanation. In either case, the fact remains that grazing fish altered nutrient availability for downstream periphyton communities which resulted in a significant increase in algal biomass in high N:P streams. A previous study conducted in a stream-like outflow of the same source-

Table 10. Mean ± 1 SE of net downstream changes in nutrient content and biomass of periphyton from fishless (-F) and fish (+F) treatments in low and high N:P streams and *t* test (df=4) results for differences between treatment effects on stoichiometric ratios and biomass. *t* values and associated *p* values are based on a generalized least squares model (GLS) allowing variance to differ by fish treatment.

	Surface water N:P ~ 11				Surface water N:P ~ 177			
	Mean (-F)	Mean (+F)	*T*	*p*	Mean (-F)	Mean (+F)	*T*	*p*
C:N	-0.013 ± 0.071	-0.041 ± 0.123	-0.19	0.852	-0.046 ± 0.019	-0.238 ± 0.079	-2.38	0.076
C:P	-0.217 ± 0.351	0.033 ± 0.037	0.71	0.518	0.119 ± 0.090	0.600 ± 0.505	0.21	0.401
N:P	-0.204 ± 0.283	0.074 ± 0.147	0.87	0.432	0.165 ± 0.072	0.838 ± 0.488	1.37	0.244
Chl. *a*	0.114 ± 0.168	-0.038 ± 0.334	-0.40	0.705	0.020 ± 0.072	1.886 ± 0.452	4.08	0.015

Table 11. Mean ± 1 SE from fishless (-F) and fish (+F) treatments in low and high N:P streams and *t* test (df=4) results for differences between treatment effects on bacterial biomass production (BBP), photosynthesis (PS) and coupling (COV) of periphyton. *t* values and associated *p* values are based on a generalized least squares model (GLS) allowing variance to differ by fish treatment.

	Surface water N:P ~ 11				Surface water N:P ~ 177			
	Mean (-F)	Mean (+F)	*T*	*p*	Mean (-F)	Mean (+F)	*T*	*p*
Area (cm^{-2})								
BBP	0.059 ± 0.009	0.128 ± 0.067	1.01	0.368	0.145 ± 0.038	0.364 ± 0.094	2.59	0.060
PS	0.048 ± 0.009	0.101 ± 0.052	1.00	0.373	0.097 ± 0.012	0.266 ± 0.062	2.69	0.055
COV	2.1^{e-5} ± 2.1^{e-5}	8.8^{e-3} ± 8.5^{e-3}	1.67	0.171	1.2^{e-3} ± 7.8^{e-4}	2.6^{e-2} ± 1.3^{e-2}	3.09	0.037
AFDM (mg^{-1})								
BBP	0.037 ± 0.008	0.096 ± 0.047	1.57	0.192	0.098 ± 0.020	0.155 ± 0.057	0.75	0.497
PS	0.032 ± 0.009	0.060 ± 0.022	1.20	0.296	0.068 ± 0.007	0.107 ± 0.035	1.10	0.333
COV	-1.0^{e-5} ± 8.7^{e-6}	2.7^{e-3} ± 2.6^{e-3}	1.57	0.192	3.9^{e-4} ± 2.2^{e-4}	3.3^{e-3} ± 1.4^{e-3}	3.15	0.035

