IMPACTS OF A HERBIVOROUS FISH, *CAMPOSTOMA ANOMALUM*

(CENTRAL STONEROLLER), ON NITROGEN

FIXATION BY BENTHIC ALGAE

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

By

Chad Robert Schwinnen
B.S., The Ohio State University, 2005

2010
Wright State University

WRIGHT STATE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

September 16, 2010

I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY Chad Robert Schwinnen
ENTITLED Impacts of a Herbivorous Fish, *Campostoma anomalum*
(central stoneroller), on Nitrogen Fixation by Benthic Algae BE
ACCEPTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF Master of Science.

_____
Yvonne Vadeboncoeur, Ph.D.
Thesis Director

_____
David Goldstein, Ph.D.
Department Chair

Committee on
Final Examination

_____
Thomas Rooney, Ph.D.

_____
John Stireman, Ph.D.

_____
Andrew T. Hsu, Ph.D.
Dean, School of Graduate Studies

ABSTRACT

Schwinnen, Chad Robert. M.S., Department of Biological Sciences, Wright State University, 2010. Impacts of a Herbivorous Fish, *Campostoma anomalum* (central stoneroller), on Nitrogen Fixation by Benthic Algae.

Herbivorous fish negatively impact algal biomass and promote the growth of

cyanobacteria. In nutrient poor conditions that sustain high levels of productivity

nitrogen fixing cyanobacteria can supply up to 28% of the available nitrogen (Higgins et

al. 2001) We investigated the role of grazing fish in sustaining high levels of

productivity in nutrient poor conditions. We used the grazing minnow, *Campostoma*

anomalum, and the acetylene reduction technique in a controlled environment to analyze

rates of nitrogen fixation and primary production by the periphyton. Fish grazing

reduced algal biomass and promoted the growth of cyanobacteria but had no impact on

overall rates of primary production and nitrogen fixation. Previous research on the

impact of fish grazing on nitrogen fixation is minimal but contradicts our results, showing

a need for future studies (Wilkinson and Sammarco 1983).

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I. INTRODUCTION

Herbivorous fish can dominate fish assemblages in some aquatic ecosystems. In most coral reef ecosystems, herbivorous fish are the most abundant trophic group and are vital to maintaining the reef system (Sabater and Tofaeono 2007; Sammarco 1983). In tropical freshwaters herbivorous fish, detritivores and their direct predators make up the majority of fish present (Winemiller 1990). Grazing armored catfish, of the family Loricariidae, are present in tropical stream pools at abundances reaching 6 individuals/m^2 (Power 1990). The central stoneroller (*Campostoma anomalum)*, an algivorous minnow, has been recorded at densities of 50 individuals/m^2 in temperate streams (Power et al. 1988). In 2007, the Ohio Environmental Protection Agency (OEPA) collected just over 1,000 *Campostoma* in a 200 meter stretch of the Little Miami River near Yellow Springs, Ohio (unpub data). Grazing fish can occur in high densities in temperate freshwaters, however, there are far fewer herbivorous fish in temperate freshwaters compared to tropical and marine environments. Since the number of herbivorous fish in temperate waters is different it is vital to understand the level of their impact on ecosystem properties.

The role of herbivorous fish has been studied primarily in marine ecosystems, where they play an important role in limiting macroalgal dominance. By removing macroalgal overstory, herbivorous fish contribute to the persistence of coral reefs (Lewis 1986). Most marine studies involving herbivorous fish focus the attention toward the fishes impact on the corals and less on the fishes impact on nutrient cycling and primary

production. In contrast, studies of herbivorous fish in tropical streams focus primarily on their role in nutrient and energy cycles. The nutrients recycled by grazing fish represent a substantial amount of the available nutrients in the stream, and therefore have a direct effect on primary production and the quality of benthic algae as a food source (Hood et al. 2005; Flecker et al. 2002). The role of herbivorous fish in temperate streams has received less attention than tropical and marine environments. Research in temperate streams has focused on the impacts macroinvertebrate grazers have on benthic algae and ecosystem processes (Vannote et al. 1980). However, grazing fish can control the distribution of benthic algae, affect primary production, and reduce the abundance of macroinvertebrate grazers (Gelwick and Matthews 1992; Power et al. 1988).

Grazing by fish in marine, tropical, and temperate environments leads to a change in community composition of benthic algae (Power et al. 1988; Sammarco 1983; Abe et al. 2007). In a stream in Oklahoma the grazing minnows, *Campostoma spp.,* immediately stripped off diatom turfs and over 11 days of natural grazing, cyanobacterial felts replaced the diatom turfs. Substrate inaccessible to grazers was overgrown with diatoms in less than 10 days (Power et al. 1988). Sammarco (1983) demonstrated a similar result in a marine environment. He exposed coral substrate to a high, intermediate, and no levels of fish grazing. The intermediate level of fish grazing was established by locating the substrate within the territory of a damselfish that excluded additional grazers from the area. The substrate representing a high level of grazing was exposed to the natural environment but not within any fish territories. The substrate protected from all grazing was composed mostly of red-algae, while the substrate within the damselfish territory (intermediate grazing) had a mixture of red-algae and cyanobacteria. The substrate

2

exposed to the highest levels of grazing was dominated by cyanobacteria. *Plecoglossus altivelis*, a common grazer in Japanese streams, was used to analyze benthic algal composition under no grazing pressure, intermediate grazing pressure (0.5 fish m^{-2}), and strong grazing pressure (2.5 fish m^{-2}). After seven days the ungrazed assemblages were composed mainly of diatoms, while the grazed benthic algae were composed mostly of upright filamentous cyanobacteria (Abe et al. 2007). No studies were found that determined the precise level of grazing needed to cause the shift in taxonomic composition, however, all of the studies' 'heavy' grazing pressures were at levels consistent with natural levels of grazing and all showed a shift to cyanobacterial mats.

The shift in community composition of benthic algae under intense grazing is due to physiological and morphological differences between the types of algae (McCormick, 1996). Studies repeatedly show that cyanobacteria are tolerant of grazing while diatoms are grazing-intolerant (Power et al. 1988; Sammarco, 1983; Abe et al. 2007). Some cyanobacteria are tolerant of grazing because of the presence of a basal heterocyst located near the point of attachment with the substrate. The basal heterocysts contain regenerative tissue allowing the cyanobacteria to be clipped or grazed above this point and still remain attached and viable (Fay, 1973). Diatoms are often single celled and the stalked filamentous species lack a basal regenerative structure. However, diatoms have faster growth and reproductive rates than cyanobacteria, which allow the diatoms to outcompete cyanobacteria for light and nutrients in the absence of grazing (Power et al. 1988). Cyanobacteria also secrete a mucus coating that may make it more difficult for grazers to eat them, however, this mucus provides a sticky substrate for diatoms and other algae to attach to. Diatoms also have a higher nutritional value and are regarded as a

better food source for grazers, possibly leading to grazers preferentially feeding on diatoms (Allan, 1995).

Many species of cyanobacteria are capable of nitrogen fixation. However, it is an energetically costly process. Nitrogen fixation involves taking up biologically unavailable N_2 and converting it to a biologically available form, ammonia. Nitrogen fixation is achieved by the enzyme nitrogenase, which is inhibited in the presence of oxygen. Cyanobacteria relegate nitrogenase activity to the heterocyst where an anoxic environment can be maintained to support the nitrogen fixing process (Stewart, 1973).

Conditions that support planktonic nitrogen fixation in lakes and estuaries have been well studied compared to the conditions promoting benthic nitrogen fixation (Vitousek et al. 2002). Planktonic nitrogen fixation usually occurs in more productive lakes, whereas benthic nitrogen fixation can be a substantial part of the nitrogen budget in very oligotrophic lakes. Most studies of benthic nitrogen fixation have occurred in marine environments. However, some studies have assessed benthic nitrogen fixation in temperate and tropical lakes.

Three freshwater lakes were fertilized with different nutrient treatments to analyze the impact nutrient levels had on benthic nitrogen fixation. Lakes fertilized with only phosphorous, had the highest rates of benthic nitrogen fixation. The addition of the phosphorus increased the nitrogen limitation and promoted the benthic algae to fix nitrogen. This shows the level of nitrogen limitation is a good predictor of the presence and rate of nitrogen fixation (Bergmann and Welch 1990).

In nitrogen limited aquatic environments, nitrogen supplied through nitrogen fixation could be vital to maintaining primary production and the organisms that rely on

4

primary producers as a food source. In Lake Malawi epilithic nitrogen fixation is responsible for 8-36% of the total annual nitrogen budget. The benthic algae patches in Lake Malawi are mostly dominated by *Calothrix*, a N-fixing cyanobacteria, with basal heterocysts (Higgins et al. 2001).

The previous studies show that grazing by herbivorous fish lead to a dramatic shift in the species composition of benthic algae towards an assemblage dominated by cyanobacteria. It is also known that cyanobacteria are active nitrogen fixers and under certain nutrient conditions can supply a large lake with a substantial portion of the nitrogen needed to support primary production. Under poor nutrient conditions grazing by fish may act to promote nitrogen fixation by cyanobacteria. To my knowledge, there is only one study that addressed this interaction. Nitrogen fixation rates of epibenthic algae on non-grazed substrates, intermediate grazed substrates, and heavily grazed substrates were measured in a coral reef ecosystem (Wilkinson and Sammarco 1983). Results showed that heavily grazed substrates fixed significantly more nitrogen than non-grazed substrates. The benthic algae on the grazed substrate were composed primarily of cyanobacteria while the non-grazed algae were mostly red algae.

We chose to study the impacts of herbivorous fish on nitrogen fixation by benthic algae because of the lack of research on the topic and the importance of nitrogen fixation to a nitrogen limited ecosystem. Our research will help researchers understand the nitrogen budget in nitrogen limited aquatic ecosystems and will analyze the role of vertebrate grazers and their impact on the production of the ecosystem.

To conduct our research we designed a laboratory experiment using *Campostoma anomalum*. We used *Campostoma anomalum* because their grazing on periphyton leads

to a shift in community composition from diatoms to cyanobacteria, which provides us with a good model to test for an effect on nitrogen fixation (Power et al. 1988). *Campostoma* is historically and currently abundant in streams throughout its range and is the only primarily herbivorous, native fish in Midwestern streams (Trautman, 1981; Gelwick and Matthews 1992). Therefore, our research may be indicative of stream dynamics that were present before streams throughout the Midwest were polluted with nitrogen from agriculture. We used periphyton collections from Lake Tahoe and cultures from the National Institute for Environmental Science (NIES) to ensure that the cyanobacteria in our experiment were capable of nitrogen fixation. We also stocked mixed assemblages of diatoms in our experiment to mimic natural periphyton communities in aquatic ecosystems.

