The Effects of Non-native and Native Anuran Tadpoles on Aquatic Ecosystem Processes

by

Robin Greene

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Masters in Science

Approved April 2015 by the
Graduate Supervisory Committee:

John Sabo, Chair
James Elser
Nancy Grimm

ARIZONA STATE UNIVERSITY

May 2015

ABSTRACT

Non-native consumers can significantly alter processes at the population, community, and ecosystem level, and they are a major concern in many aquatic systems. Although the community-level effects of non-native anuran tadpoles are well understood, their ecosystem-level effects have been less studied. Here, I tested the hypothesis that natural densities of non-native bullfrog tadpoles (*Lithobates catesbeianus*) and native Woodhouse's toad tadpoles (*Anaxyrus woodhousii*) have dissimilar effects on aquatic ecosystem processes because of differences in grazing and nutrient recycling (excretion and egestion). I measured bullfrog and Woodhouse's carbon, nitrogen, and phosphorus nutrient recycling rates. Then, I determined the impact of tadpole grazing on periphyton biomass (chlorophyll *a*) during a 39-day mesocosm experiment. Using the same experiment, I also quantified the effect of tadpole grazing and nutrient excretion on periphyton net primary production (NPP). Lastly I measured how dissolved and particulate nutrient concentrations and respiration rates changed in the presence of the two tadpole species. Per unit biomass, I found that bullfrog and Woodhouse's tadpoles excreted nitrogen and phosphorus at similar rates, though Woodhouse's tadpoles egested more carbon, nitrogen, and phosphorus. However, bullfrogs recycled nutrients at higher N:C and N:P ratios. Tadpole excretion did not cause a detectable change in dissolved nutrient concentrations. However, the percent phosphorus in mesocosm detritus was significantly higher in both tadpole treatments, compared to a tadpole-free control. Neither tadpole species decreased periphyton biomass through grazing, although bullfrog nutrient excretion increased areal NPP. This result was due to higher biomass, not higher biomass-specific productivity. Woodhouse's tadpoles significantly decreased respiration

in the mesocosm detritus, while bullfrog tadpoles had no effect. This research highlights functional differences between species by showing non-native bullfrog tadpoles and native Woodhouse's tadpoles may have different effects on arid, aquatic ecosystems. Specifically, it indicates bullfrog introductions may alter primary productivity and particulate nutrient dynamics.

ACKNOWLEDGEMENTS

During my thesis I sought to use methods and explore topics that were novel to me. Therefore, I could not have completed this work without the help and support of many people. First, I would like to thank my advisor, John Sabo. He gave me the freedom to pursue my interests and make my own mistakes, but he always provided the support and guidance necessary to keep me on track. In addition, I could not have completed my research without the valuable suggestions, lab space, and resources that my advising committee: James Elser and Nancy Grimm, provided. Sandy Anderson generously allowed me to conduct my fieldwork on her property at Gray Hawk Nature Center. She shared years of knowledge about bullfrog and Woodhouse's toad natural history with me, and she gave me many practical suggestions about my experiments.

Numerous other people provided feedback on my research and helped me with fieldwork, lab analysis, and research methods. I would particularly like to thank Jennifer Learned, Amanda Rugenski, Cathy Kochert and Blake Dirks (Goldwater Environmental Laboratory), Albert Ruhí, Lindsey Pollard, Jessica Corman, David Shanafelt, Arianna La Porte, Eric Moody, Karl Wyant, and Israel Leinbach. Aside from those who specifically helped with my research, I would also like to thank Wendi Simonson and Yvonne Delgado in the School of Life Sciences Graduate office for helping me navigate the twists and turns of graduate school. My research was funded by the generous support of the Arizona State University Graduate Professional Students Organization JumpStart Grant, the Ariel Appleton Fellowship, the Peabody Family Memorial Fellowship, the School of Life Sciences summer Graduate Research Scholarship, and the School of Life Sciences Research and Training Initiative Facilities Grant.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

Tables Page

INTRODUCTION

Aquatic consumers have significant effects on ecosystem processes through grazing and nutrient recycling (Hillebrand, 2009; Hillebrand et al., 2008; Hillebrand and Kahlert, 2001; Vanni, 2002). Grazing often decreases primary producer and detrital biomass (Flecker, 1996; Flecker et al., 1999; Holomuzki, 1998; Knoll et al., 2009; Lamberti and Resh, 1983; Mallory and Richardson, 2005; Ranvestel et al., 2004) while nutrient recycling (excretion and egestion) often increases nutrient concentrations (Benstead et al., 2010; Knoll et al., 2009; McIntyre et al., 2008; Vanni et al., 2002) and sometimes alters nutrient limitation of primary producers (Atkinson et al., 2013). Jointly, grazing and nutrient recycling often alter primary producer biomass, nutrient content, and production (Connelly et al., 2008; Cooper, 1973; Hillebrand et al., 2008; Hillebrand and Kahlert, 2001; Kupferberg, 1997a; Lamberti and Rush, 1983). Non-native, aquatic consumers can have novel and significant ecosystem impacts when they are abundant (Capps and Flecker, 2013a; Capps and Flecker, 2013b; Hall et al 2003). For example, non-native sail-fin catfish (Loricariidae: *Pterygoplichthyes*) in a Mexican river attained higher biomass and excreted more than 25 times more nitrogen and phosphorus than the native fish assemblage. As result, the catfish created biogeochemical hotspots and decreased the turnover distance for nitrogen and phosphorus (Capps and Flecker, 2013a; Capps and Flecker, 2013b). These ecosystem effects are important to understand since species spread is increasing and non-natives can have significant community and economic impacts (Lodge et al., 2006).

Recent research has highlighted the important role amphibian tadpoles play in aquatic ecosystems. They decrease algal biomass through efficient grazing (Costa and

Vonesh, 2013; Hillebrand, 2009; Kupferberg, 1997b), increase dissolved nutrient supplies via excretion (Knoll et al., 2009), alter primary productivity through a combination of grazing and nutrient excretion (Connelly et al., 2008; Kupferberg, 1997a; Kupferberg, 1997b), and decrease sediment quantity but increase sediment and biofilm quality via egestion (Connelly et al., 2014; Colón-Gaud et al., 2008; Flecker et al., 1999; Ranvestel et al., 2004). In addition, anuran species are often functionally unique (Costa and Vonesh, 2013; Kupferberg, 1997a; Kupferberg, 1997b). For example, Costa and Vonesh (2013) compared red-eyed treefrog tadpoles (Ag*alychnis callidryas*) to hourglass treefrog tadpoles (*Dendropsophus ebraccatus*), which co-occur and graze similar algal resources. However, due to differences in physiology and foraging behavior, red-eyed treefrogs decreased periphyton and increased phytoplankton biomass to a greater extent than hourglass treefrogs (Costa and Vonesh, 2013). Therefore, different species may produce diverse ecosystem responses due to variation in grazing and nutrient recycling.

While there is strong evidence for functional differences between anuran species and growing concern about the effects of aquatic non-natives, little is known about the ecosystem effects of non-native tadpoles via grazing and nutrient recycling (but see Kupferberg, 1997a and Preston et al., 2012). Three amphibians are listed in the top 100 "world's worst" invaders (ISSG, 2005). The American bullfrog (*Lithobates catesbeianus* Shaw 1802) is #76 on this list (ISSG, 2005) and many conservation managers are concerned about the effects of adult bullfrogs because of their abundance, predation and competition with native species, and their ability to carry amphibian diseases (Adams and Pearl, 2007; Kiesecker et al., 2001; Mazzoni et al., 2003; Rosen et al., 1995). Kupferberg (1997b) examined the effects of similar densities of non-native bullfrog, native yellow-

legged frog (*Rana boylii*), and native Pacific treefrog (*Hyla regilla*) tadpoles on algal resources. Tadpole assemblages that included bullfrogs decreased algae biomass to a greater extent than assemblages composed of native tadpoles only (Kupferberg, 1997b). Yet, little is known about the combined effects of bullfrog tadpole grazing and nutrient recycling on other aquatic ecosystem processes, and whether their impact differs from that of native anuran tadpoles.

Bullfrogs are originally from eastern North America but they have spread globally over the last century. By the mid-1980's, they became prevalent in permanent water bodies in southeastern Arizona (Schwalbe and Rosen, 1988) and often co-occur with native Woodhouse's toads (*Anaxyrus woodhousii* Girard 1854). Bullfrog tadpoles prefer deep, vegetated pools with densities varying from 1-52/m^2 (Kupferberg, 1997b). In contrast, Woodhouse's tadpoles can form large aggregations of over 700/m^2 (Greene unpublished data) in the shallow portions of rivers and permanent ponds. Bullfrogs have one of the longest larval periods of any anuran species, spending several months to two years as tadpoles (Wells, 2010). As a result, they can grow as large as 10-35 g (Cecil and Just, 1979). Woodhouse's tadpoles are adapted to arid and semi-arid aquatic systems that often dry or flood by mid-summer. Therefore, they have rapid and flexible developmental rates, often metamorphosing 3-8 weeks after hatching when they are less than 2 g (Greene unpublished data).

The purpose of this research was to compare the ecosystem effects of natural densities of non-native bullfrog tadpoles and native Woodhouse's toad tadpoles. Specifically, I asked two related research questions 1) How does grazing and nutrient recycling vary between bullfrog and Woodhouse's tadpoles? And 2) How does bullfrog

and Woodhouse's grazing and nutrient recycling affect dissolved and particulate nutrient concentrations and stoichiometric ratios, periphyton primary production, and microbial respiration? Based on previous research, I predicted that both tadpole species would increase dissolved and particulate nitrogen (N) and phosphorus (P) concentrations relative to carbon (C) concentrations (Knoll et al., 2009 and Seale, 1980). As a result, nutrient recycling would decrease particulate C:N and C:P ratios. Since anuran tadpoles have relatively low body P (Knoll et al., 2009 and Vanni et al., 2002), I predicted that both tadpoles would decrease the dissolved and particulate N:P ratio. Furthermore, I predicted species-specific differences in dissolved and particulate nutrient levels if bullfrogs and Woodhouse's differentially recycled C, N, or P. I predicted that Woodhouse's grazing would have a greater effect on periphyton biomass than bullfrog grazing. Furthermore, tadpole grazing and nutrient excretion would increase periphyton primary productivity as observed by Connelly et al. (2008) and others. However, the influence of grazing would be stronger than the influence of nutrient excretion because herbivore grazing often exerts stronger top-down effects than the bottom-up effects of nutrient addition, especially when nutrients are relatively high (Hillebrand et al., 2001; McIntyre et al., 2006). Lastly, I predicted that both tadpole species would increase respiration rates in detritus because tadpole egestion commonly increases the availability of nutrients that limit microbial activity (Rantala et al., 2014; Rugenski et al., 2012). I predicted species-specific differences in these patterns if there were large differences in grazing and nutrient excretion rates.

METHODS

Study Site

I conducted the fieldwork for this research in the San Pedro Riparian National Conservation Area (SPRNCA) in Cochise County, Arizona during the dry season months of April-June 2014. The SPRNA encompasses 57,000 acres of riparian habitat and approximately 40 km of the San Pedro River between the northern border of Mexico and Saint David, AZ (see Moody and Sabo, 2013 for more information).

