EFFECTS OF CATASTROPHIC DISEASE-RELATED TADPOLE DECLINES ON UPLAND NEOTROPICAL STREAM STRUCTURE AND FUNCTION

by

SCOTT J. CONNELLY

(Under the Direction of Catherine M. Pringle)

ABSTRACT

Global declines of amphibian populations are well documented, yet effects of these declines on freshwater ecosystem structure and function are poorly understood. Here we examine responses of algal primary producers and decomposition processes to tadpole extirpation over differing spatial and temporal scales. We experimentally excluded tadpoles from artificial substrata within localized areas of two streams. One stream had an intact community of frogs (*frog* stream), and the other had recently experienced a catastrophic decline (*frogless* stream), leaving virtually no tadpoles. In the *frog* stream, there were significantly greater levels of chlorophyll *a* (+111 %, P = 0.009), AFDM (+163 %, P = 0.02), inorganic sediments (+114 %, P = 0.001), and higher mean algal cell biovolume in tadpole exclusion treatments than in the tadpole access treatments. Correspondingly, overall AFDM-specific net primary production increased by 38% (P = 0.001) and chlorophyll *a*-specific NPP increased by 29% (P = 0.001) in tadpole access.. Fifteen months after our experiments, a massive amphibian decline associated with a fungal pathogen occurred in the *frog* stream, resulting in the extirpation of over 90% of tadpoles. This extirpation was followed by significant increases in levels of chlorophyll *a* (269%, P = 0.001), AFDM (+220%,

$P < 0.001$), and inorganic sediments (+140%, $P = 0.001$). Over the longer-term (3 years), the magnitude of this initial change dampened to increases of 1.9-fold in pools ($P < 0.05$) and 3.5-fold in riffles ($P < 0.05$) over pre-extirpation levels. We also experimentally excluded detritus-feeding tadpoles from localized areas. We found significantly higher mean levels of fungal biomass than in tadpole exclusion treatments ($P<0.10$; 56.9 vs 42.7 mg C g^{-1} AFDM), suggesting that Centrolenid tadpoles play a role in stimulating growth of stream fungal communities. Our experimental results, combined with algal monitoring at the reach scale, indicate that over the course of our study catastrophic amphibian losses have significant effects on stream ecosystem structure and function. Ecosystem-level impacts of tadpole extirpations were more dramatic than results from our small-scale, short-term experiments, which predicted the direction of change in response variables but underestimated the magnitude. However, the longer-term stream ecosystem responses remain unknown.

INDEX WORDS: amphibian declines, Panama, tropical streams, primary production, grazers, periphyton, time scale, decomposition

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Ecosystems across the planet are experiencing accelerating losses of biota, with largely unknown long-term consequences. In response to these losses, there has been increasing effort to better characterize and predict ecosystem-level consequences. A burgeoning literature exists to address effects of biodiversity change on ecosystem structure and function, and there is increasing evidence of significant changes in ecosystems where these losses are occurring (e.g. Tilman and Downing 1994; Hooper and others 2005; Hector and Bagchi 2007; McIntyre and others 2007). However, few studies have quantified the effects of species loss *in situ* during an actual extirpation event. What are the effects of widespread losses of entire taxonomic groups of consumers on stream ecosystems? This is an important question with respect to amphibians, which are suffering global catastrophic losses (Lips and others 2006; Whiles and others 2006). As many as 120 species of amphibians have become extinct since 1980, and currently one-third of known amphibian species are threatened (Stuart and others 2004). Amphibians are often an abundant and diverse component of both terrestrial and aquatic ecosystems. Continued widespread catastrophic declines of amphibians are expected to alter ecosystems across the globe because of the linkages that they create between aquatic and terrestrial systems (e.g., Regester and others 2006), and their potential role as keystone species (Holomuzki and others 1994; Wissinger and others 1999).

The ecological role of tadpoles in streams is poorly-studied. Experiments have shown that grazing amphibian larvae can influence the abundance and community composition of periphyton and insect assemblages (Lamberti and others 1992; Ranvestel and others 2004). However, the influence of tadpoles on ecosystem function (e.g., primary production) has rarely been studied (but see Kupferberg 1997) Moreover, experimental studies quantifying the influence of tadpoles on primary producers have been conducted on relatively small spatial scales and for short periods of time (e.g., Flecker 1996; Ranvestel and others 2004). Results of these studies have not been validated with comparisons to ecosystem response to widespread losses of tadpoles over greater spatial and temporal scales. For logistical reasons, the results of short-term studies have often been generalized to longer temporal scales, even though the limitations of this approach (e.g., time-lags in responses of indirect effects) are recognized (Sarnelle 1997).

Few data are available that document natural stream densities, feeding behavior and food requirement of many stream-dwelling tadpoles. Moreover, the degree to which the presence or absence of tadpoles may affect leaf litter decomposition dynamics and microbial and macroinvertebrate communities is relatively unknown. This is especially true of tadpoles within the glass frog (Centrolenidae) family. Species of this fossorial group are commonly found in dense leaf packs and accumulated decaying organic matter of many Neotropical streams (McDiarmid & Altig, 1999). Gut contents analysis indicates glass frog tadpoles ingest a high proportion of fine benthic detritus, although recent studies suggest the tadpoles assimilate energy primarily from microbes associated with the particles and not from the detritus itself (Hunte-Brown, 2006; Whiles *et al*.,

2006) While numerous experiments have shown that various aquatic macroinvertebrates can influence stream leaf litter decomposition dynamics, the role of locally abundant anuran larvae, with respect to litter decomposition communities and dynamics, is unknown.

