

CHANGES IN COMMUNITY STRUCTURE AND ECOSYSTEM PROCESSES IN
RESPONSE TO ARMORED CATFISH (SILURIFORMES: LORICARIIDAE)
INVASION

A Dissertation

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by

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CHANGES IN COMMUNITY STRUCTURE AND ECOSYSTEM PROCESSES IN
RESPONSE TO ARMORED CATFISH (SILURIFORMES: LORICARIIDAE)
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Krista Arminty Capps Ph. D.

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Species invasions provide an excellent opportunity to ask important ecological questions and test the effects of individual species on ecosystem structure and function. In both terrestrial and aquatic ecosystems, non-native grazing organisms alter the quality and quantity of food resources and affect nutrient storage and cycling. Low-trophic position species, such as herbivorous and detritivorous fishes, have been introduced and become established in aquatic ecosystems throughout the globe and these species can fundamentally change community structure and ecosystem processes. In Chiapas, Mexico I quantified the effects of non-native armored catfishes (Siluriformes: Loricariidae) on ecosystem structure and function. Loricariids are grazing fishes that have been introduced to many freshwater ecosystems outside of their native range due to their popularity in the aquarium trade. The purposes of this study were threefold. Initially, I examined the effects of loricariid grazing on the quality and quantity of basal food resources. Secondly, I investigated the top-down and bottom-up pathways by which loricariids affect algal biomass and gross primary productivity by quantifying the net effects of grazing and nutrient remineralization. Lastly, I examined whether loricariids are important drivers of nutrient dynamics and estimated whether they function as a source or sink of nutrients in invaded stream

reaches.

The results from this study indicate grazing by loricariids reduces the quality and quantity of benthic resources and this negatively influences higher trophic levels. Additionally, intensive grazing by high-densities of loricariids results in a negative net-effect on algal biomass and primary productivity in the Chacamax River. At a larger spatial scale, my findings suggest loricariids dramatically increase the amount of nutrients stored in fish tissue and the rate at which nutrients are remineralized via fish excretion, thereby converting the river to a system where fishes are primary drivers of nutrient dynamics. This study also demonstrated invasive organisms can simultaneously function as sources and sinks of nutrients and these effects are element-dependent. Finally, this investigation highlights the importance of quantifying the consumptive and remineralization effects of invaders to develop a comprehensive understanding of how non-native organisms influence ecosystem structure and function in invaded ecosystems.

BIOGRAPHICAL SKETCH

Krista Capps née Brewer was born in San Jose, California where she spent most of her free time playing outside with her siblings, Cameron and Arianna, and her many pets. Krista's love for animals and the outdoors was supported by her parents and these loves developed into a desire to study ecology and environmental science. In high school, Krista participated in a summer science program in which she investigated pollution and lead poisoning in a small lake. This activity piqued her interest in learning how anthropogenic activities affected the structure and function of aquatic ecosystems.

As an undergraduate student at Hope College, Krista studied biology and political science and she continued to pursue her interests in freshwater ecosystems. She participated in research and classroom activities and completed internships focused on streams and rivers. In addition, Krista took classes in comparative cultures, world religions, and Latin American studies, all of which exposed her to the challenge of managing natural resources in the developing world. During Krista's sophomore year, she traveled to India for six weeks to participate in a religion course. While visiting the Ganges River, Krista experienced the conflict between the use of water resources and the dependence of the human population on clean sources of drinking water common in many developing countries.

In chemistry class during her sophomore year, Krista met Daniel Capps. Although this meeting directly led to a lower-than-expected chemistry grade, it was her most treasured experience in college. In 1999, Krista graduated early from college

to thru-hike the Appalachian Trail with Dan. Together, they walked 3,510 km from Georgia to Maine. Miraculously, at the end of the trip, they were still friends. Krista and Dan were married on July 15, 2000 and had their wedding reception in Tropic World Africa at the Brookfield Zoo in Chicago. After the wedding, they moved to Bloomington, Indiana to pursue graduate studies.

In Bloomington, Krista received a master's degree in environmental science from Indiana University. She studied applied ecology in aquatic systems and received a research fellowship at a wetlands sanctuary on the Patuxent River. In addition to academic experiences, Krista's interests in sustainable development also shaped her desire to study aquatic ecology in Latin America. After her master's degree, she joined the Peace Corps in Honduras. As a volunteer, Krista developed projects focused on both public health and the environment. She participated in the development and construction of water systems. Death from waterborne illnesses is common in Honduras and many of these deaths were linked to environmental disturbance and pollution in watersheds. To promote watershed restoration, Krista integrated her knowledge of stream ecology and five years of teaching experience, to create an education curriculum for schools and community members in Honduras.

Experience in tropical ecosystems and conservation efforts in both Honduras and India also helped Krista appreciate the complexities that surround natural resource management in the developing world. Furthermore, the time abroad revealed the scarcity of baseline data available for these natural systems, and the lack of a basic ecological understanding of many of the relationships within tropical environments. Krista's Peace Corps service exposed her to the multitude of pressures being placed on

aquatic resources in Latin America, and reinforced her desire to pursue graduate studies in tropical aquatic ecosystems.

Krista began her dissertation studies at Cornell University in 2005 in the lab of Dr. Alexander Flecker. Her quest for a dissertation topic led her to field sites in Venezuela, Peru, Ecuador, and the Adirondacks (New York, U.S.A) before she found her field site in Mexico. In 2007, Krista viewed a YouTube video of a river invaded by armored catfish in Chiapas, Mexico. Six months later, she was collecting data at the invasion site featured in the video and beginning her self-proclaimed *lucha contra el pez diablo*. Krista continues to be inspired by the beauty of tropical aquatic ecosystems and the Mexican people and their culture. She is also excited to study the ecology of temperate ecosystems and to work in coupled human and natural systems to tackle issues in conservation and sustainable natural resource management in a changing world. Krista will begin addressing these questions as a postdoctoral fellow with the Sustainability Solutions Initiative at the University of Maine. In the future, Krista hopes to invest a lot of time and effort in developing international educational experiences for students and to continue studying the ecology and management of aquatic ecosystems in Latin America.

This work is dedicated to:

my parents, Dwaine and Arminty Brewer, who began actively supporting my love for animals at the age of 4 and who continue to support me by swimming with the fishes in

Mexico;

my husband, Dan, who has been my best friend, travel companion, and field assistant

for the last 14 years;

and the watersheds and people of Latin America who continue to intrigue and inspire me to be a better scientist and a better person.

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I am also very grateful to my committee members, Nelson Hairston, Christy Goodale, and Stuart Findlay for their counsel. Nelson's support to study tropical ecology in Hawaii allowed me to delve into the brackish-water world of the Opae'ula. I had the pleasure of taking classes from and teaching classes with Christy. Her support and advice were instrumental in being a successful student and educator of ecosystem processes. Stuart Findlay's calm demeanor and wonderful outlook on life helped me through many challenges in the lab and in the field. Moreover, Stuart's input enhanced the quality of many of my grant proposals and manuscript submissions. I also benefitted from Stuart's connection to the Carey Institute of Ecosystem Studies. Denise Schmidt and Milada Vomela of the Carey Institute's analytical lab have helped me analyze hundreds of water chemistry samples; I am so grateful for their help.

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CHAPTER 1

HIGH IMPACT OF LOW-TROPHIC POSITION INVADERS: THE EFFECTS OF NON-NATIVE GRAZING FISHES ON THE QUANTITY AND QUALITY OF FOOD RESOURCES

ABSTRACT

Low-trophic position animals, such as herbivorous and detritivorous fishes, mollusks and crustaceans, have been introduced and have become established in aquatic ecosystems throughout the globe. These species can fundamentally change community structure and ecosystem processes in invaded freshwater ecosystems. Armored catfishes (Siluriformes: Loricariidae) are grazing fishes that have been introduced to many freshwater ecosystems outside of their native range due to their popularity in the aquarium trade. High population densities of non-native loricariids have been linked to the decline of freshwater fisheries in invaded habitats, but the mechanisms driving declines are poorly understood. I coupled the results of two loricariid enclosure experiments with a comparison of invaded and uninvaded sites to measure the effects of loricariids (*Pterygoplichthys* spp.) on the quantity and quality of food resources, macroinvertebrate community structure, and primary productivity in an invaded stream ecosystem in Chiapas, Mexico. Grazing by loricariids reduced the standing stock of benthic organic matter and algal biomass in both experimental manipulations and in the site comparison. In both the experimental manipulations and the site comparison, grazing by loricariids significantly reduced the total amount of phosphorus stored in benthic organic matter. In the experimental manipulations, grazing by loricariids reduced the carbon to nitrogen ratio of benthic organic matter and increased its carbon to phosphorus and nitrogen to phosphorus ratios. Loricariid grazing stimulated chlorophyll a-specific gross primary productivity. Conversely, loricariid grazing depressed the abundance of macroinvertebrates and altered macroinvertebrate community composition. Chironomid larvae were more common in

Pterygoplichthys enclosure treatments in the experiment; however, this pattern was not evident in the site survey. Results from this study indicate that high densities of low-trophic position invaders, such as armored catfish, can significantly change both the quality and the quantity of food resources in invaded systems and these effects can be transmitted upwards in the food web to the invertebrate consumers. In particular, my findings indicate that the invasion of a P-rich grazer in a P-limited system has the potential to alter nutrient dynamics in novel systems.

INTRODUCTION

Species invasions provide unique opportunities to ask fundamental ecological questions and test the impacts of individual species on ecosystem function (Townsend 1996, Hall et al. 2003, Strayer 2006). Recent studies have demonstrated that invasive species can alter energy pathways (Hall et al. 2006, Anderson and Rosemond 2007, Strecker and Arnott 2008), shift patterns in nutrient recycling (Mack and D'Antonio 2003, Drenovsky and Batten 2007), and change labile pools of nutrients available for native organisms (Rimer and Evans 2006, Gomez-Aparicio and Canham 2008). However, the effects of invasive species on ecosystem processes may be idiosyncratic and depend on the functional group of the invasive species and the chemical, physical and biological characteristics of the system (Schutzenhofer and Valone 2006, Shelley et al. 2008). In both terrestrial and aquatic environments, studies have demonstrated that exotic grazing organisms can alter ecosystem function by changing nutrient cycling rates and primary productivity (Dukes and Mooney 2004, Hall et al. 2006).

In stream ecosystems, grazers can have direct, top-down effects through consumption. Additionally, they can indirectly influence ecosystem function by

consuming senescent cells and sediment from benthic substrates, increase the amount of light and nutrients available to primary producers (Power 1990, Flecker 1992, 1997) and potentially influencing nutrient dynamics (Knoll et al. 2009). In their native habitats, some grazing fishes have been shown to influence primary productivity (Power 1983, 1984, Gelwick and Matthews 1992, Abe et al. 2006). Additionally, fish grazing can change macroinvertebrate species abundance and richness (Flecker 1992, Flecker and Taylor 2004), primary productivity (Power 1990), ecosystem metabolism (Taylor et al. 2006), habitat heterogeneity (Flecker and Taylor 2004), epilithon composition and accrual (Flecker 1996), and nutrient remineralization (McIntyre et al. 2006, McIntyre et al. 2008, Knoll et al. 2009). These findings suggest that high densities of introduced grazers may have profound effects on community and ecosystem ecology in novel environments, especially in systems.

Armored catfishes are bottom-dwelling fishes native to Central and South America (Weber 1991, Nico and Martin 2001). Although Loricariidae (Order: Siluriformes) is one of the most diverse freshwater fish families, relatively little is understood about the ecology of many species in this family (Nelson 2002, Nonogaki et al. 2007). These fishes, known as “plecos” in the aquarium trade, are frequently released into freshwater bodies (Arthington et al. 1999, Fuller et al. 1999, Bomford and Glover 2004). Non-native populations of loricariids have been documented in several states within the United States, in the Caribbean and Pacific Islands, Australia, Europe, the Middle East, Southeast Asia, and Mexico (Courtenay et al. 1986, Fuller et al. 1999, Nico and Martin 2001, Alecke et al. 2005, Liang et al. 2005, Chavez et al. 2006, Kailola 2007, Ozdilek 2007, Hossain et al. 2008, Keszka et al. 2008, Sinha et al.

2010). Loricariids are covered in a dense, bony-plated armor. Because phosphorus (P) is an essential component of bone (Roy et al. 2002), loricariids are P-rich relative to many other fish families (Hood et al. 2005).

In Mexico, the most commonly introduced genus of loricariid is *Pterygoplichthys*. Populations of *Pterygoplichthys* have been documented in the Balsas and Mezcala Rivers in Michoacán and the Amucuzac River in Morelos (Mendoza et al. 2007). They are also established in several sites along the Usumacinta River in the states of Campeche, Tabasco, and Chiapas. In invaded habitats, *Pterygoplichthys* attain high population densities (Mendoza et al. 2009) and they are thought to compete with native fishes for basal food resources and space (Devick 1989, Hoover et al. 2004). *Pterygoplichthys* are epibenthic feeders and are classified as detritivores (German et al. 2010), characterized by a ventral sucking mouth which they use to graze hard substrates in benthic habitats (Nico and Martin 2001, Armbruster and Page 2006, German and Bittong 2009, German and Miles 2010, German et al. 2010). They consume the epilithic algal complex, a mixture of cyanobacteria, algae, fungi, bacteria, and detritus that accumulates on substrates in aquatic environments (German et al. 2010) that I refer to as “epilithon” for the remainder of this paper.

The purpose of this study was to examine the direct effects of a low-trophic position fish on the quantity and quality of basal food resources in invaded benthic habitats. I used *in situ* experimental manipulations and comparisons of high- and low-density invasion sites to study the effects of non-native *Pterygoplichthys* in the Chacamax River in Chiapas, Mexico. Myriad investigations of grazers, including

studies of armored catfish in their native range (e.g., Power 1990) have demonstrated that grazing reduces the quantity of primary producers. Therefore, I expected loricariid grazing would depress epilithon resources in invaded rivers. Conversely, grazer effects on epilithon stoichiometry are not as well documented (but see, Hillebrand et al. 2004, Evans-White and Lamberti 2005, Liess and Hillebrand 2006, Liess and Haglund 2007, Hillebrand et al. 2008). I posited that P-rich *Pterygoplichthys* would reduce the P composition and the total amount of P stored in epilithon relative to ungrazed sites. I also anticipated that loricariid grazing would depress macroinvertebrate populations that depend upon epilithon resources for food and habitat. Finally, I predicted that grazing would stimulate gross primary productivity (GPP) by removing sediment and senescent cells from growing algal biofilms and increase their exposure to ambient nutrients.

METHODS

Study Site

This study was conducted in two invaded reaches (high-density vs. low-density) of the Chacamax River, a tributary of the Usumacinta River in Chiapas, Mexico between the dry-season months of March and May in 2009 and 2010 (Appendix 1). During these months, discharge in the focal reaches was reduced to an average of approximately $1,600 \text{ L s}^{-1}$ and the stream water was generally transparent with an average depth of approximately 60 cm. However, discharge during the dry season fluctuated with precipitation and caused the depth of the river to increase up to 6 m in a 24-hour period. During periods of high discharge, turbidity increased with large sediment loads. Ambient nutrient concentrations in both of the study reaches

were typically moderate to low (average values: $\text{NH}_4^+\text{-N}$, $10 \mu\text{g L}^{-1}$; $\text{NO}_3^-\text{-N}$, $353 \mu\text{g L}^{-1}$; soluble reactive phosphorus, $< 2 \mu\text{g L}^{-1}$; total dissolved nitrogen, $387 \mu\text{g L}^{-1}$; total dissolved phosphorus, $3 \mu\text{g L}^{-1}$) and water temperature ranged from 21 to 28 °C during the study periods. Data collected from nutrient diffusing substrates (using methods outlined by Tank et al. (2006)) indicated that algal growth was limited by P availability in the Chacamax River during the study period (Appendix 2). Two species of *Pterygoplichthys*, *Pterygoplichthys pardalis* (Castelnau, 1855) and *Pterygoplichthys disjunctivis* (Weber, 1991), were first documented in the Chacamax River between 2004-2005 (Wakida-Kusunoki et al. 2007). However, many fishes did not adhere to type specimens (*personal communication*, Nathan Lujan); thus, I refer to these fishes as *Pterygoplichthys* for the remainder of this paper (Appendix 4).

Experimental and Site Comparison Design

To study the effects of *Pterygoplichthys* on benthic habitats and communities in the Chacamax River, *in situ* experimental manipulations were coupled with a survey comparing the epilithon collected from rocks downstream and upstream of a loricariid invasion front. *Pterygoplichthys* exclusion experiments were conducted in a 150 m reach (N17°29'047" W91°58'430"), with high densities of armored catfish (density (mean \pm SD): 2 ± 3 *Pterygoplichthys* m^{-2} ; biomass (mean \pm SD): 225 ± 45 g *Pterygoplichthys* m^{-2}). The survey compared benthic environments in a high-density invasion site (N17°29'047" W91°58'430") and a low-density invasion site (N17°28'226" W91°58'444") in the Chacamax River in 2010 that were separated by approximately 1.9 km of river.

Experimental Exclosure Design-The first exclusion experiment (Experiment 1) was conducted between March and April 2009 and was run for a total of 28 days, with substrate samples collected every 7 days. The experiment had four randomized complete blocks of four treatments: a *Pterygoplichthys* exclosure, a *Pterygoplichthys* enclosure (5-6 fish; $\sim 250\text{-}350\text{ g } Pterygoplichthys\text{ m}^{-2}$), a cage control (three walls and a floor to control for cage effects on sedimentation), and a stream reference (cobble placed on the stream bed without a cage). All treatments were $1.5\text{ m} \times 1.5\text{ m} \times 1.0\text{ m}$ in dimension and were constructed using poultry wire ($\sim 2.5\text{ cm}$ diameter) stabilized with rebar and cable ties (Fig. 1.1A). Each block was established in a run habitat and the bottom of each cage was lined with cobbles to standardize the substrates across treatments and blocks. Cages were built with mesh floors to prevent loricariids from burrowing in or out of the treatments. Debris was removed from the exterior of the cages twice daily for the duration of the experiment. The mesh size prevented loricariids and other large-bodied fishes from entering or escaping the enclosure and exclosure, but permitted small individuals of most native grazing species (fishes, insects, snails, and tadpoles) to access the benthic environments in each treatment. On each sample date, three rocks were randomly selected from each treatment within a block and epilithon was collected using wire brushes. Briefly, the wire brushes were used to collect all of the sediment and detritus from the top of the rocks. Aliquots of the resulting slurry were taken for chlorophyll a (5 mL), ash-free dry mass (AFDM) (10 mL), and dry mass (10 mL) and filtered onto a pre-ashed and pre-weighed Gelman A/E filter (Gelman, Ann Arbor, Michigan). The remaining slurry was frozen for C, N, and P stoichiometric analysis. Digital photos were taken of each rock with

scale bars present, and rock areas were determined using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, California). Chlorophyll a content was estimated by filtering a 5 mL subsample of the slurry and immediately placing the filter in a film canister filled with 20 mL of buffered 90% ethanol. The filters were incubated in the dark for 16h to extract chlorophyll a. Chlorophyll a samples were measured using fluorometry (AquaFluor™; Turner Designs, Sunnyvale, CA). Flooding events on day 14 of the experiment prevented the collection of rocks from blocks three and four on that sample date.

Samples for elemental analysis were dried in a convection oven to a constant mass at 45°C. For carbon (C) and nitrogen (N), ~1 to 2 mg of dried material was analyzed using an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Methionine (Costech, Valencia, California) and sulfanilic acid (Merk, Whitehouse Station, New Jersey) were used as standards. For particulate P analysis, subsamples of ~1 mg of material were combusted at 500°C, digested with 1 N HCl for 2h, and the digested solution was analyzed spectrophotometrically (Shimadzu UV 1240; Shimadzu Scientific Instruments, Columbia, Maryland) with the molybdate-blue method (Murphy and Riley 1962). Spinach was used (1570a; National Institute of Standards and Technology, Gaithersburg, Maryland) as the internal standard and 25 mg/L PO_4^{3-} solution (Ricca Company, Arlington, Texas) was used as the aqueous standard for the analysis. The total amounts of C, N, and P were estimated by multiplying area-specific dry mass by the concentration of nutrients found in the epilithon samples.

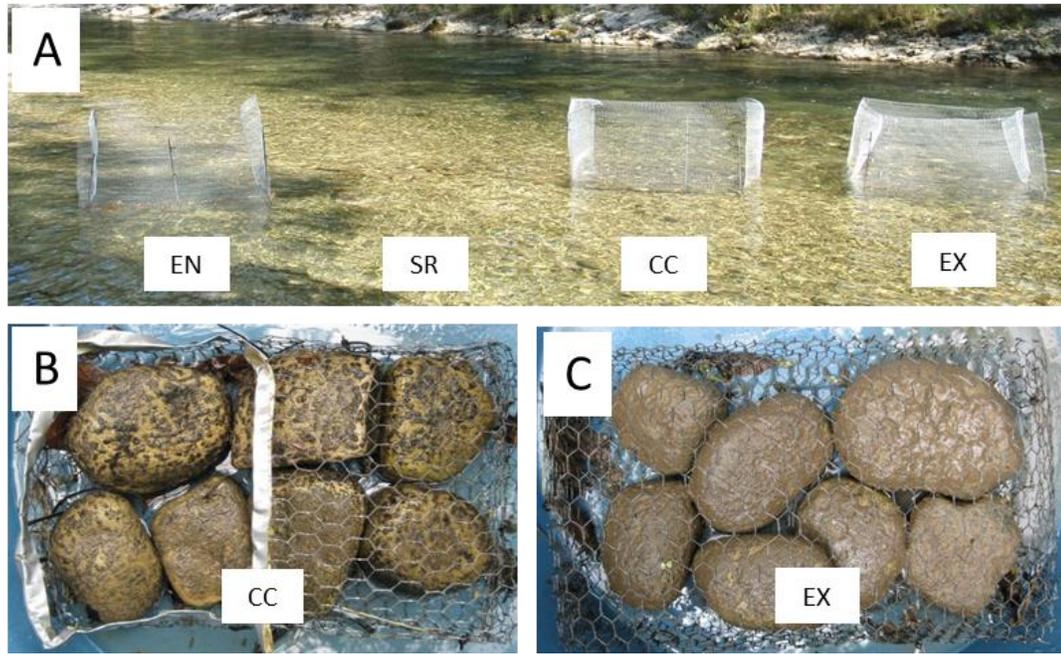


Figure 1.1. Cage design for *Pterygoplichthys* Exclosure Experiments 1 and 2. (A) Block 2 from Experiment 1. From left to right: *Pterygoplichthys* enclosure (EN), stream reference (SR), cage control (CC), and *Pterygoplichthys* exclosure (EX). (B) Cage control treatment from Experiment 2. (C) A *Pterygoplichthys* exclosure from Experiment 2.

Experiment 2 was run for a total of 30 days between April and May 2010. Small cages (dimensions: 24 cm × 48 cm × 10 cm, 2.5cm poultry wire; Fig. 1.1B-C) were completely submerged and placed in five randomized complete blocks of three grazing treatments: a cage control that permitted loricariid grazing, a *Pterygoplichthys* enclosure, and a stream reference. Experimental blocks were placed in run habitats and each of the five blocks consisted of four replicates of each of the four treatments (4 replicates × 3 treatments × 5 blocks). Flooding occurred between sample days 10 and 15 and between days 15 and 30; however, no cages were lost. Debris was removed from cages twice daily for the duration of the experiment. On four sampling dates (5, 10, 15, and 30), a single replicate of each of the treatments (an entire cage) was randomly selected and destructively sampled by pulling the cage into a Surber sampler (0.092 m², 250µm mesh size) (Wildlife Supply Company, Yulee, FL) that was placed downstream of the cage. I collected epilithon samples from two rocks from each treatment in each block on each sample day using the methods previously described. I also collected macroinvertebrates from one replicate of each treatment in each block on days 15 and 30 of the experiment. Macroinvertebrates were hand-picked from the rocks and from the Surber sampler and preserved in 95% ethanol. Subsequently, macroinvertebrates were transported to the USA and identified to family or order using Merritt and Cummings (2008).

Gross primary productivity was estimated from the epilithon of one rock from each of the four treatments in each of the five blocks on the final day (day 30) of Experiment 2. Gross primary production was estimated using a closed-chamber, non-circulating method (Bott et al. 1978, Hill et al. 1997). Briefly, a single rock was placed

in a 4.7 L clear, air-tight chamber filled with stream water. Dissolved oxygen (DO) content of the water was measured (mg L^{-1}) at time 0 using an YSI 85 Handheld Dissolved Oxygen/Conductivity Instrument (YSI, Yellow Springs, OH). The container was sealed and incubated in the stream to maintain stream temperatures for 60-90 minutes and then DO was measured again to estimate areal net ecosystem productivity (NEP). To measure community respiration (CR), the rock was removed from the clear chamber and incubated in a stream-water filled, black chamber that prevented light penetration for the same time period. Rocks were photographed with a digital camera and rock areas were determined using Adobe Photoshop CS3. Area-specific GPP ($\text{mg oxygen m}^{-2} \text{ hr}^{-1}$) was estimated by adding NEP and R (Bott 2007), and chlorophyll-specific GPP was estimated by dividing area-specific GPP by the amount of chlorophyll a (mg m^{-2}) (Kolmakov et al. 2008).

Natural Stream Comparison- A site comparison was conducted of the benthic environments of three run habitats downstream and three run habitats upstream of a loriciid invasion front. The low-density invasion site (~ 0.00025 *Pterygoplichthys* m^{-2}), sampled on 29 April 2010, was approximately 1.9 km upstream on the Chacamax River from the high-density site (~ 2 *Pterygoplichthys* m^{-2}) sampled 5 May 2010; there were no barriers between the sites that would prevent *Pterygoplichthys* from migrating upstream. Therefore, it is unclear what is limiting the movement of the fish upstream. Epilithon samples were collected and analyzed and GPP was estimated from four rocks in each run habitat using the aforementioned methods. Three sub-samples of macroinvertebrates were collected, preserved, and identified from each of the run habitats. There were no rain events between the two sample dates.

Statistical Analysis- Experiment 1 was analyzed as a repeated measures model with a randomized complete block design where blocks represented different sections of the stream reach. Day was modeled as a repeated effect with an autoregressive 1 covariance matrix to account for repeated measurements of each treatment within each block. Experiment 2 was analyzed as a mixed effects model using a randomized complete block design, where blocks represented different sections of the stream reach. Treatment and day were considered fixed effects and block was considered a random effect. Epilithon data were analyzed using the PROC MIXED procedure of SAS 9.2 statistical software (SAS Institute, 2009). The epilithon response variables tested included: chlorophyll a, dry mass, AFDM, %C, %N, %P (of dry mass), total C, total N, total P, C:N, C:P, and N:P. Percent data were arcsine square-root transformed and all other responses were $\log_{10}(x)$ transformed to fit the distribution assumptions of the model. Rocks were considered subsamples of the replicate cages, thus the mean values for all of the rocks harvested from each cage were used as a single datum per cage for the statistical analysis.

Macroinvertebrate data were $\log_{10}(x+1)$ transformed. Treatment and day were considered fixed effects and block was considered a random effect to account for the interdependence of observations within the same block. In this experiment, GPP, CR, and the total number of: invertebrates, Ephemeroptera, Trichoptera, Plecoptera (EPT), Leptophlebiidae, Leptoxyphidae, Bateidae, Odonata, Elmidae, and Chironomidae were measured. All possible interaction effects were tested and then removed from the model if they were not significant. Tukey's post-hoc tests were used to identify differences among treatment combinations for each response variable in both

experiments. Gross primary productivity was analyzed using a mixed model where treatment was a fixed effect and block was considered a random-effect. Tukey's post-hoc tests were used to identify differences among the treatments. The response variables that were tested from the natural site comparisons were the same as those listed for Experiment 2. They were analyzed using a mixed effects model where subsamples were blocked by transect which was considered a random effect and site (low- or high-density of *Pterygoplichthys*) was the fixed effect. The models were run using JMP 9 statistical software (SAS Institute, 2010).

RESULTS

Caging Experiments

In both experiments, grazing by loricariids significantly altered benthic environments by modifying the algal and nutrient composition of the epilithon. As expected, loricariid grazing reduced chlorophyll a (~30-50%) and epilithon dry mass (~40-50%) in both experiments (Tables 1.1-1.2, Fig. 1.2A-B, Fig. 1.3A-B). Grazing also significantly reduced total AFDM in Experiment 2 (Table 1.2, Fig. 1.3C), but this pattern was not evident in Experiment 1 (Table 1.1, Fig. 1.2C).

Loricariid grazing changed epilithon nutrient composition relative to ungrazed treatments in both experiments. Grazing by loricariids significantly increased epilithon %C and %N in both experiments (Tables 1.1-1.2; Figs. 1.2D-E, 1.3D-E) and decreased %P in Experiment 2 (Table 1.2; Fig. 1.3F). Total C and N were not significantly affected by loricariid grazing in Experiment 1, though there was a trend of decreased carbon stock in the loricariid enclosures (Table 1.1; Fig. 1.2G-H). Conversely, in Experiment 2, both total C and N decreased in the presence of grazing

loricariids (Table 1.2; Fig. 1.3G-H). In both experiments, total P significantly decreased with loricariid grazing (Tables 1.1-1.2; Fig. 1.2I, 1.3I). Finally, grazing by loricariids altered the ratios of nutrients found in epilithon in both experiments. Loricariid grazing significantly decreased C:N and significantly increased C:P and N:P of the epilithon relative to the *Pterygoplichthys* enclosure treatments (Tables 1.1-1.2; Figs. 1.2J-L, 1.3J-L).

There were significant day and day×treatment effects in several response variables in Experiment 1 and Experiment 2 (Tables 1.1-1.2). These effects were likely driven by flooding. For example, a rain event on sample day 14 of Experiment 1 appeared to be responsible for the reduced epilithon dry mass found on rocks in many of the treatments relative to the other sample dates (Figure 1.4A). This, in turn, may have affected many of the other response variables that we tested. Similarly, in Experiment 2, multiple flooding events between dates 15 and 30 may have been responsible for the significantly smaller epilithon dry mass on day 30 relative to the other sample dates (day 5: $p < 0.0001$; day 10: $p < 0.0001$; day 15: $p < 0.0001$; Figure 1.4B).