I conducted the majority of my fieldwork in a 1-km stretch of river near Gray Hawk Nature Center (31°36'10.57"N, 110°09'22.26"W). During the spring of 2014, the river at Gray Hawk Nature Center (GHNC) was approximately 1.5-4 m wide, 5-20 cm deep, and was dominated by shallow runs and riffles. The sediment was primarily sand and gravel. The riparian forest was variable in width and dominated by Fremont's cottonwoods (*Populus fremontii*) and Goodding willow (*Salix gooddingii*). During my research (April 25-June 9, 2014), the river temperature was 17.7±1.3°C (mean±SE) and the dissolved oxygen (DO) was 9.61±0.71 mg/L (mean±SE). On the afternoon of June 4, 2014, I measured dissolved nutrient concentrations. On this date, the water temperature was 26.2±0.65°C, DO was 10.6±0.84 mg/L, total dissolved nitrogen (TdN) was 87.7±2.48 μg/L, total dissolved phosphorus (TdP) was 58.1±2.00 μg/L, total phosphorus (P) was 81.8±1.60 μg/L, and molar TdN:TdP was 3.4±0.16 (means±SE).

Both bullfrogs and Woodhouse's toads breed in the San Pedro River, though tadpole densities are variable. Woodhouse's tadpoles are abundant at GHNC (233±51.1 tadpoles/m^2 (mean±SE) of mixed developmental stages), though bullfrog tadpoles are not because they are 1) eradicated by the landowner, and 2) there are few deep, vegetated

pools (bullfrogs' preferred habitat). However, adult bullfrogs are abundant (0.2±0.08 adults/m^2 (mean±SE), and there are locally dense aggregations of tadpoles in deep pools near San Pedro House (31°32'53"N, 110°08'29.62"W). This site is on the San Pedro River, approximately 6 km south of GHNC.

Field Methods

I conducted field sampling and a mesocosm experiment to asses how natural densities of bullfrog and Woodhouse's tadpoles affect aquatic ecosystem processes through grazing and nutrient recycling. First, I measured tadpole excretion and egestion rates to evaluate the potential for each species to influence dissolved and particulate nutrient dynamics. I followed this with a field experiment to measure the effect of tadpole grazing on periphyton biomass (chlorophyll *a*). I also used the mesocosm experiment to study the effects of grazing and nutrient recycling on dissolved and particulate nutrient concentrations, periphyton primary production, and microbial respiration. I will describe each of these methods under separate headings below. All methods involving animals were permitted under Arizona State University Institutional Animal Care and Use Committee protocols 12-1262R Amendments 4-6.

Tadpole Excretion and Egestion

I collected 12, stage 25-26 (Gosner, 1960) Woodhouse's tadpoles from GHNC on May 3 and 12, stage 25-27 (Gosner, 1960) bullfrog tadpoles from the pond at the Casa de San Pedro Bed and Breakfast on May 10 (31°24'31.94"N, 110°6'22.61"W). I rinsed all tadpoles with pre-filtered river or pond water (Whatman GF/F glass-fiber filter) then placed them in a 60-mL, acid-washed centrifuge tube. I filled the tubes with pre-filtered (Whatman GF/F filter) river or pond water then incubated them for approximately two

hours in the shade. During this time, I also measured the length of each tadpole. In addition, I incubated four centrifuge tubes per species as blanks (identical except they lacked tadpoles). After two hours, I removed and froze the tadpoles. I filtered the incubation water through a Whatman GF/F filter into a second acid-washed, 60-mL centrifuge tube and immediately froze the excretion sample (liquid) and egestion sample (filter).

I dried the tadpoles at 50°C (for at least 72 hours) and weighed them to the nearest tenth of a milligram. I estimated length to dry mass regressions for each species. I used these regressions to make estimates for total tadpole biomass in calculations and the follow-up mesocosm experiment.

I analyzed the excretion samples for ammonium (NH_4^+) using a Lachat QuikChem FIA+ 8000 Series (Lachat Instruments) and TdP using the potassium persulfate method (modified from APHA 1998). During all P analysis, I used apple leaf standards (NIST 1515) as a quality control. I calculated mass-corrected excretion rates (μg nutrient g dry mass^{-1} h^{-1}) and molar excretion ratios after subtracting mean nutrient concentration in blanks for each tadpole sample.

I cut egestion filters in half and analyzed one subsample for C and N on a PE2400 Elemental Microanalyzer and the second sample for P using the potassium persulfate method (modified from APHA 1998). I calculated mass-corrected egestion rates (μg g dry mass^{-1} h^{-1}) and molar ratios as described above.

Mesocosm Experimental Set-up

I used flow-through mesocosms to study the effects of natural densities of bullfrog and Woodhouses's tadpoles on aquatic ecosystem processes. I arranged eighteen

mesocosms (114 L, clear plastic tubs, Model #: BELLW01160101-6) in six rows on the riverbank at GHNC (Figure 1). The experiment took place from April 25-June 9, 2014. Riparian vegetation shaded the mesocosms from approximately 09:00-13:00 then again after 15:00. Each mesocosm was fitted with a snap lid. I modified the lids by removing the interior surface and replacing it with a piece of Blue Hawk Green Plastic/Polyresin Perimeter Fence to keep un-wanted animals and debris out of the mesocosms.

I sewed a PacificHydroStar pump (approximately 4732 L/r, 8.8 m head lift) into a mesh bag (to prevent debris clogging) and placed it in the river, adjacent to the experiment. I connected the pump outflow valve to a 5-m x 1.27-cm garden hose that branched into three, 3.7-m segments (1.27 cm diameter). Each hose supplied river water inflow to one block of mesocosms (two rows of three mesocosms per block). I fitted a shut-off valve and a Raindrip 8-Port Combination Irrigation Manifold with Filter (irrigation splitter) to the end of each hose. The irrigation splitter divided the inflow into six, 0.64-cm black polyvinyl hoses (each 1.2-1.5 m long). These hoses fed the inflow water to the bottom of each mesocosm. The pump was on approximately four hours per day, Friday-Monday. The flow rate through the mesocosms was approximately 0.04 L/s, slower than the adjacent river but likely similar to water flow through a side pool in the river. I closed the shut-off valves the rest of the week. I inserted a piece of 1.27-cm garden hose, covered in mesh (2 mm diameter) and sealed with silicone into a 2.54-cm diameter hole at the 76 L mark of each mesocosm. This hose funneled overflow (outflow) back to the river (Figure 1).

After I filled the mesocosms, I added approximately 4.5 g dry mass (45 mL packed in 50 mL centrifuge tube) of filamentous green algae to each mesocosm as food

and cover for the tadpoles. I collected the algae from three locations at GHNC, spun the algae 50 times in a salad spinner to remove excess water and invertebrates, then measured and added the algae to each mesocosm.

I placed eight, fritted glass crucible covers, or glass discs, (Leco Corporation, ID#528-042), in each mesocosm. The glass discs had been incubated for two months in the riffles of the river to permit periphyton colonization. Out of the eight discs in each mesocosm, I encased four in mesh (2 mm diameter) bags that prevented tadpole grazing (hereafter referred to as "caged"). I placed the remaining four discs under a mesh canopy, fashioned out of a plastic-covered coat hanger and the same 2-mm diameter mesh. The canopy controlled for any effects of the mesh bag (e.g. shading). These discs were exposed to tadpole grazing and nutrient recycling (hereafter referred to as "un-caged").

I allocated the mesocosms to three treatments (n=6/treatment): no tadpoles (control), bullfrog tadpoles, and Woodhouse's tadpoles. Replicates were systematically arranged in each row (Figure 1). I added tadpoles, stage 24-26 (Gosner, 1960), to the bullfrog and Woodhouse's treatments on April 28. Very few bullfrog tadpoles survived the fall 2013 monsoons. Therefore I could not estimate natural bullfrog tadpole densities. Instead, I stocked bullfrog treatments at 14 tadpoles/m^2 (3 tadpoles/mesocosm); an intermediate density observed in the South Fork Eel River (Kupferberg, 1997a). I stocked each mesocosm with one small bullfrog tadpole (0.5-1 g) and two medium bullfrog tadpoles (3-4 g). The bullfrog tadpoles were from the same man-made pond from which I collected bullfrog tadpoles for nutrient recycling sampling. I stocked the Woodhouse's treatments at 332 tadpoles/m^2 (70 tadpoles/mesocosm), with tadpoles collected from the river at GHNC. Although this tadpole density was higher than in other experimental

studies, it was intermediate to the densities observed at GHNC during an April 18, 2014 survey.

Twice per week, I removed excess debris that had fallen into the mesocosms (insects, leaves, seeds, and branches) with a small aquarium net. During the first week of the experiment, falling branches broke three mesocosms (one bullfrog and two Woodhouse's mesocosms). I immediately replaced the broken mesocosms and built three PVC frames around the mesocosms to prevent additional branch damage.

Mesocosm Sampling

I collected water samples and primary production measurements from all the mesocosms during four sampling periods: April 25-28, May 2-5, May 16-19, and May 30-June 2. I added the tadpoles to the mesocosms immediately after the first sampling period (afternoon of April 28).

On the first day (prior to water inflow) and the last day of each sampling period (after four days of inflow), I collected duplicate water samples in 50 mL, acid-washed centrifuge tubes. I collected the samples from the top 10 cm of each mesocosm and filtered them through a Whatman GF/F filter. During water sampling, I also recorded water temperature and DO concentrations. I immediately froze samples for later nutrient analysis. I analyzed one sample, from each duplicate pair, for TdN on a Shimadzu TOC-VC/TN, and the second sample for TdP using the potassium persulfate method (modified from APHA, 1998). In addition, I analyzed a small subset of samples, collected on May 11, for NH_4^+ on a Lachat QC8000.

To determine the effect of tadpole grazing and nutrient excretion on net primary productivity, I measured periphyton oxygen production (with a YSI Pro series water

meter, YSI, Yellow Springs, OH) on the caged and un-caged glass discs (methods modified from Hall and Moll, 1975). I wedged each glass disc firmly 2.54-cm into a 60 mL clear, plastic centrifuge tube. I immediately filled the tube with un-filtered, river water that had known DO. I expelled bubbles from the tube, and suspended it in a sunny region of the adjacent river (the sample remained cool but received light). Using a Licor PAR meter, I measured photosynthetically active radiation (PAR) every half hour on the shore of the river. PAR ranged from 68-2051$\mu mol/m^2/s$ during the four, morning sampling times. After 2-3 hours, I removed the glass discs from the tubes and recorded the final DO concentrations and start and end times of all incubations. I collected the periphyton-covered discs in aluminum foil and promptly froze them for later chlorophyll *a* analysis. I incubated four blank glass discs (controls) in the same manner as the sample discs. I calculated the areal net primary production (NPP) as the change in DO during the incubation per meter squared. In addition, I calculated biomass-specific NPP as the change in DO during the incubation divided by the chlorophyll *a* biomass on each disc.

I determined biomass (as chlorophyll *a*) on each disc using a method modified from APHA (2005). I extracted chlorophyll *a* from the periphyton with 10-15 mL of 90% acetone solution buffered with 1% magnesium carbonate. I incubated each disc in the extraction solution for 16-20 hours at 4°C in acetone-washed, black film canisters. After the incubation, I transferred 3 ml of the extraction solution to a 1 cm quartz cuvette (in dim light). I recorded the absorbance (OD) at 750 nm and 664 nm in a Genesis 10S UV-VIS spectrophotometer. I acidified the sample with 0.1 mL of 0.1N hydrochloric acid. After 90 seconds, I recorded the absorbance of the acidified sample at 750 nm and 665

nm. I calculated the chlorophyll *a* in each sample with the following equation (APHA, 2005):

$$\text{Chlorophyll } (mg/m^2) = (26.7*(OD_{664}-OD_{750}) - (OD_{665}-OD_{750})*V)/A$$

where 26.7 is the absorbance correction factor, "V" is the volume of extract (L), and "A" is the area of glass disc (573 mm^2).

On June 8, I removed all the tadpoles from the mesocosms. For all bullfrog mesocosms, I recorded tadpole wet mass, length, and approximate Gosner developmental stage. I recorded the range in tadpole length and Gosner developmental stage for each Woodhouse's mesocosm. I counted the remaining Woodhouse's tadpoles in each mesocosm then returned them to the river from which they had been collected. I swept an aquarium net for six minutes through each mesocosm to remove remaining algae and large debris.