Evaluating potential ecosystem changes over appropriate timescales following extinction events is particularly relevant in light of ongoing amphibian declines. On-going catastrophic amphibian declines provided the rare opportunity to document short- and longer-term stream ecosystem-level responses following a massive decline of a dominant grazer assemblage.

Study sites, objectives, and hypotheses

Research was conducted in two Panamanian headwater streams. One stream had an intact population of amphibians, whereas the other stream was essentially devoid of amphibians due to a catastrophic extirpation associated with the fungal pathogen *Batrachochytrium dendrobatidis* in 1996 (Lips 1999). The study streams are approximately 200 km apart, and are physically similar in terms of order, discharge, geology, temperature, canopy cover, and nutrient concentrations.

The focal stream (Rio Guabal) is located in the Parque Nacional G. D. Omar Torrijos Herrera, El Copé, Coclé, Panama ($8^{o}40'$N, $80^{o}35'$W). The park is comprised of cloud forest and is situated on the continental divide at ~600 m a.s.l. At the beginning of our study, this stream drainage supported 74 documented species of amphibians, 40 of which lived in the riparian zone. Tadpoles occur in all stream habitats: pools and runs support *Rana warszewitschii* and several treefrog (Hylidae) species; *Atelopus zeteki*

(Bufonidae) frequent riffles and erosional areas; glass frogs (Centrolenidae) use leaf packs; and several species of *Colostethus* (Dendrobatidae) are found in detritus of marginal pools.

The other stream (Quebrada Chorro) drains the Reserva Forestal Fortuna, Chiriquí, Panama (8°42'N, 82°14'W). The site is ~200 km west of the *frog* stream, at ~1200 m a.s.l., and includes montane and lower montane rainforest and cloud forest habitat. Amphibian populations have been monitored at this site since 1993 (Lips 1999, Lips unpublished data). Fifty-seven species were initially documented, of which 34 were riparian species and 22 of those with stream-dwelling tadpoles. The amphibian community once included the periphyton-grazing *R. warszewitschii*, *H. palmeri*, *H. colymba*, *Atelopus* and other groups such as *Colostethus* and Centrolenids (Lips 1999) which were found in the focal stream at the onset of our study. In 1996 Lips documented a mass mortality event and subsequent population decline of the amphibians in the *frogless* stream, and by 2000 only 23 species were found, 8 of which had stream-dwelling larvae. Stream tadpole densities were documented as high as 50 m^{-2} in 1993 (Lips 1999); however, virtually none (< 0.01 tadpoles m^{-2}) have been seen since 2000.

Our objectives were to 1) quantify algal periphyton response to amphibian declines at different spatial and temporal scales. On a 'local' scale we experimentally manipulated the presence and absence of tadpoles, *in situ*, in two upland Panamanian streams. We also examined algal community composition and biomass at the reach-scale within both study streams over a two-year period, inclusive of the period during which the stream with the healthy frog population experienced a massive amphibian die-off (Chapter 2); 2) document relatively "long-term" (i.e., up to 3 years) changes in basal food

resources, and compare short-term trends (i.e., on the scale of months) to longer-term patterns, following the loss of an entire group of consumers in a freshwater system (Chapter 3); and, 3) to compare leaf litter decomposition dynamics between two neotropical streams in Panama, to characterize the Centrolenid tadpole assemblage associated with decaying leaves, and to experimentally assess how tadpole presence and absence affects leaf decomposition rates and fungal, bacterial and macroinvertebrate communities associated with decaying leaves.

The following hypotheses are tested in these chapters:

Chapter 2. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: Can small-scale experiments predict effects of tadpole loss?

We hypothesized that tadpole-exclusion experiments in a stream with an intact amphibian community would: (1) result in algal standing crop and community composition similar to that found in a stream where tadpoles had been extirpated; and (2) predict the *direction*, but under-estimate the *magnitude*, of changes in algal standing crop and community composition resulting from whole-stream tadpole extirpation.

Chapter 3. Initial versus long-term effects of tadpole extirpations on algal resources and nitrogen cycling in a Neotropical stream.

We hypothesized that: (1) Initial high levels of algal standing crop, observed 5-months post tadpole extirpation, will not be sustained over 3 years; (2) algal food quality (as measured by biofilm C:N ratios) will decrease post-extirpation in both the short- (5 months) and longer- (3 years) term; and, (3) nutrient cycling will be

altered post-extirpation, as indicated by decreased fractionation of nitrogen stable isotopes in biofilm.

Chapter 4. Experimental comparison of leaf litter decomposition dynamics in two streams- with and without tadpoles: Evidence for localized effects of glass frog (Centrolenidae) tadpoles.

We hypothesized that: (1) the stream with frogs would have significantly higher decomposition rates than the frogless stream; and (2) experimental exclusion of Centrolenid tadpoles from leaf packs in the frog stream would result in (a) reduced decomposition rates (i.e. reduced loss of leaf mass over time); (b) increased fungal and bacterial biomass on leaves; and (c) increased abundance of shredder and filter-feeding macroinvertebrates.

CHAPTER 2

CHANGES IN STREAM PRIMARY PRODUCER COMMUNITIES RESULTING FROM LARGE-SCALE CATASTROPHIC AMPHIBIAN DECLINES: CAN SMALL-SCALE EXPERIMENTS PREDICT EFFECTS OF TADPOLE LOSS?[1]

[1] Connelly, S.J., C.M. Pringle, M. R.J. Bixby, R. Brenes, M.R. Whiles, K.R. Lips, S. Kilham, and A.D. Huryn. *Ecosystems* 4:27-34. Reprinted here with permission of publisher.