Loricariids also influenced macroinvertebrate assemblages. In grazed treatments, there were fewer total macroinvertebrates, and reduced numbers of individuals in the orders of Ephemeroptera, Trichoptera, and Plecoptera, and the families Leptohiphidae (Ephemeroptera), Baetidae (Ephemeroptera), and Chironomidae (Diptera) (Table 1.3, Fig. 1.5). Community composition was dominated

Table 1.1. Effects of treatment and sample date on response variables in Experiment 1. The data were analyzed using a repeated measures model (fixed effects: treatment and day; random effect: block). Treatment×day interactions were only reported if they were significant ($p > 0.05$). Data were collected from four experimental blocks on four sample days (7, 14, 21, and 28).

Response	Parameter	F	p
Chlorophyll a	Treatment	F _(3, 43) = 4.46	0.0067
	Day	F _(3, 43) = 3.89	0.0152
Dry Mass	Treatment	F _(3, 43) = 6.20	0.0013
	Day	F _(3, 43) = 5.18	0.0038
AFDM	Day	F _(3, 43) = 4.54	0.0075
%C	Treatment	F _(3, 43) = 17.68	<0.0001
%N	Treatment	F _(3, 43) = 11.45	<0.0001
%P	Day	F _(3, 43) = 7.35	0.0004
Total C (g m ⁻²)	Day	F _(3, 43) = 3.94	0.0144
Total N (g m ⁻²)	Day	F _(3, 43) = 3.99	0.0136
Total P (g m ⁻²)	Treatment	F _(3, 43) = 4.62	0.0069
	Day	F _(3, 43) = 6.40	0.0011
C:N	Treatment	F _(3, 43) = 4.98	0.0047
C:P	Treatment	F _(3, 33) = 8.36	0.0003
	Day	F _(3, 33) = 8.30	0.0003
	Treatment×Day	F _(3, 33) = 2.25	0.0433
N:P	Treatment	F _(3, 33) = 10.05	<0.0001

Table 1.2. Effects of treatment and sample date on response variables in Experiment 2. Data were analyzed using a randomized complete block design (fixed effects: treatment and day; random effect: block). Treatment×day interactions were only reported if they were significant. Epilithon data was collected in five experimental blocks on four sample days (5, 10, 15, 30).

Response	Parameter	F	F	p
Chlorophyll a	Treatment	F _(2, 50)	= 29.84	<0.0001
	Day	F _(2, 50)	= 4.80	0.0052
Dry Mass	Treatment	F _(2, 44)	= 30.93	<0.0001
	Day	F _(2, 44)	= 15.11	<0.0001
	Treatment×Day	F _(4, 44)	= 2.55	0.0334
AFDM	Treatment	F _(2, 50)	= 6.85	0.0023
	Day	F _(2, 50)	= 13.68	<0.0001
%C	Treatment	F _(2, 50)	= 24.62	<0.0001
%N	Treatment	F _(2, 49)	= 14.71	<0.0001
	Day	F _(2, 49)	= 12.16	<0.0001
%P	Treatment	F _(2, 45)	= 7.96	0.0011
	Day	F _(2, 45)	= 3.96	0.0137
Total C (g m ⁻²)	Treatment	F _(2, 50)	= 10.45	0.0002
	Day	F _(2, 50)	= 10.83	<0.0001
Total N (g m ⁻²)	Treatment	F _(2, 49)	= 5.05	0.0102
	Day	F _(2, 49)	= 27.31	<0.0001
Total P (g m ⁻²)	Treatment	F _(2, 39)	= 37.90	<0.0001
	Day	F _(2, 39)	= 18.67	<0.0001
	Treatment×Day	F _(4, 39)	= 2.97	0.0173
C:N	Treatment	F _(2, 43)	= 5.06	0.0160
	Day	F _(2, 43)	= 26.94	<0.0001
	Treatment×Day	F _(4, 43)	= 2.72	0.0249
C:P	Treatment	F _(2, 45)	= 32.29	<0.0001
N:P	Treatment	F _(2, 44)	= 28.80	<0.0001
	Day	F _(2, 44)	= 4.64	0.0067

Table 1.3. Effects of experimental treatments on macroinvertebrates in Experiment 2 on benthic habitats in the Chacamax River in 2010 (SR: Stream Reference; CC: Cage Control; EX: *Pterygoplichthys* Enclosure). The data were analyzed using a randomized complete block design (fixed effects: treatment and day; random effect: block). Macroinvertebrate data was collected from each of the three treatments from five experimental blocks on two sample days (15 and 30) and was only reported if there were significant effects of treatment, day or treatment×day. Different letters indicate significantly different responses according to a Tukey’s post-hoc comparison.

Response	Parameter	F	p	Treatment Comparisons		
				SR	CC	EX
Total Macroinvertebrates	Treatment	F _(2, 22) = 9.78	0.0009	a	=	a < b
	Day	F _(1, 22) = 17.84	0.0003			
EPT	Treatment	F _(2, 22) = 7.25	0.0038	a	≤	ab ≤ b
	Day	F _(1, 22) = 13.42	0.0014			
Total Ephemeroptera	Treatment	F _(2, 22) = 7.40	0.0035	a	≤	ab ≤ b
	Day	F _(1, 22) = 13.65	0.0013			
Total Leptophlebiidae	Treatment	F _(2, 22) = 9.32	0.0012	a	>	b = b
	Treatment	F _(1, 22) = 15.4	<0.0001	a	<	b = b
Total Leptohiphidae	Day	F _(2, 22) = 16.30	0.0006			
	Treatment	F _(1, 22) = 15.03	0.0008	a	=	a < b
Total Chironimidae	Day	F _(2, 22) = 7.58	0.0031			
	Treatment	F _(1, 22) = 11.06	0.0005	a	<	b = b
Total Baetidae	Treatment	F _(2, 22) = 3.46	0.0493	a	≤	ab ≤ b
	Day	F _(1, 22) = 2.71	0.1139			

by Ephemeroptera in all treatments, but chironomids comprised a greater proportion of the total invertebrate densities in ungrazed treatments.

Loricariid grazing in enclosures did not significantly affect area-specific GPP, CR, or GPP:R (Table 1.4). However, chlorophyll-specific GPP was significantly greater in both the stream reference and the cage controls (where catfish had access) relative to the *Pterygoplichthys* enclosures (Table 1.2; Fig. 1.6A).

Site Comparison

Results comparing epilithon and macroinvertebrates upstream and downstream of a *Pterygoplichthys* invasion front mirrored many, but not all, of the results collected in the enclosure experiments. Similar to the experimental results, epilithon in the high-density, downstream site had significantly less chlorophyll a ($p=0.0066$, $F_{(1,22)}= 9.008$; Fig. 1.7A) and dry mass ($p=0.001$, $F_{(1,22)}= 16.22$; Fig. 1.7B) than the epilithon collected from rocks from the low-density site, upstream of the invasion front. Likewise, epilithon %C and %N in the high-density sites ($p<0.0001$, $F_{(1,22)}= 23.41$, Fig. 1.7D; $p=0.0106$, $F_{(1,22)}= 7.795$, Fig. 1.7E, respectively), and the total stock of these elements was reduced in the presence of high densities of grazing loricariids (C: $p=0.0077$, $F_{(1,22)}= 8.611$, Fig. 1.7G; N: $p=0.0181$, $F_{(1,22)}= 6.520$, Fig. 1.7H; P: $p=0.0373$, $F_{(1,22)}= 4.912$, Fig. 1.7I, respectively). There was no significant difference in AFDM between the sites ($p=0.3291$, $F_{(1,22)}= 0.9960$; Fig. 1.7C). Unlike the experiments, there was no significant difference in epilithon C:N, C:P, or N:P between the high- and low-density invasion sites ($p=0.3464$, $F_{(1,22)}= 0.9260$, Fig. 1.7J; $p=0.6633$, $F_{(1,22)}= 0.1947$, Fig. 1.7K; $p=0.9365$, $F_{(1,22)}= 0.0065$, Fig. 1.7L,

Table 1.4. Effects of treatment and sample date on response variables in Experiment 2. Data were analyzed using a randomized complete block design (fixed effects: treatment and day; random effect: block). Treatment×day interactions were only reported if they were significant. Gross primary productivity (GPP), net ecosystem productivity (NEP), and community respiration (CR) were measured on day 30 of the experiment.

Response	Parameter	F	p
GPP	Treatment	F _(2, 14) = 0.16	0.8517
NEP	Treatment	F _(2, 14) = 1.07	0.3719
CR	Treatment	F _(2, 14) = 0.20	0.8214
GPP:R	Treatment	F _(2, 14) = 0.75	0.4940
GPP:Chlorophyll a	Treatment	F _(2, 13) = 8.78	0.0053

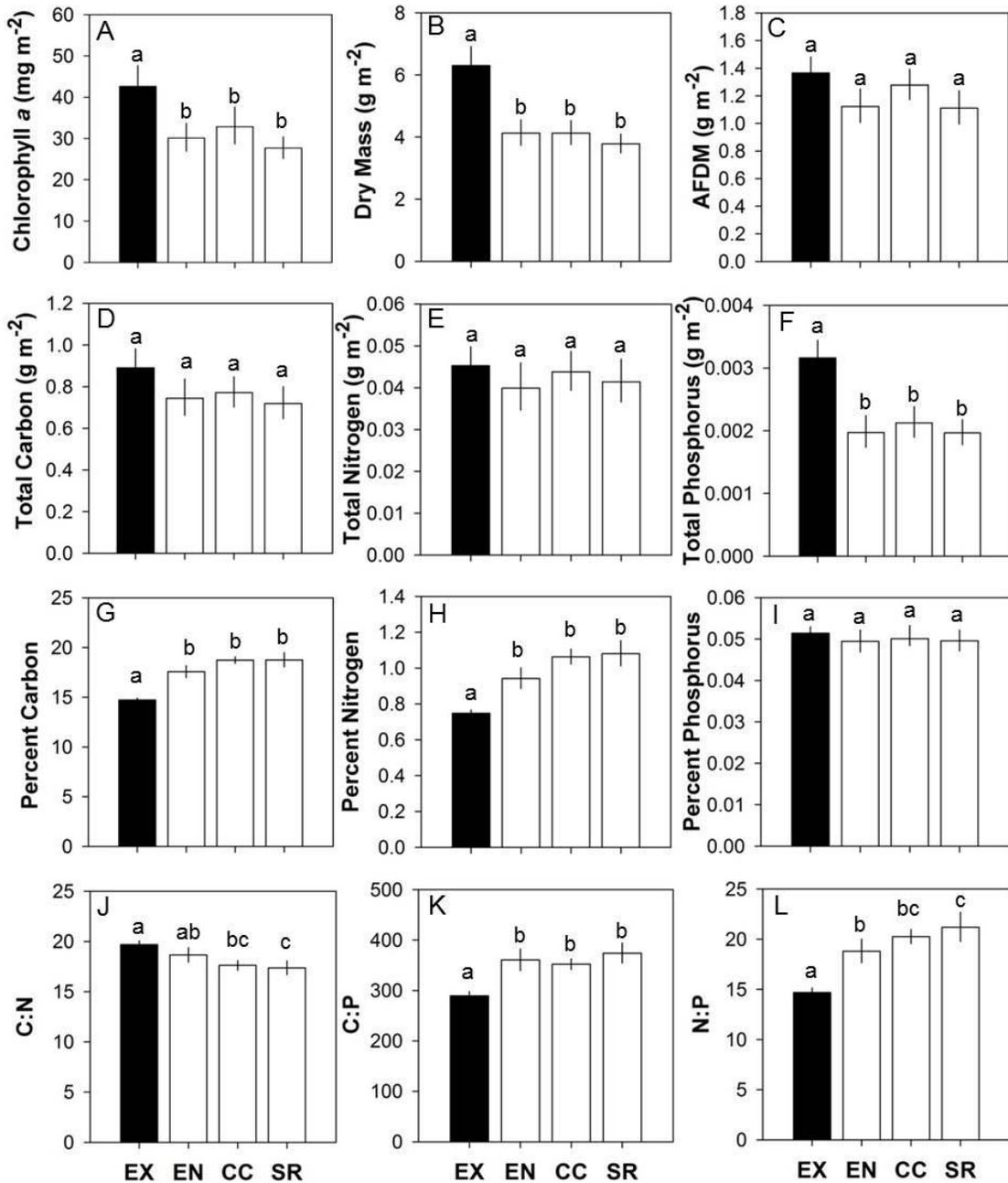


Figure 1.2. Means (± 1 S.E.) for multiple epilithion responses across all four sample dates of Experiment 1: (A) chlorophyll a, (B) dry mass, (C) AFDM, (D) percent carbon, (E) percent nitrogen, (F) percent phosphorus, (G) total carbon, (H) total nitrogen, (I) total phosphorus, (J) C:N, (K) C:P, and (L) N:P. Total nutrient values were collected from dry mass. Bars with different letters have significantly different ($p < 0.05$) values among treatments (SR: Stream Reference; CC: Cage Control; EN: *Pterygoplichthys* Enclosure; EX: *Pterygoplichthys* Enclosure) within each of the tested responses. The *Pterygoplichthys* Enclosure is represented in black.

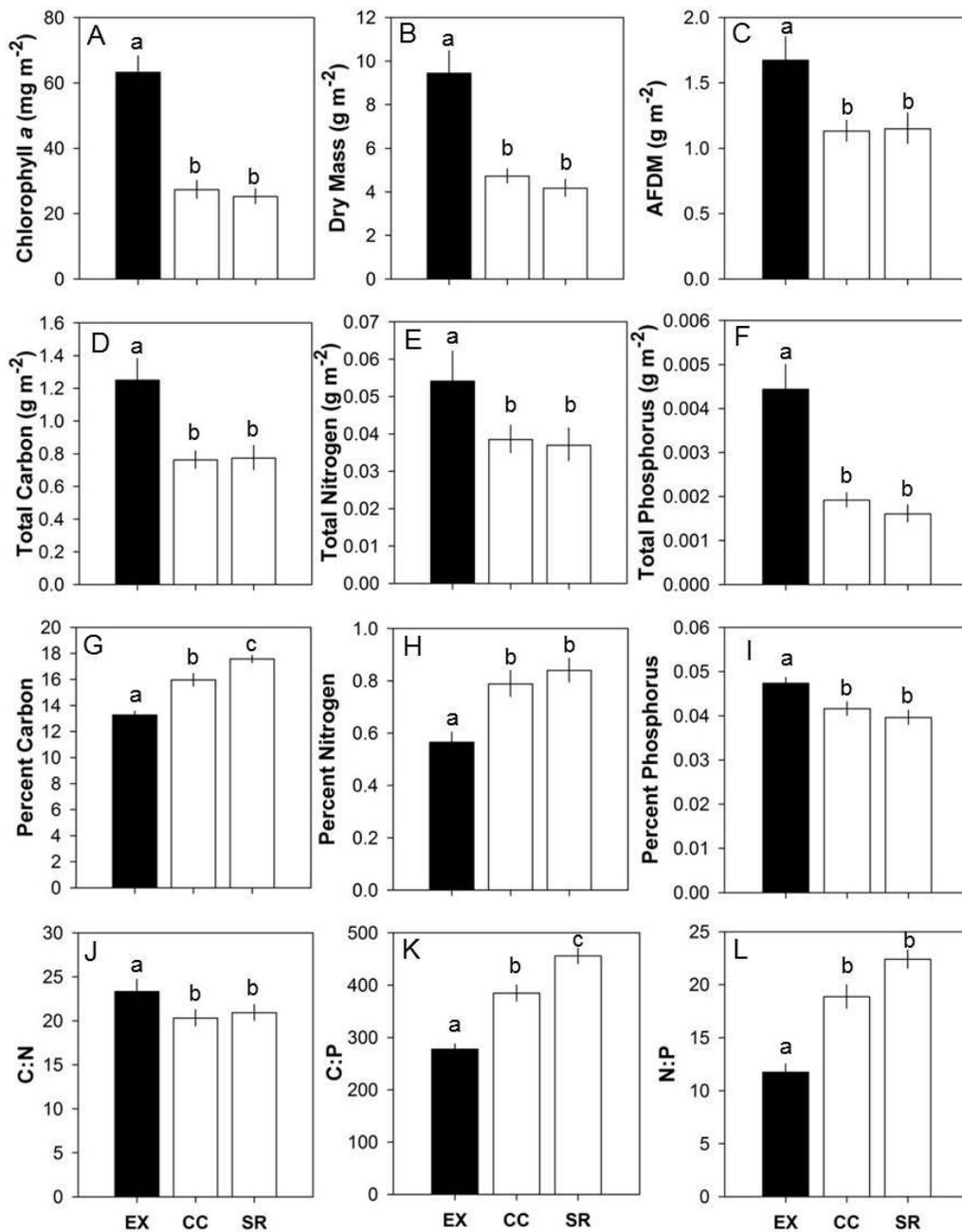


Figure 1.3. Means (± 1 S.E.) for multiple epilithon responses across all four sample dates of Experiment 2: (A) chlorophyll a, (B) dry mass, (C) AFDM, (D) percent carbon, (E) percent nitrogen, (F) percent phosphorus, (G) total carbon, (H) total nitrogen, (I) total phosphorus, (J) C:N, (K) C:P, and (L) N:P. Total nutrient values were collected from dry mass. Bars with different letters have significantly different ($p < 0.05$) values among treatments (SR: Stream Reference; CC: Cage Control; EX: *Pterygoplichthys* Exclusion) within each of the tested responses. The *Pterygoplichthys* Exclusion is represented in black.

respectively). Moreover, the epilithon %P was greater in the high-density invasion site rather the low-density invasion site ($p=0.0206$, $F_{(1, 22)}= 6.227$, Fig. 1.7F).

Macroinvertebrate abundance was significantly greater in the low-density site than in the high density site (Figure 1.5C). Similarly, the number of individuals in the orders of Ephemeroptera and Trichoptera, and Plecoptera ($p=0.0014$, $F_{(1,3.88)}= 67.68$), and Coleoptera ($p=0.0131$, $F_{(1,2.806)}= 31.97$) were significantly greater in the low-density site than in the high-density site. In addition, the numbers of individuals found in the families Leptophlebiidae ($p=0.0245$, $F_{(1,4.263)}= 11.62$) and Baetidae ($p=0.0047$, $F_{(1,3.879)}= 34.11$) tended to be greater in the low-density site. Similar to the experiments, mayflies dominated the macroinvertebrate communities of both the high- and low-density invasion sites and coleopterans (primarily the families Elmidae and Psephenidae) were more common in stream reference treatments (Fig. 1.5D). There was no significant difference in the proportion of chironomids found between the sites above and below the invasion front.

Chlorophyll-specific GPP ($p=0.0460$, $F_{(2,21)}= 6.274$) was significantly greater downstream of the invasion front, echoing the pattern seen in Experiment 2 (Fig. 1.6B). In contrast to the experiments, areal NEP ($p=0.0495$, $F_{(2,21)}= 8.251$) and CR ($p < 0.0001$, $F_{(2,18)}= 13.85$) were significantly greater in the high-density invasion site, and GPP ($p=0.0455$, $F_{(2,21)}= 4.604$) and GPP:R ($p=0.0022$, $F_{(2,21)}= 25.42$) were significantly greater in the low-density invasion site.

DISCUSSION

The results from this study document the profound effects that low-trophic position fishes such as grazers, can have on the structure and function of freshwater

ecosystems. High population densities of *Pterygoplichthys* reduced food resources and macroinvertebrate abundance in the Chacamax River. Additionally, *Pterygoplichthys* reduced the total stock of nutrients and carbon stored in epilithon and modified epilithon stoichiometry, potentially exacerbating P-limitation. Together, these results demonstrate *Pterygoplichthys* significantly reduced the quantity and quality of food resources, subsequently altering the abundance of the macroinvertebrate community and primary productivity in an invaded system.

Grazing organisms frequently reduce algal biomass through their feeding activities (Hillebrand 2002, 2009), my results support this finding. In the presence of grazing loriciids, I measured approximately 50% less algal biomass and epilithon dry mass in the site comparison and in the experiments (Figs. 1.2, 1.3, 1.7). High fish standing stocks and high per-capita consumption rates of this low-quality food combine to yield the dramatic reduction in epilithon abundance.

Nutrient stoichiometry, or the ratios of key elements such as C, N, and P, has been employed as an index of food quality in freshwater systems (Steinman 1996, Hessen et al. 2002, Sterner and Elser 2002), where low C:P and C:N indicate greater food quality. Grazer-induced structural changes to epilithon can alter epilithon stoichiometry (Frost et al. 2002, Bowman et al. 2005), and studies have demonstrated that nutrient content of algae typically increases in response to grazing (Rosemond et al. 1993, Steinman 1996). This increase in epilithon nutrient concentration may be caused by grazers removing senescent cells that are lower in nutrients (Mulholland et al. 1991) or by changes in algal community composition after grazing potentially driven by increased light and nutrient availability (Gelwick and Matthews 1992).

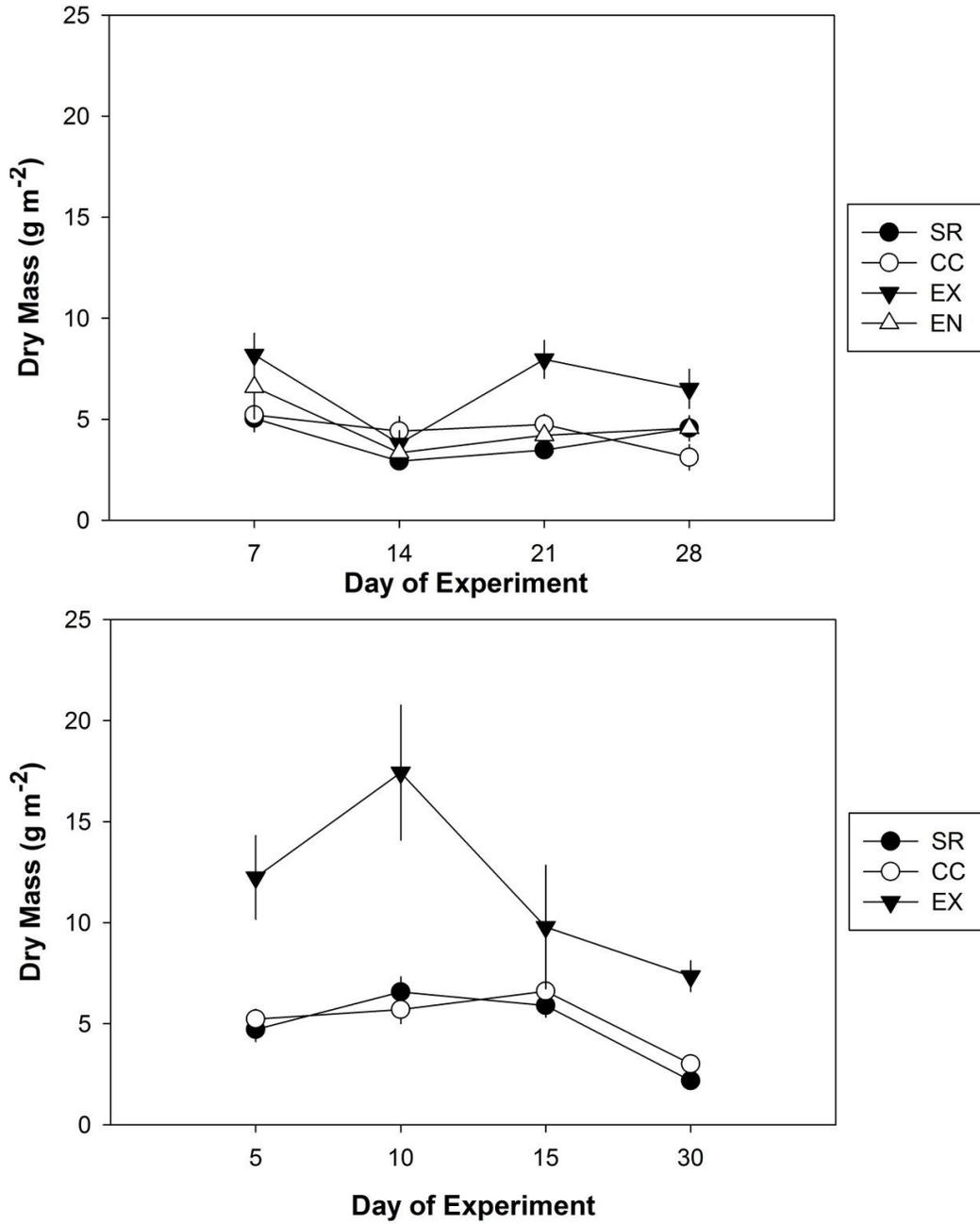


Figure 1.4. Average dry mass (± 1 S.E.) by sample date: (A) Experiment 1 (SR: Stream Reference; CC: Cage Control; EN: *Pterygoplichthys* Enclosure; EX: *Pterygoplichthys* Exclosure), (B) Experiment 2 (SR: Stream Reference; CC: Cage Control; EX: *Pterygoplichthys* Exclosure). Arrows indicate sediment-depositing flooding events during the experiments.

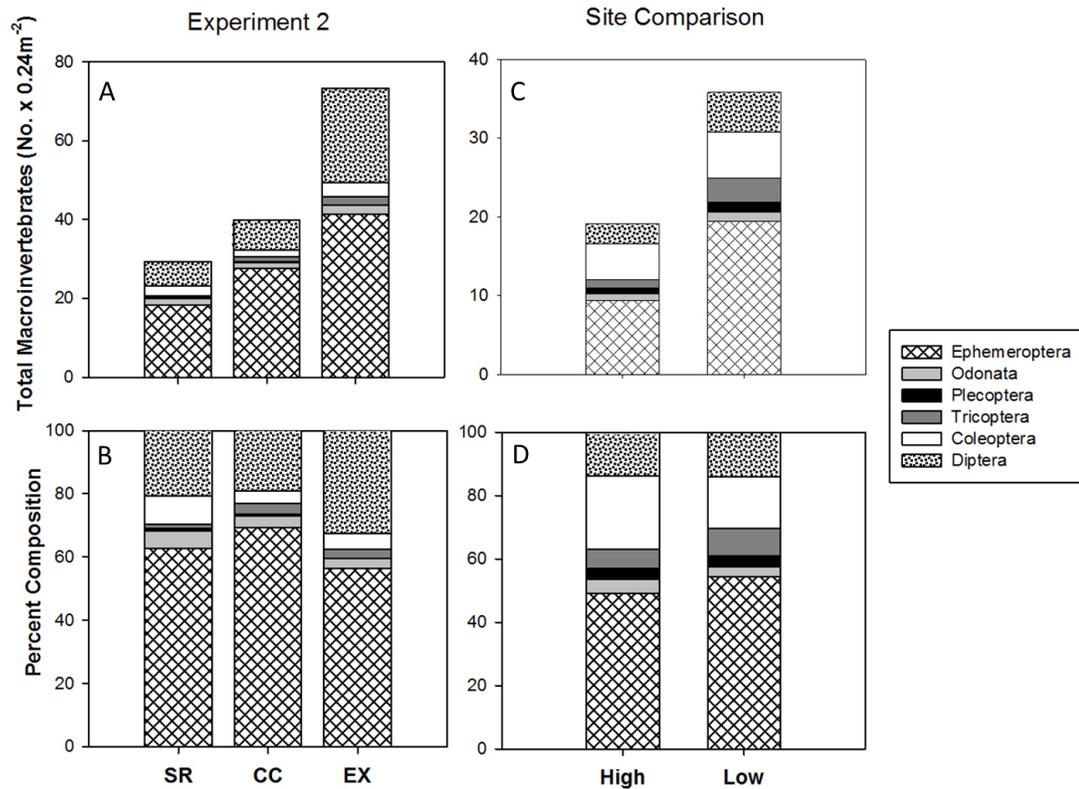


Figure 1.5. Macroinvertebrate samples from three treatments (SR: Stream Reference; CC: Cage Control; EX: *Pterygoplichthys* Exclusion) of Experiment 2 and from the site comparison (High: High-density *Pterygoplichthys* invasion; Low: Low density *Pterygoplichthys* invasion). (A) Average number of individuals found in each order in Experiment 2. (B) Percent composition of orders in Experiment 2 (C) Average number of individuals found in each order in the invasion sites. (D) Percent composition of macroinvertebrate orders in the invasion sites.

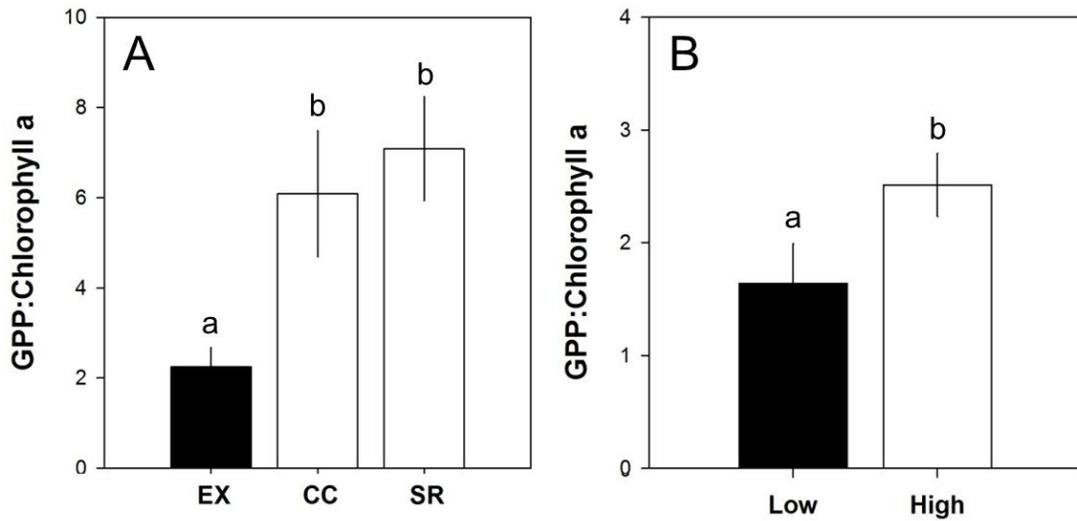


Figure 1.6. Means (± 1 S.E.) of chlorophyll-specific GPP (GPP:Chlorophyll a) from Experiment 2 ((A) SR: Stream Reference; CC: Cage Control; EX: *Pterygoplichthys* Exclosure) and the invasion sites ((B) High: High-density *Pterygoplichthys* invasion; Low: Low density *Pterygoplichthys* invasion). The *Pterygoplichthys* exclosure and the low-density site are represented in black.

respectively). Moreover, the epilithon %P was greater in the high-density invasion site rather than the low-density invasion site ($p=0.0206$, $F_{(1, 22)}= 6.227$, Fig. 1.7F).