On June 9, I collected detritus samples for later nutrient analysis. I stirred each mesocosm vigorously for 10 seconds then collected two, 30-mL water samples from each mesocosm. I filtered water samples onto Whatman GF/F filters, which I immediately froze. I dried the filters at 50°C (for at least 72 hours). I analyzed one filter from each mesocosm for C and particulate N on a CHN Elemental Analyzer PE2400 and the other filter for particulate P using the potassium persulfate method (modified from APHA 1998).

In addition to the particulate C, N, and P samples, I also measured respiration rates in a sub-set of mesocosms (approximately 4 mesocosms/treatment). First, I vigorously stirred mesocosm water for 20 seconds. Then, I filtered a 30-50 mL water sample onto a pre-ashed, pre-weighed Whatman GF/F filter. I placed the filter in a 50-mL

centrifuge tube that had been completely covered in aluminum foil. I filled each tube with filtered (Whatman GF/F filter) river water that had known DO then incubated the samples in the dark for approximately four hours. I recorded the start and end time and end DO. After the incubation, I filtered the water onto another pre-ashed, pre-weighed Whatman GF/F filter. I collected and immediately froze all filters. I also incubated four blank, pre-ashed, pre-weighed Whatman GF/F filters (controls) in the same way as the samples. I ashed (550°C for four hours) and weighed all samples and blanks. I calculated respiration as the rate of oxygen depletion divided by the ash-free dry mass. Blank filter values were used to correct all sample values.

Statistical Analysis

Tadpole Excretion and Egestion

Prior to analyzing my data, I tested the assumptions of parametric tests. I assessed normality visually (via departure from linear normal probability plots) and used Bartlett's test to determine equality of variances (α=0.05). In addition to calculating mass-corrected nutrient recycling rates, I also calculated molar ratios (excretion NH_4^+:TdP and egestion C:N, C:P, and N:P). I removed three Woodhouse's data values from the excretion dataset that were negative or extreme outliers (biologically improbable due to contamination). I compared mass-corrected excretion rates and molar ratios using Mann-Whitney U tests since all parametric assumptions were violated. I estimated areal excretion rates for both species using average excretion rates and the tadpole densities used to stock the mesocosms (bullfrog tadpoles: 14 tadpoles/m^2 and 1.81 g dry mass/m^2, Woodhouse's tadpoles: 332 tadpoles/m^2 or 1.66 g dry mass/m^2). I also calculated estimates of tadpole excretion per mesocom using average excretion rates and estimates of total tadpole dry

mass per mesocosm (calculated from length to dry mass regressions and mesocosm stocking densities). I compared per mesocosm excretion estimates using Student's t-test since all parametric assumptions were met.

I compared the dry mass of bullfrog and Woodhouse's egestion using a Mann-Whitney U test since data were non-normal and variances were unequal. I log-transformed mass-corrected egestion rates and ratios then analyzed percent C, percent N, percent P, C:P, and N:P with Student's t-tests. The egestion C:N data did not meet parametric assumptions, even after log transformation. Therefore I analyzed the un-transformed C:N ratios with a Welch's t-test. I calculated and analyzed areal egestion and per mesocosm egestion estimates in the same manner as described for excretion.

Mesocosm Experiments

Bullfrog densities remained consistent at 14 tadpoles/m^2 throughout the experiment. I estimated bullfrog and Woodhouse's dry mass (per mesocosm) at the end of the experiment using the length to dry mass regressions I calculated from the excretion and egestion samples. Estimated bullfrog dry mass was 0.67±0.07 g/mesocosm (mean±SE). Though I added 70 tadpoles to each Woodhouse's mesocosm at the beginning of the experiment (0.78±0.06 dry mass (g)/mesocosm), I found a mean (±SE) of 59.83±2.41 tadpoles/mesocosm at the end of the experiment. Based on the number of tadpoles caught at the end of the experiment, I estimated Woodhouse's dry mass to be 0.67±0.06 g/mesocosm (mean±SE). I tested for differences in tadpole biomass between bullfrog and Woodhouse's treatments (at the end of the experiment) using a Student's t-test. Since the average dry mass in the bullfrog and Woodhouse's treatments was similar (t_{10}=-1.18, p=0.27), I did not standardize mesocosm data by tadpole biomass.

Interestingly, both bullfrog and Woodhouse's tadpole development appeared to slow/halt during the experiments. This was particularly evident in the Woodhouse's treatments.

Prior to analyzing dissolved nutrient concentrations I tested assumptions of compound symmetry (using the Greenhouse-Geiser estimate) in addition to all previously mentioned assumptions of normality. I log-transformed dissolved nutrient concentrations then analyzed differences in TdN, TdP, and molar TdN:TdP with separate, repeated-measures ANOVAs (rmANOVA) using maximum likelihood estimation (rather than the default Restricted Maximum Likelihood). This analysis was carried out using the nlme package (version 3.1-117) in RStudio. I included experimental "treatment" and "day" (proxy for time) as fixed effects and "row" (mesocosm location in the experiment) and "subject" (mesocosm ID) as random effects.

To compare the grazing pressure of bullfrog and Woodhouse's tadpoles, I compared the periphyton biomass on the caged and un-caged discs. First, I log-transformed all chlorophyll *a* values to better satisfy parametric assumptions. I "standardized" these values by subtracting the un-caged and caged values in the control treatments from corresponding values in the bullfrog and Woodhouse's treatments. I subtracted standardized caged values from un-caged values. The more negative the value, the greater the effect of tadpole grazing. I analyzed these values with an rmANOVA (as described above) after confirming the data did not violate assumptions. I included "treatment" and "day" as fixed effects and "row" as a random effect. I also compared periphyton biomass between all three treatments on the last sampling date (June 1). First I log-transformed chlorophyll *a* values, then I subtracted caged values from un-caged values. I analyzed the data with a one-way analysis of variance (ANOVA) since it met

assumptions of normality. If the analysis was significant, I followed up with an appropriate post-hoc test to make multiple comparisons.

I analyzed the effect of tadpole grazing and nutrient excretion (un-caged discs) and nutrient excretion alone (caged discs) on areal NPP and biomass-specific NPP. Prior to analysis, I subtracted the NPP in the control treatments from that in the bullfrog and Woodhouse's treatments. I analyzed NPP data in the same manner as the chlorophyll *a* data using rmANOVAs (described above). I also compared areal and biomass-specific NPP on both caged and un-caged discs on the last sampling date (June 1). I used four, separate Kruskal-Wallis tests or ANOVAs depending on whether the data met parametric assumptions. When these tests were significant, I followed up with an appropriate post-hoc, pair-wise comparison test.

I analyzed particulate nutrient concentrations and ratios with non-parametric, ANOVAs since the sample sizes were small and unbalanced (control: n=4, bullfrog: n=4, bullfrog: n=3). I used separate Kruskal-Wallis tests to analyze percent C, log C:N, log C:P, and log N:P since the data violated the assumptions of normality and equal variance. I analyzed percent N and percent P with separate Welch's ANOVAs since the data met assumptions but the sample sizes were small and unbalanced. Finally, I used non-parametric Tamhanes T2 tests (in SPSS) to make post-hoc comparisons when the initial tests were significant.

The log-transformed respiration data so it met assumptions of normality and equal variance. However, the sample sizes were small and unbalanced. Therefore, I used a Welch's ANOVA to compare differences in respiration between treatments and Tamhanes T2 test (in SPSS) to make post-hoc pair-wise comparisons.

All statistical analyses were executed in RStudio version 0.98.1062 (R Core Development Team, 2015) unless otherwise noted.

RESULTS

Tadpole Excretion and Egestion

Although the larger bullfrog tadpoles excreted more nutrients (both NH_4^+ and TdP) than the small Woodhouse's tadpoles (per tadpole), there was no significant difference in biomass-specific rates of NH_4^+ or TdP excretion between the species (NH_4^+: 96.8±8.70 μg NH_4^+ g dry mass^{-1} h^{-1} (pooled mean±SE) W=63, p=0.87, TdP: 31.9±5.71 μg P g dry mass^{-1} h^{-1} (pooled mean±SE) W=44, p=0.31). Due to a slightly lower excretion TdP rate, bullfrog tadpoles excreted at a significantly higher NH_4^+:TdP molar ratio than Woodhouse's tadpoles (W=91, p=0.04, Figure 2). The variation in estimated areal excretion rates was high but there did not appear to be a difference in NH_4^+ or TdP excretion rates between species (Figure A1). There was no difference in NH_4^+ excretion per mesocosm between the tadpole species (64.2±4.41 μg NH_4^+ mesocosm^{-1} h^{-1} (pooled mean±SE), t_{10}=1.05, p=0.32). My estimates indicated that Woodhouse's tadpoles excreted significantly more TdP per mesocosm than bullfrog tadpoles (Woodhouse's: 29.7±2.61 μg P mesocosm^{-1} h^{-1}, bullfrog: 15.2±1.55 μg P mesocosm^{-1} h^{-1} (mean±SE), t_{10}=-4.81, p=0.007). In summary, bullfrog and Woodhouse's tadpole had similar biomass-specific NH_4^+ and TdP excretion rates. However, bullfrogs excreted at a significantly higher NH_4^+:TdP molar ratio due to a slightly lower TdP excretion rate.

Per gram of tadpole, bullfrog tadpoles egested more material than Woodhouse's tadpoles (W=117, p<0.001). However, Woodhouse's tadpoles egested C, N, and P at a

significantly higher rate (per gram of egestion) than bullfrog tadpoles (C egestion: t_{20}=-4.33, p=0.0003, N egestion: t_{20}=-2.68, p=0.01, P egestion: t_{20}=-6.27, p<0.001, Table 1). Therefore, Woodhouse's egestion was more nutrient-rich than bullfrog egestion. Woodhouse's egested at a significantly higher molar C:N ratio ($t_{10.98}$=-3.5, p=0.005, Figure 3) while bullfrogs egested at a higher molar N:P ratio (t_{20}=3.29, p=0.004, Figure 3). Relatively lower C and P but slightly higher N in bullfrog egestion drove these patterns. There was no difference in the egestion C:P ratio between tadpole species (t_{20}=0.5, p= 0.62). High variation in estimated areal egestion rates obscured any obvious difference in C, N, or P egestion between species (Table A1). However, I estimated that Woodhouse's tadpoles egested more C (t_{10}=-5.91, p<0.001, Table A2), N (t_{10}=-3.85, p=0.003, Table A2), and P (t_{10}=-6.38, p<0.001, Table A2) per mesocosm compared to bullfrog tadpoles. Overall, bullfrog tadpoles egested all nutrients at a lower rate (per gram of egestion) than Woodhouse's tadpoles, though they egested at higher N:C and N:P ratio.

Mesocosm Experiment

The data from the mesocosm experiment showed that bullfrog and Woodhouse's tadpoles have species-specific effects on aquatic ecosystem processes through grazing and nutrient recycling. Below I describe differences in periphyton biomass (chlorophyll *a*) attributed to tadpole grazing. I continue by outlining patterns in dissolved nutrients, NPP, particulate nutrients, and particulate respiration in the presence of the two tadpole species.

I assessed grazing pressure by bullfrog and Woodhouse's tadpoles by comparing the chlorophyll *a* biomass on the caged and un-caged discs, after standardizing by

chlorophyll *a* on the control discs. Over the course of the experiment there were visual changes in periphyton. Crustose algae communities initially colonized the discs but this community gave way to more upright, filamentous species during the last two sampling efforts. Though Woodhouse's grazing appeared to decrease chlorophyll *a*, there was not a significant "treatment*day" effect ($F_{1,29}$=1.07, p=0.31, Figure 4) in the rmANOVA. However, there was a significant effect of "day" ($F_{1,29}$=6.05, p=0.02). The post-hoc ANOVA showed there was no significant difference in chlorophyll *a* biomass between treatments on the last sampling date ($F_{2,13}$=2.74, p=0.10). Therefore, neither tadpole species significantly altered periphyton biomass through grazing.