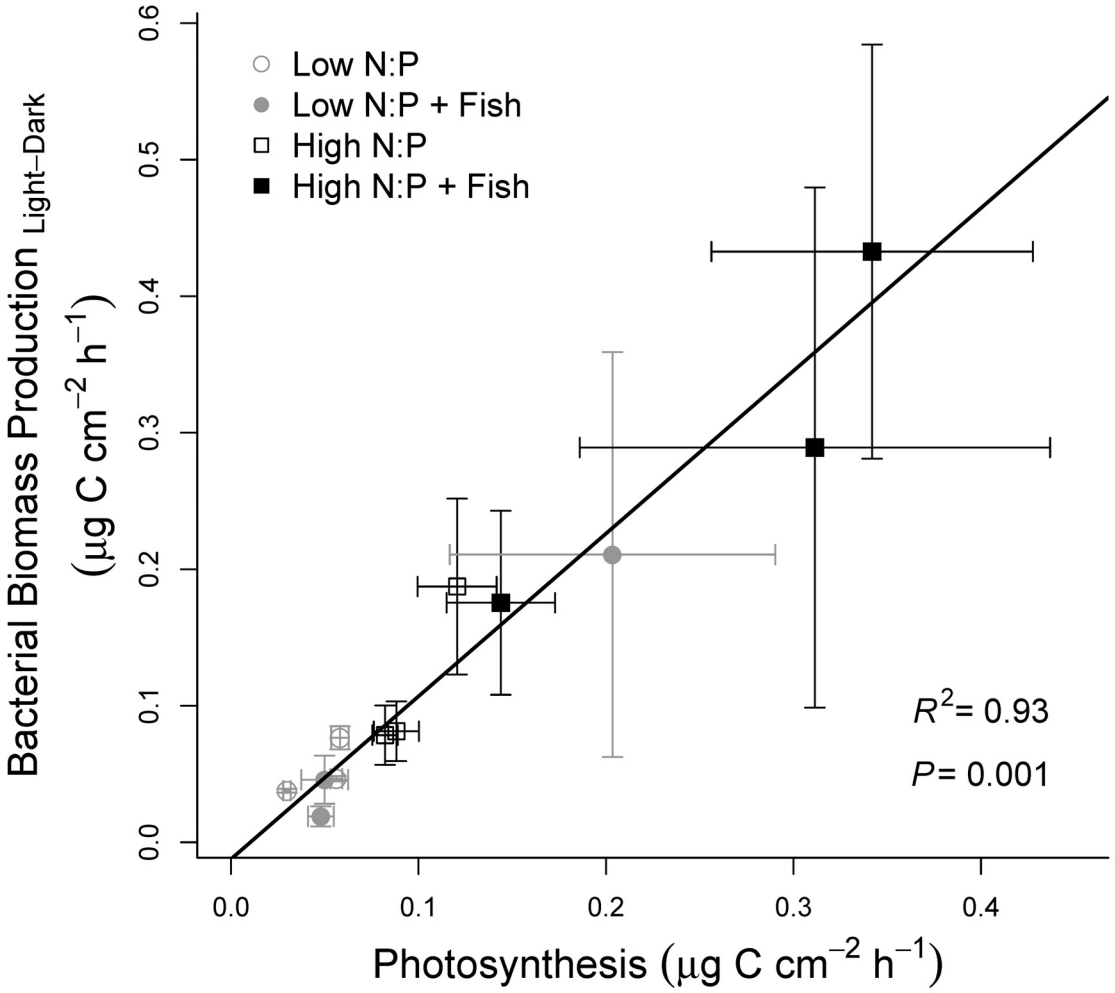

Figure 17. Major-axis regression of mean (± SE) difference between bacterial biomass production in light and dark incubations ($BBP_{Light-Dark}$) versus photosynthesis (PS) of periphyton across the mesocosm units.

water wetland for our mesocosms found that algal biomass increased with N amendments but not P (Scott et al. 2009).

Despite differences in periphyton stoichiometry, direct quantification of fish excretion rates did not differ between low-N and N-enriched streams. Nutrient excretion rate measurements are subject to error associated with effects of fasting and stress