Macroinvertebrate abundance was significantly greater in the low-density site than in the high density site (Figure 1.5C). Similarly, the number of individuals in the orders of Ephemeroptera and Trichoptera, and Plecoptera ($p=0.0014$, $F_{(1,3.88)}=$

Epilithon stoichiometry may also be affected by the stoichiometry of the consumer. Therefore, consumer stoichiometry may help predict consumer impacts on ecosystem stoichiometry and biogeochemical cycling. (Evans-White and Lamberti 2005, Knoll et al. 2009). For example, several studies examining the relationship between fish body nutrient content and the stoichiometry of excretion have documented that fish species with higher body P excrete P at a lower rate (Vanni et al. 2002, Small et al. 2011). The results from my study mirror these findings.

Conversely, in a recent review of more than 100 experiments documenting the indirect effects of grazers on periphyton nutrient content, Hillebrand et al.(2008) found that grazers significantly lowered C:N and C:P and, on average, increased periphyton N:P. Hillebrand et al. reported that grazers with high body P enhanced periphyton P content by increasing ambient P concentrations via excretion. These findings were contrary to my results which showed a reduction in epilithon P in response to grazing by an organism with high body P. Hillebrand et al. (2008) proposed that high C:P ratios, or low-body P content, in grazers indicated P-limitation of growth rather than a low P demand and that high C:P organisms may excrete less P than grazers with high body P-content. Furthermore, they posited that organisms with high C:P ratios need to be stoichiometrically flexible in order to respond to changes in food quality and

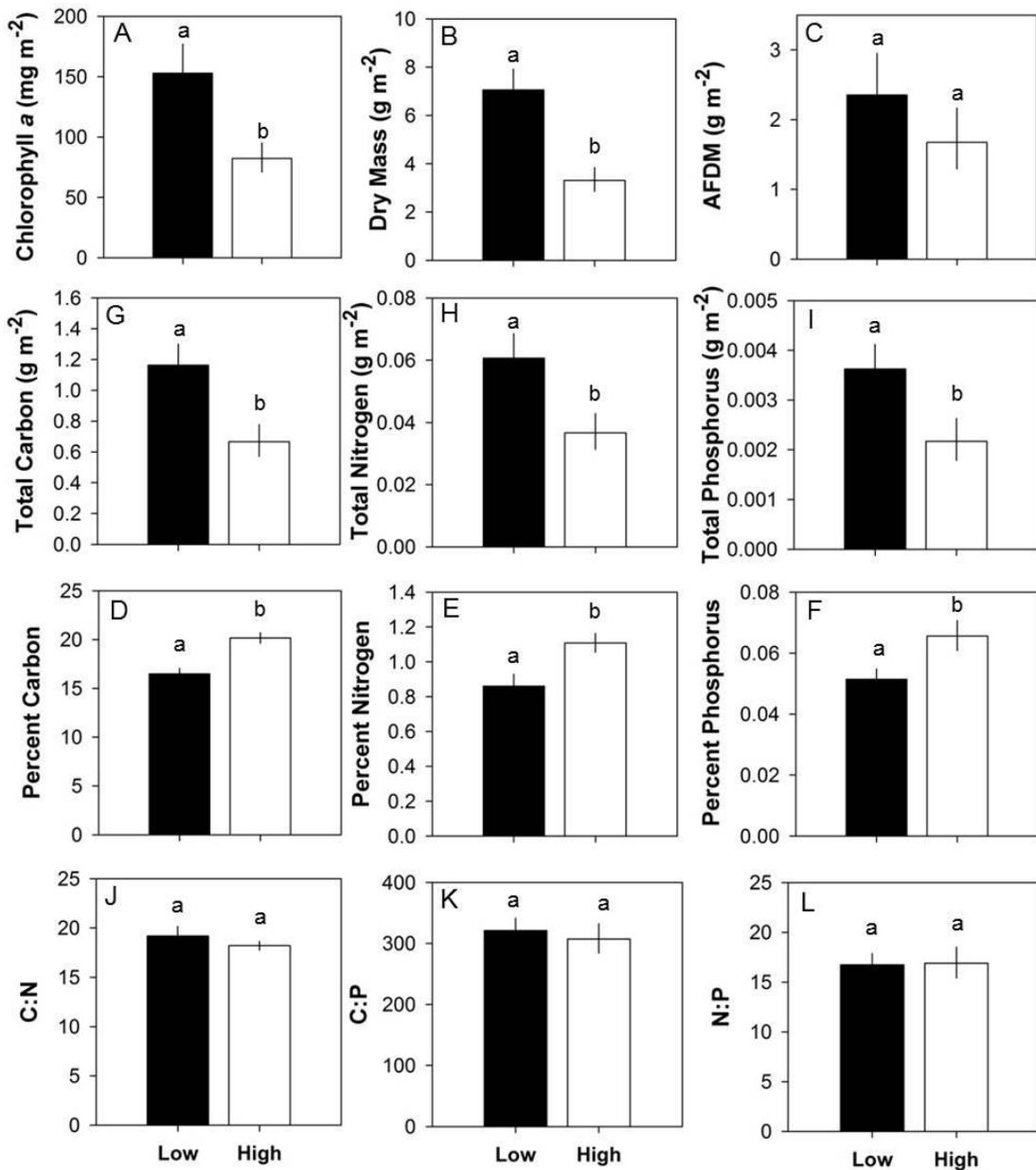


Figure 1.7. Means (± 1 S.E.) for multiple epilithon variables from the site comparison: (A) chlorophyll a, (B) dry mass, (C) AFDM, (D) percent carbon, (E) percent nitrogen, (F) percent phosphorus, (G) total carbon, (H) total nitrogen, (I) total phosphorus, (J) C:N, (K) C:P, and (L) N:P. Bars with different letters have significantly different ($p < 0.05$) values between sites (Low: Low density *Pterygoplichthys* invasion site; High: High density *Pterygoplichthys* invasion site) within each of the tested responses. Total nutrient values were collected from dry mass. The low-density site is represented in black.

abundance. One of the fundamental tenants of the theory of ecological stoichiometry is that animals have relatively homeostatic stoichiometry (Sterner and Elser 2002). Yet, several studies have documented stoichiometric flexibility in the body nutrient composition of arthropods and gastropods (i.e., Liess and Hillebrand 2005, 2006), the organisms that were most common in the Hillebrand et al. review (2008).

Stoichiometric flexibility, especially with body phosphorus content, is not thought to be as common in bony fishes (McIntyre and Flecker 2010).

Although stream fishes exhibit a wide range of body C:P and N:P ratios among families (McIntyre and Flecker 2010), and fishes may undergo ontogenetically-mediated shifts in body elemental composition (Pilati and Vanni 2007), the body N and P content among species within the same family are remarkably constant, suggesting that adult fishes are relatively homeostatic in their body stoichiometry (McIntyre and Flecker 2010). Loricariids are P-rich (5.5-6% body P) relative to many other fish families (2-4% body P) in the Chacamax River; consequently, their P demand may be greater than other fishes and their growth may be P-limited (Hood et al. 2005). These values are reflected in published values of loricariid body P-content (Vanni et al. 2002, Hood et al. 2005, McIntyre and Flecker 2010). In my experiments, *Pterygoplichthys* grazing significantly reduced the amount of P stored in epilithon and increased epilithon C:P and N:P, indicating that loricariid grazing reduced the quality of food resources in the Chacamax River. Reductions in total C, N, and P stored in epilithon was also seen in the site comparison where epilithon downstream of the invasion front stored less C, N, and P relative to the upstream site with few loricariids.

Phosphorus-limitation of loricariid growth may be intensified in systems with low epilithon P concentrations. Through intensive grazing and selective retention of P during consumption, *Pterygoplichthys* has the potential to exacerbate P-limitation by algae thereby increasing C:P and N:P in the epilithon of invaded systems with low ambient nutrient concentrations. Thus, *Pterygoplichthys* may reduce the amount of bioavailable P and create a bottom-up effect that alters native algal, macroinvertebrate, and fish community structure and primary productivity in invaded habitats. These findings and others indicate that grazing loricariids may be acting as a P sink and sequestering large amounts of P in their body mass, potentially altering the storage and recycling rates of P in invaded systems (Vanni et al. 2002, Hood et al. 2005).

By reducing the quality and quantity of epilithon in the Chacamax River, loricariids may directly compete with native grazers and negatively affect their populations (Gido and Franssen 2007, Mendoza-Carranza et al. 2010, Pound et al. 2011). Previous studies have demonstrated that dominant native grazers typically had strong, negative effects on other benthic organisms via resource exploitation (Feminella and Hawkins 1995). Grazers can also influence macroinvertebrate communities by changing the physical structure of an environment. For example, in tropical streams, grazing fish and shrimp reduced the number of macroinvertebrates such as chironomids, which depend on sediment resources that were used for habitat (Flecker 1992, Pringle et al. 1993, Flecker 1996). However, in my study, macroinvertebrate density (total number, EPT, Leptohyphidae, and Chironomidae) correlated positively with increasing algal biomass rather than the total abundance of sediment (Appendix 5), suggesting that loricariids reduced macroinvertebrate

populations indirectly via resource exploitation rather than by reducing the amount of available habitat.

While mayflies dominated the macroinvertebrate communities of both sites and all of the experimental treatments, there were interesting differences found between samples collected from areas with high and low densities of grazing loricariids. Grazing coleopterans (Psephenidae) were more common in samples collected from treatments and sites exposed to intense grazing by loricariids (Fig. 1.5B,D). Conversely, chironomids, many of which are gathering collectors (Cummins and Klug 1979), were more common in *Pterygoplichthys* exclosures in the experiment. However, this pattern was not seen in the site comparison. These results indicate that intensive grazing by loricariids may convert habitat and food resources that are appropriate for some organisms, such as collectors, to environments that are suitable for other functional groups, such as scrapers.

Although it was outside of the scope of this study, loricariids can also compete with native fish species for food resources. For example, in a food web study of mangrove and sea grass habitats of the Centla Wetland Biosphere Reserve, a wetland downstream from the Chacamax River in the Usumacinta Watershed, Mendoza-Carranza et al. (2010) found that introduced *Pterygoplichthys pardalis* had carbon isotope signatures similar to many co-occurring native fish species of commercial importance, implying competition for similar food resources. Additionally, in Texas springs, Pound et al. (2011) determined that the C and N isotopic signatures and gut contents of a non-native, grazing loricariid, *Hypostomous plecostomus*, overlapped with those of native grazing fishes, indicating that the invasive loricariids may

compete with and displace native herbivorous fishes, several of which were threatened or endangered. In the Chacamax River, native cichlids, including *Vieja bifasciata* and *V. intermedia*, can be seen grazing on epilithon. Additionally, many of the native fishes are insectivorous and depend on aquatic macroinvertebrate population for food resources (A. Pease, unpublished data). Therefore, invasive loricariids may indirectly affect native fish populations by consuming basal food resources upon which their prey depend. Negative effects on native fishes may also have negative socioeconomic consequences for the communities in the watershed that are dependent upon freshwater fisheries (Appendix 6).

The results from this study also indicate that loricariids influence the ecosystem function of the Chacamax River. Although there were no significant differences in areal GPP between fish enclosure and stream reference treatments in Experiment 2, areal GPP was significantly higher upstream of the invasion front, suggesting that intensive grazing by high densities of loricariids may reduce area-specific GPP, or the overall GPP in the Chacamax River. However, in Experiment 2 and the site comparison, the chlorophyll-specific GPP was significantly greater in areas with high loricariid density, indicating that grazing by loricariids may alter the algal community and promote the growth of highly productive algal species (Fig. 1.6). Notably, we only examined loricariid grazing effects on GPP when contrasting high and extremely low densities of grazing loricariids. It is possible that intermediate densities of grazing loricariids could increase GPP relative to areas with high and low densities of these fishes. For example, Power (1990) found that algae exposed to intermediate densities of native loricariid populations had higher standing stocks and

GPP than rocks that were either exposed high-densities of grazing fishes or rocks that were completely protected from loricariid grazing.

Loricariids are one of many of non-native fish families invading into low-trophic positions and attaining high-population densities in novel habitats. The results from my investigation detail some of the direct effects of non-native grazing fishes on epilithon in lentic systems and document the ability of low-trophic position invaders to fundamentally alter habitat and community structure. Fishes are not always stoichiometrically equivalent with other species; consequently, the effects of non-native grazing fishes on epilithon stoichiometry should be varied and species-dependent (Evans-White and Lamberti 2005, Knoll et al. 2009). Loricariids are P-rich and my investigation suggests that they have the potential to alter biogeochemical storage and cycling in invaded habitats, especially in systems that are P-limited. The results from this study underscore the importance of considering the stoichiometric constraints of exotic species when predicting their potential impacts on novel systems. Additionally, my investigation highlights the potential threat low-trophic position invaders present to the structure and function of invaded ecosystems by the direct and indirect effects of grazing on populations of primary producers and higher trophic levels.

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CHAPTER 2

INFLUENCES OF INVASIVE FISHES ON ECOSYSTEM STRUCTURE AND FUNCTION: SEPARATING THE EFFECTS OF GRAZING AND NUTRIENT RECYCLING

ABSTRACT

Separating the effects of consumption and nutrient remineralization via excretion on algal biomass and primary productivity is important for understanding the role of grazing species as drivers of ecosystem processes. This may be especially important in ecosystems threatened by the invasion of non-native grazing species. In this study, I coupled the results of mesocosm and *in situ* experimental manipulations with measurements of nutrient remineralization rates from individual fish and aggregations of fish to estimate the effects of a non-native grazer (Loricariidae: *Pterygoplichthys*) on algal biomass and primary productivity in an invaded stream ecosystem. Loricariid grazing depressed algal biomass in mesocosm and *in situ* experiments. Conversely, the remineralization of nutrients via loricariid excretion stimulated algal growth and primary productivity in mesocosms relative to control treatments without loricariids. Excretion by aggregations of loricariids also generated hotspots of nutrient production compared to ambient nutrient concentrations in the study reach. In sum, intensive grazing by high-densities of loricariids results in a negative net-effect of loricariids on algal biomass and primary productivity in the Chacamax River. This study demonstrates the need to quantify the consumptive and non-consumptive effects of invaders in order to develop a comprehensive understanding of how non-native organisms influence ecosystem structure and function in invaded ecosystems.

INTRODUCTION

In both terrestrial and aquatic ecosystems, grazers can have profound effects on the structure and function of ecosystems by influencing primary producers simultaneously through consumptive and nutrient-mediated pathways (Frank and Evans 1997, Frank et al. 2000, McIntyre et al. 2006, Knoll et al. 2009). Initially, grazers affect the biomass of primary producers in terrestrial and aquatic systems through their feeding activities (Gruner et al. 2008). In a meta-analysis of 85 studies of the top-down and bottom-up controls of periphyton biomass, Hillebrand (2002) documented the consumptive effects of grazing always reduced biomass. Similar results were observed in a recent, cross-system analysis of the effects of consumer controls on the biomass of primary producers where herbivore removal typically promoted the growth of producers in marine and freshwater ecosystems (Gruner et al. 2008).

Producer biomass often increases in response to fertilization by nutrients; thus organisms can also influence the growth and production of primary producers through nutrient remineralization via excretion and egestion (Gruner et al. 2008). Within ecosystems, mobile organisms can generate areas of enhanced nutrient recycling rates in space (hotspots) and time (hot moments) that may influence primary productivity (Meyer et al. 1983, McClain et al. 2003, McIntyre et al. 2008). For example, in a study of ungulates in Yellowstone Park, Frank et al. found that grazing modified nitrogen (N) cycling and created a mosaic of biogeochemical hotspots across the landscape that affected primary producers (Frank et al. 2000). Similarly, in a study examining the influence of a native fish assemblage on N and phosphorus (P) cycling in a tropical

river, McIntyre et al. (2008) found that the aggregate excretion of fishes was sufficient to turn over the entire ambient pool of N in the water column, the nutrient limiting primary productivity, in less than 0.3km. Other studies have demonstrated that consumer-driven nutrient remineralization can be significant in aquatic ecosystems and can enhance periphyton biomass and productivity (Evans-White and Lamberti 2006, Liess and Hillebrand 2006, Knoll et al. 2009).

In stream ecosystems, myriad studies have examined the consumptive effects of grazing on ecosystems and the recycling effects that organisms can have on algal biomass and gross primary productivity (GPP) through nutrient remineralization; yet, many of these investigations have not actively separated consumptive and nutrient remineralization effects of grazers. There has been a recent effort to tease apart the conflicting effects of grazing and excretion to determine the net effect of grazers on aquatic ecosystems (i.e., Knoll et al. 2009, Reisinger et al. 2011). However, many of these studies focused on grazers in their native ranges (but see Caraco et al. 2006, Dzialowski and Jessie 2009).

Non-native grazing organisms can have strong top-down effects by their feeding activities and can have bottom-up effects that can alter natural energy pathways that lead to higher-level consumers. For example, zebra mussel (*Dreissena polymorpha*) invasion has been linked to decreases in phytoplankton populations (top-down effects) and the collapse of consumers dependent upon phytoplankton (bottom-up effects) (Strayer 2009, 2010). Given the disproportionate success of many grazing invaders such as loricariids, carp (i.e., *Ctenopharyngodon idellus*), and mollusks (i.e., zebra mussels and *Potamopyrgus antipodarum*) there is a pressing need to expand

invasive species research to include other primary consumers that have the potential to alter biodiversity and ecosystem function (Davis et al. 2011).

Armored catfishes are native to Central and South America (Weber 1991, Nico and Martin 2001), but introduced populations of loricariids have been documented in freshwater ecosystems throughout the globe (Courtenay et al. 1986, Arthington et al. 1999, Fuller et al. 1999, Nico and Martin 2001, Bomford and Glover 2004, Alecke et al. 2005, Liang et al. 2005, Chavez et al. 2006, Kailola 2007, Ozdilek 2007, Hossain et al. 2008, Keszka et al. 2008, Sinha et al. 2010, Capps et al. 2011). In invaded ecosystems, loricariids attain high population densities and create large aggregations during daylight hours (Mendoza et al. 2009). They are thought to compete with native organisms for food resources and space (Devick 1989, Hoover et al. 2004).

The purpose of this study was to investigate the top-down and bottom-up pathways by which invasive loricariids affect algal biomass and gross primary productivity (GPP) by quantifying the net effects of grazing and nutrient remineralization. I used a combination of observational data and experimental manipulations to assess the impact of loricariids on primary producers in the Chacamax River in southern Mexico. I predicted that nutrient remineralization by loricariids would represent a substantial flux of N and P in the Chacamax River and loricariid aggregations would create hotspots of nutrient release in the river channel. Additionally, I posited nutrient remineralization by loricariids would stimulate algal growth and GPP in experimental mesocosms. However, I hypothesized high population densities of grazing armored catfish would have a negative net-effect of loricariid grazing and nutrient remineralization on algal biomass and GPP.

METHODS

The field work for this study was conducted in the Chacamax River (N17°29'047" W91°58'430") in Chiapas, Mexico during the dry season months of March-May 2008-2010 (Appendix 1). Water temperature in the river ranged from 21 to 28°C throughout the investigation, and ambient nutrient concentrations in the study reaches were moderate to low (average values: $\text{NH}_4^+\text{-N}$, $10 \mu\text{g L}^{-1}$; $\text{NO}_3^-\text{-N}$, $353 \mu\text{g L}^{-1}$; soluble reactive phosphorus, $< 2 \mu\text{g L}^{-1}$; total dissolved nitrogen, $387 \mu\text{g L}^{-1}$; total dissolved phosphorus, $3 \mu\text{g L}^{-1}$). Stream discharge averaged $\sim 1,600 \text{ L s}^{-1}$ throughout the study. Average chlorophyll abundance in epilithon was $16.2 \pm 11.7 \text{ mg m}^{-2}$ (mean \pm SD) and mean epilithon dry mass was $7.19 \pm 2.94 \text{ g m}^{-2}$ (mean \pm SD). Loricariid density was $2.3 \pm 3.4 \text{ m}^{-2}$ (mean \pm SD) and loricariid areal biomass was $225 \pm 45 \text{ g m}^{-2}$ (mean \pm SD) in the study reach in 2010 (Fig. 3.3).

In Mexico, the most common introduced genus of loricariid is *Pterygoplichthys*. *Pterygoplichthys* were first documented in the Chacamax River in 2004 (Wakida-Kusunoki et al. 2007). *Pterygoplichthys pardalis* (Castelnaud, 1855), *Pterygoplichthys disjunctivis* (Weber, 1991) and *Pterygoplichthys* that do not adhere to type specimens are found in the Chacamax River and were the subjects of this investigation (Appendices 3-4).

I employed a combination of observational data and mesocosm and *in situ* manipulations to study the effects of loricariid grazing and increased nutrient availability on algal biomass and primary productivity. This approach allowed me to estimate the separate and combined effects of loricariid grazing and nutrient

remineralization on the structure and function of primary producers in the Chacamax.

Nutrient Remineralization and Hotspot Sampling

To determine nutrient remineralization rates of loricariids and estimate the effects of their remineralization on ambient water chemistry, I conducted fish excretion incubations and I sampled water within and outside of aggregations of loricariids in the Chacamax River. Twenty *Pterygoplichthys* were collected using hand nets and immediately incubated in 15L plastic tubs for approximately 1h. Ten incubations were conducted between 1200 and 1500hrs (afternoon) and 10 incubations occurred between 1900 and 2100 hours (evening). Two additional fish-free tubs were maintained as controls during each incubation. Tubs were filled with 10L of filtered stream water and placed in the shade for the duration of the incubation. At the end of the incubation, I collected filtered water samples for NH_4^+ and total dissolved phosphorus (TDP) analysis. Fish nutrient recycling rates were estimated based on the difference in dissolved N and P concentrations between plastic tubs incubated with and without *Pterygoplichthys* (Vanni et al. 2002, McIntyre et al. 2008). At the end of the incubation period, samples were filtered through glass-fiber filters (Gelman A/E) and were either acidified and shipped to the USA for P analysis, or were analyzed in the field for NH_4^+ . I used standard colorimetric methods to analyze TDP and (soluble reactive phosphorus) SRP samples (APHA 1998) using a Lachat QuickChem 8000 (Lachat Instruments, Loveland, Colorado). All NH_4^+ samples were refrigerated and analyzed in the field using the flurometric methods outlined by Taylor et al. (2007).

To estimate the influence of time of day (afternoon/evening) on loricariid feeding behavior, 77 fish were harvested and weighed during afternoon (22 fish; 1200-

1730 hrs) or evening (55 fish; 1900-0400 hrs) hours, euthanized using an overdose of MS-222, and their gut contents were collected, dried, and weighed according to methods outlined in German and Bittong (2009) under IACUC protocol number: 2006-0169, Cornell University. All values were expressed as the ratio of gut content dry mass (g) to fish wet mass (g) to account for size variation in the fishes I sampled.

To ascertain if *Pterygoplichthys* generated hotspots of nutrient remineralization relative to ambient water chemistry, I collected paired stream water samples within and outside of aggregations of loricariids in the Chacamax River in 2008 and 2010. I sampled aggregations of *Pterygoplichthys* with minimum areas of 3m² with at least 50 *Pterygoplichthys* m⁻² (Fig. 2.1A-B). Paired sites were matched for similar depth (min=0.5m, max=1.5m, mean=0.9m) and water velocity (min=0.04 m s⁻¹, max=0.09 m s⁻¹, mean=0.07m s⁻¹). Water samples collected in and outside of fish aggregations were collected and analyzed for NH₄⁺ and SRP using the aforementioned methods.

Pterygoplichthys Exclosure Construction

To measure the consumptive effect of loricariid grazing on algal biomass and sediment dry mass, I constructed five *Pterygoplichthys* exclosures and five cage controls (dimensions: 24cm × 48cm × 10cm, 2.5cm poultry wire; Fig. 2.2A) that were randomly paired and completely submerged in five locations in a 25m reach of the Chacamax River in May 2009. This mesh size prevented grazing by loricariids but permitted smaller individuals of the common fish species and the native grazing tadpoles and snails to graze on rocks in the *Pterygoplichthys* exclosures. After a 10-day incubation in the stream, epilithon from three rocks from each treatment in each treatment pair was collected using wire brushes. Aliquots of the resulting slurry were

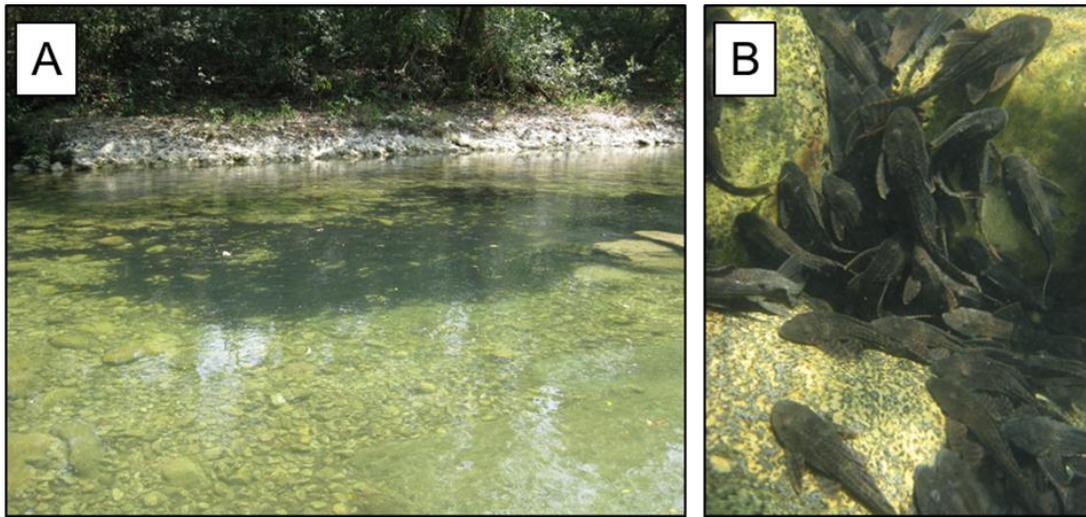


Figure 2.1. *Pterygoplichthys* in the Chacamax River (N17°29'047" W91°58'430") during daylight hours. (A) Aggregation of loricariids and (B) underwater photo of loricariid aggregation. The bedrock and cobble substrate in the river is predominantly limestone and light in color; the dark area in photo (A) is an aggregation of loricariids. Photo credit: K. A. Capps.

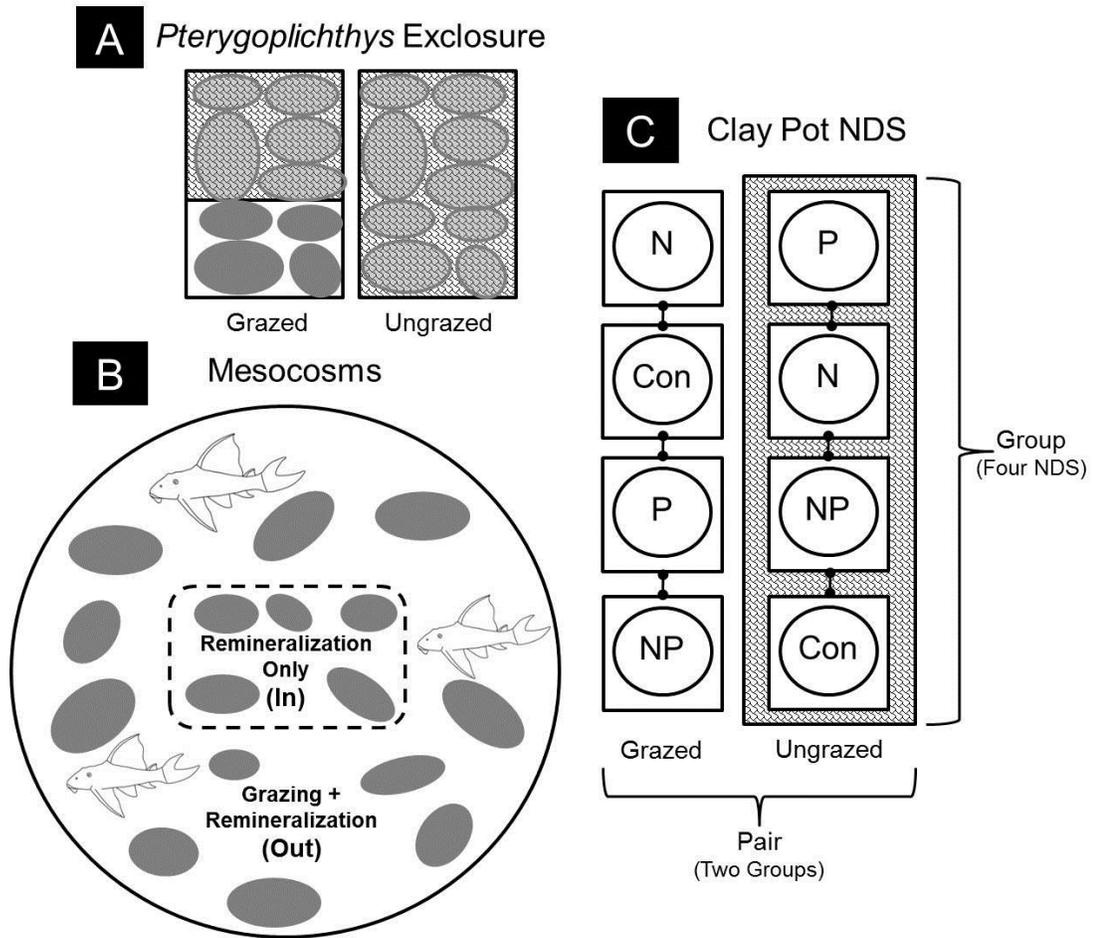


Figure 2.2. Experimental design: (A) *In situ* loricariid exclusion experiment (CC-Cage Control, EX-*Pterygoplichthys* Exclusion); (B) Low-density loricariid treatment of mesocosm experiment (In-Rocks placed in basket within mesocosm that were exposed to nutrients via recycling, but were protected from grazing, Out-Rocks outside of basket that were exposed to nutrients via recycling and to grazing); (C) One group of eight grazed/ungrazed *in situ* clay pot nutrient diffusing substrata (Con-control (no nutrients), N-nitrogen (NH_4Cl), P-phosphorus (K_3PO_4), N+P ($\text{NH}_4\text{Cl}+\text{K}_3\text{PO}_4$)). Figures are not drawn to scale. Loricariid drawing credit: T. Vigliotta.

filtered onto pre-ashed and pre-weighed Gelman A/E filters (Gelman, Ann Arbor, Michigan) for chlorophyll a (5mL), ash-free dry mass (AFDM) (10mL), and dry mass (10mL). Digital photos were taken of each rock and rock areas were quantified using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, California). Chlorophyll a content was estimated by filtering subsamples of the slurry from each rock and immediately placing the filter in an opaque film canister filled with 20 mL of buffered 90% ethanol. The filters were incubated in the dark for 16h to extract chlorophyll a. Chlorophyll a samples were measured using fluorometry (AquaFluor™; Turner Designs, Sunnyvale, CA).