ABSTRACT

Global declines of amphibian populations are well documented, yet effects of these declines on freshwater ecosystem structure and function are poorly understood. Here we examine responses of algal primary producers to tadpole extirpation over differing spatial and temporal scales. We experimentally excluded tadpoles from artificial substrata within localized areas (0.25 m^2) of two streams. One stream had an intact community of frogs (*frog* stream), and the other had recently experienced a catastrophic decline (*frogless* stream), leaving virtually no tadpoles. In the *frog* stream, there were significantly greater levels of chlorophyll *a* (+111 %, P = 0.009), AFDM (+163 %, P = 0.02), inorganic sediments (+114 %, P = 0.001), and higher mean algal cell biovolume in tadpole exclusion treatments than in the tadpole access treatments. Correspondingly, overall AFDM-specific net primary production increased by 38% (P = 0.001) and chlorophyll *a*-specific NPP increased by 29% (P = 0.001) in tadpole access treatments compared to tadpole exclusion treatments. Areal-specific NPP did not differ between treatments. There were no significant differences in chlorophyll *a*, AFDM, inorganic sediments, algal cell biovolume, or biomass-specific NPP between treatments in the *frogless* stream. Fifteen months after our experiments, a massive amphibian decline associated with a fungal pathogen occurred in the *frog* stream, resulting in the extirpation of over 90% of tadpoles. This extirpation was followed by significant increases in levels of chlorophyll *a* (269%, P = 0.001), AFDM (+220%, P < 0.001), and inorganic sediments (+140%, P = 0.001). Reach-scale net primary production increased from -1587 mg DO m^{-2} d^{-1} to -810 mg DO m^{-2} d^{-1}. Additionally, algal community composition shifted from a dominance of small adnate diatoms (pre-decline) to a dominance of large upright algal

species (post-decline). Our experimental results, combined with algal monitoring at the reach scale, indicate that over the course of our study catastrophic amphibian losses have significant effects on stream ecosystem structure and function. Ecosystem-level impacts of tadpole extirpations were more dramatic than results from our small-scale, short-term experiments, which predicted the direction of change in response variables but underestimated the magnitude. However, the longer-term stream ecosystem responses remain unknown.

Key words: tadpoles; amphibian declines; Panama; tropical streams; primary production; grazing; periphyton

INTRODUCTION

Biodiversity is declining on a global scale and across taxonomically diverse groups (Chapin and others 1998). Many freshwater taxa, such as fishes, mussels, crayfishes, and amphibians, are disproportionately imperiled (Ricciardi and Rasmussen 1999). A burgeoning literature exists to address effects of biodiversity change on ecosystem structure and function, and there is increasing evidence of significant changes in ecosystems where these losses are occurring (e.g. Tilman and Downing 1994; Hooper and others 2005; Hector and Bagchi 2007; McIntyre and others 2007). However, few studies have quantified the effects of species loss *in situ* during an actual extirpation event.

What are the effects of widespread losses of entire taxonomic groups of consumers on stream ecosystems? This is a key question with respect to amphibians,

which are suffering global catastrophic losses (Lips and others 2006; Whiles and others 2006). As many as 120 species of amphibians have become extinct since 1980, and currently one-third of known amphibian species are threatened (Stuart and others 2004). Amphibians are often an abundant and diverse component of both terrestrial and aquatic ecosystems. They are the most common land vertebrate throughout parts of the Neotropics (Stebbins and Cohen 1995), with densities of adult frog populations as high as 1.35 individuals m^{-2} in riparian habitats in Panama (Lips and others 2003). Continued widespread catastrophic declines of amphibians are expected to alter ecosystems across the globe because of the linkages that they create between aquatic and terrestrial systems (e.g., Regester and others 2006), and their potential role as keystone species (Holomuzki and others 1994; Wissinger and others 1999).

In this study we focus on effects of this biotic impoverishment (i.e., the loss of the entire frog assemblage) on algal primary producers in Neotropical streams. While the importance of other grazing taxa, such as invertebrates and fishes, is well documented in lotic systems (e.g., Rosemond and others 1993; Pringle and Hamazaki 1997; March and others 2002), the ecological role of tadpoles in streams is less-studied. Experiments have shown that grazing amphibian larvae can influence the abundance and community composition of periphyton and insect assemblages (Lamberti and others 1992; Ranvestel and others 2004). However, the influence of tadpoles on ecosystem function (e.g., primary production) has rarely been studied (but see Kupferberg 1997), and the extent to which tadpoles alter periphyton communities can be difficult to predict, as their influence on periphyton can be the result of complex interactions among a number of biotic and abiotic variables (Mallory and Richardson 2005). Moreover, experimental studies

quantifying the influence of tadpoles on primary producers have been conducted on relatively small spatial scales and for short periods of time (e.g., Flecker 1996; Ranvestel and others 2004). Results of these studies have not been validated with comparisons to ecosystem response to widespread losses of tadpoles over greater spatial and temporal scales. Short-term, small-scale experimental tadpole exclusion studies have the potential to underestimate large-scale ecosystem responses to tadpole losses as there would possibly be a lack of sufficient time for algal community composition to change. Additionally, flat experimental tiles do not fully replicate the more complex structure of natural stream substata, which could result in higher rates of sloughing of periphyton from within our treatments than might be occurring in the stream.