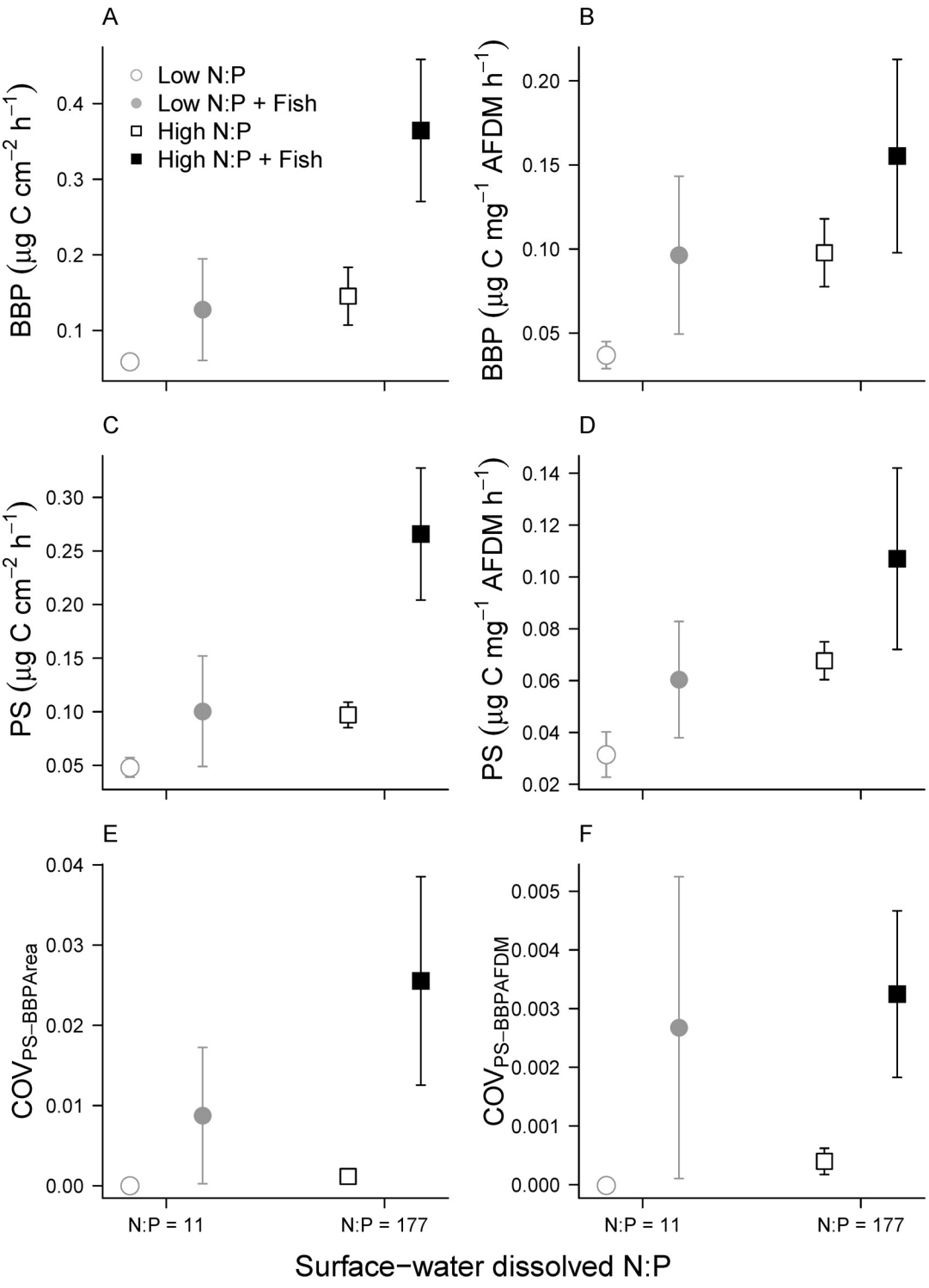

Figure 18. Mean (± SE) BBP_{Area} (A), BBP_{AFDM} (B), PS_{Area} (C), PS_{AFDM} (D), $COV_{BBP\text{-}PS,\ Area}$ (E), and $COV_{BBP\text{-}PS,\ AFDM}$ (F) in streams representing different N-enrichment and fish treatments.