Mesocosm Construction

To separate and measure the consumptive effects of grazing on algal growth and production from the non-consumptive effects of nutrient remineralization, I constructed twelve mesocosms by filling plastic wading pools (1.52 m diameter × 25cm) with 400 liters of stream water. Plastic baskets (46cm × 23cm × 25cm) were placed in the center of each pool to create a grazing treatment within each fish treatment (Fig. 2.2B). Twenty-six stream rocks were collected from the Chacamax River and immediately placed in each mesocosm, 20 rocks were placed outside of the baskets to measure the combined effects of grazing and nutrient recycling (Out) and six rocks were positioned inside the baskets (In) to measure the effects of nutrient recycling alone (Fig. 2.2B). Four replicates of three fish treatments were randomly assigned to the twelve mesocosms: control (no fish), low-density (range: 2.8-3.3 fish m⁻², 129 ± 18g fish m⁻²), and high density (range: 5.0-5.5 fish m⁻², 253 ± 19g fish m⁻²). At the end of 10 days, water chemistry samples and three rocks were collected from

both grazing treatments (In, Out) in each of the twelve mesocosms. I measured chlorophyll a concentrations and water chemistry (NH_4^+ and SRP using the previously described methods. Water temperature ranged from 28.3 ± 2.3 °C (mean \pm SD) in the mesocosms during the experiment.

I also estimated GPP using a closed-chamber, non-circulating method (Bott et al. 1978, Hill et al. 1997). Briefly, a single rock was placed in a 4.7L clear, air-tight chamber filled with stream water. Dissolved oxygen (DO) content of the water was measured (mg L^{-1}) at time 0 using a YSI 85 Handheld Dissolved Oxygen/Conductivity Instrument (YSI, Yellow Springs, OH). The chamber was incubated in the mesocosm for 60-90 minutes and then DO was measured again to estimate areal NEP. To measure respiration (R), the rock was transferred to a stream-water filled, black chamber and incubated for the same time period. I photographed rocks with a digital camera and rock areas were determined using Adobe Photoshop CS3. Area-specific GPP ($\text{mg oxygen m}^{-2} \text{ hr}^{-1}$) was estimated by adding NEP and R (Bott 2007). I also measured water temperature in mesocosms and the change in fish biomass throughout the study.

Nutrient Diffusing Substrata Construction

Clay pot nutrient diffusing substrata (NDS) were made using methods modified from Capps et al. (2011). Briefly, I filled 144 clay pots with approximately 100mL of agar (controls) or nutrient amended agar (nutrient treatments) and affixed them to plexiglass squares (Con: Control; N: NH_4Cl , P: KH_2PO_4 , N+P: $\text{NH}_4\text{Cl} + \text{KH}_2\text{PO}_4$). I randomly placed one pot of each nutrient treatment into groups the four treatments (Group); half of the pots were exposed to loricariid grazing (Grazed) and

half of the pots was placed in an exclusion constructed from 2.5cm poultry wire that was protected from loricariid grazing (Ungrazed; Fig. 2.2C). Eighteen replicate pairs of groups of grazed and ungrazed pots were randomly divided and were placed in one of three experimental blocks (a total of six pairs per block). The NDS were incubated for 14 days in run habitats in a 50m reach of the Chacamax River. At the end of the 14-day incubation, I harvested all of the pots and collected and analyzed chlorophyll a from the pots using the previously described methods. I also estimated GPP from five pots randomly selected from each of the eight nutrient/grazing combinations using the methods outlined above.

To ensure NDS were still diffusing at the end of the experiment, three additional pots of each treatment were incubated *in situ* for the duration of the experiment. At the end of the 14-day period, pots were harvested from the river and incubated for one hour in three liters of filtered stream water. Diffusion rate estimates were made by subtracting the diffusion rate of nutrient amended pots from the rate of control pots. On day 14 (mean \pm SE (mol m⁻² hr⁻¹) N: $6.8 \times 10^{-5} \pm 1.8 \times 10^{-6}$; P: $5.3 \times 10^{-4} \pm 2.7 \times 10^{-5}$; N+P: (N) $8.2 \times 10^{-6} \pm 1.9 \times 10^{-6}$, (P) $9.2 \times 10^{-5} \pm 1.9 \times 10^{-6}$), most treatments were diffusing several orders of magnitude less than they were on day 0 (mean \pm SE (mol m⁻² hr⁻¹) N: $2.9 \times 10^{-2} \pm 2.8 \times 10^{-4}$; P: $5.3 \times 10^{-3} \pm 7.7 \times 10^{-5}$; N+P: (N) $3.5 \times 10^{-2} \pm 2.6 \times 10^{-4}$, (P) $8.0 \times 10^{-3} \pm 6.9 \times 10^{-5}$).

Statistical Analysis

Nutrient recycling rates and gut content time comparisons (Afternoon/Evening) were made using one-way ANOVAs, where time was the fixed factor. I used a mixed model to estimate the effects of fish size on nutrient recycling

rates, where wet mass was considered the fixed factor and time and wet mass \times time were considered random factors. Hotspot/river comparisons were made using a two-way ANOVA where sample site, year, and the interaction term were considered fixed factors and sample pair (hotspot/river) was considered a random factor.

I analyzed the response variables (chlorophyll a and dry mass) from three rocks in each treatment in the *Pterygoplichthys* enclosure experiment with one-way ANOVAs where treatment (grazed/ungrazed) was the fixed factor and basket number and block (one set of grazed/ungrazed baskets) were considered random factors (Fig. 2.2A). Mesocosm and NDS experiments were analyzed using two-way ANOVAs followed by contrast tests with Bonferroni corrections (Tables 2.1 and 2.2). The fixed effects in both of the ANOVAs were nutrient (NDS: control, N, P, NP; Mesocosm: control, low-density fish, high-density fish), grazing (grazed/ungrazed), and nutrient \times grazing interaction. Mesocosm number and the interaction term were considered random effects in the mesocosm experiment (Fig. 2.2B). Water chemistry samples from each mesocosm were analyzed using a one-way ANOVA followed by Tukey's posthoc tests where nutrient (control, low-density fish, high-density fish) was considered the fixed factor. Experimental block, pair (one set of grazed/ungrazed groups), and group number were considered random effects in the NDS experiment (Fig. 2.2C). All data were \log_{10} transformed to meet the assumptions of the models and analyzed using JMP 9 statistical software (SAS Institute, 2010).

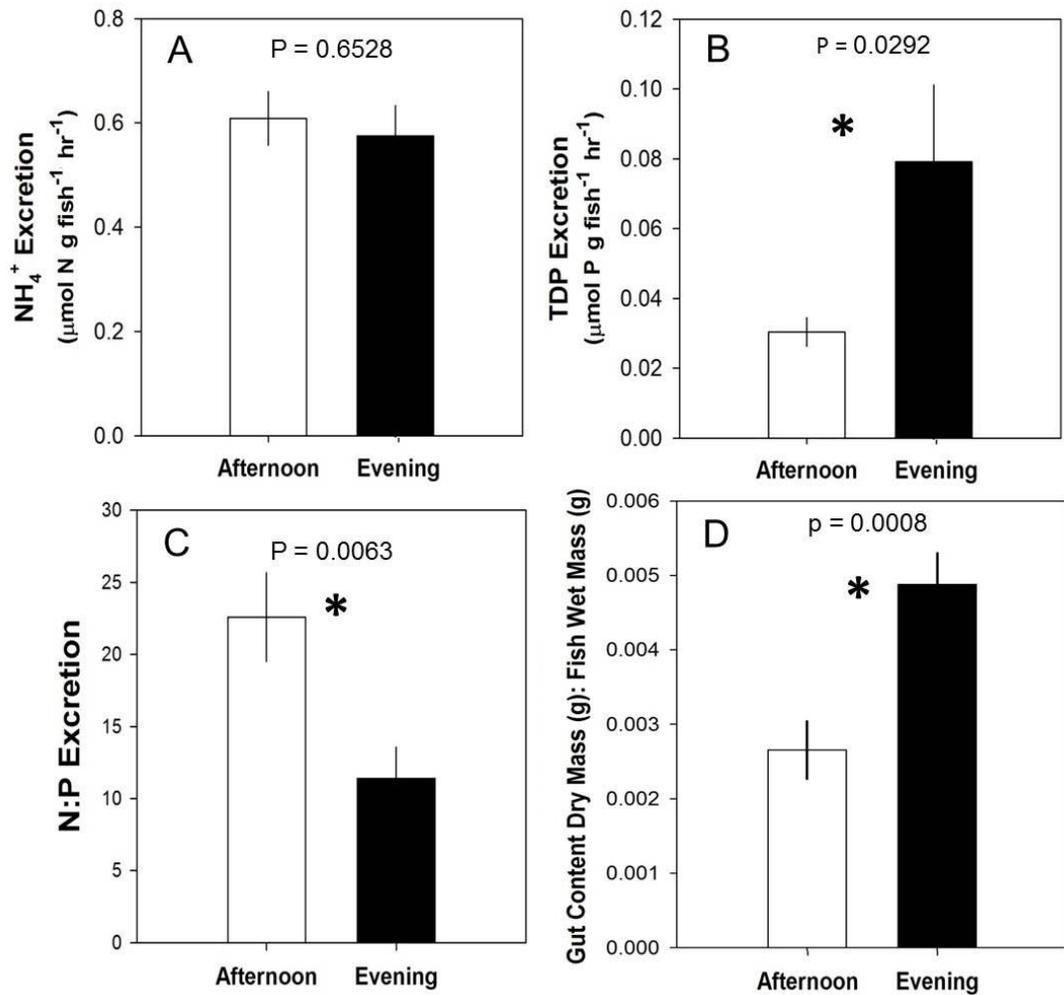


Figure 2.3. Means of *Pterygoplichthys* excretion and gut content mass during afternoon (1000-1500h) and evening hours (1900-0400h). (A) *Pterygoplichthys* NH_4^+ -N excretion rates; (B) *Pterygoplichthys* total dissolved phosphorus excretion rates; (C) N:P of *Pterygoplichthys* excretion; (D) gut content dry mass per wet mass of *Pterygoplichthys*. Error bars represent ± 1 SE.

RESULTS

Nutrient Recycling and Hotspot Observations

Average N excretion was approximately $0.6 \mu\text{mol NH}_4^+\text{-N g wet mass}^{-1} \text{ hr}^{-1}$ and did not differ between the afternoon and evening sample periods ($F_{(1, 18)}=0.209$, $p = 0.6528$; Fig. 2.3A). In contrast, average P excretion was twice as high in samples collected during evening sampling periods (approximately $0.077 \mu\text{mol TDP-P g wet mass}^{-1} \text{ hr}^{-1}$) than those collected in the afternoon (approximately $0.031 \mu\text{mol TDP-P g wet mass}^{-1} \text{ hr}^{-1}$; $F_{(1, 18)}=5.61$, $p=0.0292$; Fig. 2.3B). This resulted in a significant decrease in the N:P ratio of excretion from an average of 23 in the afternoon to approximately 12 in the evening ($F_{(1, 75)}=9.57$, $p = 0.006$; Fig. 2.3C). This pattern may have been driven by nocturnal loricariid feeding behavior, evidenced by more amorphous detritus found in loricariid guts during evening sampling hours ($F_{(1, 75)}=12.09$, $p = 0.0008$; Fig. 2.3D). Loricariid size also influenced excretion rates, as larger fishes tended to excrete less N and P per gram of fish than smaller loricariids ($F_{(1,18)}=7.52$, $p = 0.013$ and $F_{(1, 18)}=6.67$, $p = 0.019$, respectively). However, loricariid size did not influence excretion stoichiometry ($F_{(1, 18)}=0.633$, $p = 0.6966$). By multiplying loricariid excretion rates by their average areal biomass, I estimate that loricariids remineralize approximately $7 \mu\text{mol P m}^{-2} \text{ hr}^{-1}$ during daylight hours, $18 \mu\text{mol P m}^{-2} \text{ hr}^{-1}$ during evening hours, and $135 \mu\text{mol N m}^{-2} \text{ hr}^{-1}$ at both time periods.

Aggregations of loricariids (Fig. 2.1) generated hotspots of nutrient recycling relative to ambient water chemistry in paired river sites. Water samples collected within aggregations of loricariids (hotspots) had 41% higher concentrations of $\text{NH}_4^+\text{-N}$

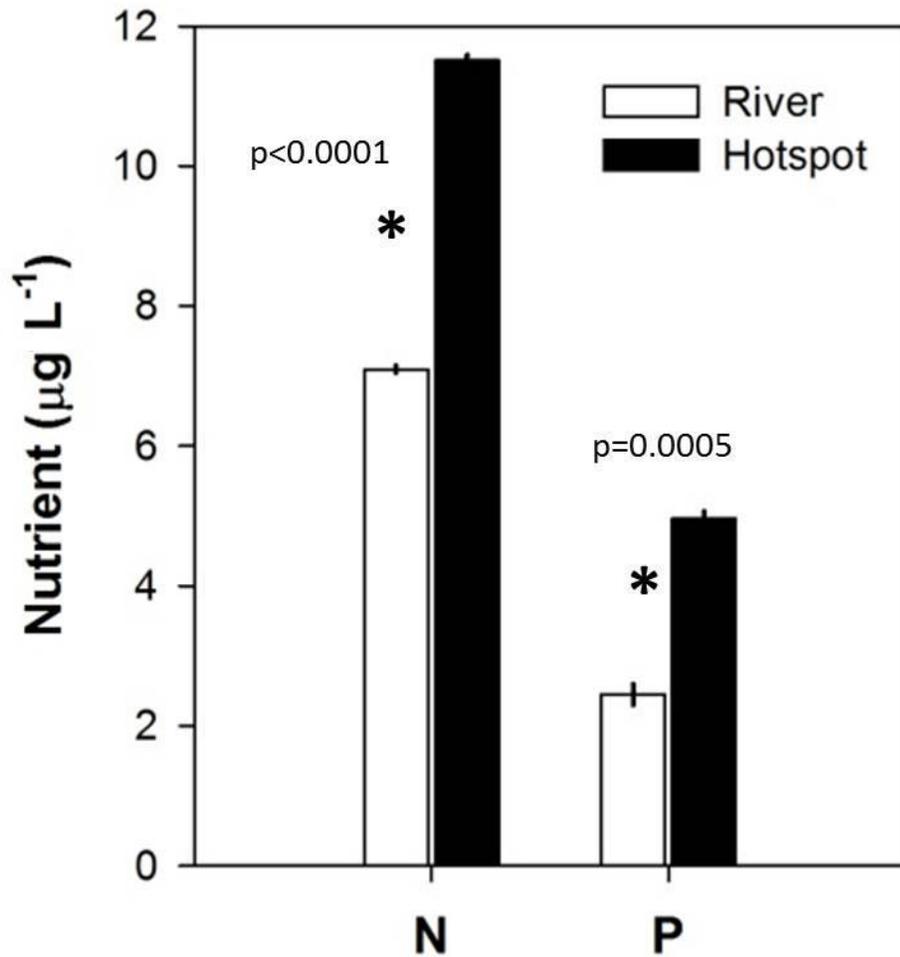


Figure 2.4. Means of $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ (± 1 SE) samples taken from paired sites within and outside of loricariid aggregations in the Chacamax River in 2008 (n=16 river sites, n=16 hotspot sites) and 2010 (n=16 river sites, n=16 hotspot sites). Hotspots were defined as water samples taken within aggregations of *Pterygoplichthys* that had an area of at least 5m^2 with at least 40 *Pterygoplichthys* per m^2 . River samples were collected from sites parallel to the hotspots without immediate upstream aggregations of loricariids.

($p < 0.0001$, $F_{(3, 60)} = 9.63$, Fig. 2.4) and 66% higher concentrations of SRP ($p = 0.0005$, $F_{(3, 60)} = 11.7$, Fig. 2.4) relative to paired river sites.

Experimental Manipulations

Loricariid grazing negatively affected algal biomass and GPP in all of the experimental manipulations. Conversely, when it was measured, exposure to nutrients via remineralization or NDS diffusion enhanced algal biomass and GPP. Initially, in the loricariid enclosure experiment (Fig. 2.2A), grazing significantly reduced algal areal biomass approximately 22%, from $\sim 45 \text{ mg m}^{-2}$ to $\sim 35 \text{ mg m}^{-2}$ and epilithon areal dry mass approximately 50%, from $\sim 22 \text{ g m}^{-2}$ to $\sim 11 \text{ g m}^{-2}$ in cage controls relative to the loricariid enclosures (Fig. 2.5).

Loricariid grazing and nutrient recycling had contrasting effects on algal biomass in mesocosms (Fig. 2.2B). There were significant, negative effects of loricariid grazing ($p < 0.0001$) and a significant interaction between grazing and nutrient remineralization ($p = 0.0011$) on algal areal biomass (Table 1; Fig. 2.6A). Rocks exposed to loricariid grazing and nutrient remineralization in the mesocosm experiments had 55% less algal areal biomass than rocks in the control treatments ($p = 0.0004$; Table 2.1). Conversely, rocks that were exposed to loricariid nutrient remineralization but were protected from grazing had an average of 42% more algal biomass than both rocks in the control treatments ($p = 0.0002$; Table 2.1) and rocks exposed to both grazing and remineralization ($p < 0.0001$; Table 2.1).

The presence of loricariids significantly increased ambient water chemistry in the mesocosm experiments. Ammonium ((mean \pm SD) control: $3.3 \mu\text{g L}^{-1} \pm 1.9$; low:

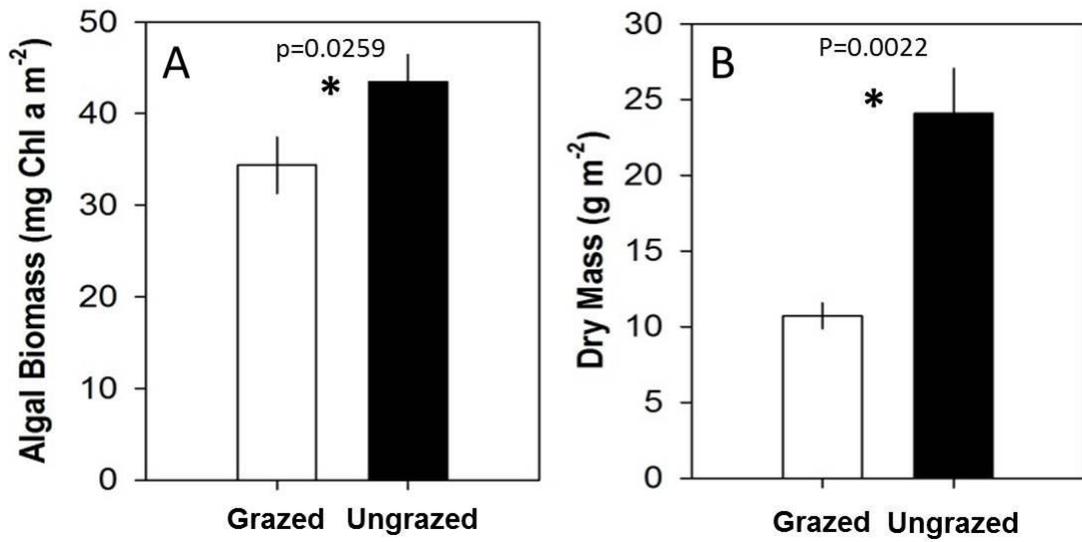


Figure 2.5. Means (± 1 S.E.) for epilithon responses from *Pterygoplichthys* enclosure experiment: (A) chlorophyll a and (B) dry mass (CC: Cage Control; EX: *Pterygoplichthys* Enclosure) within each of the tested responses. The *Pterygoplichthys* Enclosure is represented in black.

Table 2.1. Summary of probabilities for ANOVAs testing overall treatment effects and contrasts with Bonferroni corrections for the mesocosm experiment. Significant results are presented in bold, italicized font.

Mesocosm Experiment			
<i>Algal Biomass (mg m⁻²)</i>			
<i>Whole Model</i>			<i>p-value</i>
Fish	F _(2, 17) =	0.169	0.8452
Grazing	F _(1, 17) =	84.03	<0.0001
Interaction	F _(2, 17) =	10.26	0.0011
<i>Contrast Tests</i>			<i>p-value</i>
Con _(In) + Con _(Out) vs. Low _(In) +High _(In)	F _(1, 17) =	27.49	0.0002
Con _(In) + Con _(Out) vs. Low _(Out) +High _(Out)	F _(1, 18) =	23.46	0.0004
Low _(In) +High _(In) vs. Low _(Out) +High _(Out)	F _(1, 17) =	102.7	<0.0001
<i>GPP (mg O m⁻² h⁻¹)</i>			
<i>Whole Model</i>			<i>p-value</i>
Fish	F _(2, 9) =	4.309	0.0481
Grazing	F _(1, 9) =	7.329	0.0243
Interaction	F _(2, 9) =	2.997	0.1012
<i>Contrast Tests</i>			<i>p-value</i>
Con _(In) + Con _(Out) vs. Low _(In) +High _(In)	F _(1, 12) =	10.87	0.0186
Con _(In) + Con _(Out) vs. Low _(Out) +High _(Out)	F _(1, 13) =	0.1959	1.0000
Low _(In) +High _(In) vs. Low _(Out) +High _(Out)	F _(1, 9) =	10.125	0.0327

Table 2.2. Summary of probabilities for ANOVAs testing overall treatment effects and contrasts with Bonferroni corrections for the nutrient diffusing substrate experiment. Significant results are presented in bold, italicized font.

Nutrient Diffusing Substrate Experiment		
<i>Algal Biomass (mg m⁻²)</i>		
<i>Whole Model</i>		<i>p-value</i>
Nutrient	F _(3, 121) = 8.752	< <i>0.0001</i>
Grazing	F _(1, 2) = 31.49	<i>0.0306</i>
Interaction	F _(3, 121) = 1.681	0.1755
<i>Contrast Tests</i>		<i>p-value</i>
Con _(In) +N _(In) vs. P _(In) +NP _(In)	F _(1, 121) = 18.86	<i>0.0001</i>
Con _(Out) +N _(Out) vs. P _(Out) +NP _(Out)	F _(1, 121) = 2.051	1.0000
Con _(In) +N _(In) vs. Con _(Out) +N _(Out)	F _(1, 3) = 15.76	0.0996
P _(In) +NP _(In) vs. P _(Out) +NP _(Out)	F _(1, 3) = 35.35	<i>0.0313</i>
Con _(In) +N _(In) +P _(In) +NP _(In) vs. Con _(Out) +N _(Out) + P _(Out) +NP _(Out)	F _(1, 2) = 31.49	0.1525
<i>GPP (mg O m⁻² h⁻¹)</i>		
<i>Whole Model</i>		<i>p-value</i>
Nutrient	F _(3,26) = 13.31	< <i>0.0001</i>
Grazing	F _(1, 2) = 0.860	0.4457
Interaction	F _(3, 26) = 4.650	<i>0.0100</i>
<i>Contrast Tests</i>		<i>p-value</i>
Con _(In) +N _(In) vs. P _(In) +NP _(In)	F _(1, 27) = 38.73	< <i>0.0001</i>
Con _(Out) +N _(Out) vs. P _(Out) +NP _(Out)	F _(1, 24) = 1.777	1.0000
Con _(In) +N _(In) vs. Con _(Out) +N _(Out)	F _(1, 4) = 0.787	1.0000
P _(In) +NP _(In) vs. P _(Out) +NP _(Out)	F _(1, 4) = 6.288	0.3590
Con _(In) +N _(In) +P _(In) +NP _(In) vs. Con _(Out) +N _(Out) + P _(Out) +NP _(Out)	F _(1, 2) = 0.860	1.0000

47 $\mu\text{g L}^{-1} \pm 47$; high: 267 $\mu\text{g L}^{-1} \pm 184$) and phosphorus (control: 0.5 $\mu\text{g L}^{-1} \pm 1$; low: 2.5 $\mu\text{g L}^{-1} \pm 0.6$; high: 2.5 $\mu\text{g L}^{-1} \pm 0.6$) were greater in treatment mesocosms relative to the control treatments (N: $F_{(2, 11)} = 20.7$, $p = 0.0004$; P: $F_{(2, 11)} = 9.32$, $p = 0.0064$); however, there was no difference in water chemistry between the high- and low-density fish treatments.

Loricariid grazing and nutrient remineralization significantly affected GPP ($p = 0.0243$ and $p = 0.0481$, respectively) in mesocosms (Table 2.1; Fig. 2.6B), but there was no significant grazing \times remineralization interaction ($p = 0.1012$; Table 2.1; Fig. 2.6B). Contrary to the response of algal biomass, the GPP measured on rocks exposed to loricariid grazing and nutrient remineralization did not differ from the rocks in fishless control treatments ($p = 1.000$; Table 2.1). However, rocks exposed to loricariid nutrient remineralization but protected from grazing had significantly greater GPP than rocks in the control treatments ($p = 0.0186$; Table 2.1), and rocks exposed to both grazing and remineralization ($p = 0.0327$; Table 2.1). This pattern was largely driven by the GPP measured in the high-density loricariid mesocosms (Figure 2.6B).

Notably, loricariid biomass decreased 9.7% \pm 0.9% (mean \pm SD) in both high and low-density mesocosms during the 10-day incubation period, indicating the fishes may have been food-limited or otherwise stressed during the experiment.

Loricariid grazing and the addition of nutrients also affected algal biomass and GPP in the NDS experiment (Fig. 2.2C). There were significant effects of loricariid grazing ($p = 0.0306$) and nutrient treatment ($p < 0.0001$) on algal areal biomass on NDS, though there was interaction effect ($p = 0.1755$; Table 2.2; Fig 2.7A). Nutrient diffusing substrates that were protected from grazing and infused with P (P, N+P) had

significantly more algal areal biomass than control NDS and NDS solely infused with N ($p = 0.0001$; Table 2.2), indicating the primary producers were limited by P availability. These results were supported by an additional NDS deployment in 2010 that demonstrated algae in the Chacamax were P-limited (Appendix 2). However, loricariid grazing eliminated the pattern elicited by nutrient addition, and there were no significant differences between nutrient treatments on the grazed pots ($p = 1.000$; Table 2.2).

Phosphorus addition also stimulated GPP on the NDS. There were significant effects of loricariid nutrient treatment ($p < 0.0001$) and the interaction between grazing and nutrient treatment ($p = 0.0100$) on GPP collected from NDS (Table 2.2; Fig. 7A). However, there was no whole-model effect grazing on GPP ($p = 0.4457$; Table 2.2; Fig 2.7A). Similar to the pattern seen with algal biomass, P-infused NDS (P, N+P) protected from grazing had significantly greater GPP than NDS infused with N and the control NDS in the ungrazed treatments ($p < 0.0001$; Table 2.2), but the pattern was eliminated when the NDS were exposed to loricariid grazing ($p = 1.000$; Table 2.2).

DISCUSSION

In this study, loricariid grazing depressed algal biomass and GPP in mesocosm and *in situ* NDS and enclosure experiments. In contrast, excretion by loricariids generated hotspots of nutrients in the Chacamax River and exposure to nutrients via remineralization by fish or amended nutrients in NDS stimulated primary productivity. Grazing by loricariids overshadowed the stimulation of algal growth by nutrient

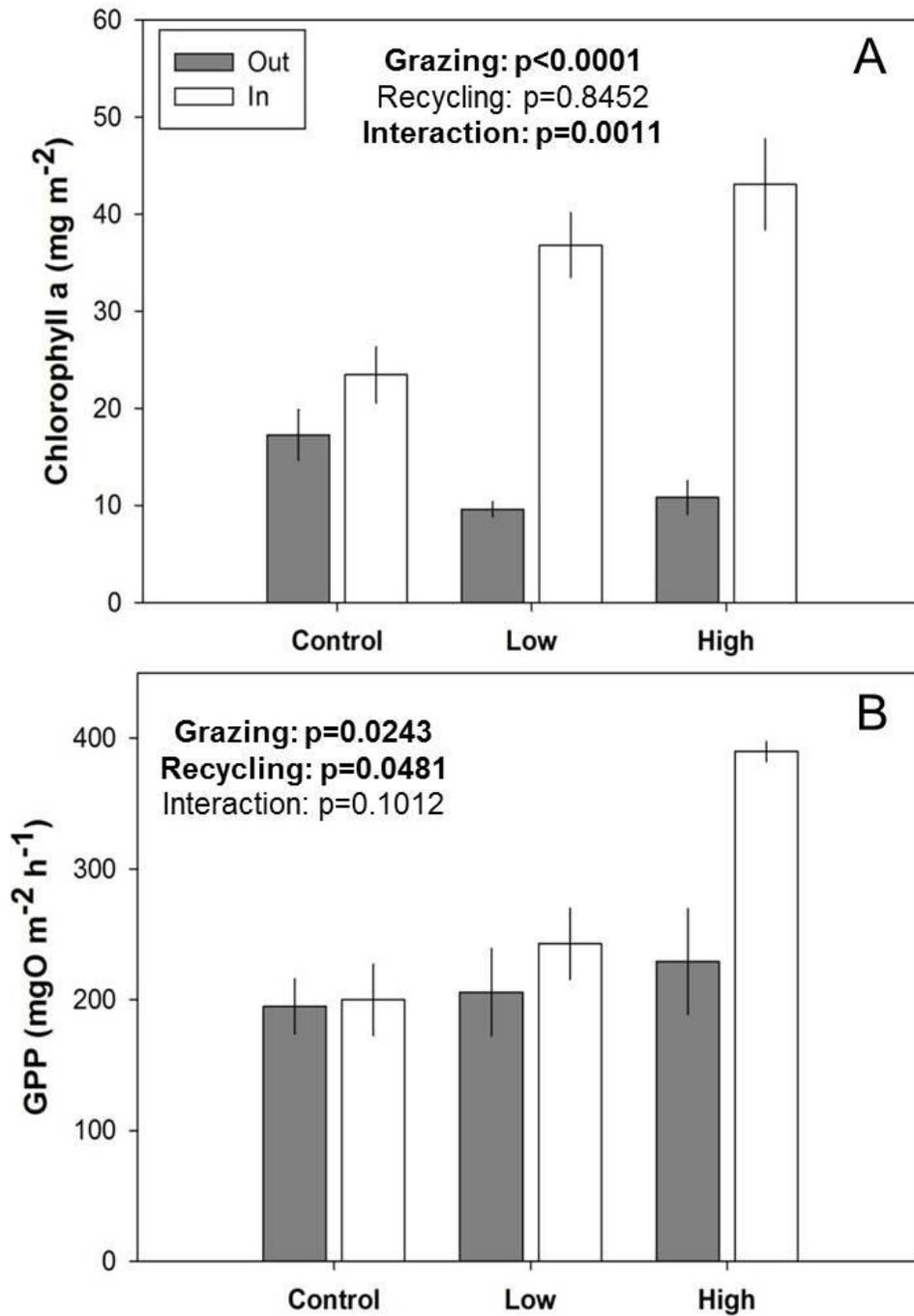


Figure 2.6. Mean (± 1 SE) of (A) algal biomass and (B) Areal GPP measured on rocks in three *Pterygoplichthys*-density treatments in mesocosms (Control (no *Pterygoplichthys*), low (5-6 *Pterygoplichthys*), and high (10-11 *Pterygoplichthys*)).