We hypothesize that periphyton released from grazing by *Campostoma anomalum* will increase in biomass and be dominated by diatoms. We also predict that periphyton released from fish grazing will increase area-specific production and decrease biomass-specific production. Finally, we tested whether rates of area-specific nitrogen fixation is higher under grazed conditions compared to when periphyton is protected from grazing

II. METHODS

Experimental Design

Four 200 L aquaria were setup on a flow through system for the experiment. The water temperature was maintained at 19°C with the use of a Frigid Units (LC A419) chiller. The entire system was fertilized with a nitrogen deficient culture medium (see Culture Medium) and conditioned using Seachem Cichlid Lake Salt to mimic natural water conditions for local streams. Eight 36 cm^2 square clay tiles were placed in each tank. The tiles were arranged in sets of 4 on white lattice to elevate the tiles off the gravel. The sets of tiles were placed on opposite ends of the tank. Periphyton communities of cyanobacteria, green algae, and diatoms were cultured on the tiles before placing them in the tank (see algal culturing methods, below). Ten stonerollers collected from Massie's Creek, a small tributary of the Little Miami River, (Xenia, Ohio, Greene Co., 39° 44′ 24″ N, 83° 56′ 24″) were placed in each tank two days after the water system was conditioned. Ten stonerollers per tank is an average number of stonerollers per unit area in natural conditions (Power et al. 1988; OEPA unpub data). The only food source for the stonerollers was the algae growing inside the tanks.

The aquaria water was conditioned and fertilized on June 22, 2009. On June 23rd, 2009 the tiles were placed in the aquaria. On June 24th, 2009 the fish were placed in each tank. The fish grazed the algae on the tiles for nineteen days before analysis of the periphyton communities began. On July 17th analysis of the periphyton samples began and we consider this day 0 of our experiment. On July 17th, 2009 after analyzing primary

production, chlorophyll concentration, community composition, and nitrogen fixation the fish were excluded from one half of each aquaria for the duration of the experiment. The exclusions were the treatments in our experiment and the tiles with fish grazing were the controls. Black, mesh netting (1/4″) was used to divide the aquaria. We removed tank 2 data from all analyses due to fish moving into the exclosure before the final analyses. Analysis of the periphyton communities was carried out for the next twenty days and each analysis is described below. Figure A describes the timing of each analysis and Figure B describes the tiles each analysis was performed on.
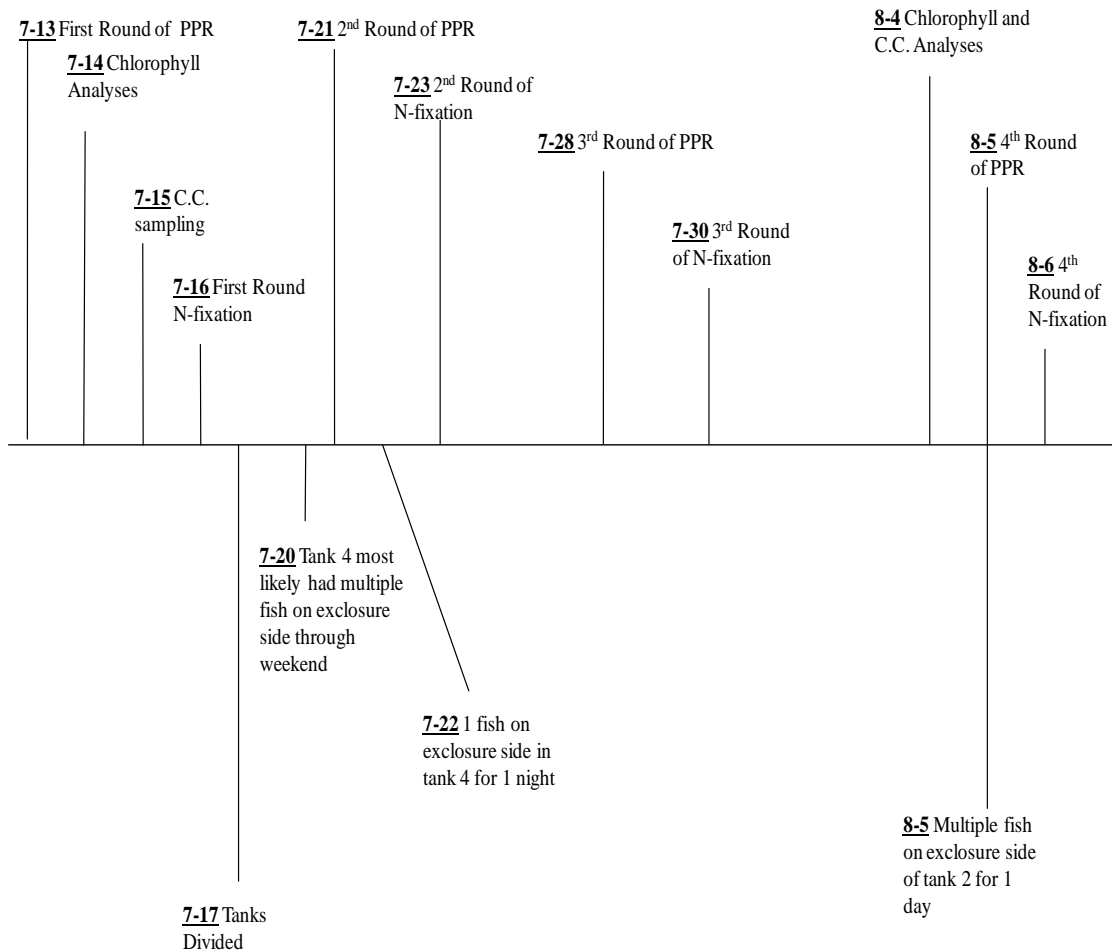
Figure. A. The top portion of the timeline includes the dates analyses were performed. The bottom portion includes changes made to the tanks and unintended fish movements into the excluded portion of the aquaria. Due to the movement of fish into the exclosure in tank 2 we removed this tank from all analyses. PPR stands for primary production. C.C. stands for community composition. N-fixation stands for nitrogen fixation.
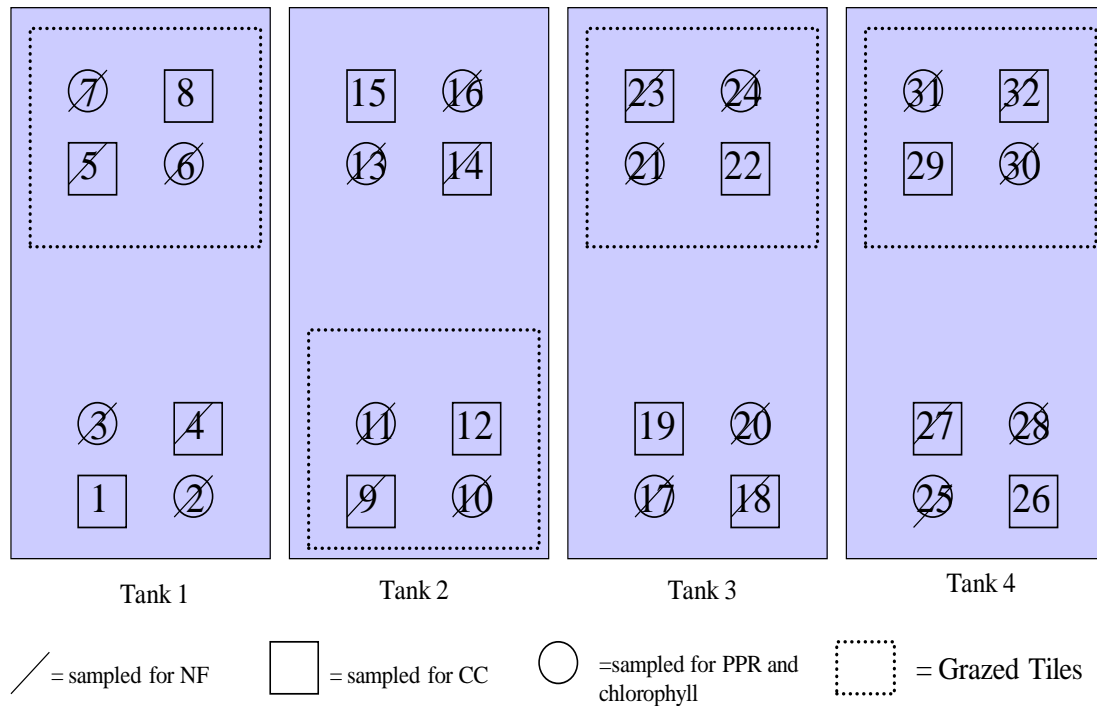
Figure. B. Diagram of the type of analysis performed on each tile and the grazing treatment in each aquarium.

Periphyton Culture

To obtain a mixture of diatoms and nitrogen fixing cyanobacteria we incubated different sources of algae in 20L glass carboys at 20°C and constant light. We sampled local streams for nitrogen fixing cyanobacteria, but did not find any active nitrogen fixing cyanobacteria. Ohio streams are nitrogen saturated eliminating the need for cyanobacteria to fix nitrogen. The algal sources included an unialgal, non-axenic culture of *Calothrix brevissima* (National Institute for Environmental Science(NIES)-2097), a periphyton sample from Lake Tahoe, and a mixed diatom assemblage (Carolina #151287). Periphyton was cultured from Lake Tahoe because Reuter et al. (1986) reported an abundance of nitrogen fixing cyanobacteria and Brant Allen (UC Davis Lake Tahoe Research Group) was able to collect a sample and quickly ship it to us. Once the cultures were established in the glass carboys we siphoned a portion of the culture into a fertilized incubation tank. Tiles were incubated in the incubation tank until a mat of algae was present on the tiles. We microscopically verified the presence of the desired diatoms and nitrogen fixing cyanobacteria before beginning our experiment.