The objective of our study was to quantify algal periphyton response to amphibian declines at different spatial and temporal scales. On a 'local' scale we experimentally manipulated the presence and absence of tadpoles, *in situ*, in two upland Panamanian streams. One stream had a healthy population of stream-dwelling tadpoles (at the onset of our studies), and the other had virtually no tadpoles as a result of a fungal pathogen-related amphibian population decline in 1996. (Lips 1999). We also examined algal community composition and biomass at the reach-scale within both study streams over a two-year period, inclusive of the period during which the stream with the healthy frog population experienced a massive amphibian die-off. This extirpation provided the rare opportunity to interpret results of our short-term, small-scale exclusion experiments in the context of longer-term, reach-scale responses. We hypothesized that tadpole-exclusion experiments in a stream with an intact amphibian community would: (1) result in algal standing crop and community composition similar to that found in a stream where

tadpoles had been extirpated; and (2) predict the *direction*, but under-estimate the *magnitude*, of changes in algal standing crop and community composition resulting from whole-stream tadpole extirpation.

METHODS

Study Sites

Research was conducted in two Panamanian headwater streams. The *frog* stream had an intact population of amphibians, whereas the *frogless* stream was essentially devoid of amphibians due to a catastrophic extirpation associated with the fungal pathogen *Batrachochytrium dendrobatidis* in 1996 (Lips 1999). The study streams are approximately 200 km apart, and are physically similar in terms of order, discharge, geology, temperature, canopy cover, and nutrient concentrations.

The *frog* stream (Rio Guabal) is located in the Parque Nacional G. D. Omar Torrijos Herrera, El Copé, Coclé, Panama ($8^{o}40'$N, $80^{o}35'$W). The park is comprised of cloud forest and is situated on the continental divide at ~600 m a.s.l. Our study reach is part of a high gradient stream characterized by distinct pool-run-riffle sequences, with substrates consisting of pebbles and gravel, with frequent cobbles, boulders, and depositional sandy areas. Riparian canopy cover is generally dense (>80%), with occasional tree-fall gaps.

The *frog* stream drainage supports 74 documented species of amphibians, 40 of which live in the riparian zone. Of these, 23 have stream-dwelling larvae (Lips and others 2003). Adult frog abundance is high throughout the year, with mean capture rates of 0.36 (± 0.05 SE) frogs m^{-1} of stream and 0.13 (± 0.0004) m^{-1} of trail walked (Lips

unpublished data). Tadpoles occur in all stream habitats: pools and runs support *Rana warszewitschii* and several treefrog (Hylidae) species; *Atelopus zeteki* (Bufonidae) frequent riffles and erosional areas; glass frogs (Centrolenidae) use leaf packs; and several species of *Colostethus* (Dendrobatidae) are found in detritus of marginal pools. Tadpoles of *R. warszewitschii, Hyla palmeri*, and *H. colymba* are numerous and prominent, achieving densities of up to 50 m^{-2}, and tadpoles are the only vertebrate grazers in this stream at this elevation (Ranvestel and others 2004). Two water column-feeding fish species (*Brachyrhaphis roswithae* and *Trichomycterus striatus*), one *Macrobrachium* shrimp species, one crab species (*Pseudothelphusa*), and 26 families of aquatic insects are found within park streams (Ranvestel 2002, Colon-Gaud and others unpublished data).

The *frogless* stream (Quebrada Chorro) drains the Reserva Forestal Fortuna, Chiriquí, Panama (8°42'N, 82°14'W). The site is ~200 km west of the *frog* stream, at ~1200 m a.s.l., and includes montane and lower montane rainforest and cloud forest habitat. The stream has a moderate gradient and, like the *frog* stream, is characterized by pool-run-riffle sequences and flows over mainly pebble, gravel, and sand substrates, with silt in depositional areas. The stream also has dense canopy cover (>80%).

Amphibian populations have been monitored at the *frogless* site since 1993 (Lips 1999, Lips unpublished data). Fifty-seven species were initially documented, of which 34 were riparian species and 22 of those with stream-dwelling tadpoles. The amphibian community once included the periphyton-grazing *R. warszewitschii*, *H. palmeri*, *H. colymba*, *Atelopus* and other groups such as *Colostethus* and Centrolenids (Lips 1999) which were found in the the *frog* stream at the onset of our study. In 1996 Lips

documented a mass mortality event and subsequent population decline of the amphibians in the *frogless* stream, and by 2000 only 23 species were found, 8 of which had stream-dwelling larvae. Stream tadpole densities were documented as high as 50 m^{-2} in 1993 (Lips 1999); however, virtually none (< 0.01 tadpoles m^{-2}) have been seen since 2000. Stream fish and invertebrate communities are similar to those of the *frog* stream (de Sousa 1999).

Small-scale tadpole exclusion experiments

Tadpole exclusion experiments were run consecutively at each site during the rainy season, from June 12 to July 22, 2003 at the *frog* stream and from July 25 to September 3, 2003 at the *frogless* stream. Mean daily rainfall during the 40 day study at the *frog* site was 3.6 mm d^{-1} (range = 0-49 mm d^{-1}), mean stream pH was 7.3, mean water temperature was 21.4^{o}C, NO_3-N was 0.16 mg/L and PO_4-P was 0.02 mg/L (one-time nutrient concentrations measured during the experiment, at baseflow conditions). At the *frogless* site, mean daily rainfall during the study was 2.7 mm d^{-1} (range = 0-10.8 mm d^{-1}), stream pH was 8.1, mean water temperature was 18.5^{o}C, NO_3-N was 0.12 mg/L and PO_4-P was 0.02 mg/L.