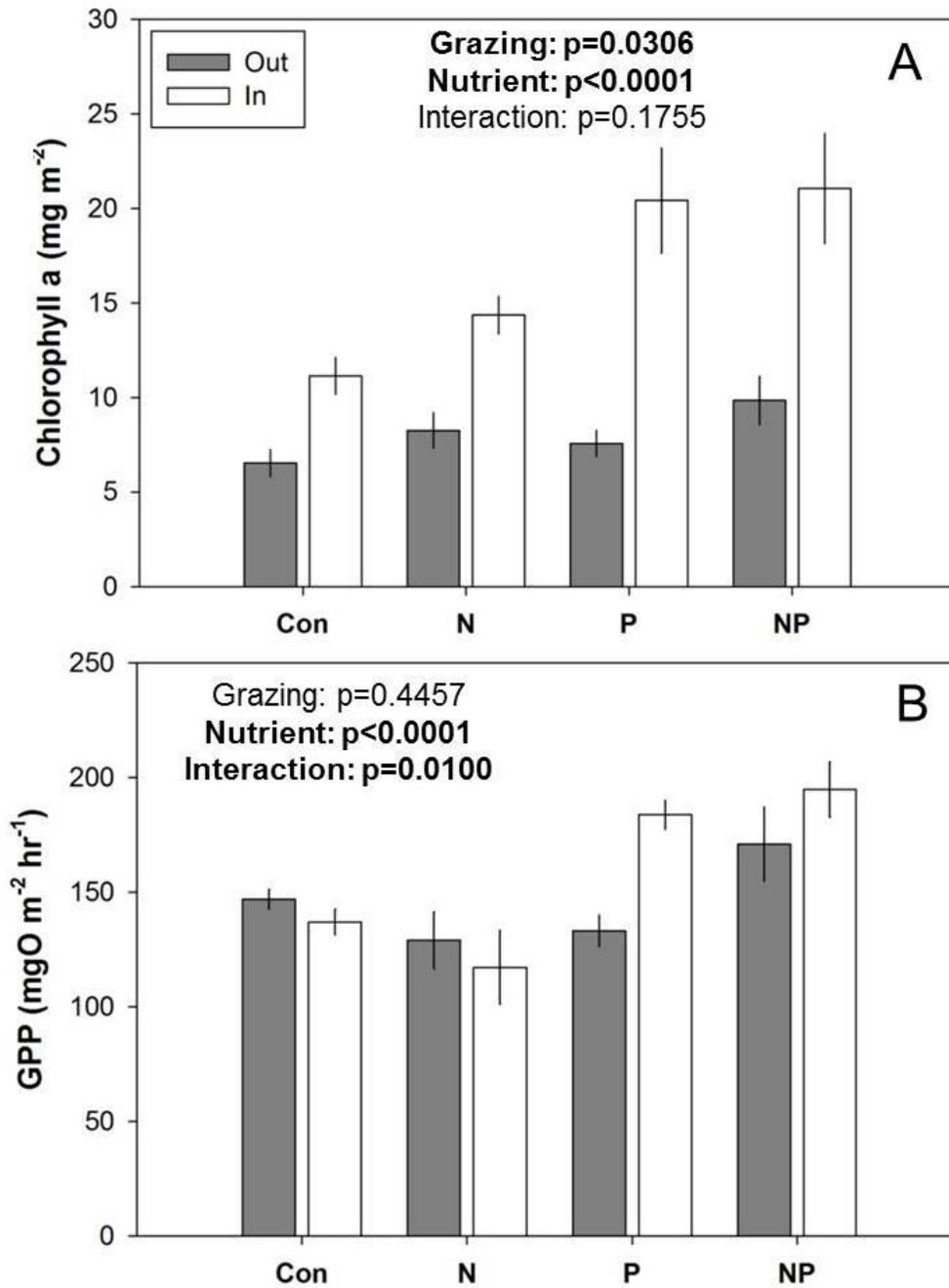


Figure 2.7. Mean (± 1 SE) of (A) algal biomass and (B) collected from grazed (A) and ungrazed (B) nutrient diffusing substrata for each of four nutrient treatments (control (Con), nitrogen (N), phosphorus (P), nitrogen and phosphorus (N + P)).

Remineralization or addition, suggesting introduced loricariids have a negative net-impact on stream algal biomass and GPP.

Consumptive Effects of Loricariid Grazing

In all experiments, grazing by loricariids had substantial, negative effects on algal biomass. These findings agree with myriad investigations of freshwater and marine systems where herbivore removal results in increased algal biomass (Gruner et al. 2008). The results of loricariid grazing on algal productivity were not as clear. In the mesocosm experiment, there was a positive, significant effect of grazer remineralization on areal GPP (Fig. 2.6); however this pattern was only seen in mesocosms with high densities of armored catfish. Conversely, there was no significant effect of grazer exclusion on areal GPP in the NDS experiment. Though I did not always document significant responses to GPP once grazers were removed, this may have been due to the colonization of grazed substrates by highly productive species that function better in environments without nutrient or light limitation as GPP is influenced by algal species identity (Steinman 1996).

Nutrient Remineralization and Biogeochemical Hotspots

My data support findings from other studies documenting the important role consumer nutrient recycling can play in stream ecosystems (McIntyre et al. 2008, Benstead et al. 2010, Reisinger et al. 2011). For example, fish excretion of the limiting nutrient N exceeded N demand in a Venezuelan stream (McIntyre et al. 2008). Similarly, freshwater shrimp excretion was equivalent to approximately 20% of the N uptake and 5% of the P uptake in Puerto Rican streams (Benstead et al. 2010). In my study, excretion of N and P by high densities of loricariids appears to be a large and

Potentially important flux of nutrients in the Chacamax River that is spatially and temporally heterogeneous.

For organisms to generate areas of enriched nutrient recycling rates in space (hotspots) and time (hot moments) across a landscape, their distribution must also change through space and/or time within a landscape (McClain et al. 2003, McIntyre et al. 2008). I observed diurnal aggregating behavior of loricariids (Fig. 2.1) and temporal variation in fish excretion rates (Fig. 2.3), thereby creating a mechanism for armored catfish to generate biogeochemical hotspots and hot moments in the Chacamax River. Additionally, to be important drivers of nutrient cycling within a system, the contribution of nutrient remineralization must be significant at the ecosystem-level (McClain et al. 2003, McIntyre et al. 2008). My data suggest that loricariids strongly influence nutrient remineralization rates in the Chacamax River. Water samples collected within loricariid aggregations had roughly double the concentration of N and P than water collected from outside the aggregations (Fig. 2.4), suggesting that loricariids generate large pulses of nutrients downstream from aggregations during daylight hours when primary producers are photosynthesizing. Additionally, the areal excretion estimates for loricariids were much higher than ambient water chemistry values indicating that remineralization by loricariids may be an important flux of nutrients within the river. Notably, loricariid areal biomass estimates were at least two orders of magnitude larger than the native fishes (Fig. 3.5). Thus, loricariid invasion may have shifted the Chacamax River from a system where fishes were not central drivers of biogeochemical processes to a system where remineralization of nutrients by fishes is a large and important flux of nutrients.

Species-specific characteristics and areal biomass are important factors to consider when predicting if an organism could be a significant driver of nutrient recycling or if they will increase or relax nutrient limitation (McIntyre et al. 2008, Small et al. 2011). Because P-excretion rates are highly variable among fish species (Vanni et al. 2002, Small et al. 2011), P-cycling in P-limited systems, such as the Chacamax River, may be strongly influenced by changes in fish communities (Small et al. 2009). In such a system, the introduction of a P-rich invader such as loricariids (Hood et al. 2005) may intensify P-limitation of primary producers and microbial heterotrophs, and influence nutrient cycling rates in streams. The elemental composition of an invader may also influence the impact of biogeochemical hotspots across an ecosystem. Although loricariid invasion may exacerbate P-limitation throughout the Chacamax River, hotspots of nutrient remineralization created by aggregations of loricariids may supply enough P to locally enhance algal biomass as I demonstrated in the mesocosm experiment. However, loricariid aggregations are not fixed in space or time and they occur in different locations in the river each day. Therefore, any increase in algal biomass or GPP driven by proximity to an aggregation of loricariids is likely removed quickly by nocturnal loricariid grazing and would be difficult to detect.

Well-mixed rivers like the Chacamax add complexity to studying the formation of biogeochemical hotspots, for unlike terrestrial environments, hotspots may not have discrete boundaries. Hence, it is important to note that loricariid remineralization contributed to the samples I collected within and outside of fish aggregations. Ambient water chemistry data on the Chacamax were not collected prior

to loriciid invasion, so I cannot determine the potential additive effects of loriciid nutrient recycling to ambient solute concentrations.

Net Effects of Grazing and Nutrient Remineralization by Loriciids

I observed significant interactions between nutrient supply and grazer cropping of algal biomass in the mesocosm experiment and GPP in the NDS experiment. Our data indicate grazing pressures were too high for nutrient enrichment to compensate for consumption as there was no significant interaction between nutrient supply and grazer presence in the NDS experiment. Similarly, this argument could also be made for algal biomass in the mesocosms where the presence of grazing fishes eliminated all evidence of stimulation by remineralization. These results were similar to those found in McIntyre et al. (2006) where grazing fishes in Lake Tanganyika consumed all additional algal biomass that was stimulated by nutrient diffusion through NDS.

Although I did not analyze changes in algal community composition in response to grazing or nutrient supply, some of the patterns I saw in biomass and GPP could have been due to changes in the algal community composition. For instance, grazers may have selected against long filamentous algae, resulting in a different algal community (Power et al. 1985, Murdock et al. 2010). Conversely, grazing may have selected for algal species that are more productive and result in positive responses in GPP in the presence of grazers (McIntyre et al. 2006).

The nutrient-mediated and consumptive effects of consumers occur simultaneously; hence, it can be challenging to determine how these factors interact with one another to influence the structure and function of algal communities and nutrient fluxes within ecosystems (Knoll et al. 2009). Studies of lakes indicate that the

effects of non-native consumers (i.e., *Dreissena polymorpha*) can mask the consequences of increased nutrient supply (Dzialowski and Jessie 2009). In the P-limited Chacamax River, a non-native, P-rich grazer has had profound effects on ecosystem structure and function that would have been difficult to estimate without separating the top-down and bottom-up influences of this species. Most likely, the nutrient-mediated and consumptive effects of grazers are species and ecosystem-specific, and the strength of these effects is determined by the trophic status and body stoichiometry of the grazer and the nutrient-limitation status of the ecosystem. Therefore, the results from this study highlight the need to conduct species-specific research that separates consumptive and non-consumptive effects of invading organisms to understand the resulting changes to ecosystem structure and function.

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CHAPTER 3

CHANGING NUTRIENT DYNAMICS AFTER INVASION: THE INFLUENCE OF NON-NATIVE FISHES ON THE REMINERALIZATION AND SEQUESTRATION OF NITROGEN AND PHOSPHORUS

ABSTRACT

In both terrestrial and aquatic ecosystems, organisms directly affect nutrient storage and cycling by sequestering nutrients through growth and remineralizing nutrients via excretion and egestion. Research has suggested that fishes can be important drivers of nutrient dynamics in freshwater ecosystems, but much of this work has been focused on understanding the role of native species. Introduced fishes threaten freshwater systems throughout the globe. They can attain high population densities in novel environments and have the potential to restructure nutrient storage and cycling. The purpose of this study was to examine the impact of a non-native grazer (Loricariidae: *Pterygoplichthys*) on nitrogen (N) and phosphorus (P) storage and cycling in an invaded stream ecosystem. Ecological stoichiometry provides a useful framework to study the potential of non-native organisms to play important roles in nutrient dynamics in invaded systems. High densities of P-rich loricariids sequestered large amounts of P in body tissues and produced an important pool of P in the system relative to other major nutrient pools. Loricariids were significantly richer in P than native fish species and this produced significantly different body and excretion stoichiometry than found in native fishes. My results indicated spatially heterogeneous aggregations of loricariids significantly elevated dissolved nutrient concentrations via excretion relative to ambient N and P, creating biogeochemical hotspots in the study site. At a larger spatial scale, my findings suggest loricariids dramatically increased the amount of N and P stored in fish tissue and the rate of N and P remineralized in fish excretion, thereby converting the river from a system where fish nutrient sequestration and remineralization were minimal to a system where fishes were primary drivers of

nutrient dynamics. Finally, my results indicate loricariids may be acting as a net source of N to the water column via excretion but a net sink of P via nutrient sequestration in my study site. My study highlights the importance of taxon-specific research to understand the effects of invasive species nutrient cycling on novel environments.

INTRODUCTION

Animals can be important drivers of nutrient dynamics in terrestrial and aquatic ecosystems (Frank and Evans 1997, Vanni 2002). Animals can influence nutrient storage and remineralization directly and indirectly through nutrient sequestration via consumption and nutrient remineralization via excretion and egestion, subsequently affecting the structure and function of ecosystems (Elser et al. 2000). For example, schools of grunts (*Haemulon flavolineatum* and *H. plumieri*) excrete large quantities of dissolved and particulate nutrients into nutrient-poor waters and can significantly enhance coral growth rates (Meyer et al. 1983). Similarly, in a study of ungulate grazing in Yellowstone Park, Frank et al. (2000) found that ungulates affect primary producers by modifying nutrient cycling rates, creating a mosaic of biogeochemical hotspots, or areas of intensified remineralization across the landscape.

Ecological stoichiometry describes the mass balance of key elements, such as carbon (C), nitrogen (N), and phosphorus (P) in consumer-resource dynamics (Elser and Urabe 1999, Elser et al. 2000, Sterner and Elser 2002). Stoichiometric theory illustrates the relationship between nutrient requirements of a species, nutrient resource availability within ecosystems, and potential competitiveness of a species for a given element. Hence, organismal stoichiometry can be a valuable approach to explain patterns of invasion (Gonzalez et al. 2010) and potentially predict the consequences of

invasion. Ecological stoichiometry also explains how the mass balance of elements in a consumer can potentially affect nutrient pools and fluxes in ecosystems (Cross et al. 2005, Frost et al. 2005, Evans-White and Lamberti 2006). Therefore, stoichiometric theory may also provide a useful framework for predicting and assessing the effects of invading organisms on ecosystem processes in an invaded system.

The ability of a species to affect the storage and cycling of nutrients in an ecosystem is determined by the biotic and abiotic characteristics of the system and the biology and stoichiometry of the species (McIntyre and Flecker 2010). Species with high dietary requirements of a limiting nutrient invading oligotrophic systems where there is intense competition for a limiting nutrient would be expected to alter nutrient dynamics. Additionally, invasive organisms that attain high areal biomass relative to native species may influence nutrient dynamics due to their ability to sequester or remineralize large quantities of nutrients at higher rates than native species. For instance, large populations of non-native alewives restructured phosphorus storage in the Great Lakes (Kitchell et al. 1975, Kraft 1993). Species that attain high population biomass and that are stoichiometrically different than native species would also be expected to alter nutrient dynamics after invasion. In a study examining native grazing fishes and tadpoles within their native ranges, Knoll et al. (2009) found species-specific body stoichiometry differentially influenced rates of N and P remineralization that subsequently affected algal stoichiometry in mesocosms. That is, tadpoles with high N requirements recycled N at much lower rates, whereas grazing catfish with high P requirements recycled much less P per unit biomass (Knoll et al. 2009). Finally, invasive species that have high dietary requirements for the limiting nutrient and are

stoichiometrically imbalanced with their food would be expected to have greater effects on ecosystem structure and nutrient cycling than organisms consuming stoichiometrically similar food items. Such species would be expected to consume a large amount of food to meet their minimum requirements of limiting nutrients. Herbivorous and detritivorous species are typically stoichiometrically imbalanced with their food (McIntyre and Flecker 2010). They compensate for this disparity by consuming large quantities of plant matter and detritus and recycling non-limiting nutrients via excretion and egestion (Elser 2006). Stoichiometric theory suggests invading organisms with all of the aforementioned characteristics have the potential to restructure the chemical environment of an ecosystem by altering nutrient storage, cycling, and demand.

Grazing organisms can also indirectly influence nutrient dynamics. By stimulating or retarding the growth and production of primary producers and microbes through nutrient remineralization or consumption, grazing organisms have the potential to alter nutrient demand and nutrient uptake rates in an ecosystem (Gruner et al. 2008).

Recent investigations of the role of fishes in freshwater ecosystem function demonstrate that fishes can play important roles in biogeochemical cycling through nutrient sequestration (e.g., Griffiths 2006, Sereda et al. 2008) and nutrient remineralization (e.g., Vander Zanden and Vadeboncoeur 2002, Vanni et al. 2002, McIntyre et al. 2008). Fishes can be important sinks of nutrients in freshwater systems (Kraft 1993, Sereda et al. 2008). For example, Sereda et al. (2008) found that fish biomass retained more than 50% of the epilimnetic P in two lakes in southern Ontario. Nutrient remineralization by fishes can also be important in freshwater systems (Vanni

et al. 2002, McIntyre et al. 2007, McIntyre et al. 2008, Small et al. 2011). In a study examining the influence of a native fish assemblage on N and P cycling in a tropical river, McIntyre et al. (2008) found that the aggregate excretion of fishes was sufficient to turn over the entire ambient pool of N, the nutrient limiting primary productivity, in the water column in less than 0.3km. Though previous investigations have linked ecosystem processes such as biogeochemical cycling with native fishes (e.g., McIntyre et al. 2007, McIntyre et al. 2008, Reisinger et al. 2011, Small et al. 2011), few studies have demonstrated how the effects of fish invasion alter these processes (but see, Kitchell et al. 1975, Kraft 1993, Bunnell et al. 2005).

In this study, I examined the role of a non-native suckermouth armored catfish (Loricariidae: *Pterygoplichthys*) in nutrient storage and cycling in an invaded stream ecosystem in southern Mexico. Armored catfishes are bottom-dwelling fishes native to South and Central America (Weber 1991, Nico and Martin 2001). Loricariids are covered with a bony-plated armor that is rich in phosphorus (P); therefore, they typically have high body P content relative to other fish species (Vanni et al. 2002, Hood et al. 2005). These fishes are common in the aquarium trade, and are frequently released and become established in warm freshwater bodies throughout the globe (Courtenay et al. 1986, Fuller et al. 1999, Nico and Martin 2001, Alecke et al. 2005, Liang et al. 2005, Chavez et al. 2006, Kailola 2007, Ozdilek 2007, Hossain et al. 2008, Keszka et al. 2008, Sinha et al. 2010, Capps et al. 2011). *Pterygoplichthys* (Siluriformes: Loricariidae) feed on organic matter, including algae and fine detritus (Fuller et al. 1999), and are thought to compete with native fishes for basal food

resources and space (Nico and Martin 2001). *Pterygoplichthys* were first documented in my study site, the Chacamax River, in 2004 (Wakida-Kusunoki et al. 2007).

The purposes of this study were fourfold. Initially, I compared the role of loricariids in nutrient sequestration relative to other nutrient pools in the system. Secondly, I determined whether loricariids were stoichiometrically different from the most common native fish species and assessed whether these differences led to a change in the role of fishes in biogeochemical cycling via nutrient remineralization in the ecosystem. Third, I estimated whether diel changes in loricariid behavior could generate biogeochemical hotspots and hot moments in the river. Lastly, I estimated whether loricariids functioned as a net source of nutrients via excretion or a net sink of nutrients via sequestration in body tissues, and I evaluated whether their effect on storage and cycling could be important at larger spatial scales. I predicted high densities of P-rich loricariids would sequester large amounts of P in body tissues and create an important pool of P relative to other nutrient pools in the system. I also posited loricariids would be significantly richer in P than native fish species and this characteristic would yield significantly different body and excretion stoichiometry than native fishes.

Aggregations of native fish assemblages can generate biogeochemical hotspots in stream ecosystems (McIntyre et al. 2008); therefore, I hypothesized that loricariid aggregations would create mosaics of biogeochemical hotspots in invaded river systems. Moreover, I predicted high densities of loricariids would convert the river from a system where fish biomass and remineralization did not play an important role in nutrient dynamics to a system where fishes were one of the primary drivers of nutrient

cycling. Finally, I predicted that the growing, dense population of loricariids would create a net sink for nitrogen (N) and P and alter nutrient dynamics throughout the river.

METHODS

Study Site--Field work was conducted in the Chacamax River (N17°29'047" W91°58'430") in Chiapas, Mexico during the dry season months of March-May of 2008-2010 (Appendix 1). Base flow averaged $\sim 1,600 \text{ L s}^{-1}$ during the study and ambient nutrient concentrations were typically moderate to low (average values: NH_4^+ -N, $10 \mu\text{g L}^{-1}$; NO_3^- -N, $353 \mu\text{g L}^{-1}$; soluble reactive phosphorus (SRP), $< 2 \mu\text{g L}^{-1}$; total dissolved nitrogen, $387 \mu\text{g L}^{-1}$; total dissolved phosphorus, $3 \mu\text{g L}^{-1}$), and water temperature ranged from 21 to 28°C. Nutrient diffusing substrates indicated the growth of primary producers in the Chacamax was limited by phosphorus availability (Appendix 2). *Pterygoplichthys* were first documented in the Chacamax River in 2004 (Wakida-Kusunoki et al. 2007), and at least two species have been found (*P. pardalis* Castelnau, 1855, *P. disjunctivis* Weber, 1991, as well as potential hybrids (Nathan Lujan, *personal communication*); thus, we refer to these fishes collectively as *Pterygoplichthys* or loricariids (Appendices 3 & 4).

To elucidate the effects of loricariids on nutrient storage and cycling, we estimated the amount of carbon (C), N, and P stored in loricariids relative to other nutrient pools in the system. Additionally, we estimated the flux of NH_4^+ -N and SRP produced by loricariids relative to native fishes and the ambient concentrations in the water column.

Fish Behavior and Nutrient Storage—To describe diel patterns in *Pterygoplichthys* behavior, I counted the number of fish found in five $1\text{m} \times 1\text{m}$ quadrats

along the edge of a 100m reach of stream during daylight and evening hours. I counted *Pterygoplichthys* every four hours over a period of three days in March 2010. To compare the density of *Pterygoplichthys* to native fishes, I conducted snorkeling surveys on two dates (10 March 2010 and 05 May 2010) along transects in a 550m reach of stream using methods modified from Thurow (1994). Briefly, I divided the reach into 25m sections. Two snorkelers traveled upstream at an angle from one end of the section to the other, creating a zigzag pattern and crossing the stream twice every 50m. I counted all fishes within 1m of both sides of each transect and the average values from both snorkelers for each transect were used to make density estimates. Individual fishes were collected for C, N, P analysis using standard electroshocking (ABP-3-600 Electrofishing Backpack System, Electrofishing, LLC, Verona, Wisconsin) and seining techniques (Hicks 2003, Hauer and Lamberti 2006) and they were euthanized using an overdose of MS-222 (IACUC protocol number 2006-0169 from Cornell University).

I assessed nutrient storage by *Pterygoplichthys* relative to other major nutrient pools in the Chacamax River ecosystem on two sample dates (09 March 2010 and 04 May 2010). I chose these dates to bracket the dry season in 2010 that ranged from approximately 01 March 2010 to 15 May 2010. On each of these dates, I collected three subsamples of epilithon, macroinvertebrates, and benthic organic matter (BOM) from each of three runs and three riffles. Epilithon from three rocks was collected using wire brushes. Aliquots (10mL) of the resulting slurry were filtered on to a 47 mm pre-ashed and pre-weighed Gelman A/E filter (Gelman, Ann Arbor, Michigan) to estimate ash-free dry mass (AFDM) and dry mass. Any remaining slurry was frozen for C, N, and P

analysis. Digital photos were taken of each rock and rock areas were determined using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, California). I collected the subsamples of macroinvertebrates using a Surber sampler (0.092 m², 250µm mesh size) (Wildlife Supply Company, Yulee, FL). To estimate standing stocks of BOM, I placed a plastic cylinder (diameter = 26.7cm) over three sections of streambed in each transect. I measured the depth at five locations within the cylinder to calculate average depth. I vigorously disturbed the sediment and rocks, collected a one liter sample of the water within the cylinder, and filtered a subsample of the water (100-500mL) onto pre-ashed, pre-weighed filters using the methods described above and dried the remaining sediment for C, N, and P analysis. I collected five subsamples of coarse particulate organic matter (CPOM) from each run and riffle by placing a weighted PVC quadrat (0.092 m²) on the streambed and harvested all of the CPOM within the quadrat. CPOM samples were dried, weighed, and ground and subsamples were analyzed for C, N, and P content.

To measure the C, N, and P content of the major nutrient pools, samples were dried to a constant mass at 45°C, weighed, and ground to a fine powder for elemental analysis. For C and N, ~2-8 mg of dried material was analyzed using an Elementar Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). For particulate P analysis, subsamples of ~1-2 mg of material were combusted at 500°C, digested with 1 N HCl for 2 h, and the digested solution was analyzed spectrophotometrically (Shimadzu UV 1240; Shimadzu Scientific Instruments, Columbia, Maryland) using the molybdate-blue method (Murphy and Riley 1962).

Total mass of C, N, and P were estimated by multiplying area-specific dry mass by the percentage of nutrients found in the epilithon samples.

Nutrient Dynamics—Nutrient demand of primary producers and the microbial community was estimated using nutrient additions in 2010 after methods outlined in Hall and Tank (2003). We measured NH_4^+ and SRP uptake in the river to evaluate the potential of loricariid nutrient recycling to meet ecosystem nutrient demand. Thus, in April 2010 I conducted two additions of NH_4^+ (NH_4Cl) and two additions of PO_4^{-3} (KH_2PO_4) using NaBr as a conservative tracer. Each addition lasted ~ 3h. On both sample dates, we conducted one NH_4^+ and one PO_4^{-3} release. I measured uptake as the difference between baseline water column nutrient concentrations and plateau water column nutrient concentrations values of solute concentrations at 10 stations along a 1 km reach of the Chacamax River (Station distance: 78 m, 103 m, 128 m, 178 m, 278 m, 378 m, 478 m, 578 m, 778 m, 978 m downstream from the pump). On each of the two sample dates, I conducted one NH_4^+ and one PO_4^{-3} release. To document diel fluctuations in water chemistry, I collected duplicate water samples from the thalweg of the stream in single a run habitat for NH_4^+ and PO_4^{-3} analysis every four hours on three dates in 2010.

Fish excretion measurements—Fish nutrient recycling rates were estimated based on the difference in dissolved N and P concentrations between plastic tubs incubated with and without fishes (Vanni et al. 2002, McIntyre et al. 2008). Fishes were collected for recycling measurements using a backpack electroshocker (Smith-Root Model 15-C; Smith-Root, Inc. Vancouver, WA 98686, USA), seines, and dip nets. After collection, I immediately incubated five individuals from the seven most common

native fish genera (*Astyanax*, *Monopterus*, *Poecillia*, *Rhamdia*, *Theraps*, *Thorichthys*, and *Vieja*,) and five *Pterygoplichthys* in 10L plastic tubs for 1h. Each tub was filled with 2-7L of stream water that was filtered twice through a 125 μm sieve, covered with 90% shade cloth, and placed in the shade for the duration of the incubation. At the end of the incubation, I collected water samples for NH_4^+ and soluble reactive phosphorus (SRP) analysis.

Water samples were filtered through glass-fiber filters (Gelman A/E) to remove particles and feces. Water samples collected for P analysis were acidified with 2N H_2SO_4 (< pH 2) for preservation and shipped to the USA for analysis. I employed standard colorimetric methods to analyze P samples (APHA 1998) using a Lachat QuickChem 8000 (Lachat Instruments, Loveland, Colorado). All NH_4^+ samples were refrigerated and analyzed in the field using the flurometric methods outlined by Taylor et al. (2007).

To understand the potential contributions of loricariid and native fish excretion relative to other nutrient pools within the Chacamax River, I calculated volumetric fish excretion using the equation: $E_V = E_A \times A \times T \times V^{-1}$, where E_A was the areal excretion rate ($\text{mol nutrient m}^{-2} \text{ h}^{-1}$), A was reach area (m^2), T was the travel time through each reach, and V was volume of water in the stream reach (m^3) (McIntyre et al. 2008, Benstead et al. 2010). E_V , as described by McIntyre et al. (2008), estimates the mean addition of nutrients excreted by fishes to the water column as it moves along a given reach. E_V assumes perfect mixing and no uptake along the reach (McIntyre et al. 2008). I also calculated excretion turnover distance, or the distance required for excretion to completely replace the ambient nutrient pool, by dividing the ambient nutrient

concentration by E_v and multiplying by reach length. I employed a 100 m reach length in my calculations after Benstead et al. (2010). Loricariids were uncommon in riffle habitats during daylight and evening hours, so I assumed that only 10% of the total loricariid biomass was found in riffles along the 550m reach at any given time. To estimate the effects of aggregating behavior on how E_v changed through the stream reach between daylight and nighttime, I identified and measured the locations of loricariid aggregations and estimated the area covered by loricariid aggregations larger than 15m² within the reach on two sample days in 2010. I incorporated the locations and aggregation biomass estimates into the daytime E_v estimates. Loricariids excrete less P during evening hours (daytime: 0.03 $\mu\text{mol g fish}^{-1} \text{ hr}^{-1}$; nighttime: 0.08 $\mu\text{mol g fish}^{-1} \text{ hr}^{-1}$; $p=0.029$ (total dissolved phosphorus)), but there is no diel variation in N excretion (daytime and nighttime average: 0.81 $\mu\text{mol g fish}^{-1} \text{ hr}^{-1}$; $p= 0.653$) (K. Capps, Fig. 2.3). Therefore, I calculated both day and night excretion values for P but only a single N excretion value to estimate E_v .

To evaluate whether loricariids were acting as sources or sinks of nutrients in the Chacamax River, I subtracted the amount of nutrients produced via nutrient remineralization from the amount of nutrients stored in loricariid tissues through growth between 2008-2010. To make these calculations, I assumed there was little migration from downstream habitats.