A culture medium modified from Kruskopf and Du Plessis (2006) was used to grow the periphyton on the tiles and to fertilize the aquaria prior to placing the tiles in the aquaria. We modified their culture medium by removing the nitrogen source and adding a phosphorous source to encourage the growth of nitrogen fixing cyanobacteria, adding silica to ensure diatom growth, and adding boron to promote heterocyst growth and nitrogenase activity in the cyanobacteria (Bonilla et al, 1990). The following compounds were dissolved in 4.5 liters of distilled water to fertilize the entire flow through system (1700 liters). $MgSO_4$ (50 g), $CaCl_2$ (23.4 g), Na_2CO_3 (26 g), citric acid (7.8 g), $FeSO_4$

(6.5 g), vitamin B12 (dash), and $Co(NO_3)_2$ (dash). We added phosphorous to the system by adding a solution of Na_2PO_4 (25.5 g) and 850 mls of distilled water. We added silica to the system by adding a solution of Na_2SiO_3 (40 g) and 750 mls of distilled water.

Chlorophyll

Chlorophyll measurements were used to assess algal biomass. The periphyton was aspirated from 200.96 mm2 of the clay tile. Aspiration was conducted by connecting a 500-ml side arm flask to the wall mounted vaccum system. A rubber stopper with a rubber hose (1/8 inch ID) inserted into it was placed into the flask. The end of the hose opposite the flask end was fitted with a pointed, hard plastic piece to scrape the tile. The tile was scraped and aspirated inside the area sectioned off with the use of a rubber O-ring (16mm ID) fixed to a bottle cap. Aspiration lasted until the periphyton inside the O-ring was completely removed. The water inside the flask was filtered onto a Whatman 47 mm glass microfiber filter. The filter was placed inside a Corning 15 mL centrifuge tube and stored in a freezer until extraction. Extraction was completed 24 hours before flourometric analysis with 95% ethanol. Twenty four hours after extraction the samples were analyzed on a TD-700 fluorometer.

Community Composition

Community composition samples were collected from the tiles by the same method that chlorophyll samples were collected (see above). Samples were preserved in .25 % gluteraldehyde solution, refrigerated in the dark, and sonnicated. Five milliliters of sample was vaccum filtered through a 0.45 μm Millipore membrane filter (cat no. HAWP02500). The filters were placed on microscope slides and cleared with immersion

oil.   We counted and identified the algal cells in 10 fields of view or at least 300 individuals with a Nikon Optiphot at 400x.

Primary Production

Two tiles from each side of each tank were analyzed for primary production. The tiles were incubated in clear, acrylic (2355 ml) chambers. All tiles were incubated inside their chambers for 2 hours in a 750 liter incubation tank that was kept the same temperature as the experimental tanks. After the incubation time, the water in the chamber was siphoned into a 300 ml BOD bottle, which was stored in the dark and analyzed between 20 and 24 hours later. The oxygen concentration in the water was analyzed using the Winkler method. Three water samples were taken immediately before, during, and after the tiles were removed from each experimental tank to provide oxygen levels in the water column. All tiles were incubated in the light and dark to correct for oxygen consumed during respiration.

Nitrogen Fixation

Nitrogen fixation rates were measured on days 3,10, 17, and 24 of the experiment. We analyzed three randomly chosen tiles from each side of the tank divider for each aquaria. Nitrogen fixation rates were analyzed using a modification of the acetylene reduction technique (Stewart et al. 1967). This technique is based on the ability of cyanobacteria to reduce acetylene to ethylene at a rate comparable to the rate of nitrogen fixation.

The clay tiles were incubated in clear, (2355 ml) acrylic chambers for 2 hours. The chambers were fitted with upper and lower plugs that provided a gas-tight seal inside the chamber. The upper plug had a built-in stirring device and two holes fitted with

rubber septa. Each tile was placed inside the chamber after submerging the chamber in the tank. After placing the tile in the chamber, the upper plug was inserted into the chamber until the top of the plug was flush with the top of chamber. The resulting water volume inside the chamber was 600 milliliters. Rubber septa were placed in the holes on the upper plug to make the chamber gas-tight. The chambers were removed from the tank and sixty milliliters of water was removed from the chamber using a 60-ml Becton-Dickson (BD) syringe. Sixty milliliters of acetylene-saturated water was injected into the chamber using a BD syringe to make a 10% acetylene saturated water solution inside the chamber. The acetylene saturated water was made by bubbling 99.9% pure acetylene (AirGas Atomic Absorption grade 99.8%) through DI water in a 500 milliliter Erlenmeyer flask. The chambers were placed in a 750 liter incubation tank that was the same temperature as the experimental tanks. The chambers were incubated for approximately two hours. Twelve tiles were incubated each round and two rounds of incubations were conducted each day nitrogen fixation was analyzed. Preliminary analyses showed no trace of ethylene production after incubating only water from the tanks and incubating tiles without periphyton growth. Therefore, no blanks were incubated along with the tiles. The chambers were stirred every 15 minutes. After the incubations, the chambers were removed from the tank and approximately 30 milliliters of water was pulled from the chambers using a 50-ml SGE gas-tight syringe. Fifteen milliliters of the sample inside the gas-tight syringe was distributed equally into three 7-ml Becton-Dickson vacutainers. The vacutainers were shaken vigorously for two minutes and then set aside to equilibrate at room temperature.

The headspace in each vacutainer was analyzed less than five hours later for acetylene and ethylene production using a HP 5890 Gas Chromatograph. Fifty microliters of headspace from the vacutainer was injected into the GC using a 100 microliter Hamilton gas tight syringe. The GC was fitted with a 0.53 GS-Alumina-Megabore 30 meter column, helium carrier gas, and a flame ionization detector. Ethylene values were determined using standard curves generated using a 5% ethylene in helium specialty gas. Standard curves were generated twice during the experiment. The values of the nitrogen fixation analyses performed on July 16th were based on a standard curve generated on July 7th (Figure C). The values of nitrogen fixation analyses performed on July 23rd, July 30th, and August 6th were based on a standard curve generated July 27th (Figure D). There is 71% variance between the peak areas at a concentration of 44.6 nM of ethylene on each standard curve, making it difficult to compare nitrogen fixation values between time periods.
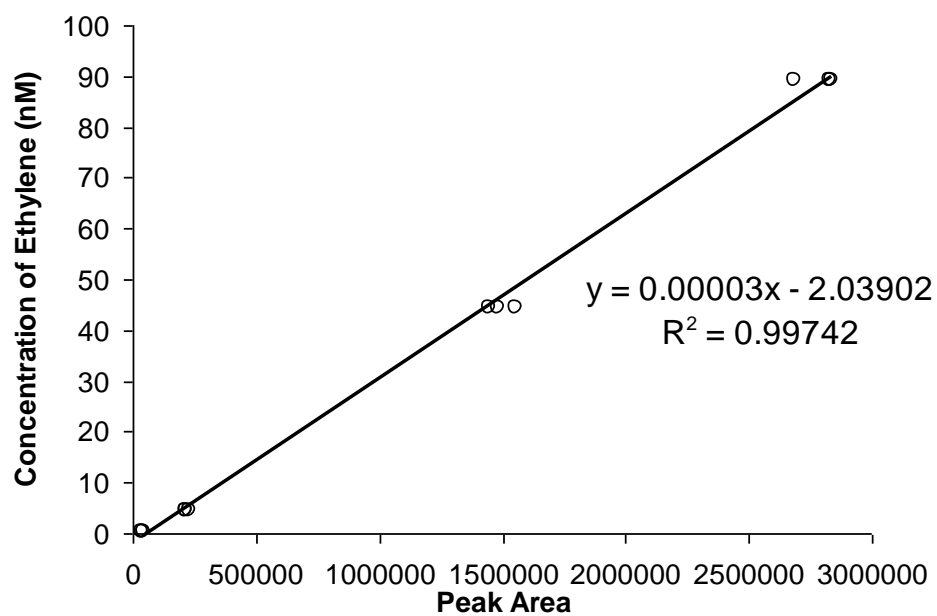
Figure C. Standard curve generated on July 7th. This standard curve was used to calculate
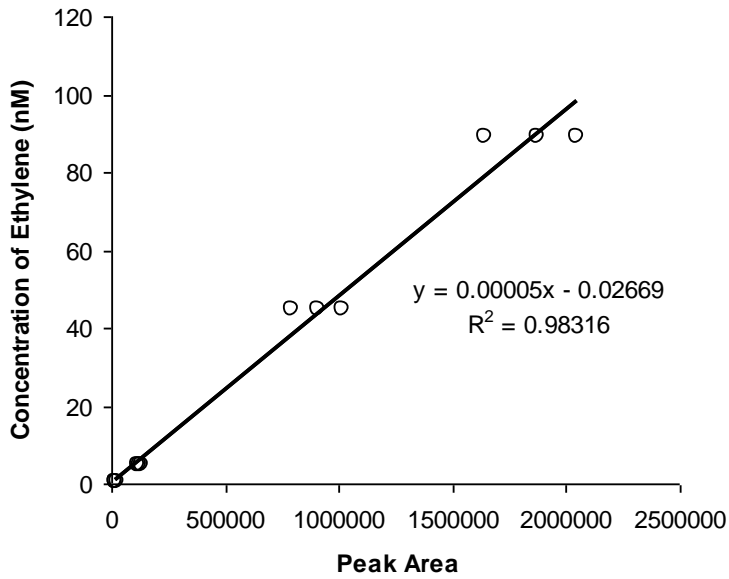
nitrogen fixation values for July 16th.

Figure D. Standard curve generated on July 27th. This standard curve was used to calculate nitrogen fixation values for July 23rd, July 30th, and August 6th.

Standard Curve Procedures

The standard curve used to evaluate ethylene levels was conducted using serial

dilutions of 5% ethylene in helium. A gas bulb fitted with a septum and filled with the

ethylene gas mixture serves as the source of ethylene for the dilutions. The standard

curve ranged from 0.46 nM of ethylene to 89.2 nM of ethylene. The amount of ethylene

in each injection was based on Avogadro's law that one mole is equal to 22.4 liters of

gas. We did not calibrate for temperature and pressure. Each data point on the standard

curve was the average of three injections. Table 1 lists the concentration values for each

point on the standard curve and the volume of ethylene gas required for each point. We

created the serial dilutions by pulling ethylene and air into our syringe and waiting 30

seconds for the gasses to mix. Our final injection volume was 10 microliters.