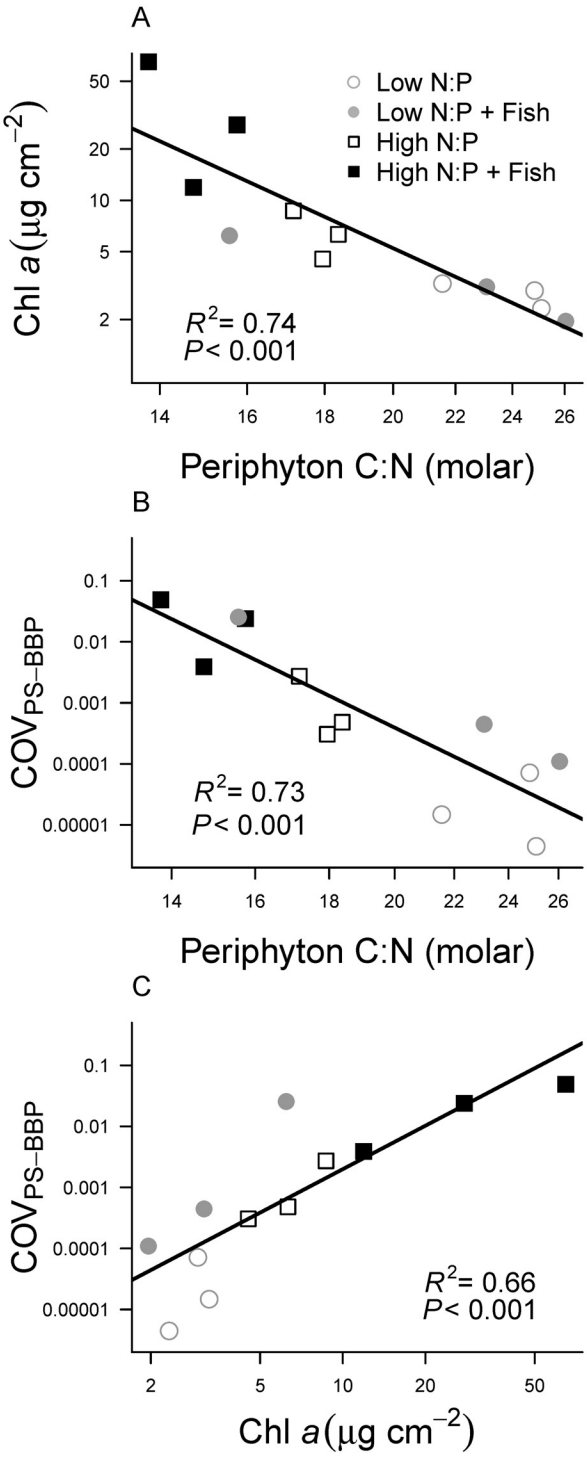

Figure 19. Linear regression of chlorophyll *a* biomass vs periphyton C:N ratios (A), the covariance of photosynthesis (PS) and bacterial biomass production in the light (BBP_L) (COVPS–BBP,AFDM) vs periphyton nutrient ratios (B), and vs chlorophyll *a* biomass (C).

(Whiles et al. 2009) and recent investigations that compared stream fish excretion rates across enrichment gradients found little difference in N excretion rates between individuals of the same species feeding on resources of differing quality (Kohler et al. 2011; Wilson and Xenopoulos 2011). Lack of differences in direct excretion measures are potentially due to differences in feeding behavior (Kohler et al. 2011) or growth rates (Sterner and Elser 2002) between low and high N:P streams, or simply because fish behaved similarly while in excretion incubations, regardless of their respective streams. We did not quantify differences in diet, nor did we measure growth rates to avoid handling stress in extreme temperatures while establishing the experiment. Our direct excretion measures were variable and only represent a single one hour period within a 42 day experiment, whereas excretion rates likely varied in response to changes in water chemistry, resource stoichiometry, temperature, and growth rates through time during our study. Following Knoll et al. (2009), we used periphyton stoichiometry to measure consumer-mediated recycling effects because it should be a stronger measure of nutrient status in aquatic systems. Periphyton stoichiometry integrates environmental variation over larger time scales (Gaiser et al. 2006; Scott et al. 2008), and we argue that comparing upstream and downstream periphyton stoichiometry captured cumulative effects of differential fish excretion during the study, providing a more integrative measure of consumer-mediated nutrient recycling. Additionally, using large mesocosms as whole replicates necessarily reduced our statistical power for detecting effects. Our statistical power was too low to detect fish effects on longitudinal changes in periphyton C:P and N:P ratios despite trends suggesting these ratios increased in the presence of fish in high N:P streams.

Elemental imbalances between food resources and aquatic herbivores are governed by water column nutrient availability which can lead to P limitation of growth in herbivorous fish (Hood et al. 2005). Central stoneroller body P content (% DM) in our study was lower (1.4 vs. 2.2%) and body N:P was higher (18.8 vs. 9.4) than those reported for stonerollers by McManamay et al. (2011). These differences may be due to different fish preparation and tissue digestion methods between the two studies (i.e. we removed intestines and did not ash before digestion). However, results from the current study agreed with previous findings that cyprinid fishes have relatively low P content compared to other fish families (Sterner and George 2000, Hendrixson et al. 2007). Central stoneroller body P content was intermediate between benthic vertebrate grazers used in previous nutrient recycling studies (Knoll et al. 2009). In N-limited systems, nutrient-recycling by P-rich grazing fish (*Ancistrus triradiatus*, N:P = 4) increased algal C:P and N:P, while algae exposed to nutrients recycled by vertebrate grazers with low P content (*Rana palmipes,* N:P = 23) have lower N:P ratios (Vanni et al. 2002, Hood et al. 2005, Knoll et al. 2009). In contrast, fish that feed primarily on P-rich terrestrial inputs (e.g., insects) are not governed by water column nutrient availability but their influence on nutrient cycling in streams is still determined by background nutrient availability (Small et al. 2011). Based on periphyton stoichiometry within fish cages during our study, central stonerollers were likely P-limited in low and high N:P streams. Despite this limitation, we did not observe strong fish effects on longitudinal changes in N or P in our low N:P streams. However, when nutrients were unbalanced and there was an excess of N, streams with central stonerollers had significantly lower downstream periphyton C:N ratios and higher, although not significant, C:P and N:P ratios, evidence that fish

were recycling more N and potentially hoarding P. Our observations highlight the fact that while previous studies have demonstrated that stream fish excretion can meet benthic demand for limiting nutrients (Grimm 1988, Vanni et al. 2002, McIntyre et al. 2008, Small et al. 2011, Wilson and Xenopoulos 2011), P-rich consumers can also decouple the downstream availability of limiting and non-limiting nutrients when N is in excess and P is in short supply (Small et al. 2009).