Mesocosm water temperature and DO were similar between treatments over the course of the experiment (Figure A2 and A3). I found that neither tadpole species altered dissolved nutrient concentrations in the mesocosms (Figure A4a and A4b), although there was an effect of "day" for TdP ($F_{2,97}$=64.51p<0.001). For all treatments, TdN was extremely high on day 36 (May 30). Similarly, TdP was very low on day 36 (May 30) and very high on day 1 (April 25). The molar TdN:TdP ratio was similar between treatments but it varied over time. Excluding the April 25 and May 30 values, the molar TdN:TdP ratio was 8.48±0.45 (mean ±SE). Molar TdN:TdP ratios on April 25 and May 30 were 3.96±0.39 and 41.43±1.22 respectively (mean±SE). NH_4^+ concentrations were below detectable limits on May 11.

I assessed the effects of bullfrog and Woodhouse's grazing and nutrient excretion on areal NPP by comparing NPP on the caged and un-caged discs. There was a significant "treatment*day" effect for areal NPP on the caged discs ($F_{1,38}$=4.19, p=0.05, Figure 5). This implied bullfrog and Woodhouse's excretion affected NPP in unique

ways over time. The Kruskal-Wallis test for the last sampling date (June 1) was also significant (χ^2_2=6.21, p=0.05), though one Woodhouse's data point was an extreme outlier. Cook's test for leverage indicated that this observation had high influence on the statistical outcome. Therefore, I re-ran the test with this point excluded (F_2=14.37, p<0.001). I used a Tamhanes's T2 test (in SPSS) to make the post-hoc multiple comparisons since the dataset was unbalanced. Prior to removing the outlier data point, areal NPP was higher in the bullfrog treatments compared to the control treatments (p=0.048), though neither tadpole treatment was significantly different from each other. After removing the Woodhouse's data point, areal NPP was higher in the bullfrog treatments compared to both the control (p=0.048) and Woodhouse's treatments (p=0.001). Below, I rely most on this latter result, but temper the discussion, where necessary, with the caveat that the significance of tadpole effects differed with and without one influential observation. Although, there was no significant difference in areal NPP between treatments for the un-caged discs, there was a trend of increasing NPP in the bullfrog treatments. However, high within-treatment variation obscured possible differences.

There were no differences in biomass-specific NPP between treatments on the caged or un-caged discs. This implied that changes in areal NPP were not due to higher biomass-specific NPP rates, but changes in overall biomass. I used the methods described above to run an rmANOVA to compare chlorophyll *a* biomass between treatments on the caged discs (after first subtracting chlorophyll *a* biomass in control treatments from that in bullfrog and Woodhouse's treatments). There was a significant "day" effect ($F_{1,34}$=29.12, p<0.001), and a marginally significant "treatment*day" effect ($F_{1,34}$=3.97,

p=0.05). A Kruskal-Wallis test showed that chlorophyll *a* biomass was different between treatments on last sampling date (χ^2_2=8.26, p=0.02). The Tamhane's, multiple comparisons test revealed that the chlorophyll *a* biomass was greater in the bullfrog treatments compared to the control treatments (p=0.049). However, the chlorophyll *a* biomass in the Woodhouse's treatments was similar to that in the bullfrog and control treatments. Therefore, changes in areal NPP were likely due to changes in chlorophyll *a* biomass.

Percent particulate C and N at the end of the experiment were similar between treatments (C: 54.8±2.88 % (pooled mean±SE), χ^2_2=1.66, p=0.0.44, N: 3.5±0.2 % (pooled mean±SE), $F_{2/6.338}$=0.97, p=0.43). However, I found that percent particulate P was significantly different between treatments ($F_{2,7.52}$=15.89, p=0.002). The post-hoc multiple comparisons test showed that percent particulate P was higher in both tadpole treatments compared to the control but there was no difference between tadpole treatments (Figure 6). There was no difference in particulate molar C:N ratios between treatments (18.46±0.52 (pooled mean±SE), $F_{2/7.788}$=0.75, p=0.50), though there were significant differences in molar C:P ratios (χ^2_2=8.41, p=0.01) and molar N:P ratios (χ^2_2=8.91, p=0.01) between treatments. Differences in C:P and N:P were driven by differences in particulate P between the control and tadpole treatments. Post hoc tests showed that particulate C:P and N:P ratios were significantly lower in the bullfrog and Woodhouse's treatments, compared to the control treatments (Table 2).

Microbial respiration in the mesocosm detritus was significantly different between treatments ($F_{2,3.653}$=18.37, p=0.01). The post-hoc comparison showed that

particulate respiration was similar in bullfrog and control treatments but significantly lower (47%) in the Woodhouse's treatments (Figure 7).

In general, tadpoles most strongly affected NPP, particulate nutrient concentrations, and particulate respiration. While bullfrog and Woodhouse's tadpoles had the same effect on particulate nutrient concentrations, there were species-specific responses in NPP and respiration rates.

DISCUSSION

Over the last ten years, an increasing number of studies have documented the important roles amphibians play in aquatic and terrestrial ecosystem processes (e.g. Costa and Vonesh, 2013; Preston et al., 2012; Rantala et al., 2014; Semlitsch et al., 2014; Whiles et al., 2006). However, while community-level effects of non-native anuran tadpoles are well documented (Boone et al., 2007; Kiesecker and Blaustein, 1997; Kupferberg, 1997a), there is less known about their ecosystem impacts. In this paper, I seek to help fill this knowledge gap. Overall, I found that natural densities of non-native bullfrog and native Woodhouse's tadpoles had variable effects on aquatic ecosystem processes. While there was no difference in grazing pressure between the two species, Woodhouse's tadpoles egested more nutrients than bullfrog tadpoles. However, non-native bullfrogs excreted and egested relatively more N than native Woodhouse's tadpoles. Although both tadpole species increased particulate P, bullfrog nutrient excretion had a greater positive effect on areal NPP while Woodhouse's significantly decreased particulate respiration.

Tadpole Grazing

Neither bullfrog nor Woodhouse's grazing altered periphyton biomass. However, I observed a qualitative trend of decreasing chlorophyll *a* in the Woodhouse's treatments. Though bullfrog and Woodhouse's tadpoles are both grazers, Woodhouse's tadpoles are exclusively grazers, while bullfrog tadpoles are also efficient at filtering seston from the water column. Woodouse's tadpoles are such efficient grazers, that I could often identify where they had been foraging in the river, since they left cleared patches in algal mats (Greene, personal observation). Furthermore, though the tadpole biomass was similar between bullfrog and Woodhouse's treatment, there were 20x more tadpoles in the Woodhouse's treatments than the bullfrog treatments. High consumer density often results in higher grazing rates (Knoll et al., 2009; Mallory and Richardson, 2005). Therefore, I was surprised to see to effect of grazing, especially in the Woodhouse's treatments.

Both tadpole species feed on a wide variety of organic detritus. Therefore, I might have underestimated grazing pressure by only measuring changes in chlorophyll *a* biomass. A better estimate of tadpole grazing may have been to measure changes in total organic dry mass.

Tadpole Nutrient Recycling

Both bullfrog and Woodhouse's tadpoles excreted significant quantities of NH_4^+ and TdP. Though there is variation in published tadpole excretion rates, the rates I report were only slightly higher than those recorded for several tropical and temperature anuran species (Vanni et al., 2002; Whiles et al., 2006; Whiles et al., 2009). However, the bullfrog NH_4^+ excretion rates I calculated were on the low end of the range reported for

bullfrog tadpoles in the South Fork Eel River in California (Kupferberg, 1997b). Though excretion rates were similar between species, bullfrog tadpoles excreted at a slightly higher NH_4^+:TdP ratio. In contrast, there were significant differences in species-specific egestion rates. Native Woodhouse's tadpoles egested substantially more C, N, and P (per unit biomass) than non-native bullfrog tadpoles, though these values were 2-3 times lower than those reported for tropical anuran tadpoles (Rugenski, 2013). In addition, bullfrog tadpoles egested at a lower C:N and higher N:P ratio than native Woodhouse's tadpoles, implying bullfrogs egested relatively more N (and conserved P) compared to Woodhouse's.

Differences in nutrient recycling were most likely due to differences in diet and body composition (Moody et al., 2014; Sterner and Elser, 2002). While the Woodhouse's tadpoles (for excretion samples) were collected from the San Pedro River at GHNC, the bullfrog tadpoles were collected from a eutrophic, man-made pond at the Casa de San Pedro Bed and Breakfast. Tadpoles feed on a variety of primary producers and organic detritus, which have flexible nutrient stoichiometry (Sterner and Elser, 2002). Therefore, it is likely that the food resources available to the Woodhouse's and bullfrog tadpoles had dissimilar and variable nutrient content and stoichiometry. Since tadpoles, and other metazoans, maintain stoichiometric homeostasis (Sterner and Elser, 2002), they recycle nutrients in excess of their physiological demands. Therefore, the nutrient recycling rates I measured, may have been less indicative of species differences than habitat-specific resource stoichiometry. Furthermore, the tadpoles in the experiment may have excreted at different rates than the tadpoles I used for the nutrient recycling samples. Lastly, potential coprophagy may have caused me to underestimate nutrient recycling rates.

Ecosystem Effects of Grazing and Nutrient Recycling

Dissolved and Particulate Nutrients

In contrast to my initial prediction, I did not find differences between bullfrog and Woodhouse's nutrient recycling reflected in dissolved and particulate nutrient concentrations or ratios. However, since particulate P was significantly higher in both tadpole treatments compared to the control treatments, it is apparent that tadpole egestion influenced particulate nutrient content. Therefore, it appears that the ecosystem effect of bullfrog and Woodhouse's nutrient recycling was similar.

Although tadpoles increased particulate P concentrations, I was surprised that there was no effect of tadpole excretion on TdN concentrations. Both species recycled significant quantities of NH_4^+, and aquatic consumers (including tadpoles) often affect both N and P in sediment and biofilm (Knoll et al., 2009; Rugenski et al., 2012; Whiles et al., 2006) even though NH_4^+ is often a small component of TdN. N limitation of primary producers could help explain this phenomenon. N limitation is a widespread pattern in desert streams (Grimm et al., 1981; Grimm and Fisher, 1986). The San Pedro River, specifically at GHNC, has high NO_3^- uptake efficiency during the dry season (Martin et al., 2011). In addition, I observed extremely low TdN relative to TdP, in the river water (molar TdN:TdP, 3.4). Jointly, this suggests N limitation at GHNC in 2014. Whereas high ambient TdP may have swamped TdP excretion, N-limited primary producers may have rapidly sequestered TdN excretion. As a consequence, I did not detect elevated TdN concentrations.

Connelly et al. (2014) similarly found no difference in biofilm N content in the presence and absence of tadpoles. Instead, they found that the isotopic ratio of particulate

N changed after a catastrophic tadpole decline (Connelly et al., 2014). Higher $\delta^{15}N$ in the biofilm, prior to the amphibian decline, indicated that the N had been biologically processed (Connelly et al., 2014). Therefore, though tadpoles did not alter the quantity of particulate N, tadpole N egestion did contribute to biofilm N (Connelly et al., 2014). Based on the Connelly et al. (2014) results, I predict similar changes in the isotopic ratio of the particulate N in the mesocosm experiment.