Concentration of Ethylene (nM)	Volume of Ethylene in Syringe (μl)
0.446	0.01
4.46	0.1
44.6	1
89.2	2

Table 1. Concentration of ethylene (nM) and the volume of ethylene (μl) in the syringe at

each point on the standard curve

18

Converting Ethylene Values to Nitrogen Values

We generated ethylene values based on Capone (1993). The equation is as follows.

(Peak area unknown/Peak area standard) * Standard *GPV*SC   where

Pk. area unknown = average value of the area under the curve for three injections

Pk. area std.= area under the curve for best-fit standard at a concentration of 44.6 nM

Std = concentration of the standard at the midpoint of the standard curve (nM)

GPV = gas phase volume in sample vacutainer (2 ml)

SC = solubility constant, $1 + (\alpha * A/B)$ where

$\qquad \alpha = 0.122$ (Breitbarth et al., 2004)

$\qquad A$ = volume of aqueous phase in sample container (5 ml)

$\qquad B$ = volume of gaseous phase in sample container (2 ml)

Ethylene fixation values were converted to nitrogen fixation values by assuming a 3:1 conversion ratio of ethylene fixed to nitrogen fixed. We used a 3:1 ratio in order to compare our nitrogen fixation rates with studies evaluating similar biological components.

Statistics

Effect Size

We calculated the log response ratio (R) to analyze the relative differences between the control and treatment in each tank. The log response ratio is used to quantify the proportional change resulting from an experimental manipulation and is reported as the effect size (Hedges et al. 1999). We calculated the log response ratio as $R = \ln(X_t/X_c)$. X_t = the mean for treatment tiles (fish not grazing) and X_c = the mean for the

control tiles (fish grazing). The mean effect size from each tank was used to determine

average effect size. In our case, a positive effect size indicates higher values on the

treatment tiles (excluded from fish grazing) and a negative effect size indicates higher

values on the control tiles (subjected to fish grazing).

T-tests

We used paired t-tests to analyze data that was collected at the beginning and end

of the experiment. We report the *t* statistic, degrees of freedom, and *p* value for each test.

All t-tests were conducted on Fathom version 2 statistical software.

RMANOVA

We used a repeated measures analysis of variance (RMANOVA) to analyze data

that was sampled over four different time intervals. The data analyzed by RMANOVA

includes area-specific primary production, area-specific respiration, and area-specific

nitrogen fixation. The RMANOVA's were conducted on SAS statistical software.

III. RESULTS

At the beginning of the experiment (Day 0), we saw no difference in algal

biomass between treatment groups because all tiles were grazed (paired $t = 0.49$, df $= 5$, p

$= 0.64$). The tiles that were grazed throughout the experiment showed no change in

biomass over time (paired $t = 0.63$, df $= 5$, $p = 0.56$). Algal biomass (chlorophyll) on the

ungrazed tiles doubled when released from fish grazing (paired $t = -2.48$, df $= 5$, $p =$

0.06), and was higher, but not significantly so, on the ungrazed tiles than the grazed tiles

at the end of experiment (paired $t = -1.57$, df $= 5$, $p = 0.18$).

The absence of fish grazing caused a shift in the community composition of the

periphyton. Fig 2A shows that fish grazing (Initial Grazed, Initial Ungrazed, Final

Grazed) resulted in no difference in the composition of the periphyton. The removal of

fish (Final Ungrazed) resulted in a periphyton community composed mostly of diatoms.

There is a significant difference in the numbers of diatoms and *Calothrix spp*. after the

treatment on the ungrazed tiles (paired $t = 7.09$, df $= 5$, $p < 0.001$). This significant result

allows us to reject the null hypotheses that the removal of fish grazing will not impact the

community composition of the periphyton.   The difference between numbers of

Calothrix spp. on the initial ungrazed tiles and the final ungrazed tiles is also significant

(paired $t = 3.94$, df $= 5$, $p = 0.01$). Therefore, the removal of fish grazing has a negative

impact on the abundance of *Calothrix spp.*

The effect sizes at the end of the experiment indicate *Calothrix spp.* were more

abundant on the tiles subjected to fish grazing and diatoms were more abundant on the

tiles excluded from fish grazing. The decrease in the effect size of the *Calothrix spp*

during the experiment is significant (paired $t = -4.41$, df = 2, $p = 0.048$) and indicates

Calothrix spp. are more abundant during grazed conditions.

Fish affected the structure of periphyton by reducing the biomass and promoting

the presence of cyanobacteria. Our next step was to analyze how the changes in structure

affect the functioning of the periphyton. Fig 3A. indicates that fish grazing has no effect

on the rates of area-specific primary production. We used a repeated measures analysis

of variance (RMANOVA) to test for a significant grazing effect, grazing * time effect,

tank effect, and tank * time effect. We found the removal of fish grazing had no

significant effects on any of the response variables. Therefore the removal of fish

grazing had no impact on the amount of area-specific gross primary production during

the experiment. We also analyzed the grazing effect on area-specific respiration (Fig.

3B).

The changes in the structure of the periphyton had no impact on the area-specific

productivity of the periphyton. However, the biomass became less efficient when fish

grazing was removed. Biomass-specific primary production is a measure of efficiency

because it analyses the amount of production (mg C hr^{-1}) per unit biomass (chlorophyll *a*)

in the periphyton. Figure 4 shows the biomass-specific primary production did not

change on tiles that were grazed throughout the experiment. However, when fish grazing

was removed the amount of biomass-specific primary production decreased. The

decrease in biomass-specific primary production was not statistically significant (paired *t*

$= -2.191$, df = 5, $p = 0.08$).

Figure 5 shows the effect sizes for analyzing the structure and function of the biofilm. Only the chlorophyll *a* effect size is statistically significant (paired $t = 6.23$, df = 2, $p = 0.03$). There was an increase in the biomass-specific primary production on the grazed tiles, but the difference in effect size was not significant (paired $t = -2.11$, df = 2, $p = 0.17$).

To further investigate the functioning of the periphyton in response to fish grazing we analyzed nitrogen fixation by the periphyton. Figure 6 shows areal nitrogen fixation was not impacted by the removal of fish grazing during the experiment. A repeated measures ANOVA showed no significant grazing effect or grazing*time effect. Nitrogen fixation rates over time may not be accurate because the measurements were not standardized with a standard curve each day the measurements were taken. Therefore, we use effect sizes for each day because this measurement compares the nitrogen fixation values within the day and does not compare nitrogen fixation measurements between sampling intervals. Figure 7 uses the effect sizes and shows that the area-specific nitrogen fixation did not change over time between the grazed and ungrazed tiles. There is a significant difference between the response ratios on day 3 and day 10 (paired $t = 16.66$, df = 2, $p = 0.004$), however the difference did not persist throughout the experiment.

Although the area-specific nitrogen fixation was not affected by the removal of fish grazing, the biomass-specific nitrogen fixation decreased when fish grazing was removed. The response ratio for biomass-specific nitrogen fixation became more negative, meaning the periphyton that was grazed increased biomass-specific nitrogen fixation compared to the periphyton without fish grazing (paired $t = -3.89$, df = 2, $p =$

0.06) (Fig. 8). Fish grazing reduced the algal biomass, but did not impact the rate that nitrogen was fixed.

To investigate the decrease in the efficiency of nitrogen fixation with the removal of fish grazing, we analyzed the community composition of the periphyton. With the removal of fish grazing diatoms made up 83% of the cells present in the periphyton community (Fig. 9) which was significantly different from the ungrazed tiles at the beginning of the experiment (paired $t = 5.37$, df $= 5$, $p = 0.003$). Fish grazing promoted a more balanced periphyton community with diatoms never making up more than 54% of the cells in the biofilm. The removal of fish grazing led to a biofilm dominated in numbers by diatoms, but the shift in community composition did not reduce nitrogen fixation.