Statistical Analysis- To estimate the change in cross-species relationships between nutrient excretion and body mass and nutrient excretion and body nutrient content after *Pterygoplichthys* invasion, I performed linear regressions with and without *Pterygoplichthys* included in the calculations. I analyzed the effect of taxon on fish

body nutrient concentration and fish excretion rate (per gram of fish) using a generalized linear model (PROC GLM), with Tukey's adjustment for multiple comparisons. All data were \log_{10} transformed to address non-uniform variance. Fish body and excretion data were analyzed using SAS 9.2 and all other analysis were conducted using JMP 9 statistical software (SAS Institute, 2010).

RESULTS

Fish Behavior and Density—Loricariids formed large aggregations in the main channel of the Chacamax River during the day, but spread out to graze the entire riverbed at night (Figures 3.1-2). Concurrent increases in ambient NH_4^+ occurred at night when loricariids were broadly dispersed and most active; however, there was no similar increase in ambient PO_4^{3-} (Fig. 3.2A). Importantly, all of the PO_4^{3-} samples were at or near detection; thus, any change in ambient levels would have been difficult to detect.

Loricariid density in the Chacamax River increased from approximately 1.2 fish m^{-2} in 2008 to 3.4 fish m^{-2} in 2010. Additionally, there was a significant change in the size of individual *Pterygoplichthys* counted during surveys between 2008 -2010, shifting from a population dominated by small individuals (<15cm standard length (SL)) in 2008 and 2009 ($F_{(2,6)}=22.96$, $p=0.0015$) to a population primarily composed of medium-bodied fishes (15-25cm SL) in 2010 ($F_{(2,6)}=23.29$, $p=0.0015$). Increasing *Pterygoplichthys* density and average size resulted in a gain in *Pterygoplichthys* areal biomass from approximately 50g m^{-2} in 2008 to approximately 225g m^{-2} in 2010 (Fig. 3.3A-B).

Body Size and Nutrient Storage—Loricariids were approximately 10 times larger in biomass (mean: 101 g) than the common native fish genera (mean: 11g) ($p < 0.0001$, $F_{(7, 32)} = 27.13$). Additionally, they had significantly lower body C ($p < 0.0001$, $F_{(7, 32)} = 10.54$; Fig. 3.4A) and N ($p < 0.0001$, $F_{(7, 32)} = 27.55$; Fig. 3.4B) concentration relative to the other fishes I included in my comparison (C mean: 32% versus 41% and N mean: 8% versus 11%, respectively). Notably, the mean *Pterygoplichthys* body carbon values are some of the lowest reported for freshwater fishes (McIntyre and Flecker 2010). Conversely, *Pterygoplichthys* were almost twice as rich in P (mean: 5.7%) relative to the other fishes (mean: 3.3%) sampled ($p < 0.0001$, $F_{(7, 32)} = 12.01$; Fig. 4C).

Although there were no significant differences in molar C:N ($p = 0.0856$, $F_{(7, 32)} = 2.00$; Fig. 3.4D) among fish genera, loricariids had significantly lower molar C:P ($p < 0.0001$, $F_{(7, 32)} = 12.63$; Fig. 3.4E) and molar N:P ($p < 0.0001$, $F_{(7, 32)} = 19.59$; Fig. 3.4F) than native fishes. Loricariid molar C:P and N:P ratios averaged 5.5 and 1.4 respectively, compared to the 13.1 and 3.5 for native fishes.

Relative to other biotic pools in the Chacamax River (dominant native fishes, macroinvertebrates, CPOM, BOM, and epilithon), loricariids comprised a large amount of the biomass and stored a substantial proportion of the nutrients we measured (Fig. 3.5). I estimated that *Pterygoplichthys* stored 20 g N m^{-2} (Fig. 3.5C) and 10 g P m^{-2} (Fig. 3.5D), or approximately 64% of the total N and 97% of the total P in pools I measured. By multiplying the change in areal biomass between 2008 (Fig. 3.3B) by the average body nutrient composition of *Pterygoplichthys* (Fig. 3.4B-C), I estimated loricariids sequestered 16.7 g N m^{-2} and 12.3 g P m^{-2} in their body tissue during the two-year study period at a rate of approximately $1,440 \text{ } \mu\text{mol N m}^{-2} \text{ day}^{-1}$ and $480 \text{ } \mu\text{mol P m}^{-2} \text{ day}^{-1}$.

Nutrient Recycling—Fish body size is known to affect nutrient excretion rates (Vanni et al. 2002, McIntyre et al. 2008) and loricariids were significantly larger than the native fishes I included in excretion trials; hence, I compared excretion rates per gram of fish to account for the size differences. Overall, larger fishes tended to excrete less N and P per gram of fish relative to smaller fishes (Fig. 3.6A, C). *Pterygoplichthys* excreted significantly less N per gram of body mass compared to all other genera measured except the swamp eel, Synbranchidae: *Monopterus* sp. ($p < 0.0001$, $F_{(7, 32)} = 20.83$). However, P excretion per gram of *Pterygoplichthys* was less than the characid, *Astyanax aeneus*, the molly, *Poecilia mexicana*, and the native pimelodid catfish, *Rhamdia guatemalensis*, but did not significantly differ from the other fishes I sampled ($p < 0.0001$, $F_{(7, 32)} = 23.50$). *Pterygoplichthys* excretion ratio of N to P was significantly less than the cichlid, *Theraps* sp., and significantly greater than *Astyanax aeneus*, but did not differ from the other genera sampled ($p < 0.0001$, $F_{(7, 32)} = 23.50$).

The cross-species relationship between P excretion and body mass and body P content remained significant with or without *Pterygoplichthys* included in the analysis, but the relationship was stronger when *Pterygoplichthys* was included (Table 3.1; Fig. 3.6 C, D). However, this was not the case with N excretion. When *Pterygoplichthys* were included in the N analysis, the relationships between N excretion and body mass and body N content were significant (Table 3.1; Fig. 3.6A, B); yet, when they were excluded from the analysis, there was no relationship between N excretion and N body content (Table 3.1). The relationships of N to P excretion ratio to body mass and to body N to P ratio were also significant (Table 3.1; Fig. 3.6 E- F). However, these

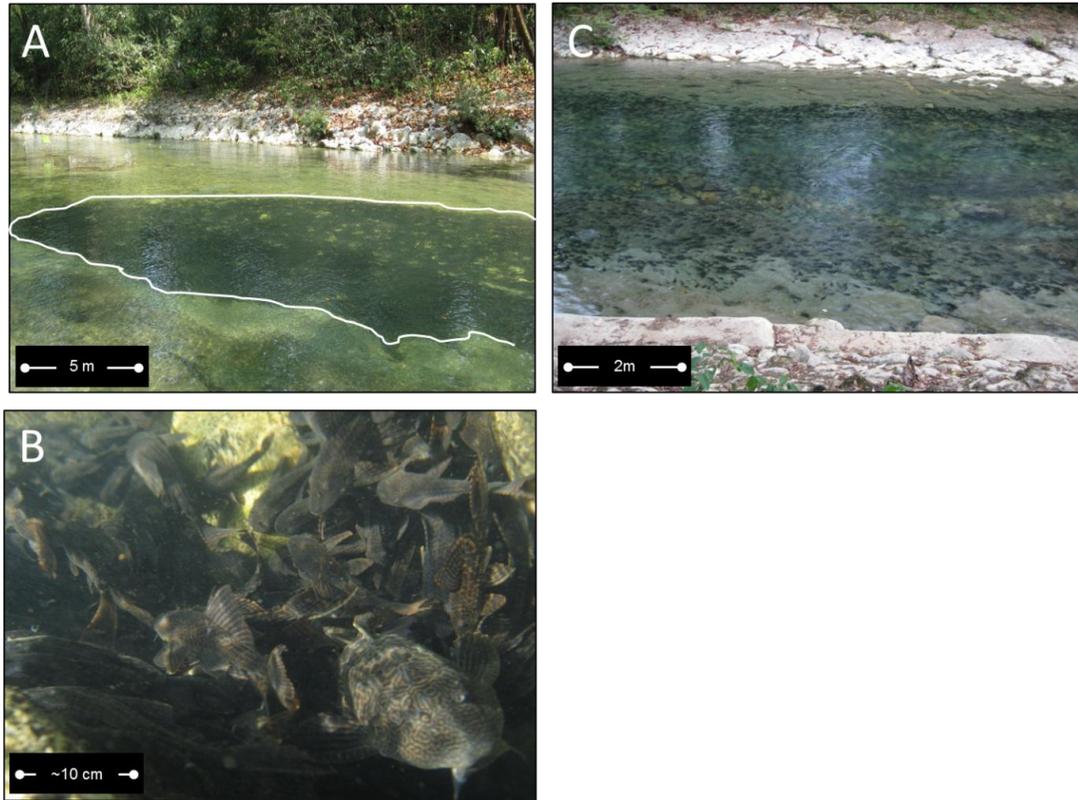


Figure 3.1. *Pterygoplichthys* in the Chacamax River (N17°29'047" W91°58'430"). (A) Daytime aggregation of loricariids. The white line outlines the aggregation boundary. (B) Underwater photo of loricariid aggregation. (C) Loricariids spreading out from aggregation to begin evening feeding. Each dark spot (C) is at least one *Pterygoplichthys*. Photo credit: K. A. Capps.

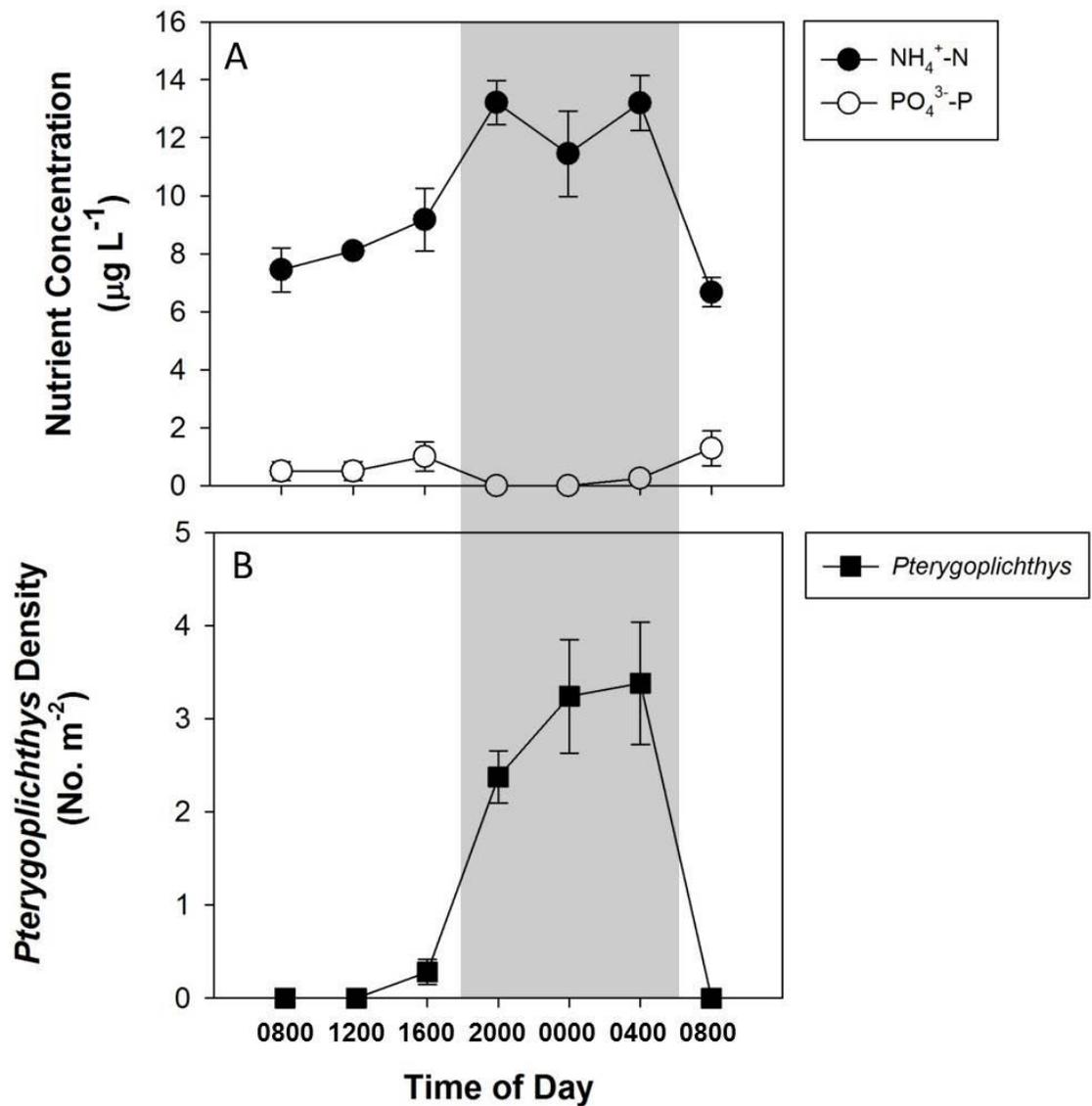


Figure 3.2. Diel water chemistry values and *Pterygoplichthys* behavior data collected in 2008 and 2010 (± 1 SE). (A) $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ concentrations over time; (B) number of *Pterygoplichthys* counted in 1m^2 quadrats near the stream bank (within 24 cm) over time. The shaded areas represent night-time sampling hours.

relationships were stronger when *Pterygoplichthys* was removed from the analysis (Table 3.1).

Daytime areal excretion estimates indicated that loricariids excreted more than 25 times the amount of N ($191 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ vs. $7.5 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and P ($4.5 \mu\text{mol P m}^{-2} \text{hr}^{-1}$ vs. $0.18 \mu\text{mol P m}^{-2} \text{hr}^{-1}$) than what was excreted by the native fish assemblage.

Nutrient Limitation and Demand—Volumetric excretion (E_V) of N and P by loricariids was much greater than the native fish assemblage (Figure 3.7A-B) and differed according to time of day. Hence, the N:P ratio of loricariid E_V increased during the day when primary producers are actively taking up N and P (Figure 3.7A-B). Additionally, the aggregating behavior of loricariids creates pulses of nutrient flux along a stream reach during the day, whereas excretion at night was relatively constant throughout the stream when loricariids are spread out and actively grazing (Fig. 3.1A-B, Fig. 3.7 A-B). Average nutrient uptake rates in the Chacamax were approximately $75 \mu\text{mol NH}_4^+\text{-N m}^{-2} \text{hr}^{-1}$ and $7 \mu\text{mol PO}_4^{3-}\text{-P m}^{-2} \text{hr}^{-1}$. Consequently, loricariid excretion was equivalent to approximately 255% of the NH_4^+ and 70% of the P demand in the Chacamax. By contrast, excretion by native fishes was equivalent to approximately 10% of the NH_4^+ and 3% of the P demand (Fig. 3.8A). Moreover, loricariids also reduced the fish excretion turnover distance of NH_4^+ and P by more than 95% from 21 to 0.8 km NH_4^+ and 102 to 4.06km P (Fig. 3.8B).

To estimate whether loricariids were having greater sequestration or remineralization effects on nutrients in the Chacamax, I subtracted the nutrients

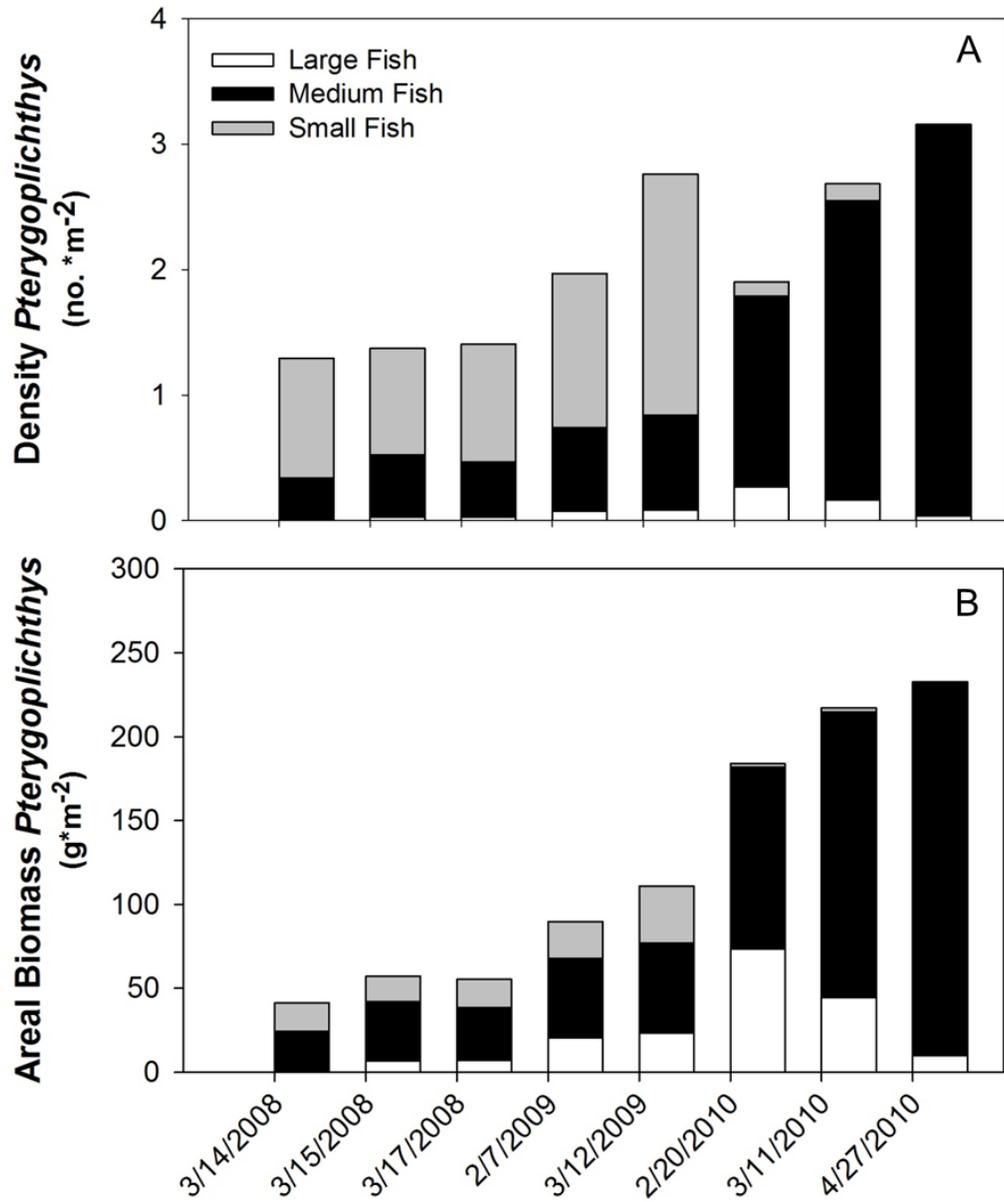


Figure 3.3. *Pterygoplichthys* counted in surveys of a 550m reach of the Chacamax River (N17°29'047" W91°58'430") between 2008 and 2010. (A) Density of *Pterygoplichthys*. (B) Areal biomass of *Pterygoplichthys*. Small fish (<15cm SL) are represented in grey, medium fish (15-25cm SL) in black, and large fish (>25cm SL) in white.

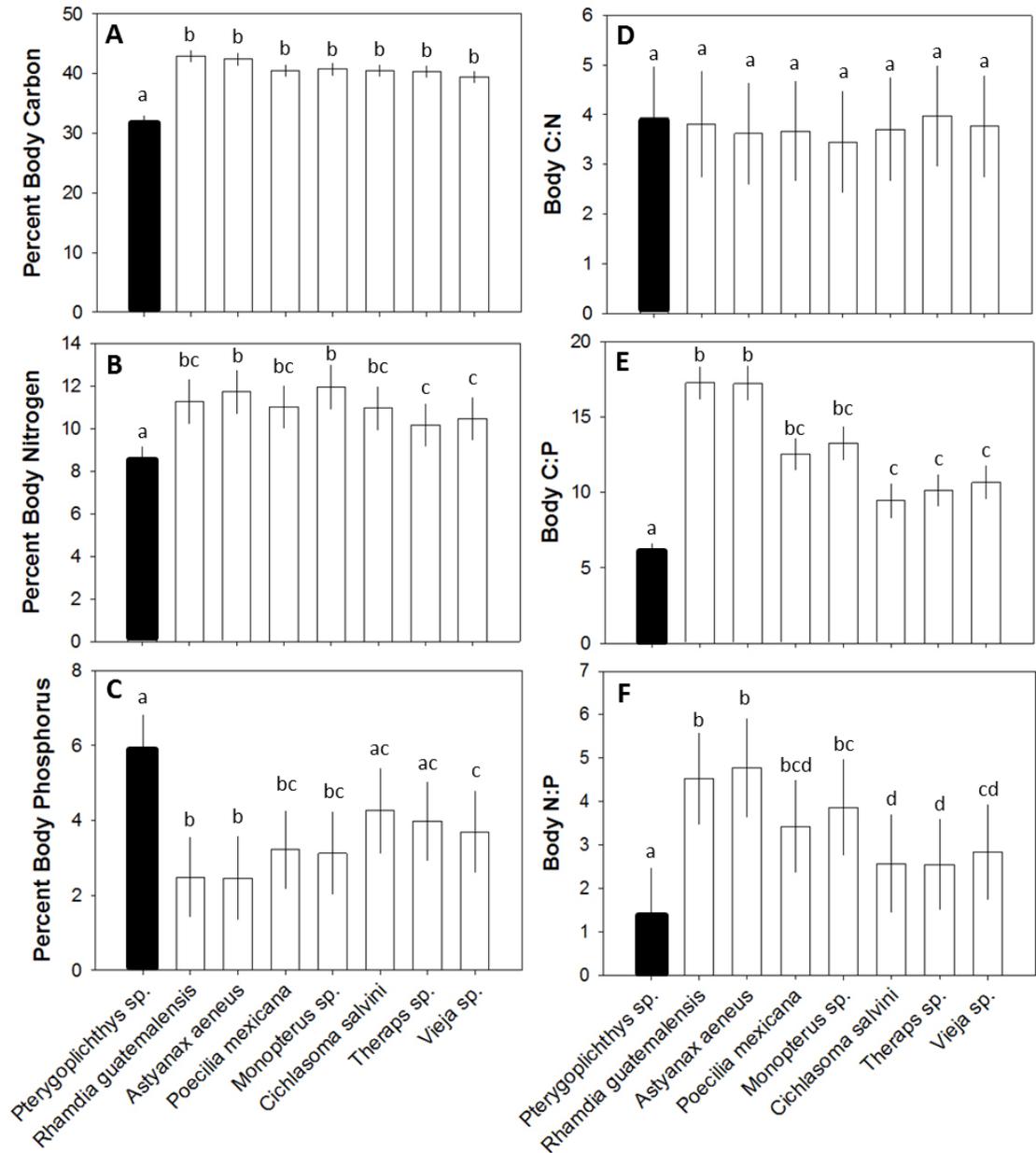


Figure 3.4. Means (± 1 SE) of fish body nutrient content based on 5 individuals of each genus. (A) percent body carbon; (B) percent body nitrogen; (C) percent body phosphorus; (D) carbon to nitrogen ratio; (E) carbon to phosphorus ratio; (F) nitrogen to phosphorus ratio. Bars with different letters have significantly different nutrient contents or ratios according to Tukey's Honestly Significant Difference test. Black bars represent *Pterygoplichthys* and open bars are common native species.

produced via nutrient remineralization ($191 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ and $7.5 \mu\text{mol P m}^{-2} \text{hr}^{-1}$) from the nutrients sequestered in loricariid tissues through growth ($60 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ and $20 \mu\text{mol P m}^{-2} \text{hr}^{-1}$). When combined, these results suggest that loricariids were remineralizing more N than they were sequestering through population growth. Conversely, they were sequestering more P than they were releasing through remineralization.

DISCUSSION

My results indicate non-native loricariids exerted a strong influence on nutrient dynamics and their role in nutrient storage and cycling was influenced by their population density, body stoichiometry, and by limiting ambient nutrient conditions. Additionally, these data suggest loricariid invasion converted the upper Chacamax River from a system where nutrient recycling and storage by fishes was negligible to a system where fishes form an important pool of particulate nutrients. Loricariid remineralization was a significant flux of inorganic N and P to the water column and excretion could turn over the ambient nutrient pool in relatively short distances. My findings show the role of a species in nutrient storage and cycling differs between nutrients, suggesting organisms can simultaneously function as a net source of one nutrient, while functioning as a net sink of another.

Biogeochemical Hotspots and Hot Moments Generated by Loricariids

Nutrient remineralization by animals can play an important role in nutrient dynamics in stream ecosystems (McIntyre et al. 2008, Benstead et al. 2010, Reisinger et al. 2011, Small et al. 2011). For example, nutrient excretion by freshwater shrimp was

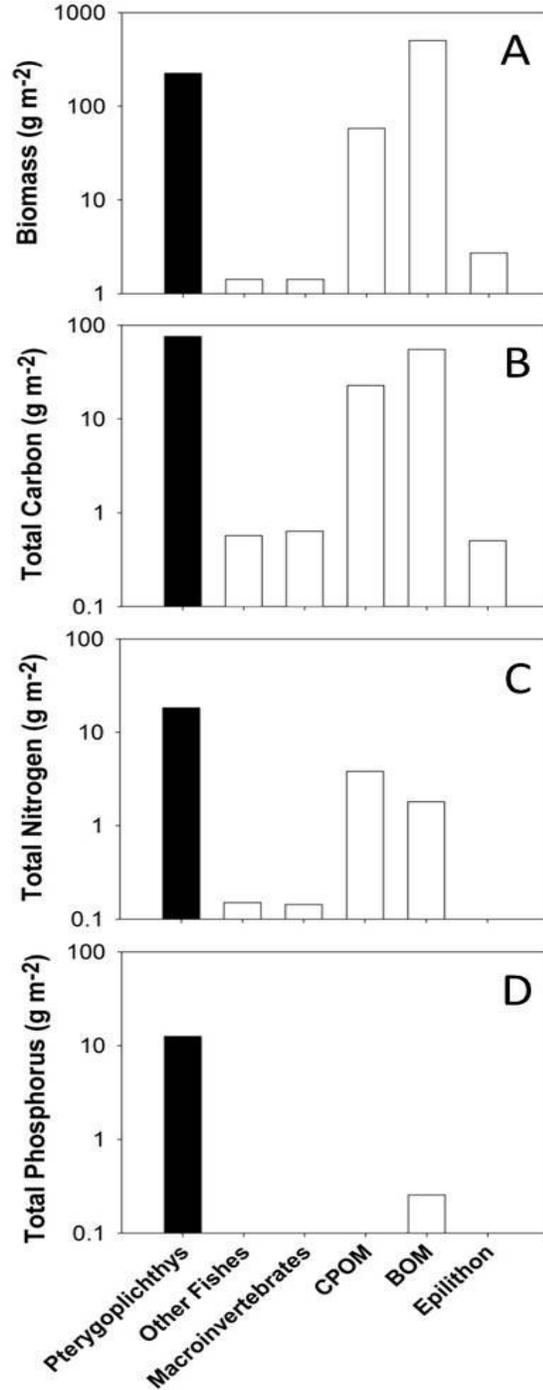


Figure 3.5. Estimated biomass and nutrient composition of major nutrient pools (*Pterygoplichthys*, other fishes, macroinvertebrates, coarse particulate organic matter (CPOM), benthic organic matter (BOM), and epilithon) in the Chacamax River. (A) biomass, (B) estimated carbon, (C) estimated nitrogen, and (D) estimated phosphorus. The x-axes are on log scales. Black bars represent *Pterygoplichthys*.

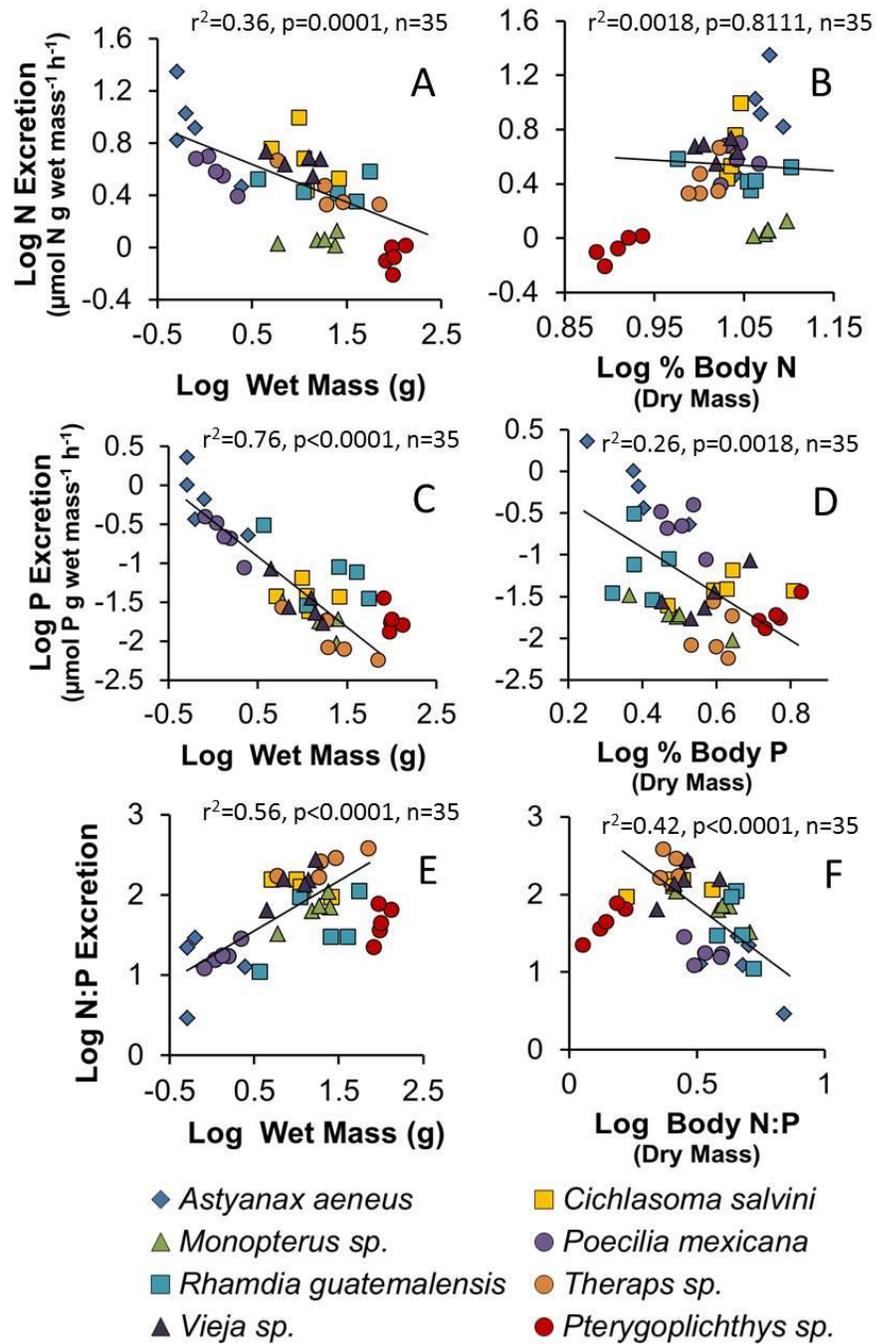


Figure 3.6. Log₁₀-transformed molar excretion rates and ratio versus body mass and body nutrient (n=five individuals per genus) in the Chacamax River. Non-native *Pterygoplichthys* were not included in the regression. (A) nitrogen excretion versus wet mass, (B) nitrogen excretion versus body nitrogen, (C) phosphorus excretion versus wet mass, (D) phosphorus excretion versus body phosphorus, (E) excretion nitrogen to phosphorus ratio versus wet mass, (F) excretion nitrogen to phosphorus ratio versus body nitrogen to phosphorus ratio.

equivalent to approximately 5% of the P uptake and 20% of the N uptake in a Puerto Rican stream (Benstead et al. 2010).