While aquatic consumers often alter dissolved and particulate nutrient concentrations, stoichiometry, and nutrient limitation (Atkinson et al., 2013; Capps and Flecker, 2013; Knoll et al., 2009; McIntyre et al., 2008; Seale, 1980), several studies have found that tadpoles, including bullfrogs, have no effect (Connelly et al., 2014; Kupferberg, 1997a; Preston et al., 2012; Schaus et al., 1997). Therefore, the impact of nutrient recycling likely depends on research methods, environment, tadpole species, nutrient recycling rates, and tadpole densities. In my experiment, frequent flushing (inflow) and possible N limitation could have diluted the effects of tadpole nutrient recycling on dissolved and particulate nutrient concentrations.

Net Primary Productivity

I found that areal NPP increased in the presence of bullfrog nutrient excretion (caged discs). This pattern was due to an increase in biomass, not an in increase in biomass-specific productivity. Relatively higher TdN excretion by bullfrogs may have stimulated growth of N limited periphyton. Surprisingly, Woodhouse's excretion did not cause a similar outcome even though Woodhouse's excreted similar concentrations of N and P compared to bullfrogs. However, areal NPP may not have increased in the presence of Woodhouse's since they excreted at a lower N:P ratio.

In contrast, areal NPP did not respond to tadpole grazing and nutrient excretion (un-caged discs). These results contrast with my initial prediction and previous findings. For example, Lamberti and Resh (1983) found that caddisfly grazers decreased algal biomass but the grazed algae was more productive. In mesocosm experiments, the combination of grazing and nutrient excretion frequently stimulates algal productivity (Connelly et al., 2008; Kupferberg, 1997a).

In the context of the San Pedro River, my results indicate that bullfrog excretion may stimulate N limited NPP in river pools. However, this prediction is tempered by the fact that bullfrog grazing may obscure this pattern.

Respiration

Lastly, I compared the effect of bullfrog and Woodhouse's tadpoles on respiration in the mesocosm detritus. Since microbes rely on labile nutrients, tadpole egestion often increases microbial activity (Rantala et al., 2014; Rugenski et al., 2012; Whiles et al., 2013). However, I observed a very different response. Respiration was significantly lower in Woodhouse's treatments compared to both bullfrog and control treatments. Though there were species-specific differences in respiration, the direction of change was opposite to what I predicted. High densities of Woodhouse's tadpoles, along with efficient grazing behavior, likely drove this pattern. During the experiment, I observed many Woodhouse's tadpoles feeding in the benthic sediment. In addition, the Woodhouse's mesocosms were visually less turbid than the control or bullfrog mesocosms. Though I did not quantify these observations, there appeared to be less benthic detritus, higher water clarity, and less floating algae in the Woodhouse's treatments. Therefore, Woodhouse's tadpoles, especially at the high densities observed

during early developmental stages, may exhaust local resources and turn to feeding on detritus, thus decreasing microbial respiration rates.

The Mesocosm Results in Context

In the semi-arid Southwest, fall monsoons often eradicate bullfrog tadpoles living in river systems. However, in ponds or non-monsoon river systems bullfrog tadpoles may be present all year around. Therefore, they may affect the aquatic ecosystem over a longer temporal scale than other native anurans, whose tadpoles develop and leave the water within weeks to months (e.g. Woodhouse's toads).

I observed the effects of two tadpole species on ecosystem processes over a short temporal and spatial scale. Studies looking at whole ecosystems over longer time scales will be needed to better understand the role of anuran tadpoles on ecosystem processes (Connelly et al., 2008; Connelly et al., 2014; Rantala et al., 2014). For example, Connelly et al. (2008 and 2014) found that short-term changes in chlorophyll biomass, NPP, and biofilm inorganic and organic biomass did not persist over the long term (3 years). Therefore, it would be informative to study bullfrog and Woodhouse's tadpoles in the river itself over a longer time scale. In addition, it is important to investigate the effects of different densities of bullfrog and Woodhouse's tadpoles on ecosystem processes. Such research could provide data to build predictive model for the ecosystem effects of bullfrog invasion or removal.

Conclusions

My research highlights functional differences between two anuran tadpoles. It suggests that bullfrog tadpoles, which are non-native in the San Pedro River, have different grazing and nutrient recycling effects than native Woodhouse's tadpoles.

Bullfrog tadpoles appeared to have the strongest ecosystem impacts on primary productivity through nutrient excretion and particulate nutrients through grazing. This information, in combination with research describing the community effects of bullfrogs, can begin to inform managers about possible consequences of bullfrog invasion. Future research should continue to examine the ecosystem effects of variable densities of bullfrog, and other non-native tadpoles, on different aquatic ecosystems over longer time scales.

WORKS CITED

Adams, M. J., & Pearl, C. A. (2007). Problems and opportunities managing non-native Bullfrogs: is there any hope? In *Biological invaders in inland waters: Profiles, distribution, and threats* (pp. 679-693). Springer Netherlands.

Atkinson, C. L., Vaughn, C. C., Forshay, K. J., & Cooper, J. T. (2013). Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. *Ecology*, *94*(6), 1359-1369.

Benstead, J. P., Cross, W. F., March, J. G., McDOWELL, W. H., Ramirez, A., & Covich, A. P. (2010). Biotic and abiotic controls on the ecosystem significance of consumer excretion in two contrasting tropical streams. *Freshwater Biology*,*55*(10), 2047-2061.

Boone, M. D., Semlitsch, R. D., Little, E. E., & Doyle, M. C. (2007). Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. *Ecological Applications*, *17*(1), 291-301.

Capps, K. A., & Flecker, A. S. (2013a). Non-native fishes generate biogeochemical hotspots in a nutrient-limited system. *PLoS One*, *8*(1), e54093.

Capps, K. A., & Flecker, A. S. (2013b). Invasive aquarium fish transform ecosystem nutrient dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1769), 20131520.

Cecil, S. G., & Just, J. J. (1979). Survival rate, population density and development of a naturally occurring anuran larvae (*Rana catesbeiana*).*Copeia*, 447-453.

Colón-Gaud, C., Peterson, S., Whiles, M. R., Kilham, S. S., Lips, K. R., & Pringle, C. M. (2008). Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: potential effects of larval amphibians on organic matter dynamics. *Hydrobiologia*, *603*(1), 301-312.

Connelly, S., Pringle, C. M., Bixby, R. J., Brenes, R., Whiles, M. R., Lips, K. R., ... & Huryn, A. D. (2008). Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? *Ecosystems*, *11*(8), 1262-1276.

Connelly, S., Pringle, C. M., Barnum, T., Hunte-Brown, M., Kilham, S., Whiles, M. R., ... & Brenes, R. (2014). Initial versus longer-term effects of tadpole declines on algae in a Neotropical stream. *Freshwater biology*, *59*(6), 1113-1122.

Cooper, D. C. (1973). Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. *Limnology and Oceanography*, *18*(1), 31-37.

Costa, Z. J., & Vonesh, J. R. (2013). Prey subsidy or predator cue? Direct and indirect effects of caged predators on aquatic consumers and resources. *Oecologia*, *173*(4), 1481-1490.

Federation, W. E., & American Public Health Association. (1998). Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*.

Federation, W. E., & American Public Health Association. (2005). Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*.

Flecker, A. S. (1996). Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology*, *77*(6), 1845-1854.

Flecker, A. S., Feifarek, B. P., & Taylor, B. W. (1999). Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. *Copeia*, 495-500.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 183-190.

Grimm, N. B., Fisher, S. G., & Minckley, W. L. (1981). Nitrogen and phosphorus dynamics in hot desert streams of Southwestern USA. *Hydrobiologia*, *83*(2), 303-312.

Grimm, N. B., & Fisher, S. G. (1986). Nitrogen limitation in a Sonoran Desert stream. *Journal of the North American Benthological Society*, 2-15.

Hall, C. A., & Moll, R. (1975). Methods of assessing aquatic primary productivity. In *Primary productivity of the biosphere* (pp. 19-53). Springer Berlin Heidelberg.

Hall Jr, R. O., Tank, J. L., & Dybdahl, M. F. (2003). Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Frontiers in Ecology and the Environment*, *1*(8), 407-411.

Hillebrand, H. (2009). Meta-analysis of grazer control of periphyton biomass across aquatic ecosystems. *Journal of Phycology*, *45*(4), 798-806.

Hillebrand, H., Frost, P., & Liess, A. (2008). Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia*, *155*(3), 619-630.

Hillebrand, H., & Kahlert, M. (2001). Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnology and Oceanography*, *46*(8), 1881-1898.

Holomuzki, J. R. (1998). Grazing effects by green frog tadpoles (Rana clamitans) in a woodland pond. *Journal of Freshwater Ecology*, *13*(1), 1-8.

ISSG. (2005). Ecology of Lithobates catesbeianus (Publication from National Biological Information Infrastructure (NBII).

Kiesecker, J. M., & Blaustein, A. R. (1997). Population differences in responses of red-legged frogs (Rana aurora) to introduced bullfrogs. *Ecology*, *78*(6), 1752-1760.

Kiesecker, J. M., Blaustein, A. R., & Miller, C. L. (2001). Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfrogs. *Ecology*, *82*(7), 1964-1970.

Knoll, L. B., McIntyre, P. B., Vanni, M. J., & Flecker, A. S. (2009). Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects. *Oikos*, *118*(11), 1732-1742.

Kupferberg, S. (1997a). Facilitation of periphyton production by tadpole grazing: functional differences between species. *Freshwater Biology*, *37*(2), 427-439.

Kupferberg, S. J. (1997b). Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. *Ecology*, *78*(6), 1736-1751.

Lamberti, G. A., & Resh, V. H. (1983). Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. *Ecology*, 1124-1135.

Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., ... & McMichael, A. (2006). Biological invasions: recommendations for US policy and management. *Ecological Applications*, *16*(6), 2035-2054.

Mallory, M. A., & Richardson, J. S. (2005). Complex interactions of light, nutrients and consumer density in a stream periphyton–grazer (tailed frog tadpoles) system. *Journal of Animal Ecology*, *74*(6), 1020-1028.

Martin, R. A., Harms, T. K., & Grimm, N. B. (2011). Chronic N loading reduces N retention across varying base flows in a desert river. *Journal of the North American Benthological Society*, *30*(2), 559-572.

Mazzoni, R., Cunningham, A. A., Daszak, P., Apolo, A., Perdomo, E., & Speranza, G. (2003). Emerging Pathogen of Wild Amphibians in Frogs (*Rana catesbeiana*) Farmed for. *Emerging infectious diseases*, *9*(8), 995.

McIntyre, P. B., Michel, E., & Olsgard, M. (2006). Top-down and bottom-up controls on periphyton biomass and productivity in Lake Tanganyika. *Limnology and Oceanography*, *51*(3), 1514-1523.

McIntyre, P. B., Flecker, A. S., Vanni, M. J., Hood, J. M., Taylor, B. W., & Thomas, S. A. (2008). Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots. *Ecology*, *89*(8), 2335-2346.

Moody, E. K., & Sabo, J. L. (2013). Crayfish impact desert river ecosystem function and litter-dwelling invertebrate communities through association with novel detrital resources. *PloS one*, *8*(5), e63274.

Moody, E. K., Corman, J. R., Elser, J. J., & Sabo, J. L. (2014). Diet composition affects the rate and N: P ratio of fish excretion. *Freshwater Biology*.

Preston, D. L., Henderson, J. S., & Johnson, P. T. (2012). Community ecology of invasions: direct and indirect effects of multiple invasive species on aquatic communities. *Ecology*, *93*(6), 1254-1261.

Rantala, H. M., Nelson, A. M., Fulgoni, J. N., Whiles, M. R., Hall, R. O., Dodds, W. K., ... & Connelly, S. (2014). Long-term changes in structure and function of a tropical headwater stream following a disease-driven amphibian decline. *Freshwater Biology*, *60*(3): 575-589.

Ranvestel, A. W., Lips, K. R., Pringle, C. M., Whiles, M. R., & Bixby, R. J. (2004). Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. *Freshwater Biology*, *49*(3), 274-285.