The rate of nitrogen fixed did not decrease with the amount of nitrogen fixing cyanobacteria present in the absence of fish grazing. Therefore, we investigated the amount of nitrogen fixed per heterocyst. Fig. 10 indicates the amount of nitrogen fixed for each heterocyst was higher in the ungrazed biofilms. However, our data were highly variable around the mean, which makes it difficult to draw conclusions (paired $t = 1.02$, df $= 2$, $p = 0.41$).
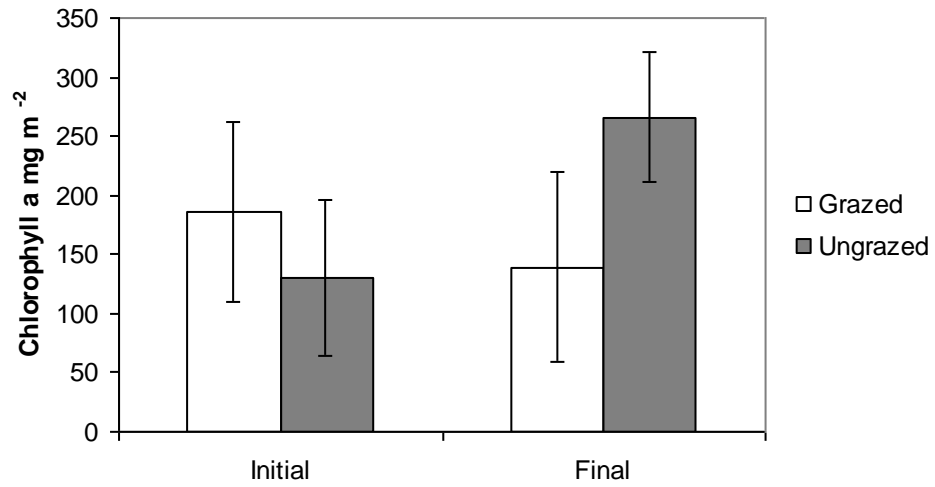
Figure. 1. Chlorophyll a concentrations for grazed and ungrazed treatments (+-SE). At the time of initial samples all tiles had been exposed to fish grazing for 20 days. The final samples were collected 18 days after dividing the tanks. The chlorophyll values were not corrected for pheophytin concentration. Fish density doubled after dividing the tanks causing the slight reduction in algal biomass in the grazed treatment. Algal biomass doubled when released from grazing.
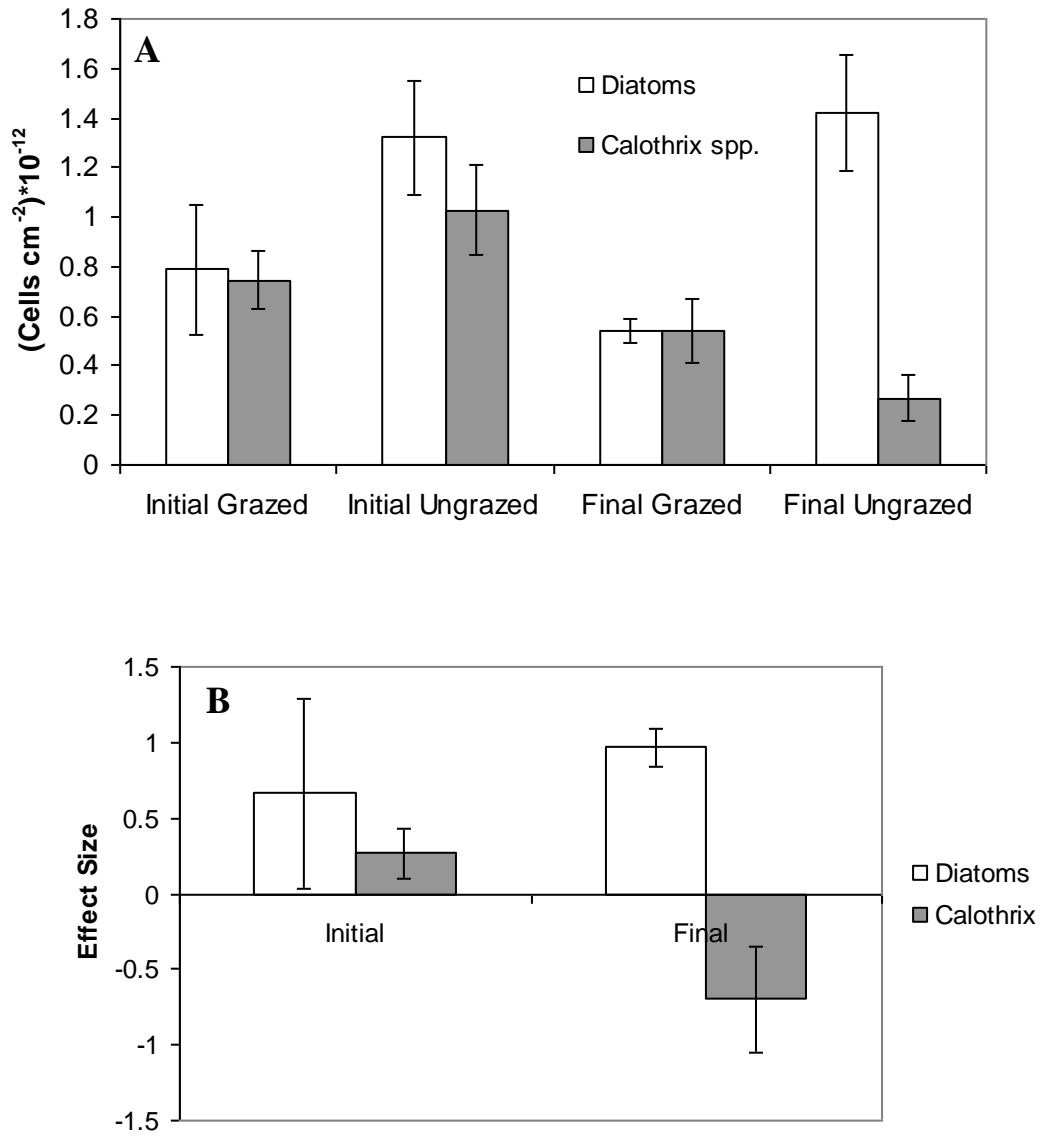
Figure. 2 (A). Community composition of the periphyton (+-SE). Diatoms were

identified to genus. Calothrix always made up at least 76% of the cyanobacteria present.

Green algae never made up more than 2% of the periphyton community. Absence of fish

grazing caused a drop in the abundance of cyanobacteria and an increase in the number of

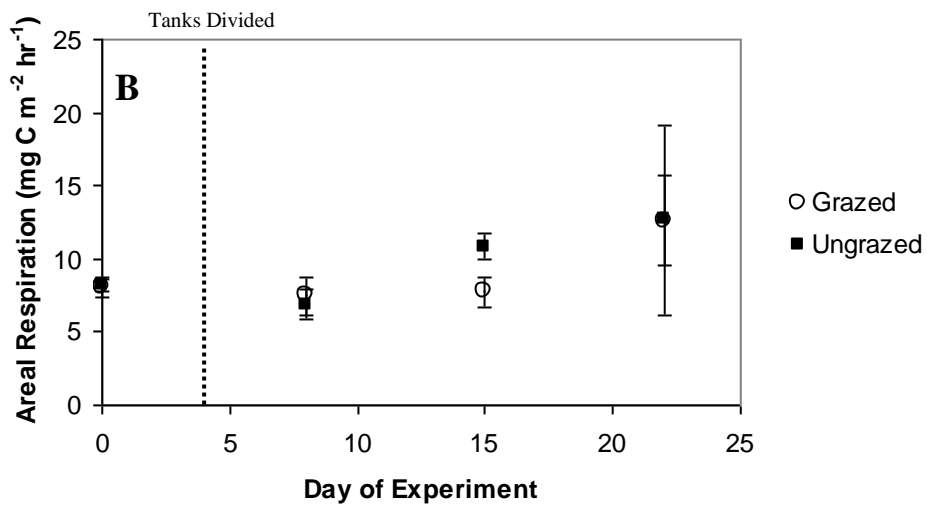diatoms. (B). Response ratio for the community composition of the periphyton (+-SE).
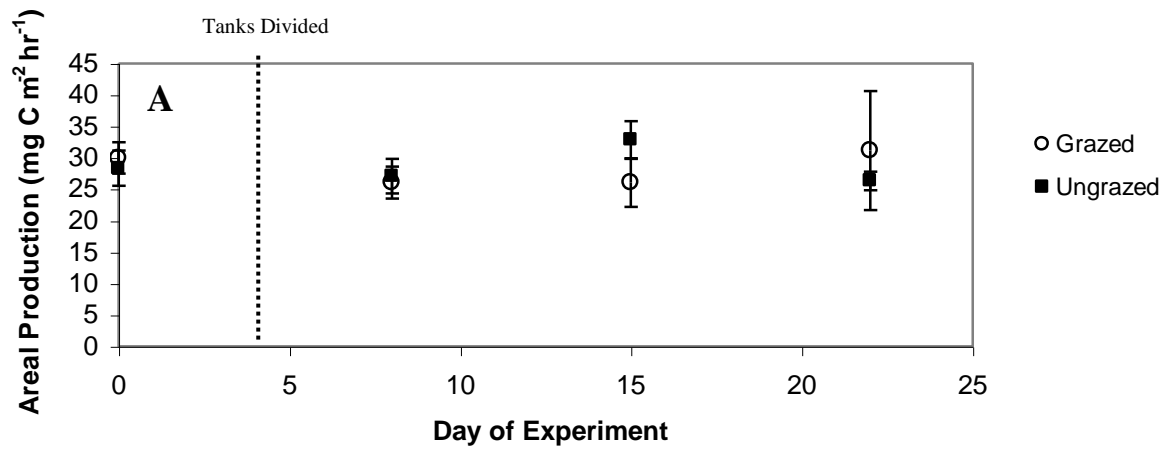
Figure. 3 (A). Rates of gross primary production for grazed and ungrazed tiles (+-SE).

Neither the doubling of grazing intensity associated with tank division nor the exclusion

of fish affected the primary production. (B). Rates of area-specific respiration for grazed
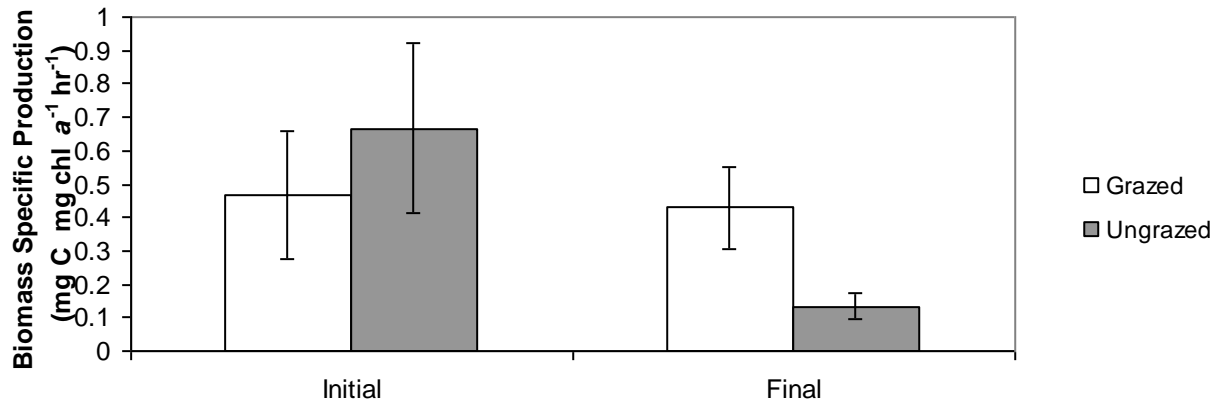
and ungrazed tiles (+-SE).

Figure. 4. Biomass-specific primary production for grazed and ungrazed tiles (+-SE).