Similarly, in a study examining the influence of a native fish, *Astyanax aeneus*, on P-cycling in Costa Rican streams, Small et al. (2011) suggested fish were keystone nutrient recyclers as they had disproportionately large effects on P remineralization rates relative to their biomass. In these systems, they found that aggregate excretion by *Astyanax* was equivalent to 90% of stream P demand (Small et al. 2011).

Correspondingly, in my study, loricariid excretion was equivalent to approximately 255% of the NH_4^+ demand and 70% of the P demand in the Chacamax River compared to 10% of the NH_4^+ demand and 3% of the P demand from native fish excretion (Fig. 3.8A). However, the intensified recycling estimates I documented were most likely due to the fact that *Pterygoplichthys* comprises a high biomass and different stoichiometry relative to the native fish biomass in the Chacamax River rather than a disproportionate effect on nutrient remineralization rates.

Analogous to the results from other studies, *Pterygoplichthys* in the Chacamax River attained high population densities (Nico and Martin 2001, Chavez et al. 2006) and exhibited diel shifts in behavior (Nico 2010). The areal biomass of *Pterygoplichthys* in the Chacamax River was at least two orders of magnitude greater than the native fish community in the Chacamax River (Fig. 3.3 & 3.5; $\sim 225\text{g m}^{-2}$ and $\sim 1.4\text{g m}^{-2}$, respectively). These data are especially striking considering loricariids were first reported in the Chacamax River in 2004, only four years prior to the onset of this study (Wakida-Kusunoki et al. 2007).

Table 3.1. Results of regressions relating nutrient excretion rates to body mass and body nutrient content and relating nitrogen excretion rates to phosphorus excretion rates in fishes from the Chacamax River. Regressions were conducted with and without *Pterygoplichthys* included in the calculation. The p values of significant correlations are presented in bold italics.

Parameter		Estimate (SE)	R ²	F-Ratio	Sum of Squares	p	
Log N Excretion Rate ($\mu\text{mol N} \cdot \text{g wet mass}^{-1} \cdot \text{h}^{-1}$)	With <i>Pterygoplichthys</i>	Intercept	0.81956 (0.0660)	-	-	-	-
		Log Body Mass	-0.36314 (0.0549)	0.5350	43.716	2.475	<0.0001
		Log Body N (% Dry Mass)	3.1235 (0.9069)	0.2379	11.862	1.007	0.0014
	Without <i>Pterygoplichthys</i>	Intercept	0.7805 (0.0689)	-	-	-	-
		Log Body Mass	-0.2881 (0.0665)	0.3628	18.792	1.065	0.0001
		Log Body N (% Dry Mass)	-0.3939 (1.6354)	0.0018	0.058	0.005	0.8111
Log P Excretion Rate ($\mu\text{mol P} \cdot \text{g wet mass}^{-1} \cdot \text{h}^{-1}$)	With <i>Pterygoplichthys</i>	Intercept	-0.54154 (0.0945)	-	-	-	-
		Log Body Mass	-0.75666 (0.0787)	0.7089	92.550	10.746	<0.0001
		Log Body P (% Dry Mass)	-2.4255 (0.6154)	0.2925	15.536	4.399	0.0003
	Without <i>Pterygoplichthys</i>	Intercept	-0.4624 (0.0929)	-	-	-	-
		Log Body Mass	-0.9074 (0.0896)	0.7565	102.499	10.566	<0.0001
		Log Body P (% Dry Mass)	0.1968 (0.4309)	-	-	-	-
Log NP Excretion (molar)	With <i>Pterygoplichthys</i>	Intercept	1.3618 (0.1113)	-	-	-	-
		Log Body Mass	0.39468 (0.0926)	0.3233	18.158	2.924	0.0001
		Log Body NP (molar)	-0.9780 (0.4022)	0.1344	5.900	1.215	0.0200
	Without <i>Pterygoplichthys</i>	Intercept	1.2429 (0.0986)	-	-	-	-
		Log Body Mass	0.6219 (0.0951)	0.5642	42.719	4.963	<0.0001
		Log Body NP (molar)	-2.4532 (0.5040)	0.4179	23.689	3.67	<0.0001
Log N Excretion Rate ($\mu\text{mol N} \cdot \text{g wet mass}^{-1} \cdot \text{h}^{-1}$)	With <i>Pterygoplichthys</i>	Intercept	0.9179 (0.9813)	-	-	-	-
		Log P Excretion ($\mu\text{mol P} \cdot \text{g wet mass}^{-1} \cdot \text{h}^{-1}$)	0.3546 (0.0687)	0.4122	26.643	1.906	<0.0001
	Without <i>Pterygoplichthys</i>	Intercept	0.8942 (0.0852)	-	-	-	-
		Log P Excretion ($\mu\text{mol P} \cdot \text{g wet mass}^{-1} \cdot \text{h}^{-1}$)	0.2905 (0.0617)	0.4015	22.139	1.178	<0.0001

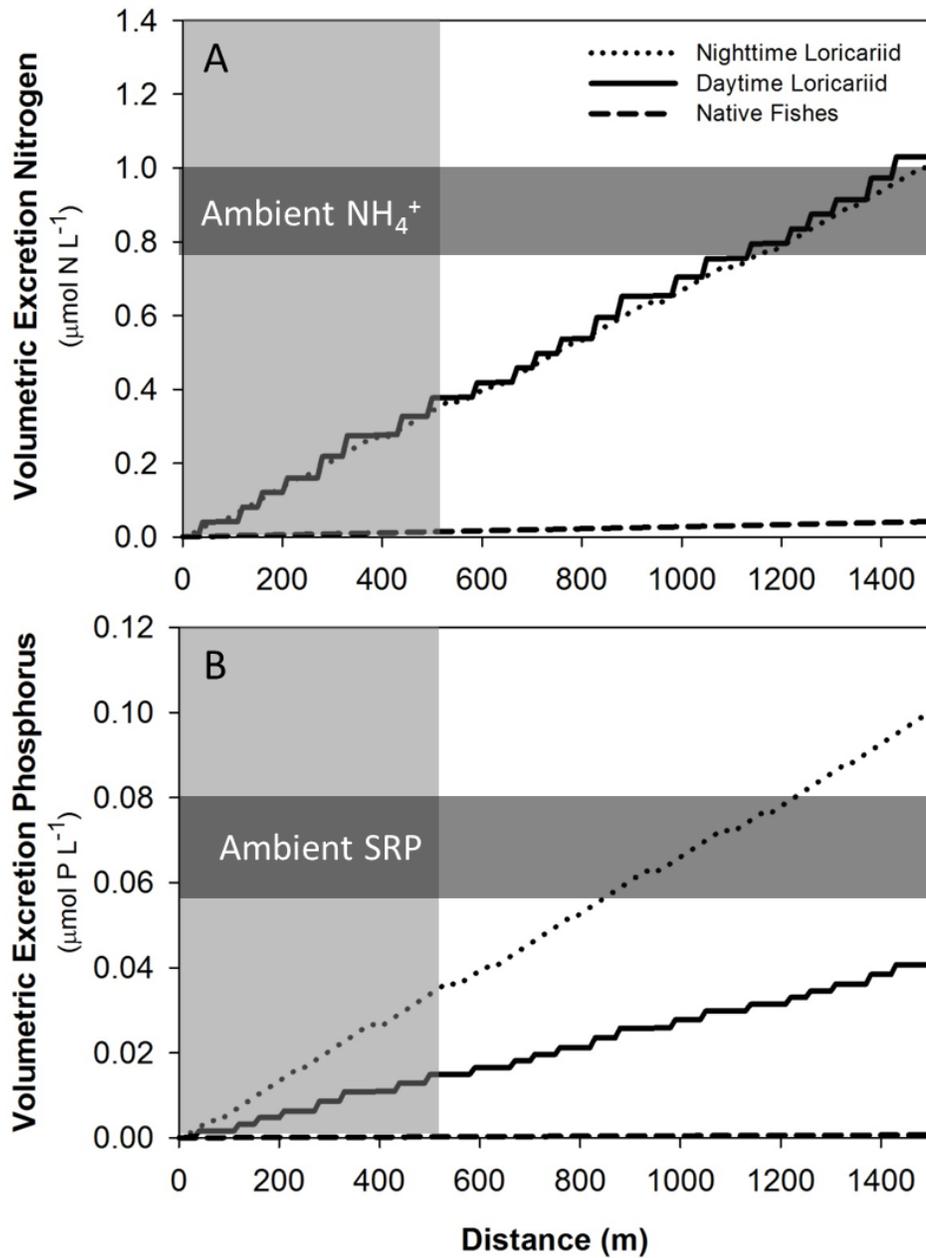


Figure 3.7. Volumetric excretion rates of (A) NH_4^+ -N and (B) SRP by loricariids and the native fish community in the Chacamax River over a 1.5km stream reach. The black solid line represents daytime excretion estimates for loricariids, the dashed line represents nighttime excretion estimates for loricariids, and the black dotted line represents excretion estimates for native fishes. The lines in the light gray shading were measured in the 550m experimental reach. The dark grey shading represents ambient chemistry concentrations.

Loricariid excretion greatly increased nutrient recycling by fishes within my study reach (Fig. 3.8) and potentially generated hotspots and hot moments of biogeochemical activity (Fig. 2.4). McClain et al. (2003) defined biogeochemical hotspots as small areas within a landscape matrix that show comparably high reaction rates relative to the surrounding areas. Further, they defined hot moments as brief periods of time that display disproportionately great reaction rates relative to lengthier, subsequent periods of time (McClain et al. 2003). For organisms to generate biogeochemical hotspots or hot moments within ecosystems, their population densities must change through space and/or time and the contribution of the species to nutrient remineralization rates must be significant relative to ecosystem demand (McIntyre et al. 2008). This study documents diel shifts in loricariid aggregating behavior that created areas and periods of time, hotspots and hot moments, of high nutrient release rates in the Chacamax.

Similar to observations reported for loricariids in their native ranges (Casatti and Castro 2006) and introduced populations of *Pterygoplichthys* in Florida. (Nico 2010), *Pterygoplichthys* in the Chacamax River were nocturnal. Nocturnal behavior may have evolved to minimize predation from day-active predators, as diel changes in behavior has been attributed to predator avoidance in native populations of loricariids (Appendix 7). In my study, loricariids formed large aggregations within the main channel during the day, but spread out to graze the entire riverbed at night (Fig. 3.1A-C; Fig 3.2B). Aggregations of loricariids generated pulses of nutrient remineralization during the day, whereas excretion at night was relatively even throughout the stream when loricariids are spread out and actively grazing (Fig. 3.1A-B, Fig. 3.7 A-B).

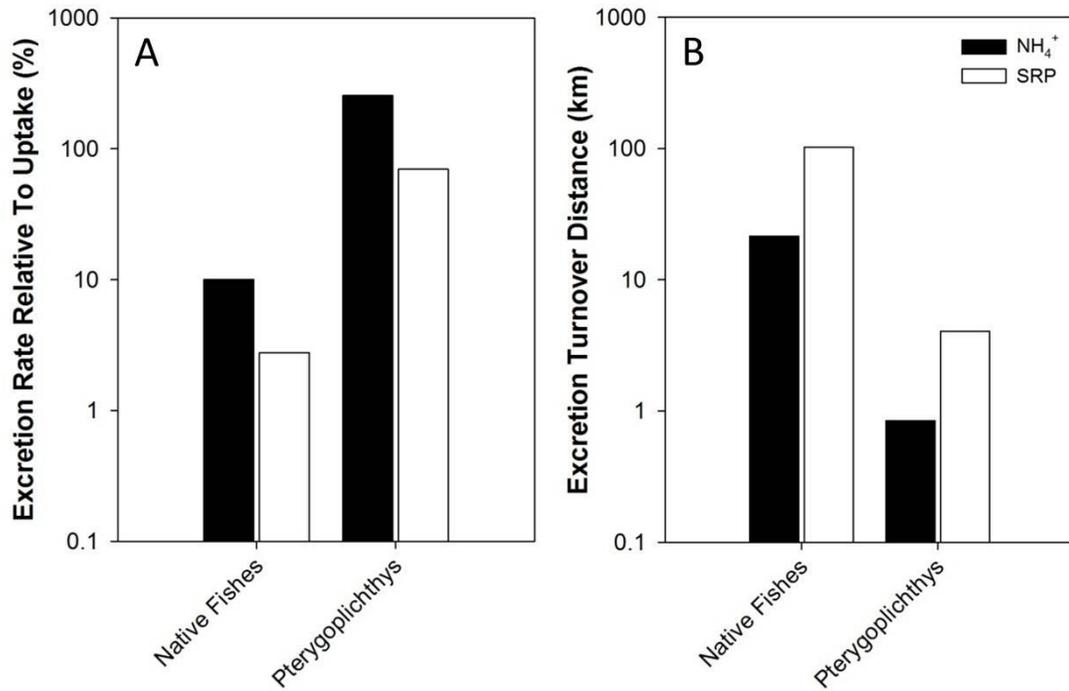


Figure 3.8. (A) Excretion turnover distance (km) by loricariids and native fishes in the Chacamax River. (B) Nutrient excretion rate relative to stream nutrient uptake (%) over a 100m reach in the Chacamax River. Note that the y-axes are on a log scale.

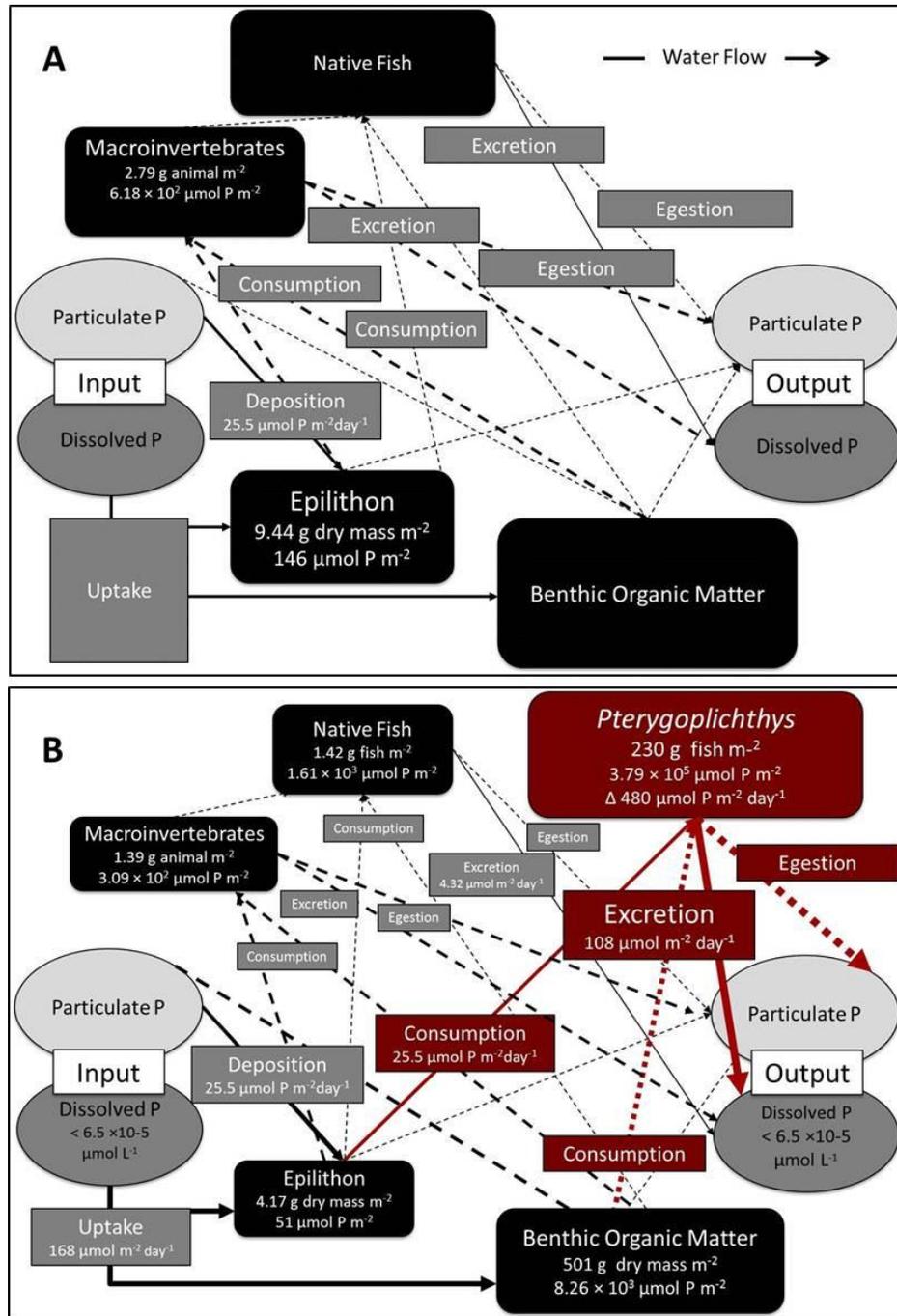


Fig 3.9 Simplified model of phosphorus (P) storage and cycling before (A) and after (B) *Pterygoplichthys* invasion. Black boxes represent pools in both figures. Arrows represent P flows through the system and measured values are presented in grey boxes. Solid arrows have been estimated, dashed arrows are unknown. Larger arrows correspond with larger fluxes. Changes in macroinvertebrate and epilithon areal biomass were estimated using data from Experiment 2 enclosures (Figs. 1.3, 1.5). Deposition rate was estimated as the rate of dry mass accrual in the first 5 days of Experiment 2 (Fig. 1.3). In (B), *Pterygoplichthys* nutrient storage, consumption, and waste production are denoted in red.

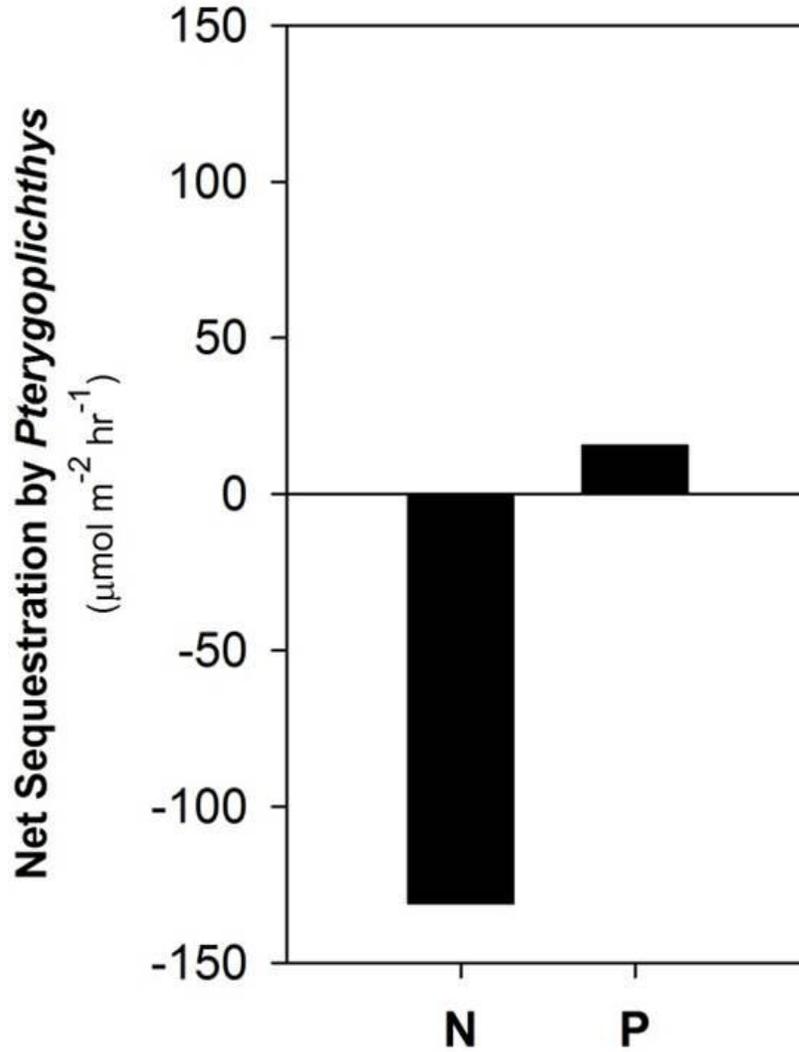


Figure 3.10. Nutrient sequestration estimates of nutrients by loricariids in the Chacamax River. The values were obtained by subtracting the areal nutrient recycling rates of loricariids from the areal rate of nutrient sequestration by loricariids in the Chacamax River. Positive values indicate that loricariids are acting as a sink of nutrients through sequestration in body tissue and negative values indicate that loricariids are acting as net recycler of nutrients via remineralization.

Nutrient remineralization by loricariids also seemed to be important at larger spatial scales in the Chacamax River. Daytime areal excretion estimates indicated that loricariids excreted more than 25 times the amount of N ($191 \mu\text{mol N m}^{-2} \text{hr}^{-1}$ vs. $7.5 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and P ($4.5 \mu\text{mol P m}^{-2} \text{hr}^{-1}$ vs. $0.18 \mu\text{mol P m}^{-2} \text{hr}^{-1}$) than was excreted by the native fish community. Additionally, the volumetric excretion estimates of N and P by loricariids were much greater than for the native fish assemblage (Figure 3.7A-B) and were influenced by loricariid behavior. This led to a 95% reduction in the distance required for fish excretion to turn over the ambient pools of NH_4^+ and SRP (Fig. 3.8B). Thus, loricariid invasion may have transformed the Chacamax River from a system where fishes were not key drivers of biogeochemical processes to a system where remineralization of nutrients by fishes represented a large and important flux of nutrients. The effect of loricariids on nutrient cycling may not be limited to an increase in the amount of nutrients that are recycled by fishes. Loricariids may also be influencing the turnover time of nutrients in the Chacamax River.

Loricariids as Novel Nutrient Sinks

Fish can be important nutrient sinks in aquatic systems (Kitchell et al. 1975, Kairesalo and Seppala 1987, Kraft 1993, Sereda et al. 2008). For example, Kitchell et al. (1975) determined that most of the P in the pelagic zone of a highly productive lake was stored in fish biomass and Kraft (1993) found that alewives (*Alosa pseudoharengus*) in Lake Michigan sequestered a large amount of particulate P in the system. In my study, loricariids created an important pool of nutrients in the Chacamax River. The areal biomass of loricariids increased steadily throughout the

study period (Fig. 3.3), growing at a rate of approximately $100 \text{ g } Pterygoplichthys \text{ m}^{-2} \text{ yr}^{-1}$. This increase in biomass resulted in large amounts of nutrients being sequestered in loriciid body tissue at a rate of approximately $8 \text{ g N m}^{-2} \text{ yr}^{-1}$ and $6 \text{ g P m}^{-2} \text{ yr}^{-1}$. Although the lifespan of *Pterygoplichthys* has not been described in either native or introduced ranges, data from other genera of loriciids indicate that armored catfishes may live longer than 15 years (Secutti and Trajano 2009). Coupled with a lack of natural predators (Appendix 7), this lifespan estimate suggests that nutrients sequestered by loriciids will not be available to primary producers for years, if not decades. Moreover, most of the P stored in loriciids is retained in bone, which can take months to degrade in water (Premke et al. 2010). This was evidenced downstream in the Chacamax River, where large piles of loriciids that were created by fishermen on the riverbanks were still decomposing after several months of exposure to scavengers and weather (Appendix 6: Fig.3 6A).

Loriciids created important sinks of nutrients after invasion. High densities of loriciids sequestered approximately 50% of the carbon, 75% of the N, and 97% of the P measured in dominant pools in the system (Fig. 3.5). As I predicted, loriciids dominated the particulate P pool due to their high body P content (Figure 3.9); however, my data also indicated loriciids stored half of the carbon and the majority of the N in the pools we sampled. Once again, these results are remarkable considering the short period of time loriciids have been documented in the Chacamax River. My data also indicate loriciids need to consume more P than is stored in epilithon on rocks in the Chacamax River to maintain the rate of population biomass increase as the population is sequestering approximately $480 \mu\text{mol P m}^{-2} \text{ day}^{-1}$ and excreting

approximately $110 \mu\text{mol P m}^{-2} \text{ day}^{-1}$ but only consuming $26 \mu\text{mol P m}^{-2} \text{ day}^{-1}$ from the epilithon (Fig. 3.9). Most likely, the loricariids are not limiting their feeding to rock surfaces and are accessing algae and detritus (BOM) found in interstitial spaces between rocks.

Stoichiometric Implications of Invasion

Nutrient excretion and egestion rates depend on the elemental composition of the consumer (Elser and Urabe 1999, Elser et al. 2000, Sterner and Elser 2002). In this study, differences between loricariid and native fish species elemental composition yielded different body and excretion stoichiometry. The elemental composition values we reported here are similar to those published for Loricariidae and the native fish families I sampled (Vanni et al. 2002, McIntyre and Flecker 2010). As I predicted, loricariids were significantly richer in P than native species (Fig. 3.4C). Interestingly, they also had significantly less C and N than native fishes (Fig. 3.4A-B). Lower C and N content coupled with higher P content increased the stoichiometric differences between native fishes and loricariids.

Loricariids recycled nutrients via excretion and sequestered nutrients in body tissues in the Chacamax River; however they influenced N and P differently. Phosphorus-limitation of loricariid growth may be intensified in P-limited systems such as the Chacamax River. By subtracting rates of nutrient sequestration through increase in population biomass from nutrient recycling from excretion, my results suggest that loricariids may act as a net recycler of N ($131 \mu\text{mol N m}^{-2} \text{ hr}^{-1}$), but a net sink of P ($-12.5 \mu\text{mol P m}^{-2} \text{ hr}^{-1}$) in the Chacamax River (Fig. 3.10). Importantly, these values were based on daytime excretion estimates from loricariids. However, even

when I employed evening excretion estimates in the calculation, loricariids appeared to be a net sink for P ($-6.25 \mu\text{mol P m}^{-2} \text{hr}^{-1}$) in the Chacamax River.

Through intensive grazing and selective retention of P during consumption, *Pterygoplichthys* has the potential to exacerbate P-limitation of epilithon. Furthermore, loricariids in the Chacamax River excrete more P during evening hours when their guts are full; thus, the N:P ratio of loricariid E_v increases during the day when primary producers are actively taking up N and P (Figure 3.7A-B). Hence, *Pterygoplichthys* may reduce bioavailable P content and may increase the C:P and N:P of epilithon through grazing and reduced daytime P excretion rates, thereby reducing the quality of epilithon available to primary consumers. This may create a bottom-up effect that alters native algal, macroinvertebrate, and fish community structure and primary productivity in invaded habitats.

It has been suggested that stoichiometric constraints of invaders could limit invasion success (Gonzalez et al. 2010). If true, this would indicate that loricariid population growth in the Chacamax River might be P-limited. This idea is supported by studies of loricariids in their native ranges that suggest loricariids may be at or near P-limitation of growth. For example, Hood et al. (2005) reported that growth of two loricariid species (*Ancistrus triradiatus* and *Chaetostoma milesi*) was P limited in a tropical stream where primary producers were N limited. To support their findings, they reported the stoichiometric imbalance of loricariid diet relative to their body stoichiometry, low levels of P-excretion by both loricariid species, and the fact that *Ancistrus* did not consume enough P to account for the measured growth during experimental trials. Importantly, though near P-limitation, both loricariid species

exhibited positive growth rates during the study and continued to excrete and egest P (Hood et al. 2005). These findings suggest stoichiometric limitation may not constrain loricariid invasion and positive population growth in invaded systems. Similarly, Naddafi et al. (2009) reported that the fitness of introduced zebra mussels (*Dreissena polymorpha*) in Swedish lakes was not limited by stoichiometry. They suggested the lack of imbalance between food resources and zebra mussel body stoichiometry and the ability of the mussels to alter their body stoichiometry in response to nutrient availability may have enabled the mussels to overcome potential stoichiometric constraints (Naddafi et al. 2009). Body stoichiometry of fishes is considered to be more fixed than the stoichiometry of plants and invertebrates (McIntyre and Flecker 2010); therefore, nutrient limitation of growth might be a greater constraint on invading populations of fishes.

Context-Dependent Consequences of Invaders on Nutrient Dynamics

The results from this investigation demonstrate the importance of taxon-specific research to understand the effects of invasive species on ecosystem processes in novel environments (Ehrenfeld 2010). The functional role of species in modifying the storage and cycling of nutrients is determined by the biotic and abiotic characteristics of the ecosystem and the biology and stoichiometry of the species (McIntyre and Flecker 2010). Because P-excretion rates are highly variable among fish species (Vanni et al. 2002, Small et al. 2011), P-cycling, may be strongly influenced by changes in fish communities (Small et al. 2009). Additionally, the strong influence of loricariids on nutrient dynamics in the Chacamax River was almost certainly linked to the low ambient P in the system. In a system with low P

availability, the introduction of a P-rich invader, such as loricariids may have intensified P-limitation of primary producers and influenced nutrient recycling and uptake rates within the stream. Most likely, loricariid influence would vary across ecosystems with greater ambient nutrient concentrations and different nutrient limitation scenarios (Small et al. 2011, Wilson and Xenopoulos 2011).