Rosen, P. C., & Schwalbe, C. R. (1995). Bullfrogs: introduced predators in southwestern wetlands. *Our living resources: a report to the nation on the distribution, abundance, and health of US plants, animals, and ecosystems. US Department of the Interior, National Biological Service, Washington, DC*, 452-454.

Rugenski, A. T., Murria, C., & Whiles, M. R. (2012). Tadpoles enhance microbial activity and leaf decomposition in a Neotropical headwater stream. *Freshwater Biology*, *57*(9), 1904-1913.

Rugenski, A. T. (2013). Influences of Disease-driven Amphibian Declines on Ecosystem Structure and Function in Panamanian Headwater Streams (Unpublished doctoral dissertation). Southern Illinois University, Carbondale, IL

Semlitsch, R. D., O'Donnell, K. M., & Thompson III, F. R. (2014). Abundance, biomass production, nutrient content, and the possible role of terrestrial salamanders in Missouri Ozark forest ecosystems. *Canadian Journal of Zoology*, *92*(12), 997-1004.

Schwalbe, C. R., & Rosen, P. C. (1988). Preliminary report on effect of bullfrogs on wetland herpetofaunas in southeastern Arizona. *General technical report RM-Rocky Mountain Forest and Range Experiment Station, US Department of Agriculture, Forest Service (USA)*.

Seale, D. B. (1980). Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology*, 1531-1550.

Schaus, M. H., Vanni, M. J., Wissing, T. E., Bremigan, M. T., Garvey, J. E., & Stein, R. A. (1997). Nitrogen and phosphorus excretion by detritivorous gizzard shad in a reservoir ecosystem. *Limnology and Oceanography*, *42*(6), 1386-1397.

Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press.

Vanni, M. J. (2002). Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, 341-370.

Vanni, M. J., Flecker, A. S., Hood, J. M., & Headworth, J. L. (2002). Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecology Letters*, *5*(2), 285-293.

Wells, K. D. (2010). *The ecology and behavior of amphibians*. University of Chicago Press.

Whiles, M. R., Lips, K. R., Pringle, C. M., Kilham, S. S., Bixby, R. J., Brenes, R., ... & Peterson, S. (2006). The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. *Frontiers in Ecology and the Environment*, *4*(1), 27-34.

Whiles, M. R., Huryn, A. D., Taylor, B. W., & Reeve, J. D. (2009). Influence of handling stress and fasting on estimates of ammonium excretion by tadpoles and fish: recommendations for designing excretion experiments. *Limnology and Oceanography: Methods*, *7*(1), 1-7.

Whiles, M. R., Hall Jr, R. O., Dodds, W. K., Verburg, P., Huryn, A. D., Pringle, C. M., ... & Connelly, S. (2013). Disease-driven amphibian declines alter ecosystem processes in a tropical stream. *Ecosystems*, *16*(1), 146-15.

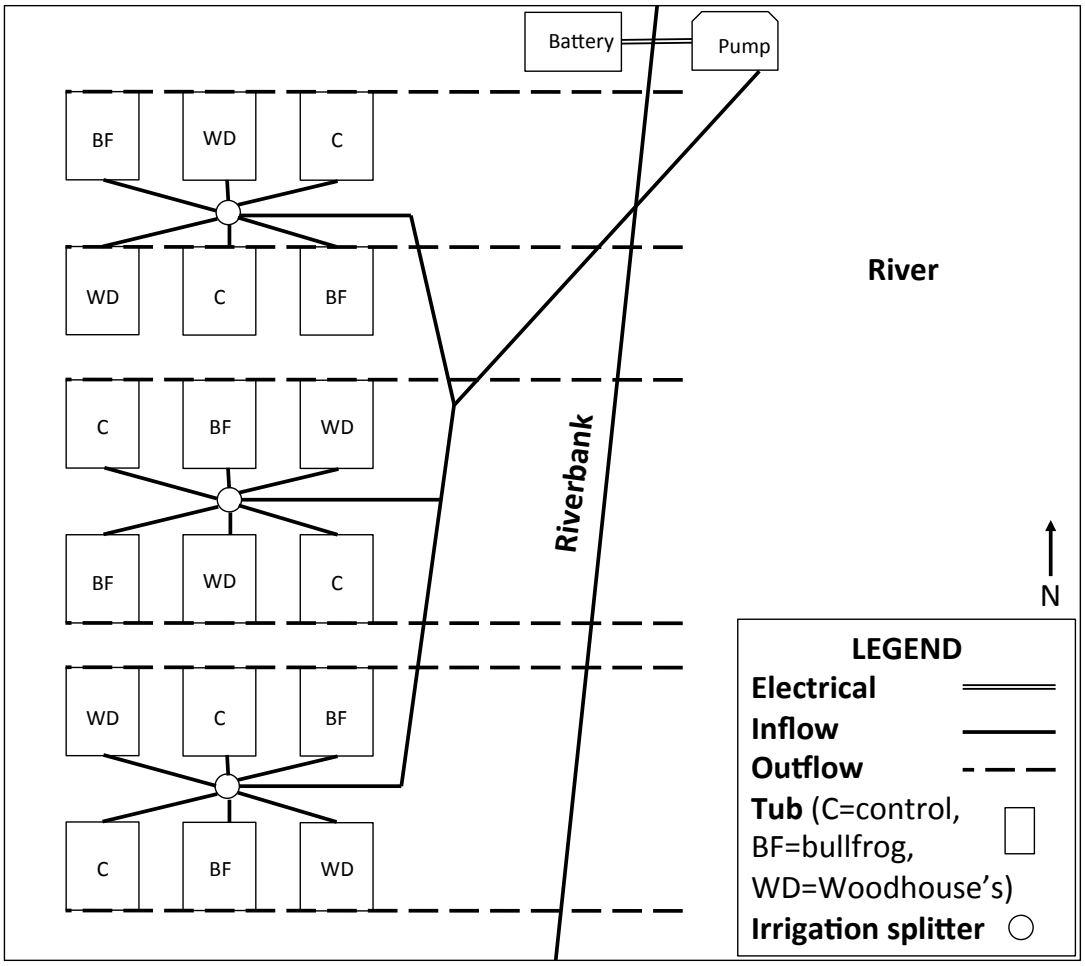

Figure 1: Diagram of flow-through mesocosm experiment at GHNC (April-June 2014).

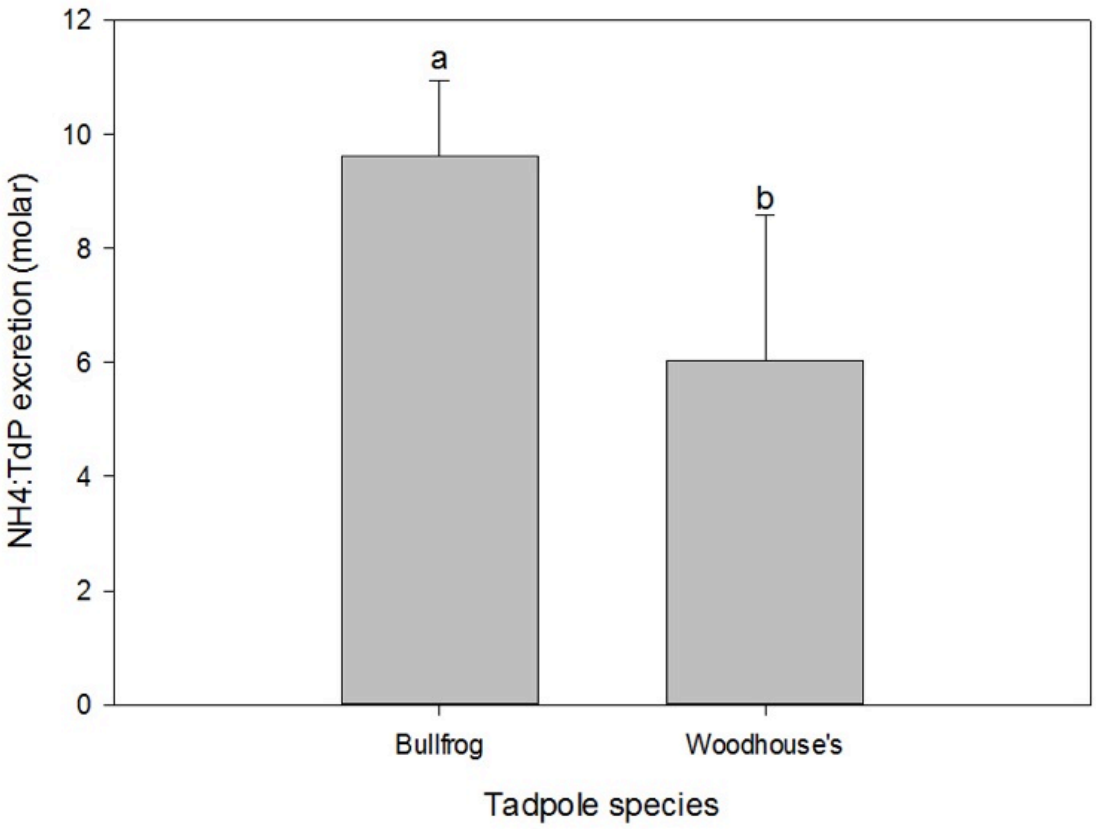

Figure 2: Mean (±SE) tadpole excretion NH_4^+:TdP molar ratio. Bullfrog n=12, Woodhouse's n=10. Means that are statistically different have different letters above bars ($p<0.05$, Mann-Whitney U test).

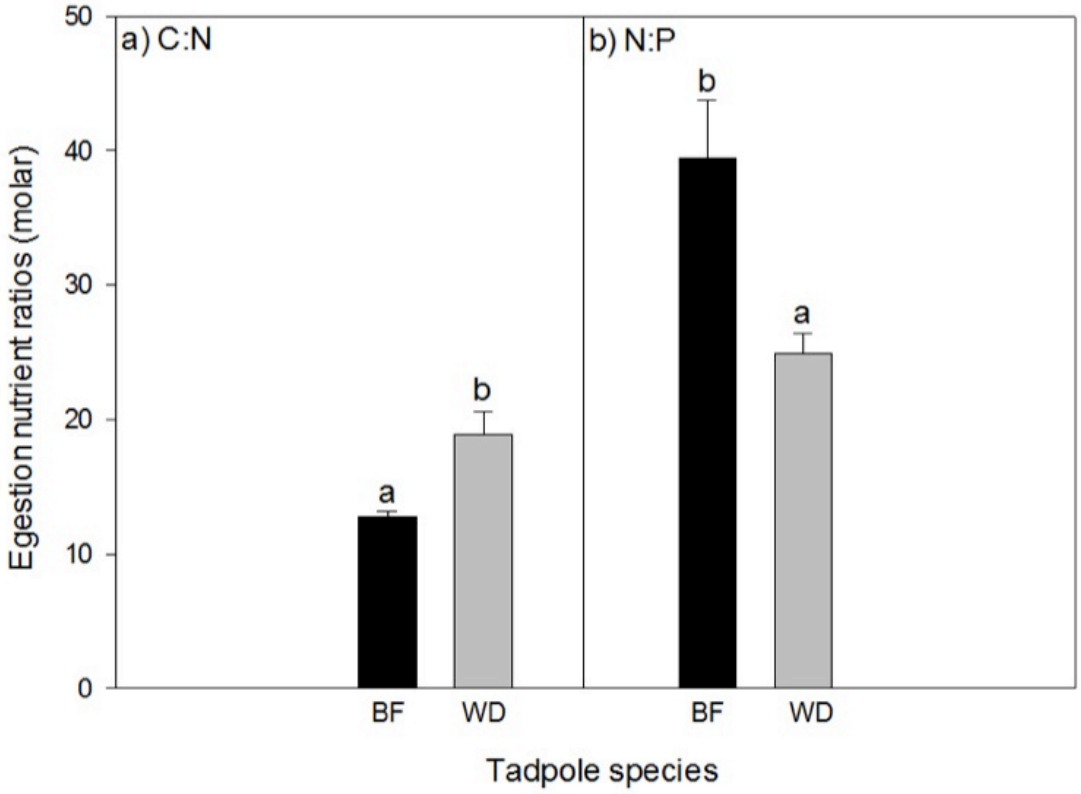

Figure 3: Mean (±SE) tadpole egestion molar ratios. BF=bullfrog tadpoles (n=11) and WD=Woodhouse's tadpoles (n=11). Statistically different means have different letters above the bars ($p<0.05$, Student's tests).