Fish-Mediated Effects on Coupling of Algal-Bacterial Production

We linked effects of fish-mediated nutrient recycling to ecosystem function by comparing BBP and PS rates downstream of enclosures in low and high N:P streams. Areal BBP and PS were greater downstream of enclosures containing fish in high N:P streams but there were no significant differences in production rates between fish treatments in low N:P streams. These differences were driven by higher overall algal biomass, not biomass-specific production rates. Other studies have demonstrated that direct grazing effects of fish can increase biomass-specific production rates by increasing periphyton turnover associated with biomass removal or by removing light limitation associated with sediment deposition (Stewart 1987; Power 1990; Gelwick and Matthews 1992). Our observations support previous findings that grazing fish also influence benthic algal communities through indirect nutrient recycling pathways and can increase benthic algal biomass accrual when separated from direct grazing effects (Knoll et al. 2009). Algal biomass responds more strongly to increased N than P in our system (Scott et al. 2009). Reisinger et al. (2011) found that NH_4^+ amendments (simulation of fish excretion) increased community respiration in stream mesocosms but grazing and actual excretion by *C. amomalum* had no effect. Their study observed no differences in gross

primary production between control, streams with fish, or NH_4^+ enriched treatments. Results from the current study suggest that when combined with N enrichment, fish-mediated nutrient recycling increases the overall amount of autotrophic and heterotrophic production occurring on downstream benthic surfaces, demonstrating that N enrichment and grazing fish may interact to change stream ecosystem function.

There was strong congruence in patterns of algal and bacterial production rates across our treatments and higher $COV_{BBP\text{-}PS}$ downstream of fish enclosures in high N:P streams, indicating that fish-mediated differences in cycling of nutrients influences coupling of algae and bacterial microbial processes. Periphyton communities in our study system have been shown to be co-limited by both N and P with the potential for differential limitation among organisms occupying different trophic levels (Scott et al. 2009). Inorganic nutrients can influence bacteria biomass indirectly by increasing algal biomass, thereby increasing substrate for bacterial colonization (Rier and Stevenson 2001; Carr et al. 2005). Alternatively, particularly when organisms within the periphyton matrix exhibit differential nutrient limitation, linkages between algae and bacteria are also derived from the exchange of inorganic C and recycling of inorganic nutrients and micronutrients within the periphyton matrix (Haack and Mcfeters 1982; Espeland and Wetzel 2001; Francoeur and Wetzel 2003). Increased biomass and development of a thick polysaccharide matrix decreases the mass transfer of stream water nutrients into internal algal cells, potentially creating an isolated microenvironment where increased efficiency of C flow from autotrophs to heterotrophs and recycling of inorganic nutrients may occur (Rier and Stevenson 2002; Larned et al. 2004). Overall patterns among our treatments suggest that increased downstream algal biomass was associated with higher

periphyton N content and that algal-bacterial coupling responded positively to both increased biomass and N within the periphyton matrix. Bacterial-algal production may be tightly coupled when algal biomass is high, but relies on water column DOC when algae are limited by light or nutrients. In low-N streams, algae were presumably limited by nutrients and bacteria relied on water column DOC resulting in very low $COV_{BBP\text{-}PS}$. Despite having little influence on algal biomass or periphyton N content in two out of three cases, $COV_{BBP\text{-}PS}$ was highly variable and trended slightly higher in low-N streams with fish. High variability in $COV_{BBP\text{-}PS}$ within un-enriched streams was driven by one stream with fish having $COV_{BBP\text{-}PS}$, algal biomass, and periphyton C:N ratios, similar to enriched streams (Figure 18). We attempted to maintain even fish densities across streams during the study by checking for mortality daily and replacing individuals when needed. Despite this, some variation in fish density and total biomass among streams occurred at the end of our study, and the stream in question had the highest fish biomass among streams (Table 12). This finding suggests that our densities may have been too low in low N:P streams to influence longitudinal changes in periphyton nutrient content and highlights the fact that both consumer abundance and nutrient supply can influence the role of consumer-mediated nutrient recycling on periphyton structure and function (Hillebrand et al. 2008; Knoll et al. 2009; Small et al. 2009).

Table 12. Fish density and total biomass for low and high N:P streams with fish on day 42 of the study. Instream measure for rep. 3 for low N:P streams behaved similarly to high N:P streams with fish.