Combined with published values of taxon-specific body and remineralization stoichiometry (e.g., Vanni et al. 2002, McIntyre et al. 2008, Knoll et al. 2009), the findings from this study could be applied to other nutrient-limited systems threatened by stoichiometrically unique invaders to estimate potential changes in ecosystem processes resulting from invasion. The results from this study also indicate that stoichiometric theory affords a useful framework to examine the potential of invaders to alter ecosystem processes. Importantly, my investigation suggests that introduced organisms can have contrasting effects on the storage and cycling of N and P in an invaded system, and that in relatively short periods of time after invasion, non-native vertebrates may sequester and remineralize enough nutrients to modify the storage and cycling of nutrients in aquatic ecosystems.

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APPENDICES

APPENDIX 1

The Usumacinta is the largest river in Mesoamerica and has a basin area greater than 270,000 km² (Hamman and Ankersen 1996). It is a tropical watershed characterized by wet and dry seasons and regions of lower elevation that are seasonally flooded. In Mexico, the Usumacinta flows through the states of Chiapas, Campeche, and Tabasco and it defines the Mexican border with Guatemala. The Usumacinta watershed is also one of the most biologically diverse areas in Mesoamerica (Dickinson and Lawton 2001). The watershed contains approximately 112 fish species, 50% of which are thought to be endemic. Fish fauna common to North America (e.g., ictalurid catfishes and catostomid suckers) and South America (e.g., heptapterid catfishes, characins, and cichlids) are found in the watershed, and freshwater representatives of marine-derived groups, including ariids and gobioids, are found in the Usumacinta (Rodiles-Hernandez et al. 2005).

The riparian habitats of both study reaches were characterized by a heterogeneous mixture of primary and secondary forest and agricultural development on the Chacamax River, and the substrate in the focal reaches was a mix of cobbles and bedrock (Fig. 1A.1 A-B, Fig 1A.2 A-B). All of the work for this study was conducted between the months of March and May in 2009 and 2010, the dry season in southern Mexico. Typically, during these months, the discharge in the focal reaches is reduced to an average of about 1,600 L s⁻¹ and the stream water is transparent. However, discharge during the dry season can fluctuate with precipitation and can cause the depth of the river to increase up to 6m in a 24 hour period. During these periods of high discharge, turbidity increases and the water carries large sediment loads. Additionally, high flow

can scour streambeds and reduce the amount of material found on benthic substrates.

These flooding events occurred several times each year during the studies.

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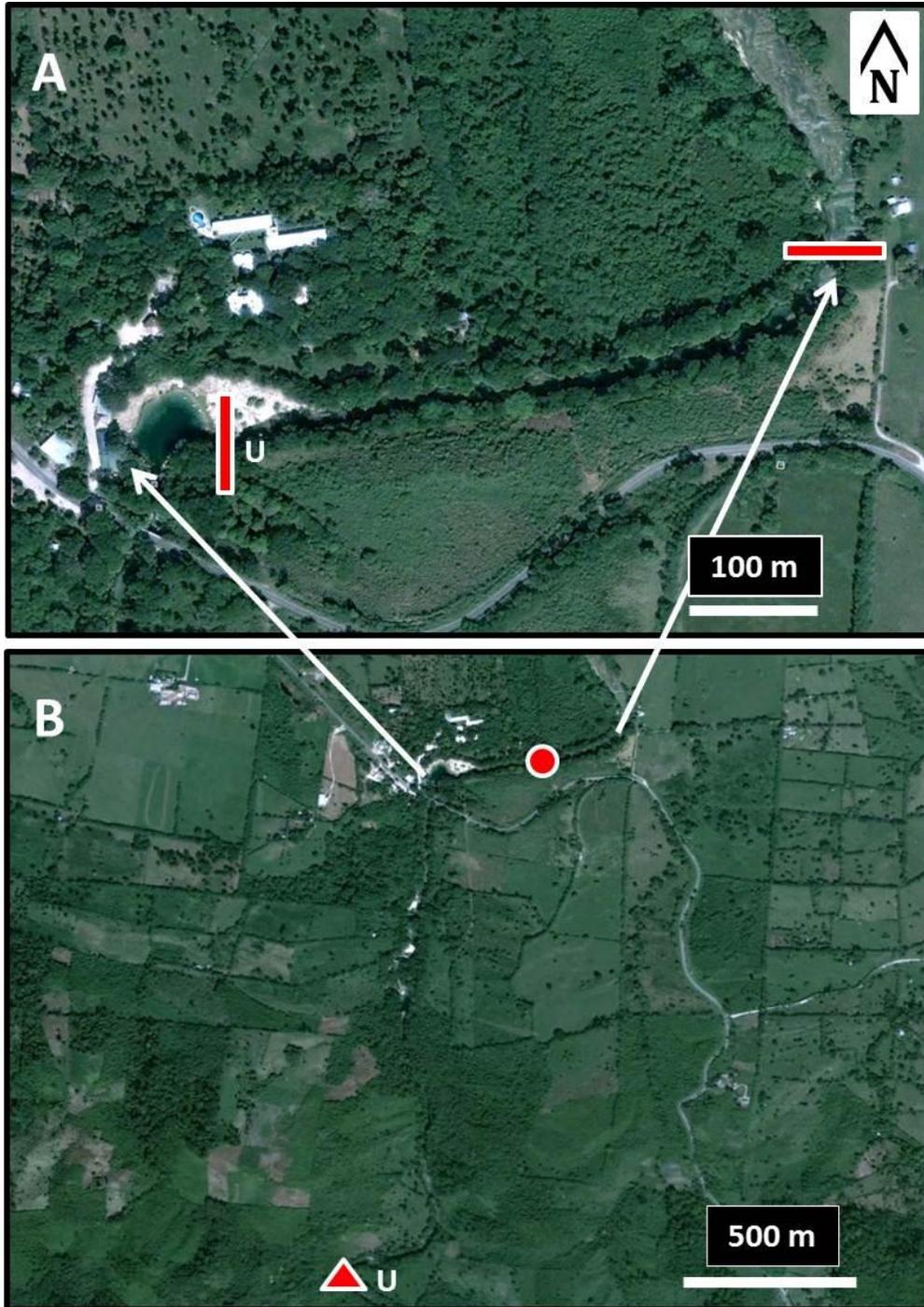


Figure 1A.1. (A) Experimental stream reach (550m: N17°29'047" W91°58'430") with high densities of armored catfish. Red bars delineate the upstream and downstream markers for the 550 m reach. (B) Two survey sites compared benthic environments in a high-density invasion (circle: N17°29'047" W91°58'430") and a low-density invasion (triangle: N17°28'226" W91°58'444") site. Upstream locations are denoted with a U. White arrow indicate the same location in the photos. Images were created in Google Earth[®] in 2011. Aerial photos were taken on 18 November 2004.

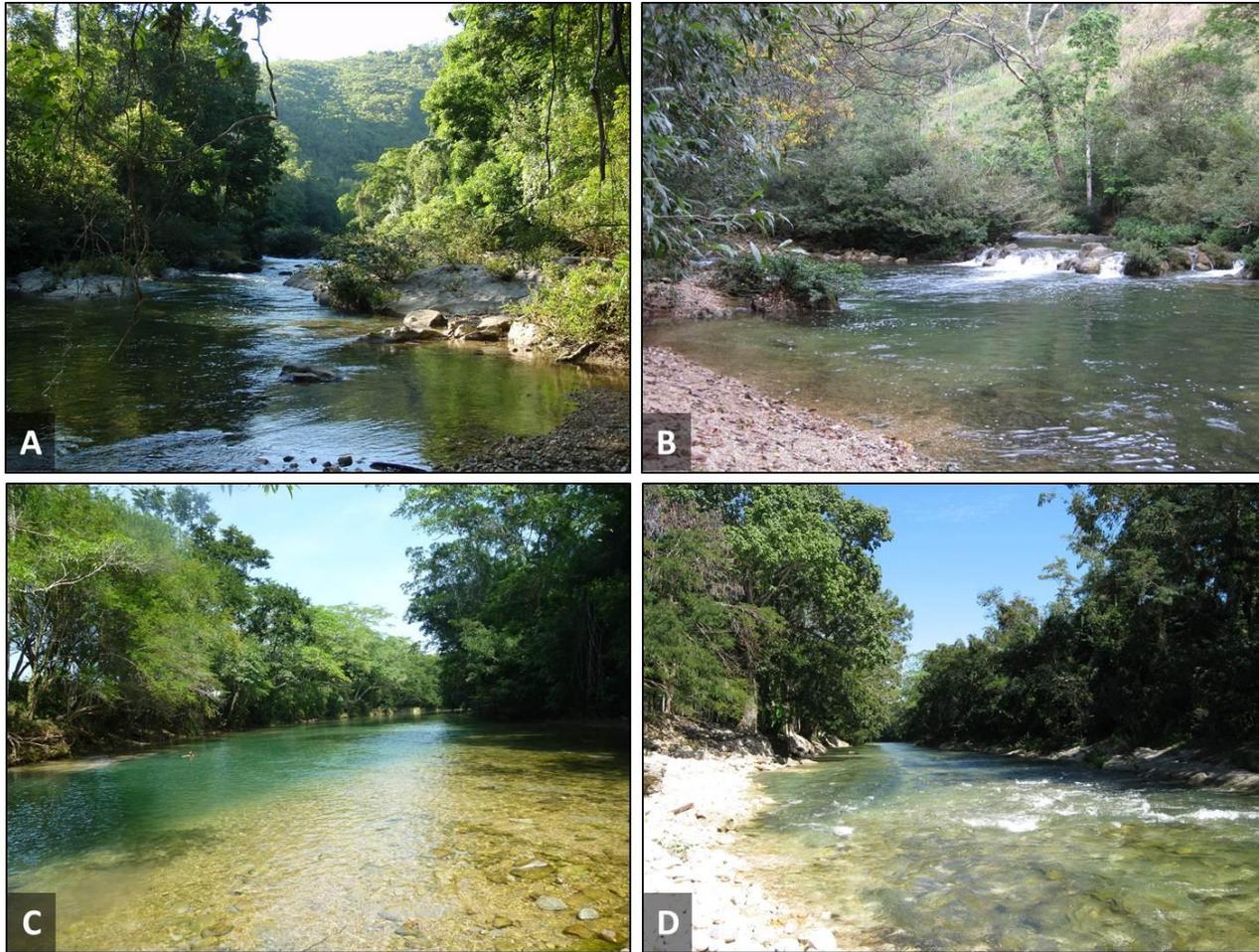


Figure 1A.2. Two survey sites compared benthic environments in an upstream, low-density invasion site (A-B: N17°28'226" W91°58'444") and a downstream high-density invasion site (C-D: N17°29'047" W91°58'430"). Photo credit: K. A. Capps.

APPENDIX 2

In 2010, I estimated nutrient limitation using nutrient diffusing substrates (NDS). I assembled the NDS based on methods outlined in Tank et al. (2006). Briefly, I filled 1-ounce (29.5 ml) hinged plastic cups (Poly-Cons®; Madan Plastics, Crawford, New Jersey) with ~30 mL of nutrient-enriched agar and covered each with a fritted-glass crucible cover (5.1 cm²) (Leco Corporation, St. Joseph, Michigan). For control (CON) and single-nutrient treatments (N, P), we added 20 g of agar per L of water and for N+P treatments I added 30 g of agar per L of water. Nitrogen and N+P treatments were amended with 26.7 g NH₄Cl L⁻¹ and P and N+P treatments were amended with 68.0 g KH₂PO₄ L⁻¹ (0.5 M) (Tank et al. 2006). I deployed 12 replicates of each nutrient treatment. At the end of a 14-day incubation period, the glass crucibles from NDS were harvested and they were immediately placed in individuals film canisters filled with 20 mL of buffered 90% ethanol. I incubated the crucibles in the dark for 16 h to extract chlorophyll a (Capps et al. 2011). Data were analyzed using a one-way ANOVA where nutrient was the fixed factor using JMP 9 statistical software (SAS Institute, 2010).

To confirm NDS were still diffusing at the end of the experiment, three additional NDS of each treatment were incubated *in situ* for the duration of the NDS deployment. At the end of the 14-day period, I collected the NDS and incubated them for one hour in one liter of filtered stream water. Diffusion rate estimates were made by subtracting the diffusion rate of nutrient amended NDS from the rate of control NDS. On day 14 (N: $7.2 \times 10^{-5} \pm 3.8 \times 10^{-6}$; P: $7.1 \times 10^{-4} \pm 3.1 \times 10^{-5}$; N+P: (N) $9.5 \times 10^{-6} \pm 2.1 \times 10^{-6}$, (P) $8.1 \times 10^{-5} \pm 1.5 \times 10^{-6}$, mean \pm SE (mol m⁻² hr⁻¹)), treatments were diffusing less than on day 0 (N: $2.1 \times 10^{-2} \pm 3.1 \times 10^{-4}$; P: $4.9 \times 10^{-3} \pm 4.3 \times 10^{-5}$; N+P: (N) $3.9 \times 10^{-2} \pm 3.1 \times 10^{-4}$,

(P) $9.1 \times 10^{-3} \pm 5.8 \times 10^{-5}$, mean \pm SE ($\text{mol m}^{-2} \text{hr}^{-1}$). The results from nutrient diffusing substrates indicated that primary producers in the Chacamax River were P-limited ($p < 0.0001$, $F_{(3,43)} = 13.6$, Fig. 2A.1).

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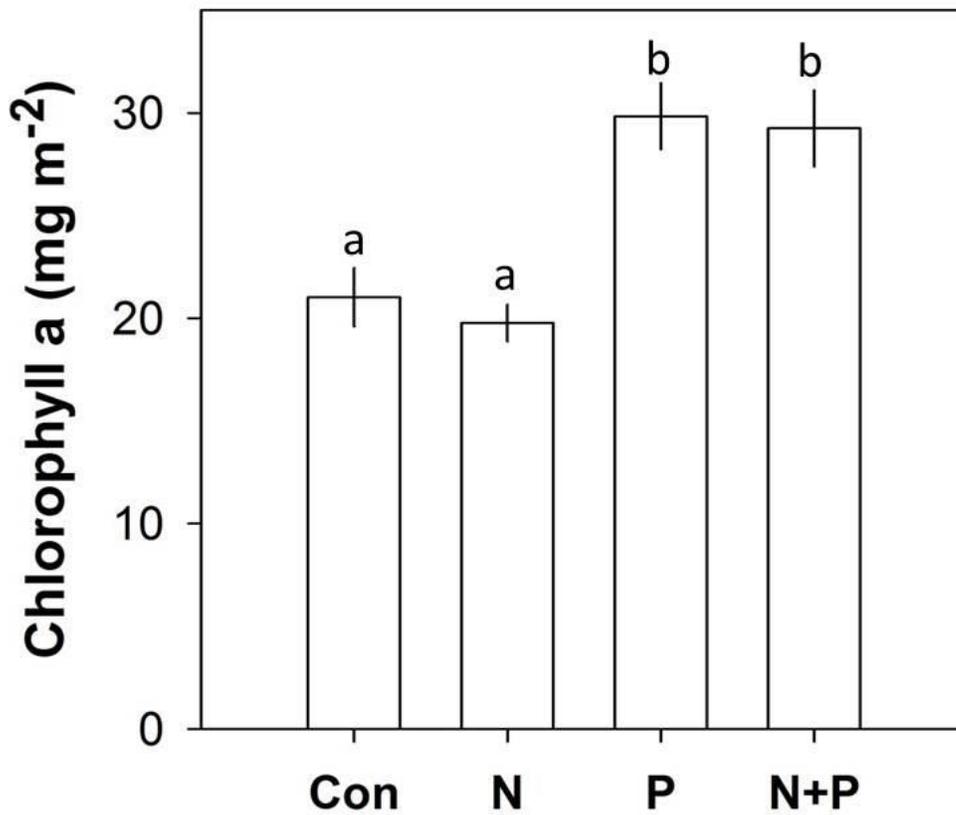


Figure 2A.1. Mean (± 1 SE) algal biomass collected from nutrient diffusing substrates for each of four nutrient treatments (control (CON), nitrogen (N), phosphorus (P), nitrogen and phosphorus (N + P)). Bars with different letters have significantly different ($p < 0.005$) algal biomasses according to Tukey's Honestly Significant Difference test.

APPENDIX 3

Length and weight measurements recorded for the *Pterygoplichthys* collected in the Chacamax River (N17°29'047'' W91°58'430'') between January 2008 and April 2010. Gillnets, cast nets, and hand nets of different mesh sizes were used to collect the fishes. Standard length was measured to the nearest 0.1 cm and total wet mass to the nearest 0.1 g for 972 fishes in the three year sample period (Fig. 3A.1).

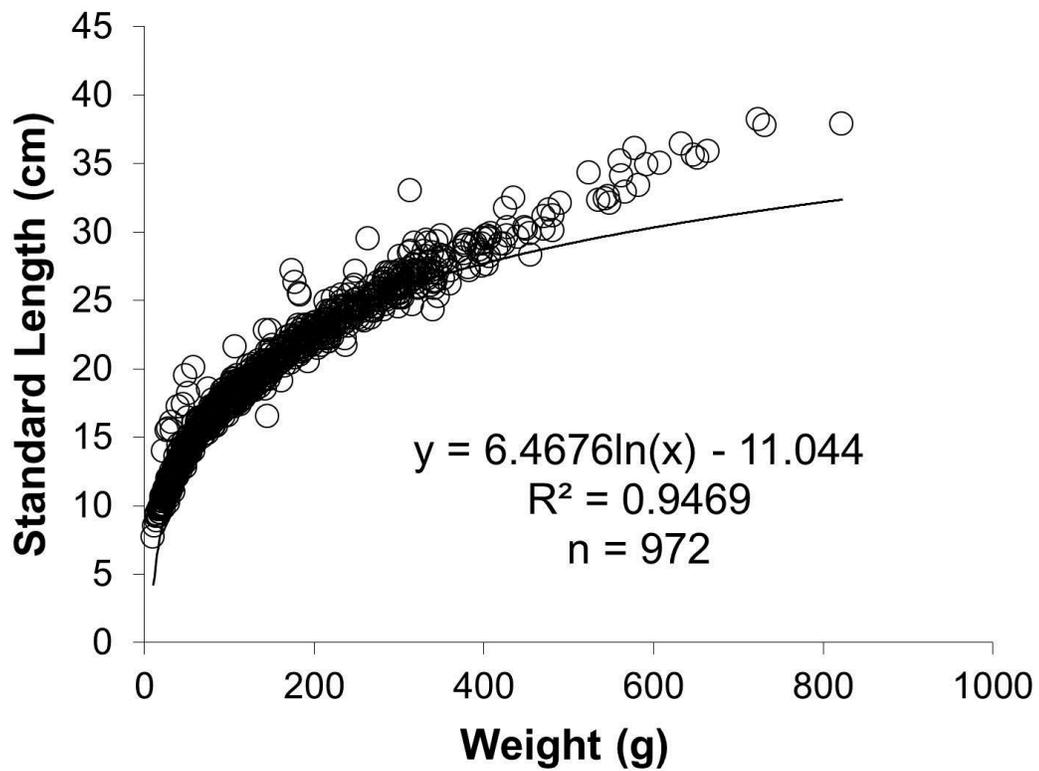


Figure 3A.1. Standard length and weight (wet mass) relationship for *Pterygoplichthys* collected in the Chacamax River (N17°29'047" W91°58'430") between 2008 and 2010.

APPENDIX 4

Pterygoplichthys pardalis is characterized by a ventral pattern of dark spots (Fig. 4A.1 (A)). Conversely, the venter of *Pterygoplichthys disjunctivus* is characterized by dark, vermiculated lines (Fig. 4A.1 (G); Armbruster & Page 2006). *Pterygoplichthys* I collected in the Chacamax River exhibited a spectrum of these characteristics (Fig. 4A.1 (A-G)). Chavez et al. (2006) observed similar intermediate ventral patterns for an introduced population of *Pterygoplichthys* in the Philippines. Similarly, I documented intermediate ventral patterns in the *Pterygoplichthys* population in the Chacamax River (Fig. 4A.1 (B-F)).

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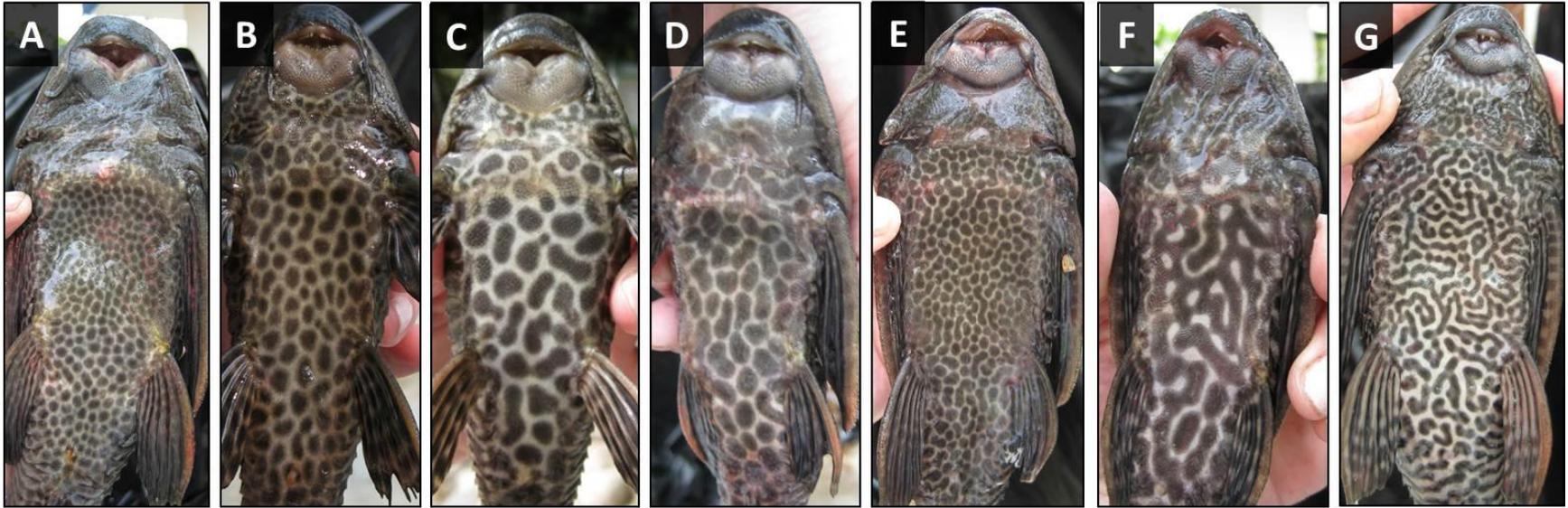


Figure 4A.1. Range of ventral patterns of *Pterygoplichthys* collected in the Chacamax River (N17°29'047" W91°58'430"). *Pterygoplichthys pardalis* is characterized by a ventral pattern of dark spots (A). *Pterygoplichthys disjunctivus* is characterized by dark, vermiculated lines (G) (Armbruster & Page 2006). Photo credit: K. A. Capps.

APPENDIX 5

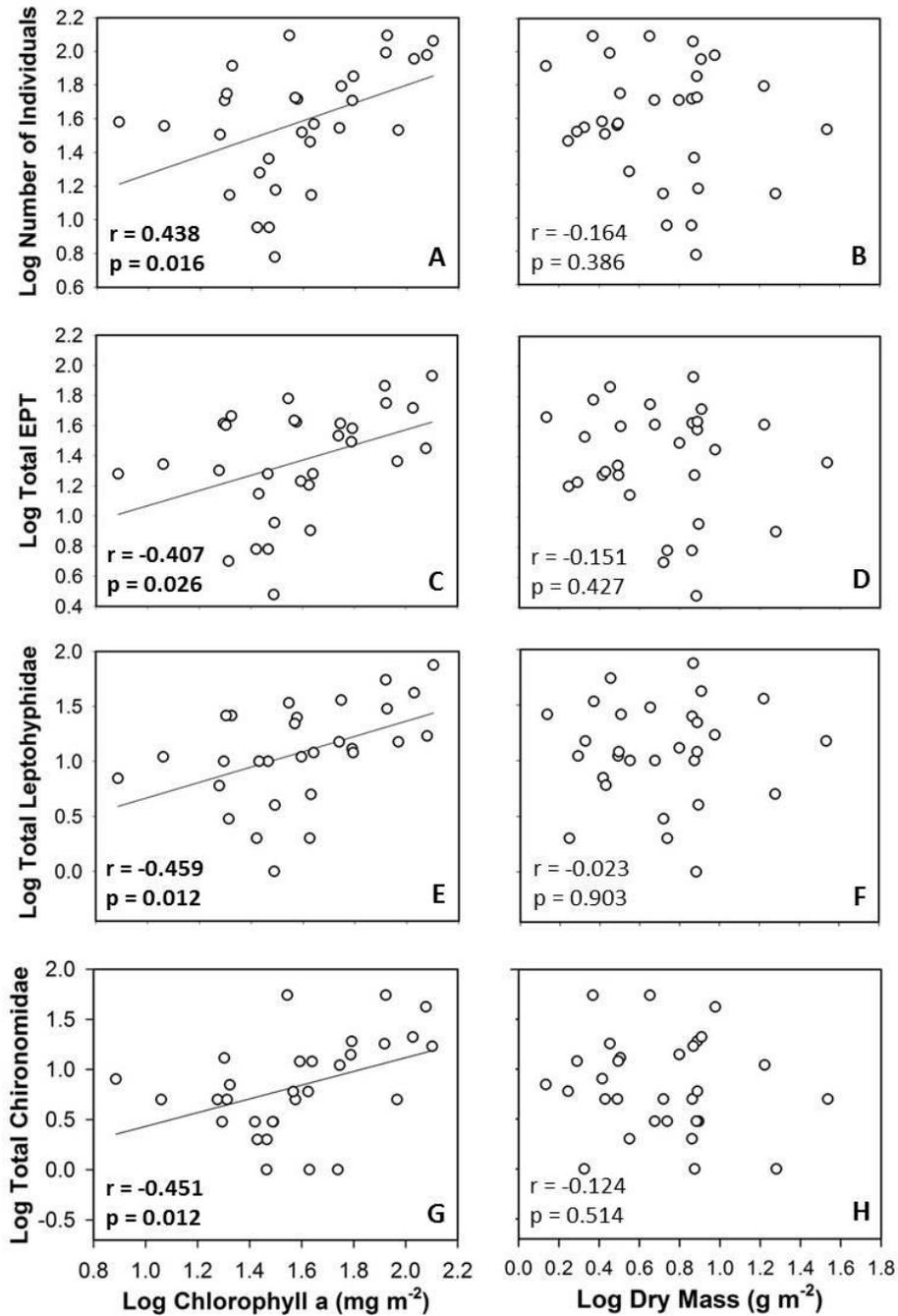


Figure 5A.1 Pearson product-moment correlation coefficients from macroinvertebrate and epilithon from three treatments (Stream Reference; Cage Control; *Pterygoplichthys* Exclosure) in Experiment 2. Significant correlations are indicated with bold-face type.

APPENDIX 6

Fishermen from the Chacamax River Fishing Cooperative in La Libertad, Chiapas claim they are catching less than the half of the fish they used to prior to the invasion. Although the impact of *Pterygoplichthys* on economically important fishes is not fully understood, declines in economically important species is common after *Pterygoplichthys* invasion (Mendoza et al. 2009). Moreover, *Pterygoplichthys* frequently become caught in fishing nets and destroy fishing equipment and boats, increasing the economic cost of fishing. Additionally, high densities of *Pterygoplichthys* caught in fishing gear increase the time required to fish, as fishermen must remove *Pterygoplichthys* from entire sections of river to effectively fish for other species (Mendoza et al. 2009; Figs. 6A1-6A3). Decomposing piles of *Pterygoplichthys* are common along the banks of heavily-fished sections of the Chacamax River (Fig. 6A.3) Carcasses can remain intact for several months, even after being subjected to consumption by scavenging species such as fire ants (*Solenopsis geminata*), vultures (*Coragyps atratus*), and feral cats (*Felis catus*) and dogs (*Canis lupus familiaris*) (Fig. 6A.3).

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Figure 6A1. *Pterygoplichthys* caught by the Chacamax River Fishing Cooperative in 2008 (17°41'16"N, 91°42'29"W). (A) *Pterygoplichthys* caught in mylar mesh nets; (B) Fishermen collecting *Pterygoplichthys* and removing them from a section of the Chacamax River. Photo credit: K. A. Capps.



Figure 6A2. *Pterygoplichthys* caught by the Chacamax River Fishing Cooperative in 2008 (17°41'16"N, 91°42'29"W). Photo credit: K. A. Capps.



Figure 6A.3. Decomposing *Pterygoplichthys* on the banks of the Chacamax River (17°41'16"N, 91°42'29"W). Photo credit: K. A. Capps.

APPENDIX 7

Loricariids formed large aggregations within the main channel during the day, but spread out to graze the entire riverbed at night (Fig. 3.1A-C; Fig 3.2B). This behavior may have evolved to prevent predation, as diel changes in behavior has been attributed to predator avoidance in native populations of loricariids. For instance, Power described changes in habitat use by loricariids to avoid predation by diurnally-active fishing birds (Power 1984, Power et al. 1989). Likewise, in a non-native population of fishes, Nico (2010) documented double-crested cormorants (*Phalacrocorax auritus*) consuming *Pterygoplichthys* from on several occasions. The fishes selected by cormorants were small (10-20 cm TL) and bird feeding activities were typically restricted to crepuscular hours. Similarly, in my study site, predation of loricariids was restricted to Neotropic cormorants (*Phalacrocorax brasilianus*) that hunted small individuals during crepuscular hours.

In conjunction with observations from the Nico study (2010), my data suggest that native predators will not effectively control growth and expansion of non-native populations of loricariids. In their native range, loricariids are consumed by many organisms including, but not limited to crocodilians (Willard 1985, Borteiro et al. 2009), the Neotropical otter (*Lontra longicaudis*) (Kasper et al. 2008), other fishes (Nico and Taphorn 1988, Duque and Winemiller 2003), and wading birds (Power 1984, Power et al. 1989). However, many of these predator populations are threatened or endangered in the watershed (Thorbjarnarson et al. 2006, Martin 2008), or they do not have ranges that extend into the Usumacinta River. Additionally, potential predators may not initially recognize loricariids as a food source. For instance, introduced

loricariid populations initially went unnoticed by fish-eating birds and otters in Florida, but as time progressed, these predators began to actively consume loricariids (Nico 2010). Unfortunately, due to the limited number of predators remaining in the Chacamax River and the large body size of *Pterygoplichthys* (up to at least 70cm SL (Froese and Pauly 2011)), it is unlikely that predation will control the loricariid population in the region.

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