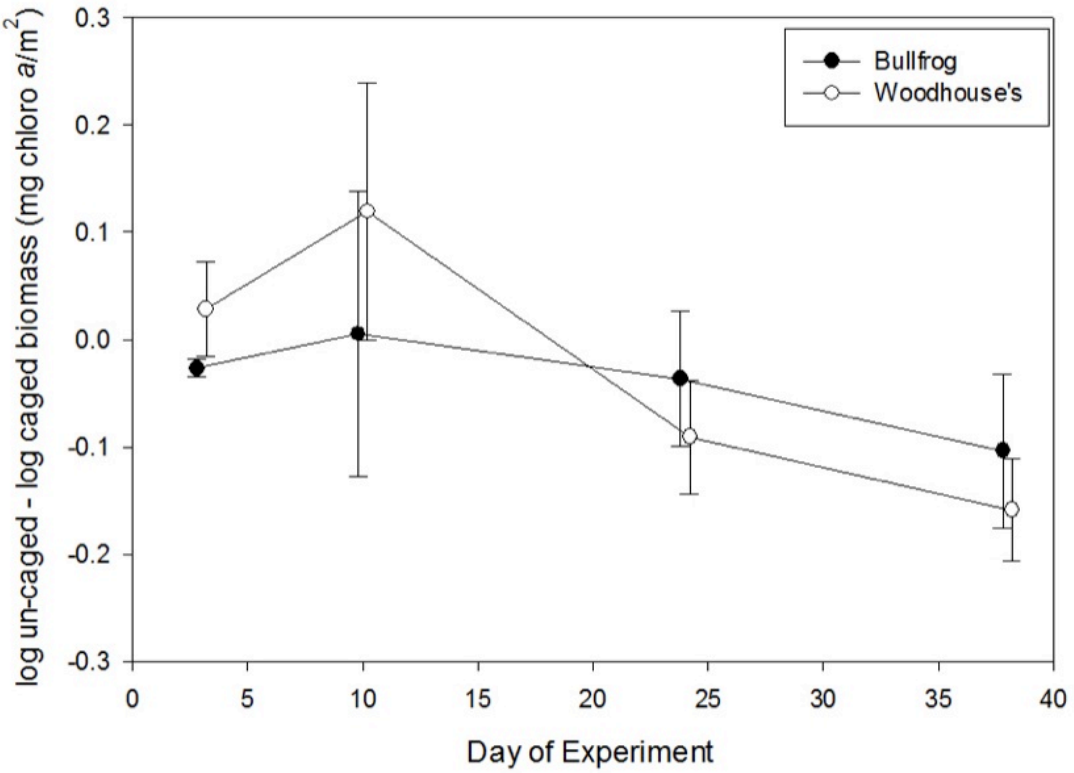

Figure 4: Effect of tadpole grazing on chlorophyll biomass throughout the mesocosm experiment. Calculations: chlorophyll values on caged and un-caged discs were log transformed. Control values, for caged and un-caged discs, were then subtracted from both bullfrog and Woodhouse's values. The "standardized" caged values were then subtracted from un-caged values. Data illustrate means±SE.

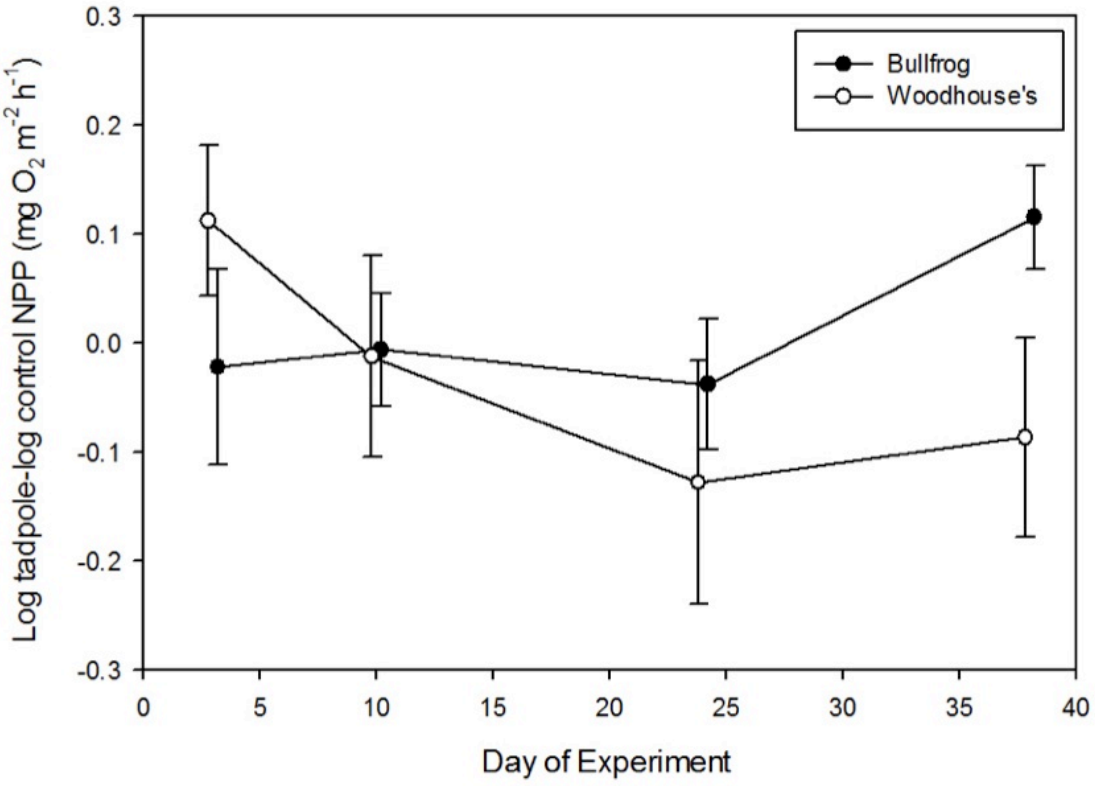

Figure 5: Mean (±SE) areal NPP on caged discs exposed to tadpole nutrient excretion. Calculations: NPP was first log transformed. The NPP for control treatments was then subtracted from NPP in both tadpole treatments.

Figure 6: Mean (±SE) percent phosphorus in mesocosm detritus in control (n=5), Bullfrog (n=6), and Woodhouse's (n=6) treatments on June 9, 2014. Statistically different means have different letters above the bars; Whelch's ANOVA and Tamhanes T2 post-hoc test ($p<0.05$).

Figure 7: Mean (±SE) Microbial respiration in Control (n=4), Bullfrog (n=4), and Woodhouse's (n=3) treatments on June 9, 2014. Statistically different means have different letters above the bars; Whelch's ANOVA and Tamhanes

Table 1: Mean (±SE) tadpole egestion rates (μg nutrient g dry mass tadpole^{-1} h^{-1})

Nutrient	Bullfrog	Woodhouse's
C	904.3±110.23	2169.3±319.02
N	81.8±9.39	139.2±19.28
P	0.2±0.01	0.4±0.04

Table 2: Mean (±SE) particulate C:P and N:P molar ratios

	Control	Bullfrog	Woodhouse's
C:P	2710.1±437.90	925.5±98.53	839.5±50.29
N:P	155.8±27.14	50.3 ±3.66	45.0±4.58

APPENDIX A

A: DATA COLLECTED: APRIL-JUNE 2014

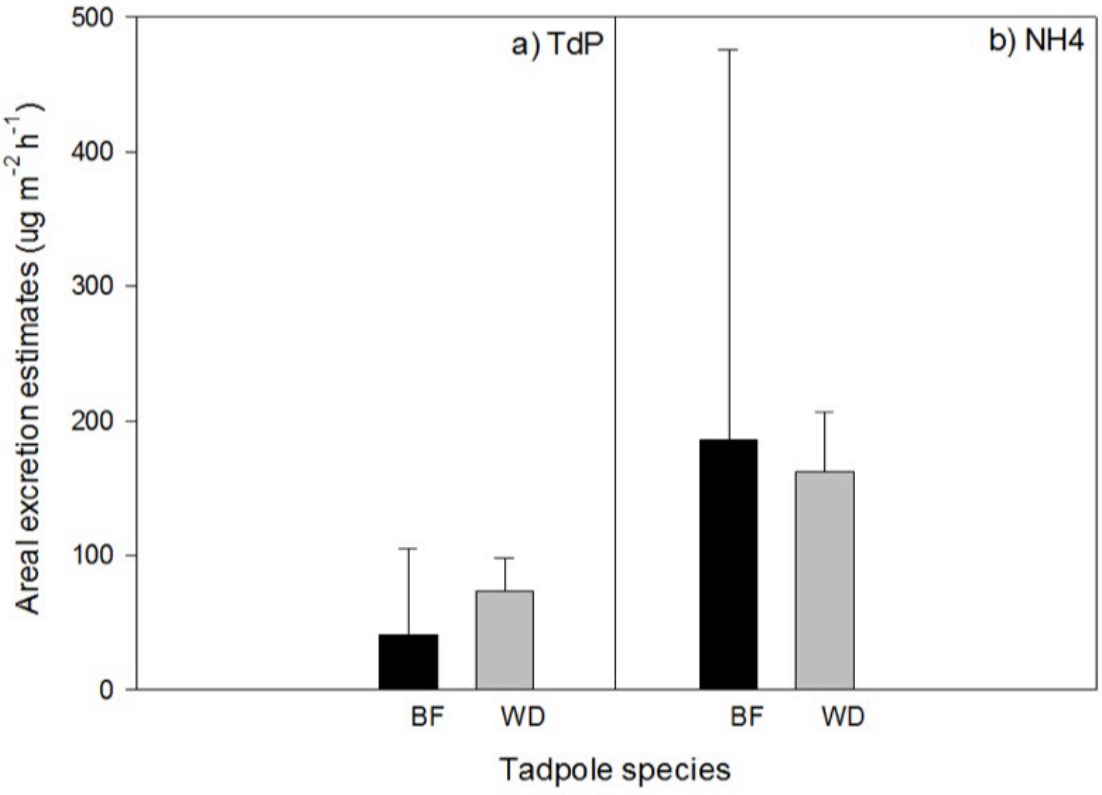

Figure A1: Estimated areal excretion for bullfrog (BF) and Woodhouse's (WD) tadpoles assuming densities same as those used to stock mesocosms (BF=14/m^2 and WD=332/m^2). Means±SE.