Stream	N:P	Density (indv. m^{-2})	Total wet mass (g m-2)
Rep. 1	11	15.0	60.9
Rep. 2	11	13.9	70.8
Rep.	11	13.9	80.1
Rep. 1	177	12.2	67.6
Rep. 2	177	15.0	63.3
Rep. 3	177	12.2	65.8

Higher primary production in response to enrichment increases nutrient demand, and can result in a shift from reliance on recycled nutrients to water column nutrients (Lyon and Ziegler 2009). In the current study, higher algal production was enhanced by fish in high N:P streams. High algal biomass was driven by development of macroalgae which requires substantial N to build chloroplasts (Sterner and Elser 2002). This was probably supplied by increased water column DIN from enrichment and fish recycling. Decoupling of algal-bacterial production can be the result of shifts in periphyton communities to attached macroalgae with high proportions of green algae. This shift can decrease bacteria cells reliance on EOC as green macroalgae are more palatable to grazers and potentially increase export of DOC from biofilms through increased herbivory (Ziegler and Lyon 2010). We did not see evidence for this process in the current study. $COV_{BBP\text{-}PS}$ increased with algal biomass associated with macroalgae growth, but grazer exclusion and low flow conditions in our stream mesocosms may have dampened the response observed by Ziegler and Lyon (2010). Observations of algal-bacterial coupling across nutrient enrichment gradients in natural streams suggest that coupling of bacterial-algal production is partially driven by internal recycling of limiting nutrients in oligotrophic systems, but rely more on water column nutrients in enriched systems (Scott et al. 2008; Lyon and Ziegler 2009). In oligotrophic systems, diurnal microbial processes capture and provide P to algal cells in the upper layers of periphyton for utilization during daylight photosynthesis (Espeland and Wetzel 2001; Sharma et al. 2005; Rier et al. 2007; Borovec et al. 2010). Scott et al. (2009) demonstrated that N enrichment increased alkaline phosphtase activity in periphyton communities within our study system, indicating that N enrichment resulted in P limitation. In the current study,

it is likely that higher N availability, P hoarding by fish, and high algal biomass contributed to increased P limitation of downstream periphyton which potentially increased APA activity and reliance on internal recycling of the limiting nutrient within the periphyton community.

Synthesis and Directions for Future Research

Our study was designed to test the role of grazing fish-mediated nutrient recycling on nutrient cycling in streams with low and high surface-water N:P and relate recycling effects to downstream microbial processes. Evidence from this study suggests that central stonerollers, a numerically abundant grazing minnow in midwestern North American streams, can play a significant role in stream nutrient cycles when surface-water limiting nutrients are unbalanced. While statistical significance may have been sacrificed in some cases due to the use of whole stream mesocosms as replicates, trends in our data support finding from simulation models that suggest that P-rich organisms decouple cycling of limiting and non-limiting nutrients when water column N is abundant compared to P (Small et al. 2009). However, in low N:P streams, one stream with higher fish biomass behaved similarly to high N:P streams with fish, supporting the notion that consumer biomass as well as abiotic factors such as temperature and stream flow influence consumer-nutrient recycling effects on stream nutrient dynamics (Vanni 2002; Hillebrand et al. 2008; Knoll et al. 2009). We conducted our experiment with high fish biomass and low flows to mimic summer conditions in Texas streams. A previous study has demonstrated that stonerollers do not constitute a major biogeochemical flux of N in prairie streams when biomass is low (Dodds et al. 2000). Additional experiments are

needed to establish the strength of relationships between grazing fish and nutrient cycles across the range of conditions and fish biomass experienced in natural streams.

We also found evidence that nitrogen enrichment effects on downstream microbial processes were enhanced by differential recycling of limiting and non-liming nutrients by central stonerollers. Patterns in our dataset support the notion that stream periphyton can be limited by both N and P (Francoeur 2001; Scott et al. 2009). Algal biomass was enhanced by increased turnover of N through fish-mediated nutrient recycling in high N:P streams. In turn, increased algal biomass combined with differential recycling of N and P by fish likely resulted in higher P limitation of downstream periphyton communities in high N:P streams with fish. Our results suggest that P requirements in this situation were potentially met by increased internal P recycling and coupling of algal-bacterial production, evidence that fish-mediated recycling can influence nutrient cycles at both benthic microbial and whole stream scales.