Electric exclusion devices (0.5 m^2 frames constructed of PVC tubing and concentric copper wire loops), modified from Pringle and Hamazaki (1997), were used to exclude tadpoles from artificial substrates placed on the stream bottom. Similar electric exclusion devices have been used effectively to exclude a variety of aquatic macroconsumers (e.g., tadpoles, fishes, shrimps, and crayfishes) from habitat patches, while not affecting movements of small aquatic insects (Pringle and Blake 1994;

Schofield and others 2001; March and others 2002; Ranvestel and others 2004). Within each stream, 10 treatment pairs (one electrified frame and one identical control frame without electricity) were placed within comparable 200 m reaches, with five pairs each in pools and riffles. Pools and riffles were selected based on similar current velocities (pools: ~0 m s^{-1}, riffles 0.15-0.33 m s^{-1}), depths (pools: 19-24 cm; riffles: 15-18 cm), and percent canopy cover (75%-85%). Current velocity was measured with a Marsh McBirney current meter and canopy cover was measured with a spherical densiometer. The two PVC frames of each pair were situated at least 0.5 m apart and anchored to the stream bottom using tent stakes and cable ties. Eight unglazed ceramic tiles (7 x 15 cm) were secured within each frame using binder clips and monofilament fishing line, for a total of 80 tiles per treatment per stream. Each frame was observed for three minutes on each of 20 days and 20 nights during the course of the experiment, for a total of 40 hours for all frames in each stream, in order to quantify tile visitation by tadpoles and to verify that other potential macroconsumer visitors (e.g., fishes and shrimps) were not accessing control treatments. Grazing tadpoles were identified to species in the cases of *R. warszewitschii* and *A. zeteki*. Due to the difficulty of species-level identification *in situ,* tadpoles of the genus *Hyla* were identified only to genus.

One randomly selected tile was removed from each frame every 5 days over a 40-day period. Each tile was placed within a fine mesh dip net (to prevent the escape of invertebrates), slowly raised to the stream surface, and placed in a Ziploc® bag along with the contents of the dip net. Tiles were placed in a cooler and transported to the laboratory where each tile was scrubbed with a toothbrush and rinsed, along with the contents of the bag, into an enamel pan. Invertebrates were removed from the

homogenate and preserved in 7% formalin for identification. The homogenate was transferred to a beaker and diluted to a known volume. While stirring, 20 ml was subsampled and preserved with 2% formalin for later analysis of algal species composition and biovolume. From the remaining homogenate two subsamples were obtained. The first 40-100 ml subsample was filtered onto a combusted and pre-weighed Whatman glass fiber filter (0.7 μm). These filters were dried at 60^{o}C for 24 h, weighed to the nearest 0.0001 g, ashed at 500^{o}C for 2 h, and reweighed to determine ash-free dry mass (AFDM) and inorganic sediments. The second 40-100 ml subsample was filtered onto a glass fiber filter (0.7 μm), placed in an aluminum foil pouch, and frozen until analysis for chlorophyll *a*. Chlorophyll *a* was estimated using a Turner Designs model 10-AU fluorometer (Turner Designs, Inc., Sunnyvale, CA, USA) using standard methods (APHA 1985).

Benthic invertebrates from control and experimental treatments were sampled using a Surber sampler after the final tiles were collected on day 40. Invertebrates were preserved in 7% formalin and identified to family or genus. Algal community composition and biovolume also was assessed on day 40 using a 20 ml formalin-preserved subsample. Densities of filamentous cyanobacteria were determined using a Palmer-Maloney counting chamber at 400x magnification (brightfield optics) on a Zeiss Universal microscope. Taxa were identified and enumerated along a transect(s) until 300 live cells/units were recorded. Some cyanobacterial filaments were counted in 10 micrometer lengths (one length = one unit). In samples with extremely low cell densities, a maximum of 10 transects were examined.

A 5 ml subsample for diatom identification was boiled in 30% hydrogen peroxide for 1 h to oxidize organic material. Samples were rinsed six times with distilled water to remove by-products and evaporated onto coverslips in 100 ml beakers to concentrate cell densities. Coverslips were mounted on microscope slides with Naphrax™ (PhycoTech, St. Joseph, MI, USA). A minimum of 500 valves was enumerated and identified along transects of the coverslip using a Zeiss Universal research microscope (Carl Zeiss, Thornwood, NY, USA) with brightfield oil immersion optics at 1000x. Identifiable valve fragments along each transect were categorized by size relative to whole valves (e.g., 25%), and mathematically reconstituted to whole-valve units and fractions. Diatoms were identified to the lowest taxonomic level (usually species) using standard taxonomic references (Patrick and Reimer 1966; Krammer and Lange-Bertalot 1986; 1988; 1991) and published Neotropical flora descriptions (Bourrelly and Manguin 1952; Foged 1984; Silva-Benavides 1996). We calculated the biovolume for each taxon by measuring dimensions of 10 cells and using published geometric equations to calculate cell biovolumes (Hillebrand and others 1999).