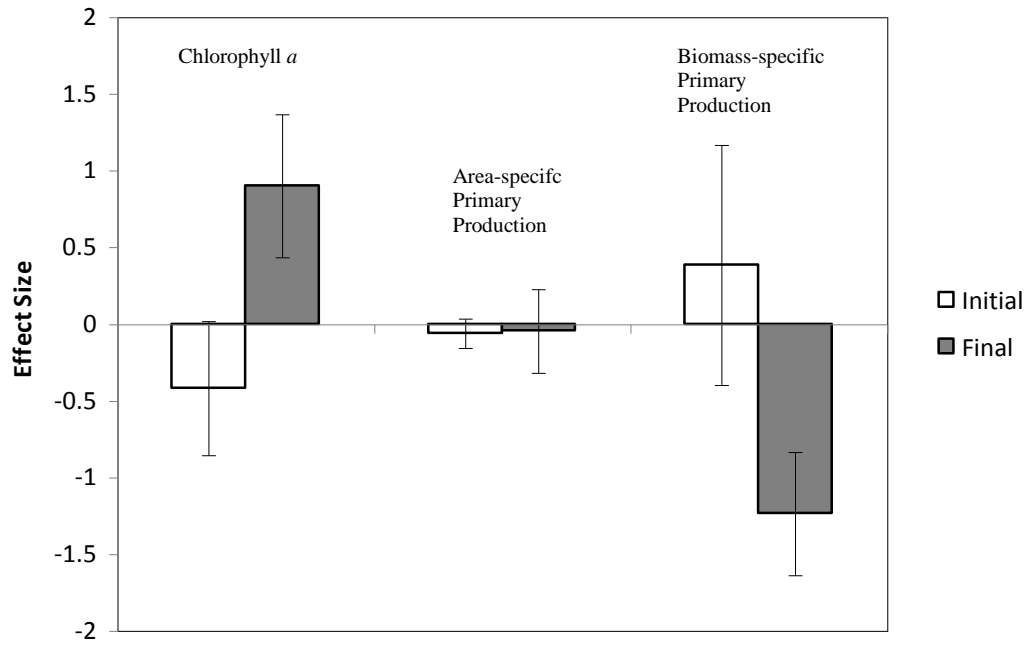The removal of grazers reduced the production efficiency of the periphyton.

Figure 5. Initial and final effect sizes for chlorophyll *a*, area-specific primary production, and biomass-specific primary production (+-SE).
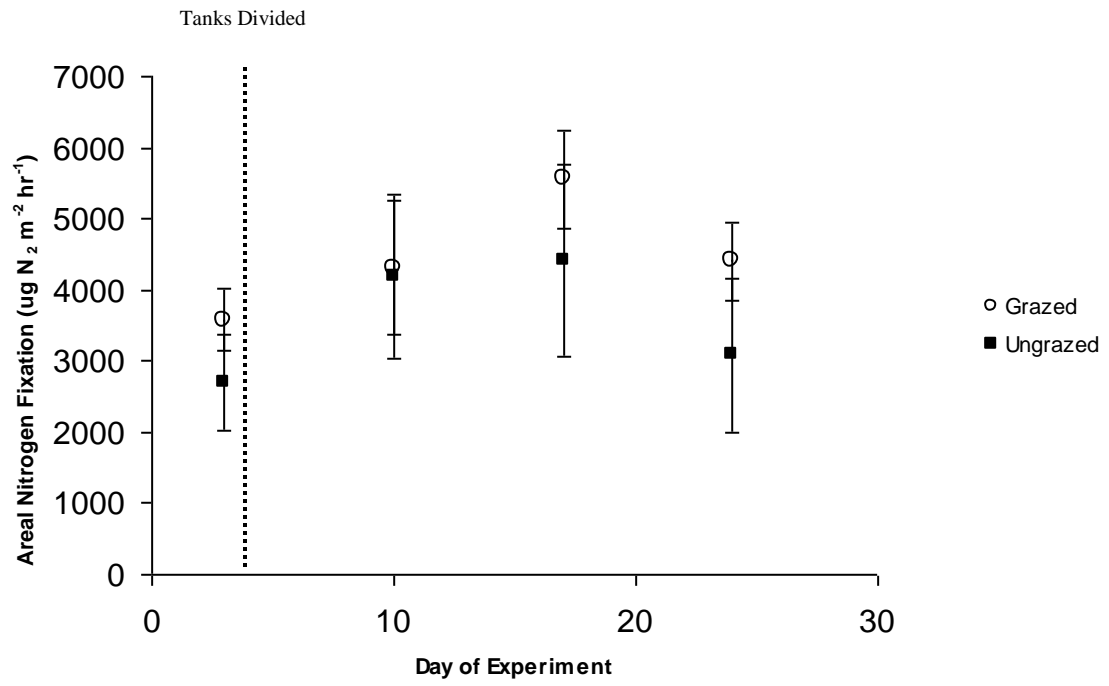
Figure. 6. Area-specific nitrogen fixation for the grazed and ungrazed tiles (+-SE).

Removal of grazers had no impact on the rates of area-specific nitrogen fixation.
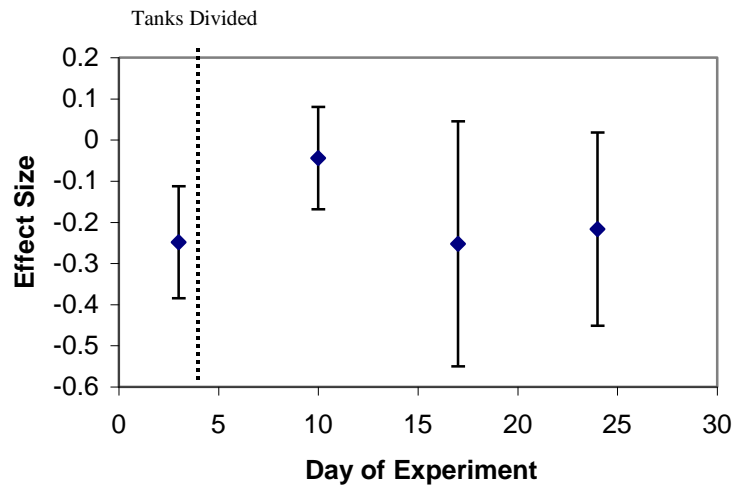
Figure. 7. Effect size for area-specific nitrogen fixation (ug of N_2 m^{-2} hr^{-1})(+-SE) . Effect size is equal to the ln(exclosure/enclosure). The effect size shows no change over time, which suggests the absence of fish does not affect area-specific nitrogen fixation.

Figure 8. Initial and final effect size for chlorophyll *a*, area-specific nitrogen fixation, and biomass-specific nitrogen fixation (+-SE).

Figure 9. Percent community composition of the periphyton (+-SE). Diatoms and

Calothrix spp. always made up at least 95% of the periphyton community.

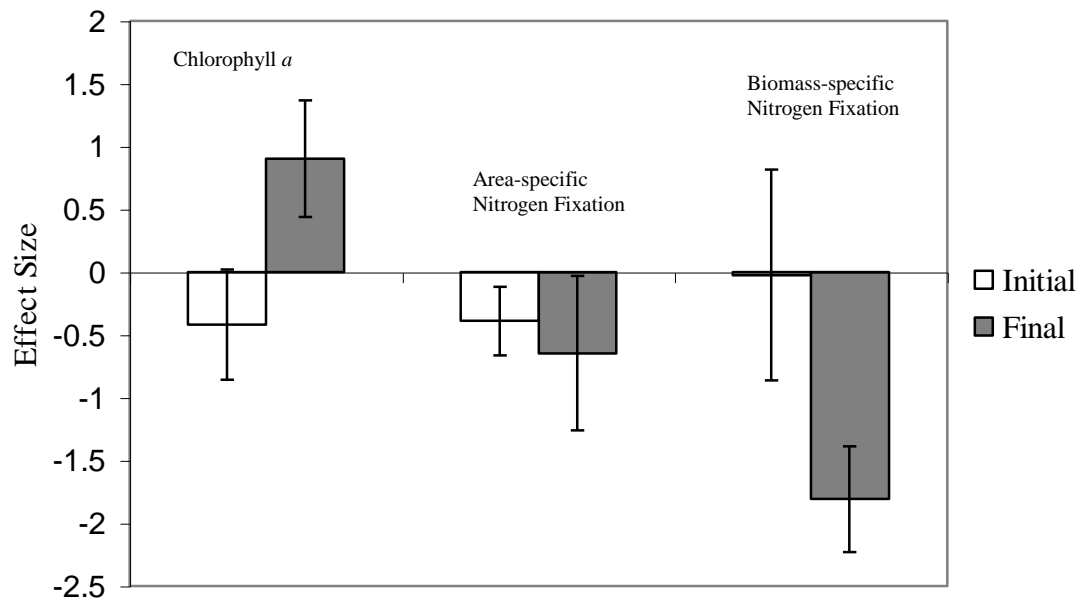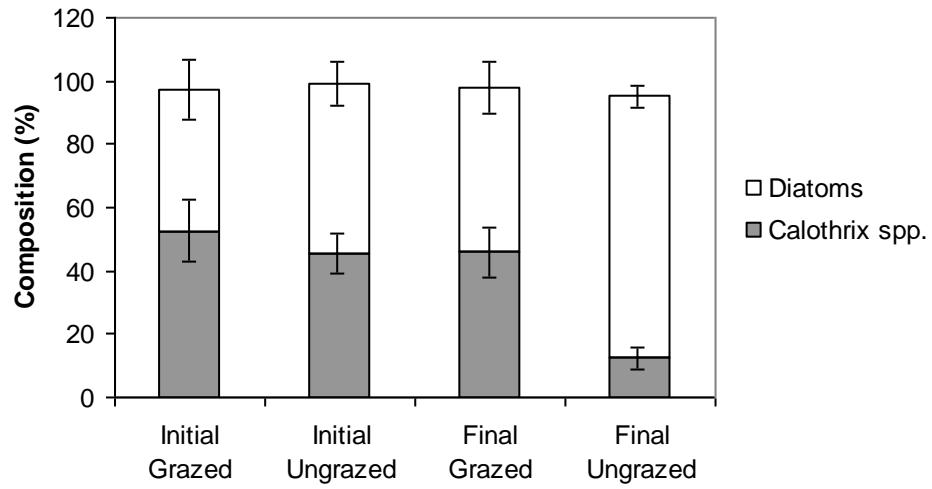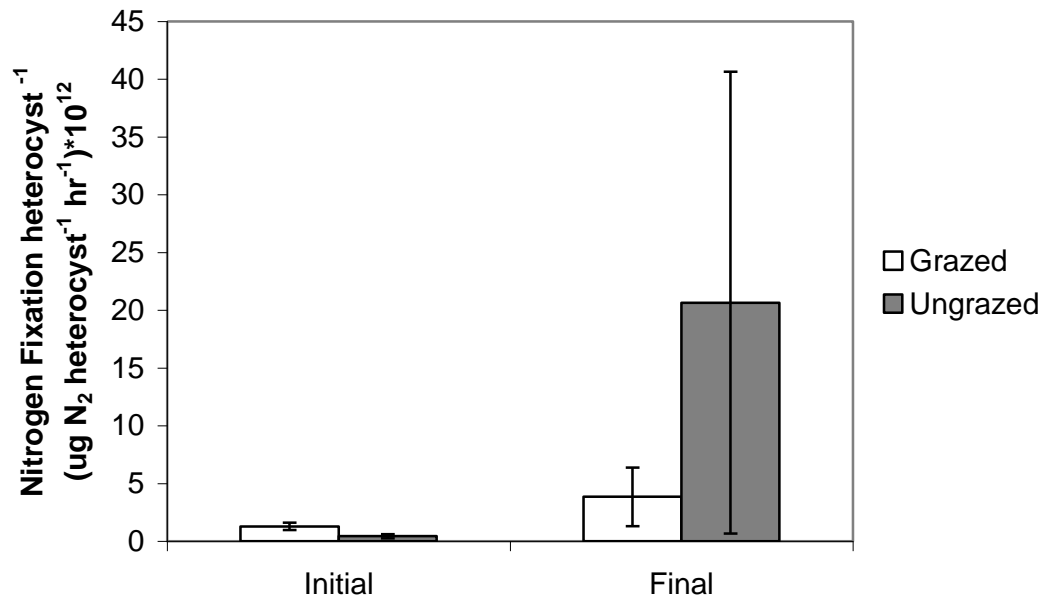Figure 10. Heterocyst specific nitrogen fixation (ug N_2 heterocyst^{-1} hr^{-1})*10^{12} on grazed and ungrazed tiles (+-SE).