These results contribute new insights to a large body of literature describing functional roles of grazing fish in stream ecosystems and provide a framework for evaluating changes in grazing fish assemblages. For example, South American armored catfish (Loricariidae) have been introduced to North American streams historically occupied by central stonerollers and other native grazing fish (Lopez-Fernandez and Winemiller 2005; Pound et al. 2011). Growth of armored catfish is P-limited due to high body P content, thus these fishes have the potential to significantly alter patterns of P limitation in invaded habitats (Hood et al. 2005). More studies that investigate the role of native and invasive fish species on stream nutrient cycles are needed to understand the

consequences of changes in grazing fish assemblages and altered nutrient regimes on ecosystem function.

CHAPTER FIVE

Summary and Conclusions

Synthesis

Results from the field study conducted as part of this dissertation present multiple lines of evidence that components of both primary producer and consumer assemblage structure change abruptly at low levels of P (TP ~ 20 μg L^{-1}) and N (TN no greater than 550 μg L^{-1}) in our study region. The experimental component of this dissertation also demonstrated that grazing by central stonerollers (*Campostoma anomalum*) maintained a higher proportion of algae in the periphyton matrix and as a result, the response of periphyton elemental composition to P enrichment was stronger on grazed substrates. Evidence from a second study suggests that grazing fish can also play a significant role in stream nutrient cycles when limiting nutrients are unbalanced due to N enrichment and that enrichment effects on downstream microbial processes are enhanced by differential recycling of limiting and non-liming nutrients by central stonerollers.

Findings from Chapter Two support the prediction that periphyton elemental and algae species composition respond abruptly to low levels of nutrient enrichment and proliferation of weedy algal species is likely when surface-water concentration exceed thresholds observed in this study. Despite the prediction that fish assemblages are less likely to respond to changes in habitat conditions associated with nutrient enrichment due to tolerances to highly variable natural conditions associated with prairie streams, results present good evidence that at least two specialist fishes, orangethroat darters (*Etheostoma*

spectabile) and central stonerollers, decline in response to low levels of nutrient enrichment and its sources. Additionally, abundance of tolerant native and invasive fishes are likely to increase when surface-water concentrations exceed observed in-stream thresholds. Results from Chapter 2 provide a weight of evidence that suggests a numerical criterion for surface-water TP of approximately 20 µg L^{-1} and TN no greater than 550 µg L^{-1} would be needed to protect natural primary producer and consumer assemblages within our study region. These findings should be of concern to water quality regulators in the region as current state laboratory protocols do not calibrate analytical equipment low enough to detect these changes in response to TP (Figure 20).

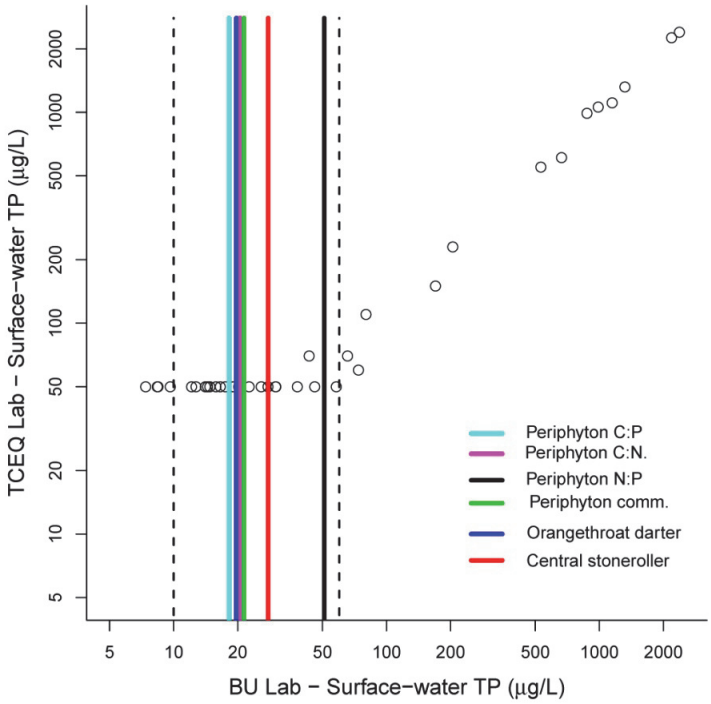

Figure 20. Relationship between surface-water TP analyses conducted by Baylor University (BU) and Texas Commission on Environmental Quality (TCEQ) labs for water samples from each sampling event. Solid vertical lines represent TITAN thresholds identified for declining portion of the periphyton assemblage, *Etheostoma spectabile* and *Campostoma anomalum*. Broken vertical lines represent the lowest and highest 5% and 95% confidence intervals identified between the three thresholds among 1000 bootstrap replicates.