On a localized scale, net primary production, as measured by oxygen production, was measured on the final sampling dates (days 39 and 40; 10 tiles per treatment per stream). Each tile was placed in a custom-made Plexiglass metabolism chamber (Rapid Creek Research, Boise, Idaho, USA), which was filled with stream water. Air bubbles were removed, the container was sealed, and a 12-volt electric re-circulating pump was used to simulate stream current at 2.4 cm s^{-1} within the chamber. Changes in dissolved oxygen over a period of 1 hour were obtained from a small chamber port using a YSI Model 58 Dissolved Oxygen Meter (Yellow Springs Instrument Co., Inc., Yellow

Springs, Ohio, USA) with a self-stirring probe. Each tile used in the metabolism chambers was processed as described above to determine the corresponding algal biomass estimates used for calculations of biomass-specific NNP.

Pre- and post-decline comparisons

To characterize changes in algal standing crop and community composition over time, and to determine whether periphyton biomass and composition associated with experimental tiles were representative of the natural stream substratum, periphyton on rocks from both streams were sampled monthly over 24 months, beginning June 2003. A benthic sampler, modified after Loeb (1981), was used to quantitatively sample periphyton from a known area of these natural substrata. Five replicate samples of benthic algae and inorganic sediments were collected from rocks, during baseflow, in each of five pools and riffles along the same 200 m reach used for the exclusion experiment. The replicate samples from each pool or riffle were pooled and homogenized. A 20 ml subsample was preserved with formalin, and the remaining homogenate was analyzed for chlorophyll *a*, AFDM, and inorganic sediments following previously described methods. Subsamples from three pools and three riffles sampled from the *frog* stream were analyzed monthly for diatom and soft algae community composition. Densities were determined using a Palmer-Maloney counting chamber at 400x magnification (brightfield optics) on a Zeiss Universal microscope. We counted 300 cells, identified the nine largest diatoms to species level, quantified the filamentous cyanobacteria cells, and categorized the remaining smaller diatom species as a group.

For samples with extremely low cell densities, a maximum of 10 transects were examined.

Tadpole abundance surveys were conducted monthly. Surveys began in June 2003 and continued through June 2005. Tadpoles in three randomly chosen pools were quantified with a stove pipe benthic corer (22 cm diameter) that was modified with external rubber flaps at the base, which helped seal the bottom of the sampler when substrate was irregular. The core sampler was pushed approximately 3 cm into the substrate, and tadpoles were removed with a dip net (15 x 10 x 10 cm), counted, identified, and released. Kick net sampling was used to quantify tadpoles in three randomly chosen riffles, according to methods described by Hauer and Resh (1996) and Heyer and others (1994).

We measured reach-scale metabolism in the *frog* stream on March 4-5, 2004 (pre-decline) and June 5-6, 2005 (post-decline), using the single-station method (Owens 1974). Prevailing weather (e.g. temperature and rainfall), staff gage height, and discharge (39 L/s; baseflow conditions), were similar during the two sampling periods. Diel oxygen curves (readings at 10-12 min intervals for 24 h) were obtained at the upstream and downstream ends of the 100 m study reach during each sampling event using YSI 600 sondes. We used the single-station approach because diel changes in dissolved oxygen in this stream were too subtle to use an upstream-downstream, two-station method. Prior to sampling, sondes were calibrated in water-saturated air at sea level and oxygen saturation values were later corrected for altitude. Reaeration was estimated by measuring decline in dissolved propane concentrations along the study reach during a steady-state propane injection performed on each sample date (Marzolf

and others 1994). A sodium chloride solution was added as a conservative tracer at the same time as the propane to account for dilution and to estimate reach travel time. Metabolism was calculated based on Marzolf and others (1994) using the corrected measure of oxygen flux via reaeration (Young and Huryn 1998). Reach-scale metabolism could not be measured at the *frogless* stream because of the discovery of a large groundwater source along the reach and adverse weather conditions during site visits.

Statistical Methods

Differences in chlorophyll *a,* AFDM, inorganic sediments, and invertebrates in the small-scale exclusion experiments were assessed using repeated measures ANOVA. Chlorophyll *a,* AFDM, and inorganic sediments values were log-transformed prior to analysis to satisfy the assumption of normality of variances. Analyses were performed on SAS System for Windows, Version 8.0 (SAS Institute, Cary, NC, USA) using $\alpha = 0.05$.

Indicator species analysis (Dufrêne and Legendre 1997) was used to determine which algal taxa were characteristic of either control or exclusion treatments in the small-scale experiment. This analysis generates an indicator value [0 (not an indicator) -100 (perfect indicator)] for each taxon based on the product of relative frequency and abundance (based on cell numbers) of each taxon in each treatment. Monte Carlo tests (1500 randomizations) were run to determine if the indicator value is greater than expected by chance. Indicator species have both an indicator value > 25 and a $p < 0.05$ (Dufrêne and Legendre 1997). This classification was calculated using PC-ORD software (Version 4.37, McCune and Mefford 1999).

Monthly whole-stream sampling was unreplicated (i.e. one reference and one treatment stream). We used Randomized Intervention Analysis (RIA, Carpenter and others 1989) to detect pre- and post-decline changes in chlorophyll *a*, AFDM, levels of inorganic sediments, and tadpole abundance in the *frog* stream relative to the *frogless* stream. RIA tested the null hypothesis that no change occurred in these variables in the *frog* stream relative to the *frogless* stream following the decline in amphibian abundance.