IV. DISCUSSION

We expected fish grazing to have a negative impact on the biomass of the periphyton on the tiles. Figure 1 shows that the removal of fish grazing caused a doubling of chlorophyll *a*, but the chlorophyll *a* on the control tiles (with fish grazing) showed little change. The effect size (Fig. 5) also shows the amount of chlorophyll *a* increased with the removal of fish grazing relative to the tiles subjected to grazing. The biomass results are evidence that the system was stable. If we saw an increase in chlorophyll *a* on the control tiles this would suggest the system was not yet stable and periphyton was continuing to colonize the tiles. We observed an increase in the amount of invertebrates (likely Chironomidae, not identified or quantified) on the tiles without fish grazing. Invertebrates reduce algal biomass, therefore our results are conservative for the tiles without fish grazing (Steinman 1996).

Fish grazing leads to a periphyton community composed mostly of cyanobacteria, while the absence of fish grazing leads to a periphyton community composed mostly of diatoms (Wilkinson and Sammarco 1983; Power et al. 1988; Gelwick and Matthews 1992; Abe et al. 2007). Our results are consistent with the shift in community composition of periphyton under grazed conditions. The treatment tiles (fish grazing removed) showed a decline in the abundance of cyanobacteria and a small increase in the abundance of diatoms (Fig. 2a). Diatoms made up 83% of the periphyton community with no fish grazing but under grazed conditions never made up more than 54% of the periphyton community. Multiple explanations exist for cyanobacteria persisting under grazed conditions compared to diatoms. Some cyanobacteria have a basal heterocyst

35

from which algal tissue can be regenerated if the filaments are clipped above this point (Fay, 1973). The cyanobacteria most abundant in our study was *Calothrix spp.*, and this genus can form basal heterocysts. Also, cyanobacteria can secrete a mucous coating that may make it difficult for grazers to consume them. In the absence of grazing, the mucous coating may provide a sticky substrate that facilitates the colonization of diatoms (Power et al. 1988).

Diatoms are faster growing than cyanobacteria and may outcompete cyanobacteria for nutrients and light when no grazing is present (Power et al. 1988). According to this explanation, productivity of the periphyton should increase when diatoms dominate the periphyton community. However, our results do not support this explanation. We found gross primary production did not change and biomass-specific production declined when diatoms dominated the periphyton community.

Diatoms also have a higher nutritional value and are regarded as a better food source for grazers, possibly leading to grazers preferentially feeding on diatoms (Allan 1995). *Plecoglossus altivelis*, a herbivorous fish in streams and rivers of Eurasia, consume a significantly higher percentage of diatoms than the diatoms represent in the periphyton community leading to a dominance of upright filamentous cyanobacteria (Abe et al. 2006). *Plecoglossus altivelis* use a comb-like mouth to remove algae, providing strong evidence that some species of fish selectively feed on diatoms (Abe et al. 2006). *Prochilodus*, a detritivorous fish, promoted the growth of *Calothrix* in a tropical stream showing non-selective feeding styles can also lead to a cyanobacteria dominated periphyton community (Flecker et al. 1996).

Campostoma remove algae by sliding their lower jaw across the substrate and don't appear to exhibit selectivity in their feeding (personal observation). However, if microstructures in the jaw are responsible for the selectivity we would not be able to observe this. Our observations and data support the idea that the shift from diatoms to cyanobacteria dominated periphyton under grazed conditions is mediated by the attributes of the algal species and not the grazer. Fish grazing by *Campostoma* caused substantial structural changes in the periphyton community. Despite the large change in community composition, there was no evidence of a negative effect of fish grazing on primary production or N-fixation. The response of periphyton to *Campostoma* grazing was analyzed in a small Oklahoma stream and net primary production values ranged from 100-450 (mg C m^{-2} hr^{-1}). Fish grazing also had a negative impact on areal production (Gelwick and Matthews 1992). These values are higher than our gross primary production values (25-35 mg C m^{-2} hr^{-1}) and may be attributed to higher algal biomass, nutrient levels, water temps, and light intensities (light and temperature were not reported). Gross primary production in response to snail grazing in oligotrophic Alaskan lakes ranged from 5-27 (mg C m^{-2} hr^{-1}) (Gettel et al. 2007). The water temperatures in the Alaskan lakes ranged from 10°-13° C which is 9° and 6° respectively below the temperature of our system and may explain the slightly lower gross primary production values. Overall, our gross primary production values are in the same range as studies from natural, benthic, periphyton communities which gives us confidence in drawing conclusions from our system.

We predicted that periphyton released from fish grazing would have higher gross primary production due to the increase in biomass. However, we expected that primary

production in the exclosures would eventually become light-limited due to shading of cells at the base of the periphyton mats. The removal of fish grazing had no impact on the gross primary production of the periphyton at any time during the experiment. Respiration rates also showed no change when released from grazing. Since the biomass on the tiles increased when fish grazing was removed and gross primary production did not change, biomass-specific primary production was substantially higher on the grazed tiles than the ungrazed tiles.

In natural stream studies, biomass-specific production decreased but areal primary production increased when periphyton was released from fish grazing (Gelwick and Matthews 1992, Abe et al. 2007, and Stewart 1987). Thus, periphyton production is more efficient when fish are grazing. This could be due to fish removing senescent algae and detritus when they feed giving active algae more access to light and nutrients. In our experiment, the increased efficiency in the biomass is not a result of recycled nutrients from the fish because all tiles were on the same flow-through system. I observed that *Campostoma* tends to graze on previously grazed periphyton patches, which supports the idea that the fish are creating and sustaining a better food source with grazing. We do not have data on the growth of our fish, but the fish maintained excellent condition throughout the experiment even after reducing the available substrate in half by dividing the tanks. These observations suggest fish grazing is important to maintaining and increasing productivity in aquatic systems. Ungulate grazing in the grasslands of the Serengeti also maintains high levels of productivity (Frank et al. 1998). In our system and the Serengeti, primary consumers that are capable of consuming large amounts of biomass may function to increase the productivity of the entire ecosystem. In these

grazed systems the grazers can lead to higher productivity levels by reducing self-shading and increasing the rate of nutrient cycling (Frank et al. 1998). Future researchers should analyze the growth of the selected grazer and the C:N ratio of the periphyton to understand the contribution of benthic algae to consumer growth and ecosystem productivity under grazed and non-grazed conditions.

Light limitation may explain the decrease in biomass-specific primary production but no change in area-specific primary production with the removal of fish grazing. The periphyton released from grazing may have been using all the light available before the tanks were divided. An experimental design with different light levels could be used to tell if the periphyton was limited by the light intensity in our experiment.

The reduction in the number of cyanobacteria present when periphyton was released from fish grazing led us to expect that rates of nitrogen fixation would also decrease. If nitrogen fixation rates are higher with grazing it would suggest grazers have a positive impact on their food source and affect the nutrient availability in the ecosystem. However, area-specific nitrogen fixation did not change when the periphyton was protected from fish grazing.

Mean nitrogen fixation rates in our experiment were near 4500 μg m^{-2} hr^{-1}, which are comparable to nitrogen fixation rates in natural ecosystems. Epilithic periphyton communities in Lake Malawi, east Africa are dominated by *Calothrix* and the average N-fixation rate in the littoral zone of Lake Malawi is 3890 μg m^{-2} hr^{-1} (Higgins et al. 2001). Epilithic periphyton in a desert stream in Arizona fixed nitrogen at a mean rate of 5280 μg m^{-2} hr^{-1} (Grimm and Petrone 1997). A patch of *Calothrix* on the Great Barrier Reef fixed nitrogen at an average rate of 3920 μg m^{-2} hr^{-1} (Larkum et al. 1988)

Similar to primary production grazing increases the efficiency of N-fixation by periphyton. Biomass-specific nitrogen fixation decreased when fish grazing was removed. The increase in diatoms associated with the release from fish grazing might have led to increased competition between diatoms and *Calothrix* for light and nutrients in the exclosures, limiting the ability of the cyanobacteria to fix nitrogen. If light and nutrients are unavailable *Calothrix* may reduce nitrogen fixation due to the high energy requirements of producing heterocysts and performing nitrogen fixation. Our data show the number of heterocysts decreased in the ungrazed periphyton but the remaining heterocysts fixed more nitrogen than the heterocysts on the grazed periphyton. This suggests competition for light and nutrients did not reduce the ability of cyanobacteria to fix nitrogen. The lack of correlation between nitrogen fixation and reduced numbers of nitrogen fixers, was surprising and is difficult to explain. It is difficult to conclude that heterocyst specific nitrogen fixation increased in ungrazed periphyton because of the large amount of variation and lack of replication. There is increasing literature that diatoms can contain endosymbiotic nitrogen fixers (Prechtl 2004). If diatoms in our periphyton culture contained endosymbiotic nitrogen fixers, this could explain a decrease in heterocysts but no change in rates of nitrogen fixation in the ungrazed periphyton.