Data from Chapter Two provides evidence that central stoneroller abundance declines in response to low levels of pasture landuse and P enrichment in natural streams. Chapter Three experimentally investigated the influence of interactions between this functionally important fish and phosphorus enrichment on benthic resources in stream mesocosms. The central finding of this study was that grazing by central stonerollers maintains a higher proportion of algae in the periphyton matrix and as a result, the response of periphyton stoichiometry to experimental P enrichment is stronger on grazed substrates. These interactions influenced periphyton nutrient ratios. C:P ratios were not influenced by central stonerollers in control or low P streams, but ratios were lower on grazed surfaces in high P streams. Periphyton C:N ratios were lower and only responded to P enrichment on grazed surfaces. Positive grazer effects were observed on periphyton N:P ratios in low P streams but diminished with P enrichment. These results contribute to the growing body of literature that suggest organisms can modify resource quality under limiting abiotic conditions (nutrients, sediments) but also present new evidence that P enrichment may alter these interactions in benthic habitats.

Nitrogen and P enrichment gradients were correlated across the field sampling sites utilized in Chapter Two and one interesting finding from that study was that sites with high N enrichment but TP below observed thresholds had periphyton and fish assemblages characteristic of low P streams. The experiment described in Chapter Four was designed to test the role of grazing fish-mediated nutrient recycling on nutrient cycling in this situation. Results from Chapter Four support predictions from simulation models that suggest that P-rich organisms decouple cycling of limiting and non-limiting nutrients when water column N is abundant compared to P (Small et al. 2009). Further,

differential recycling of limiting and non-liming nutrients by central stonerollers enhanced the influence of N enrichment on downstream algal biomass and associated microbial processes. Algal biomass was enhanced by increased turnover of N through fish-mediated nutrient recycling in high N:P streams. In turn, increased algal biomass combined with differential recycling of N and P by fish likely resulted in higher P limitation of downstream periphyton communities in high N:P streams. Our results suggest that P requirements in this situation were potentially met by increased internal P recycling and coupling of algal-bacterial production. These results suggest that fish-mediated recycling can influence nutrient cycles at both benthic microbial and whole stream scales.

Future Directions

The three studies encompassing this dissertation presents evidence that nutrient enrichment, particularly in terms of P availability, influences species composition of primary producers and consumers (fish), as well as elemental composition of periphyton, and interactions between grazing fish and periphyton structure and function. Despite garnering new insights into the influence of nutrient enrichment on stream ecosystems, results from these observational and experimental studies also highlight several important unanswered questions. At the landscape scale, two important questions emerge from the results of the field study. First, overall patterns in the data indicate that negative effects of P enrichment on the abundance of benthic fish species may, in certain streams, be mitigated by enhanced stream flows associated with high levels of permitted WWTP discharges. Future experimental studies that examine fish response to P enrichment under different flow conditions are needed to better understand this potential interaction.

Secondly, source-sink dynamics between effluent-dominated streams and their tributaries are not well understood, and could also explain the presence of declining benthic fish in streams with high WWTP discharge. Future field studies should be designed to explicitly examine the distribution of stream habitats in relation to WWTP effluent discharge and movement of fish between these different environments.

Results from the first mesocosm study indicated that sediment removal from benthic substrates was a key ecosystem process performed by central stonerollers. The study was not explicitly designed to test how interactions between light limitation and P limitation influence periphyton nutrient content, but grazing fish presumably increased light: nutrient supply ratios on grazed substrates by removal of sediments early in the study. Higher photosynthesis rates due to increased light and/or high turnover rates associated with grazing potentially induced P-limitation at concentrations below 20 μg L^{-1} PO_4-P which would explain the lack of response of periphyton C:P ratios to grazing in control and low P streams. Further studies that directly measure C fixation (^{14}C-HCO_3^- uptake), P and N uptake, and separate indirect (sediment removal) and direct effects of grazing fish are needed to test a grazer-induced, sediment-driven, light: nutrient hypothesis (Sterner et al. 1997). The second experiment was conducted with high fish biomass and low flows to mimic summer conditions in Texas streams. A previous study has demonstrated that stonerollers do not constitute a major biogeochemical flux of N in prairie streams when biomass is low (Dodds et al. 2000). Consumer biomass, as well as abiotic factors such as temperature and stream flow influence consumer-nutrient recycling effects on stream nutrient dynamics. Additional experiments are needed to

establish the strength of relationships between grazing fish and nutrient cycles across the range of conditions and fish biomass experienced in natural streams.

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