RESULTS

Small-scale tadpole exclusion experiments

Tadpole densities: In the *frog* stream, tadpoles were abundant grazers throughout the experiment. Ambient stream densities of the most common species, *R. warszewitschii,* ranged from 6.4-39.2 m^{-2} (mean = 27.6 ± 11.8) in pools and 0-28.1 m^{-2} (mean = 14.2 ± 10.2) in riffles. *Hyla* spp. and *A. zeteki* were encountered less frequently than *Rana*, with *Hyla* more common in pools (range = 1.8-5.4 m^{-2}, mean = 3.4 ± 1.4) and *A. zeteki* found only in riffles (range = 0-3.6 m^{-2}, mean = 2.0 ± 0.9). No other macroconsumers (e.g., crabs and shrimps) were observed within control treatments. Water column-feeding fishes were seen above tiles, but never in contact with tiles. The electric current effectively deterred tadpoles from exclusion treatments, and only 5 small tadpoles were observed in exclusion plots over 20 h of observation during the course of the experiment. No tadpoles were observed in control or exclusion treatments in the *frogless* stream.

Chlorophyll a, AFDM, and sediments: Tadpoles significantly reduced the amount of chlorophyll *a* in control treatments in the *frog* stream over time in pools and riffles (Fig. 1.1). During the final 20 days of the experiment, after a major rain and scouring

event (base flow increased from 25 L/s to 135 L/s), control treatments in pools and riffles had 54% ($F_{1,8}$ = 11.62, P = 0.009) and 35% ($F_{1,8}$ = 18.87, P = 0.0025) less chlorophyll *a*, respectively, than exclusion treatments.

Organic mass (AFDM) and inorganic sediments followed similar trends to chlorophyll *a* (Fig. 1.1). During the final 20 days, AFDM in pools was 70% less ($F_{1,8}$ = 39.31, P = 0.002) in controls than in exclusion treatments, and AFDM in riffles was reduced 53% ($F_{1,8}$ = 9.42, P = 0.02) in control versus exclusion treatments. During this period, inorganic sediments in pools of control treatments were reduced 51% relative to exclusion treatments ($F_{1,8}$ = 22.81, P = 0.025) and reduced 56% ($F_{1,8}$ = 26.71, P =0.003) in riffles (Fig. 1.1).

In contrast to results for the *frog* stream, in the *frogless* stream we found no differences in chlorophyll *a* between treatments in pools ($F_{1,8}$ = 1.18, P = 0.30), or in riffles ($F_{1,8}$ = 0.00, P= 0.99) (Fig. 1.1). Like the *frog* stream, the *frogless* stream experienced a high-discharge event prior to day 20, although of lesser intensity (personal observation). By day 35, levels of chlorophyll *a* on tiles in the *frogless* stream pools began to stabilize near 2.46 mg m^{-2}, similar to final levels measured in tadpole exclusion treatments in tadpole-dominated pools of the *frog* stream (mean = 2.21 mg m^{-2}). Similarly, mean chlorophyll *a,* in both exclusion and control treatments in the *frogless* stream riffles by day 35, approximated mean levels of chlorophyll *a* in the exclusion treatment in the *frog* stream (1.53 mg m^{-2} vs. 1.63 mg m^{-2}).

AFDM and sediment levels of control and exclusion treatments in the *frogless* stream were not statistically different (Fig. 1.1). Subsequent to the high-discharge event, AFDM and inorganics in exclusion and control treatments in the *frogless* stream pools

and the tadpole exclusion treatments in the *frog* stream pools were similar (*frogless* stream exclusion = 7.31 ± 3.43 g AFDM m^{-2}, *frogless* stream control = 7.44 ± 3.89 g AFDM m^{-2}, and *frog* stream exclusion = 8.44 ± 5.65 g AFDM m^{-2}; inorganic sediments data not shown).

Algal community composition: We found a total of 132 diatom taxa (31 genera) in the *frog* stream on all tiles collected on day 40 of the exclusion experiment. Of the more dominant larger and motile taxa, such as *Gyrosigma sciotoense* (Sullivant and Wormley) Cl , *Navicula* sp., *Pinnularia butantanum* (Krasske) Metzeltin & Lange-Bert, and *Rhopalodia gibberula*, cell densities were greater in tadpole exclusion treatments relative to controls. Cell densities were 59% greater in the tadpole exclusion treatment, and this difference was greater in both pools and riffles. Mean diatom biovolume was 105% greater in pool habitat and 71% greater in riffle habitat where tadpoles were excluded relative to control treatments (Table 1.1). Of the genera present, *Achnanthes, Eunotia, Navicula, Nupela, Pinnularia, Synedra,* and *Terpsinöe,* comprised 87.4% of the total biovolume on experimental tiles. In contrast, no significant differences in cell densities or biovolume were found between control and exclusion treatments in the *frogless* stream (Table 1.1).

Indicator species analysis showed a shift in algal community composition between control and exclusion treatments within the *frog* stream. This analysis showed that mostly the small-celled *Diadesmis contenta* (Grun.) D.G. Mann (indicator value 70.9, P = 0.0413), *G. sciotoense* (Sullivant & Wormley) Cl. (56.0, P = 0.0240), *Navicula pseudoarvensis* Hust. (47.5, P = 0.0553), *Nitzschia clausii* Hantzsch (56.6, P = 0.0267), and *Nupela* sp. (67.7, P = 0.0047) were indicators of control treatments with tadpole

grazing; no taxa were indicators of the tadpole exclusion treatments. Indicator species analysis showed no difference in algal community composition between control and exclusion treatments in the *frogless* stream.