Overall, we found fish grazing had no impact on the amount of biologically available nitrogen contributed to the system by the periphyton. To our knowledge, only Sammarco and Wilkinson (1983), have investigated the impact of fish grazing on nitrogen fixation. They found fish grazing increased the amount of nitrogen fixed by periphyton after 12 months, whereas our study only lasted 21 days. Our data supports the

change in community composition due to grazing and the increase in biomass-specific primary production that other studies have found (Power et al. 1988; Gelwick and Matthews, 1992; Samarco, 1983; Stewart, 1987). These experiments lasted at the most 55 days. Therefore, future studies analyzing the impacts of fish grazing on nitrogen fixation should consider longer time periods.

Epilithic nitrogen fixation in Lake Malawi could provide up to 35% of the available nitrogen to the lake (Higgins et al. 2001). Despite the large input of biologically available nitrogen to oligotrophic lakes from periphyton, the factors affecting the rates of nitrogen fixation are not well understood (Gettel et al. 2007; Vitousek et al. 2002). Fish grazing directly impacts the biomass and community composition of the periphyton mats fixing the atmospheric nitrogen. However, further research is needed to analyze the role of herbivorous fish in the supply of available nitrogen to these ecosystems. Clarifying the impact herbivorous fish have on nitrogen fixation in these lakes will improve the efforts to maintain these ecosystems, which support local economies and provide high levels of biodiversity.

We consider our experiment and the results to be exploratory due to the lack of research on the impact of grazing on nitrogen fixation by benthic algae. Our results are best suited to inform future researchers who study similar ecological relationships. A consideration is to analyze the impact of different densities of fish grazing. Although we stocked natural densities of *Campostoma* in our experiment the density may have been too high or low to produce real-world results. Our nitrogen fixation and primary production values are similar to values recorded in natural systems but we have no way to know the impact of a higher or lower density of grazers. Future research could test the

impact of different light intensities. It is possible the available light limited our rates of

nitrogen fixation and primary production, thereby limiting the impact of fish grazing on

primary production and fish grazing. The nutrient levels in the system should also be

compared to nitrogen fixation values. We added phosphorous to our system to

exacerbate the nitrogen limitation. However, we did not analyze the nutrient ratios in the

water after the addition of fish. Nitrogen fixation still occurred providing evidence of

nitrogen limitation, but it is difficult to analyze the relationship between nitrogen

limitation and the rates of primary production and nitrogen fixation in our study.

BIBLIOGRAPHY

Abe, SI, Kiso, K, Katano, O, Yamamoto, S, Nagumo, T, and Tanaka, J. Impacts of differential consumption by the grazing fish, *Plecoglossus altivelis*, on the benthic algal composition in the Chikuma River, Japan. <u>Phycological Research</u> 54 (2006): 94-98

Abe, SI, Uchida, K, Nagumo, T, and Tanaka, J. Alterations in the biomass-specific productivity of periphyton assemblages mediated by fish grazing. <u>Freshwater Biology</u> 52 (2007): 1486-93

Allan, JD. <u>Stream ecology: Structure and function of running waters</u>. (1995)

Bergmann, MA, and Welch, HE. Nitrogen fixation by epilithic periphyton in small arctic lakes in response to experimental nitrogen and phosphorus fertilization. <u>Canadian Journal of Fisheries and Aquatic Sciences</u> 47 (1990): 1545-1550

Bonilla, I, Garcia-Gonzalez, M, and Mateo, P. Boron Requirement in cyanobacteria: It's possible role in early evolution of photosynthetic organisms. <u>Plant Physiology</u> 94 (1990): 1554-60

Breitbarth, E, Mills, M.M, Friedrichs, G, and Laroche, J. The Bunsen gas solubility
coefficient of ethylene as a function of temperature and salinity and its importance
for nitrogen fixation assays. Limnology and Oceanography: Methods 2 (2004):
282-288

Capone, D.G. Determination of nitrogenase activity in aquatic samples using the
acetylene reduction procedure. In: Handbook of methods in aquatic microbial
ecology (Eds. Kemp, R.F., Cole, J.J., Sherr, B.F., and Sherr, E.B.) (1993):

Fay, P. The heterocyst. In: The biology of blue-green algae. Botanical Monographs (9).
(Eds. Carr, NG., and Whitton, BA.), (1973): 238-259

Flecker, AS. Ecosystem engineering by a dominant detritivore in a diverse tropical
stream. Ecology 77.6 (1996): 1845-54

Flecker, AS, Taylor, BW, Bernhardt, ES, Hood, JM, Cornwell, WK, and Cassatt, SR.
Interactions between herbivorous fishes and limiting nutrients in a tropical stream
ecosystem. Ecology 83.7 (2002):1831-44

Frank, DA, McNaughton, SJ, and Tracy, BF. The ecology of the Earth's grazing
ecosystems. BioScience 48.7 (1998): 513-521

Gelwick, FP, and Matthews, WJ. Effects of an algivorous minnow on temperate stream ecosystem properties. <u>Ecology</u> 73.5 (1992): 1630-45

Gettel, GM, Giblin, AE, and Howarth, RW. The effects of grazing by the snail, Lymnaea elodes, on benthic N2 fixation and primary production in oligotrophic, arctic lakes. <u>Limnology and Oceanography</u> 52.6 (2007): 2398-2409

Grimm, NB and Petrone, KC. Nitrogen fixation in a desert stream ecosystem. <u>Biogeochemistry</u> 37 (1997): 33-61

Hargrave, CW, Ramirez, R, Brooks, M, Eggleton,MA, Sutherland,K, Deaton,R, and Galbraith,H. Indirect food web interactions increase growth of an algivorous stream fish. <u>Freshwater Biology</u> 51.10 (2006):1901-10

Hedges, L.V, Gurevitch, J, and Curtis, P.S. The meta-analysis of response ratios in experimental ecology. <u>Ecology</u> 80.4 (1999): 1150-1156

Higgins, SN, Hecky, RE, and Taylor, WD Epilithic nitrogen fixation in the rocky littoral zones of Lake Malawi, Africa. <u>Limnology and Oceanography</u> 46 (2001): 976-982

Hood, JM, Vanni, MJ and Flecker, AS. Nutrient recycling by two phosphorus-rich

    grazing catfish: the potential for phosphorus-limitation of fish growth. <u>Oecologia</u>

    146.2 (2005):247-57

Kruskopf, M, and Du Plessis, S. Growth and filament length of the bloom forming

    Oscillatoria simplicissima (Oscillatoriales, Cyanophyta) in varying N and P

    concentrations. <u>Hydrobiologia</u> 556 (2006): 357-362

Larkum, A.W.D., Kennedy, I.R., and Muller, W.J. Nitrogen fixation on a coral reef.

    <u>Marine Biology</u> 98 (1988) 143-155

Lewis, SM. The role of herbivorous fishes in the organization of a Caribbean reef

    community. <u>Ecological Monographs</u> 56.3 (1986): 184-200

McCormick, PV. Resource competition and species coexistence in freshwater benthic

    algal assemblages. In: <u>Algal Ecology: Freshwater Benthic Ecosystems</u>. (Eds.

    Stevenson, RJ., Bothwell, ML., and Lowe, RL.), (1996): 229-249

Power, ME. Resource enhancement by indirect effects of grazers: armored catfish, algae,

    and sediment. <u>Ecology</u> 71.3 (1990):897-904

Power, ME, Matthews, WJ, and Stewart, AJ. Grazing minnows, piscivorous bass, and

    stream algae: dynamics of a strong interaction. <u>Ecology</u> 66.5 (1985):1448-56

Power, ME, Stewart, AJ, and Matthews, WJ. Grazer control of algae in an ozark
mountain stream: effects of short-term exclusion. <u>Ecology</u> 69.6 (1988):1894-98

Prechtl, J, Kneip, C, Lockhart, P, Wenderoth, K, and Maier, U. Intracellular spheroid
bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial
origin. <u>Molecular Biology and Evolution</u> 21.8 (2004): 1477-81

Reuter, JE, Loeb, SL, and Goldman, CR. Inorganic nitrogen uptake by epilithic
periphyton in a N-deficient lake. <u>Limnology and Oceanography</u> 31.1 (1986): 149-
160

Sabater, MG, and Tofaeono, SP. Scale and benthic composition effects on biomass and
trophic group distribution of reef fishes in American Samoa. <u>Pacific Science</u> 61.4
(2007): 503-520

Sammarco, PW. Effects of fish grazing and damselfish territoriality on coral reef algae. I.
Algal community structure. <u>Marine Ecology – Progress Series</u> 13 (1983): 1-14

Stainton, MJ, Capel, MJ, and Armstrong, FAJ. The Chemical Analysis of Fresh Water.
Environment Canada; Fisheries and Marine Service, <u>Miscellaneous Special
Publication</u> 25 (1974): 67-69

Standard Methods for the Examination of Water and Wastewater. 20th ed. United Book
Press, Inc. (1998)

Steinman, ADS. Effects of grazers on freshwater benthic algae. In: Algal Ecology:
Freshwater Benthic Ecosystems. (Eds. Stevenson, RJ., Bothwell, ML., and Lowe,
RL.), (1996): 341-366

Stewart, WDP. Nitrogen fixation. In: The Biology of Blue-Green Algae. Botanical
Monographs (9). (Eds. Carr, NG., and Whitton, BA.), (1973): 260-278

Stewart, W.D.P., Fitzgerald, G.P., Burris, R.H. In situ studies on N2 fixation using the
acetylene reduction technique. Proceedings of the National Academy of Sciences
of the United States of America 58.5 (1967) 2071-78

Trautman, MB. The Fishes of Ohio. The Ohio State University Press. (1981)

Vannote, RL, Minshall, GW, Cummins, KW, Sedell, JR, and Cushing, CE. River
continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37.1
(1980): 130-137

Vitousek, PM, Cassman, K, Cleveland, C, Crews, T, Field, CB, Grimm, NB, Howarth, RW, Marino, R, Martinelli, L, Rastetter, EB, and Sprent, JI. Towards an ecological understanding of biological nitrogen fixation. <u>Biogeochemistry</u> 57/58 (2002): 1-45

Wilkinson, CR, and Sammarco, PW. Effects of fish grazing and damselfish territoriality on coral reef algae. II Nitrogen fixation. <u>Marine Ecology – Progress Series</u> 13 (1983): 15-19

Winemiller, KO. Spatial and temporal variation in tropical fish trophic networks. <u>Ecological Monographs</u> 60.3 (1990): 331-367