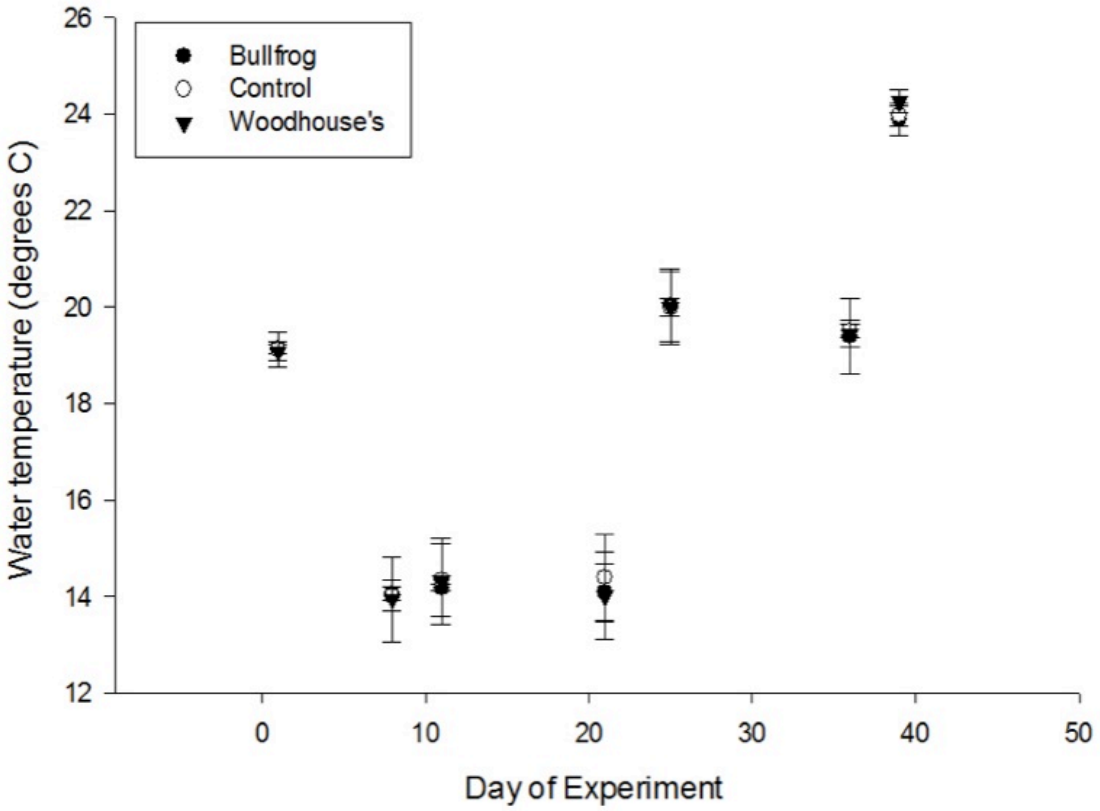

Figure A2: Mean (±SE) water temperature in the mesocosms over the 39-day experiment. River water inflow occurred between days 8-11, 21-25, and 36-39. Tadpoles were added between day 1 and day 8.

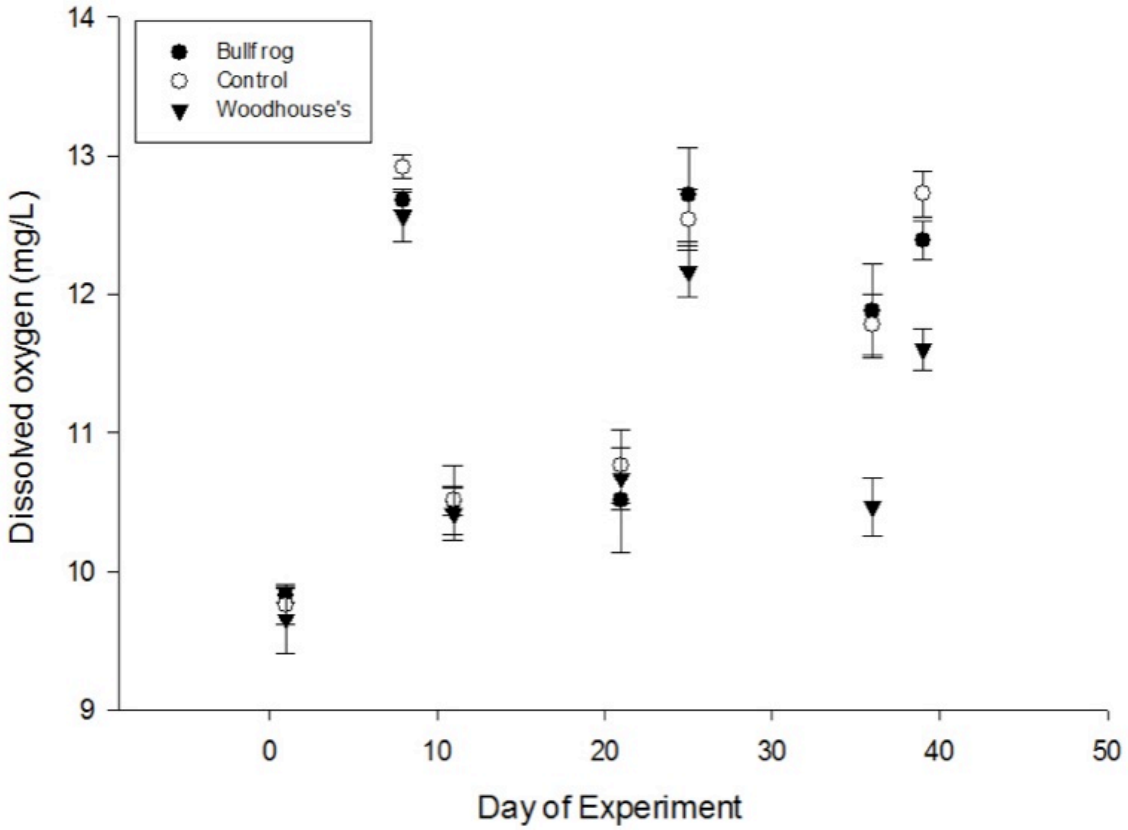

Figure A3: Mean (±SE) Dissolved oxygen (DO) in mesocosms over the 39-day experiment. River water inflow occurred between days 8-11, 21-25, and 36-39. Tadpoles were added between day 1 and day 8.

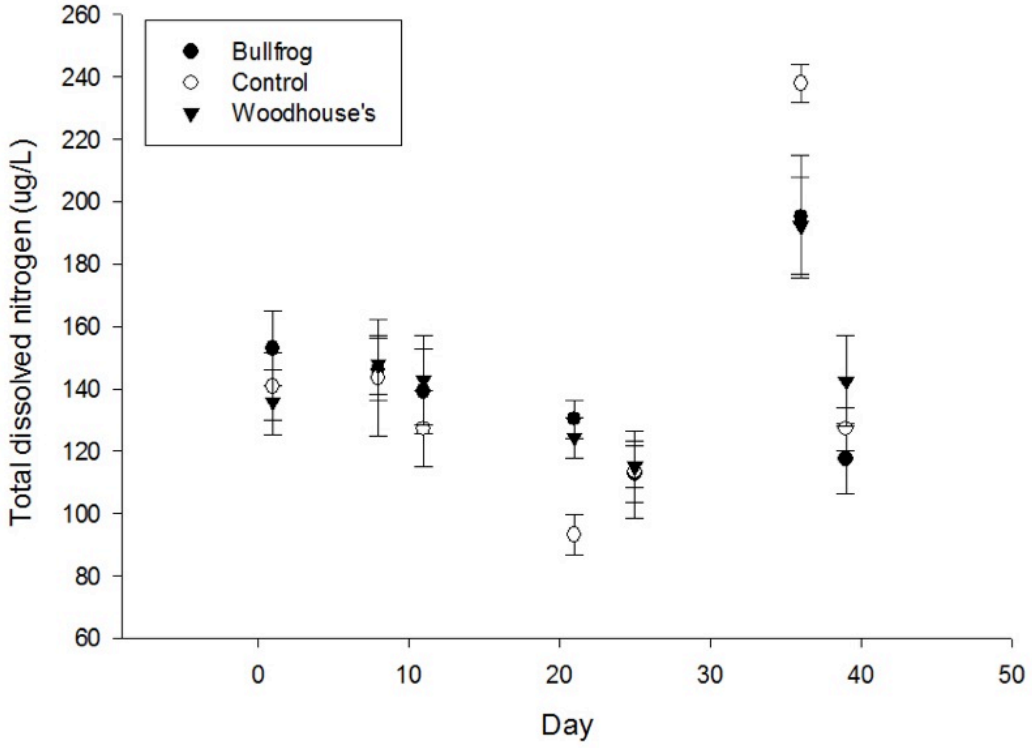

Figure A4a: Mean (±SE) total dissolved nitrogen in mesocosms over the 39-day experiment. River water inflow occurred between days 8-11, 21-25, and 36-39. Tadpoles were added between day 1 and day 8.

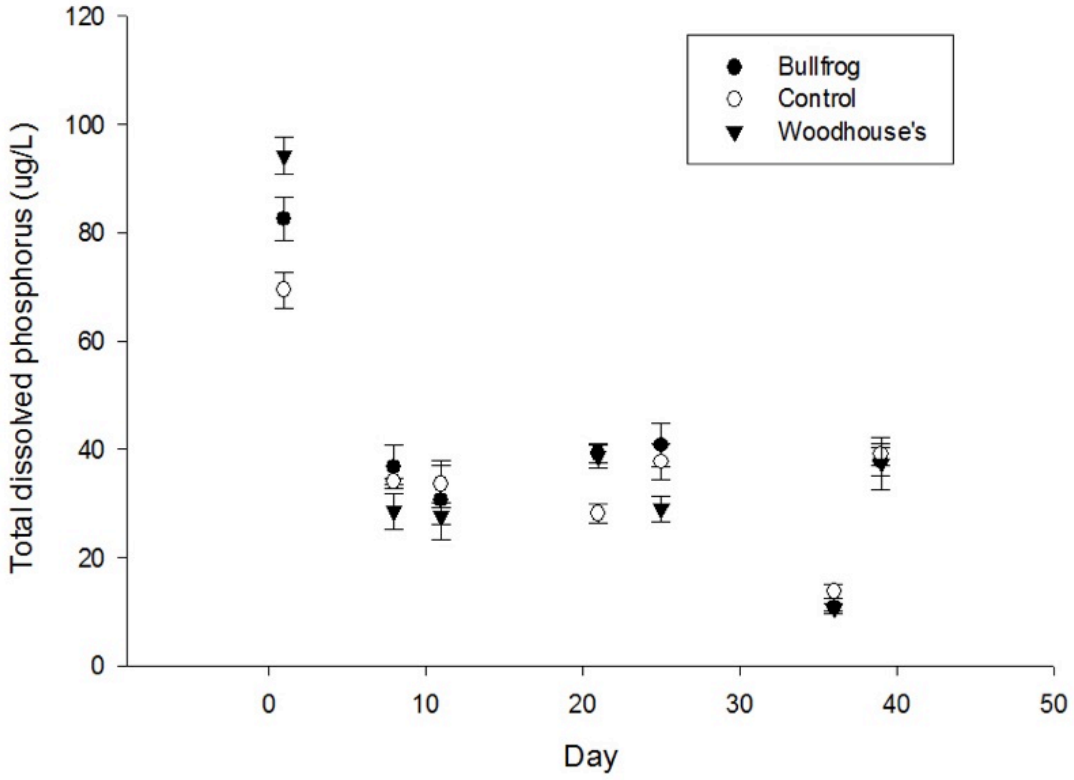

Figure A4b: Mean (±SE) total dissolved phosphorus in mesocosms over the 39-day experiment. River water inflow occurred between days 8-11, 21-25, and 36-39. Tadpoles were added between day 1 and day 8.

Table A1: Mean (±SE) estimated areal egestion rates (μg nutrient m^{-2} h^{-1})

	Bullfrog	Woodhouse's
C	1501.2±2359.05	3920.8±927.74
N	135.8±213.30	251.6±58.22
P	0.3±0.40	0.7±0.15

Table A2: Mean (±SE) estimated egestion rates per mesocosm (μg nutrient m^{-2} h^{-1})

	Bullfrog	Woodhouse's
C	607.1±62.28	1450.9±127.25
N	54.9±5.63	93.1±8.17
P	0.1±0.01	0.3±0.02