Primary production: AFDM-specific net primary production measured at a small-scale (0.25 m^2) in the *frog* stream was 37% greater on tadpole-grazed tiles within pools ($t = 4.05$, $P = 0.015$) and 55% greater in riffles ($t = 2.72$, $P = 0.048$; Fig. 1.2). Chlorophyll *a*-specific net primary production was 36% greater on grazed tiles in pools ($t = 3.73$, $P = 0.02$), and 49% greater in riffles ($t = 2.86$, $P = 0.035$). However, small-scale *areal*-specific net primary production was not significantly different between treatments in either pools or riffles. Biomass-specific net primary production was not significantly different between control and exclusion treatments when normalized for AFDM, chlorophyll *a*, or area in either pools or riffles in the *frogless* stream.

Invertebrates: Invertebrates colonizing tiles consisted almost entirely of baetid mayflies (Ephemeroptera) and chironomids (Diptera) in both the *frog* and *frogless* streams. There were no treatment differences in invertebrate abundance between control and exclusion treatments (baetids $F_{1,18} = 0.19$, $P = 0.67$; chironomids $F_{1,18} = 0.15$, $P = 0.72$) in the *frog* stream or in the *frogless* stream (baetids $F_{1,18} = 0.13$, $P = 0.72$; chironomids $F_{1,18} = 0.06$, $P = 0.81$). There were also no differences in invertebrate communities (collected by Surber sampling) from control and experimental treatments on the final day of the electric exclusion experiments in the *frog* or *frogless* streams.

Pre- and post-decline comparisons of natural substrate

Dead and dying frogs infected with a pathogenic chytrid fungus were first detected at the *frog* stream during September 2004. Subsequent adult frog mortality was high through January 2005, at which time the riparian amphibian abundance had become greatly reduced (Lips and others 2006). A mass reduction in tadpole abundance, compared with previously documented abundances, occurred nearly concurrently in the *frog* stream. During this time tadpole densities within pools declined from a mean of 8.2 tadpoles m^{-2} to 1.3 tadpoles m^{-2} ($P = 0.042$, RIA). Tadpole densities in riffles declined from 0.9 tadpoles m^{-2} to 0.0 tadpoles m^{-2} during this period, although the change was not significant ($P > 0.05$, RIA). We characterize the periods of June 2003 – September 2004 as 'pre-decline', and February 2005 – June 2005 as 'post-decline'. The transitional period (October 2004 – January 2005) was not used for our RIA analysis.

Chlorophyll a, AFDM, and sediments: Following tadpole extirpation, mean monthly levels of chlorophyll *a* and AFDM in the *frog* stream increased significantly in both pools ($P = 0.001$ chlorophyll *a*, AFDM, and sediments, RIA), and riffles ($P < 0.001$ chlorophyll *a;* $P = 0.001$ AFDM and sediments, RIA; Fig. 1.3). Monthly chlorophyll *a* increased in pools by 2.6 fold (3.00 ±1.31 mg m^{-2} to 8.31 ± 0.63 mg m^{-2}) and 5.9 fold (from 1.07 ± 1.27 mg m^{-2} to 6.74 ± 0.62 mg m^{-2}) in riffles post-decline (Fig. 1.3). AFDM showed a similar trend. Post-decline AFDM increased by 2.2 fold in pools (19.14 ± 5.04 g m^{-2} to 41.95 ± 4.98 g m^{-2}) and increased by 2.3 fold in riffles (10.74 ± 7.32 g m^{-2} to 24.25 ± 2.14 g m^{-2}). Post-decline inorganic sediments increased 1.6 fold in pools, (47.54 ± 16.14 g m^{-2} to 76.35 ± 25.98 g m^{-2}), and 1.4 fold in riffles (38.78 ± 16.62 g m^{-2} to 54.25 ± 2.14 g m^{-2}).

In Rio Chorro, where tadpoles had been extirpated 8 years earlier, there were far less dramatic increases in chlorophyll *a* (+32% in pools, +68% in riffles; Fig. 1.3), AFDM (+22% in pools, +31% in riffles), and sediments (+17% in pools, +19% in riffles) between the same periods. Randomized intervention analysis (RIA) indicated significant increases in chlorophyll *a*, AFDM, and sediments in the *frog* stream, relative to the *frogless* stream, following tadpole extirpation.

Algal community composition: The pre-decline algal community within the *frog* stream shifted from one dominated by small adnate diatom taxa, to a post-decline community with a higher percentage of large upright taxa, and included more filamentous cyanobacteria. Of the 132 diatom species identified in the *frog* stream, the nine largest species were between 1 and 3 orders of magnitude larger in biovolume than the smaller 123 species. As a group, these species increased in mean abundance from 10.1% of total diatom abundance during the pre-decline period to a post-decline mean abundance of 20.6%. *Pinnularia butantanum* and *T. musica* accounted for the largest mean increase by biovolume, increasing 155% and 67%, respectively. Filamentous cyanobacteria cell density increased by 282%, from a pre-decline mean density of 214 cells mm^2 to a post-decline mean of 605 cells mm^2.

Reach-scale primary production: Reach-scale net primary production (NPP) increased from a pre-decline mean of -1587 mg DO m^{-2} d^{-1}, in the presence of grazing tadpoles, to a post-decline mean of -810 mg DO m^{-2} d^{-1}. Reach-scale gross primary production (GPP) increased from a pre-decline mean of 113 ± 12.5 mg DO m^{-2} d^{-1} to 238 ± 35.0 mg DO m^{-2} d^{-1} after tadpole extirpation. Community respiration dropped from a mean of 1700 ± 345.0 mg DO m^{-2} d^{-1} to 1048 ± 276.4 mg DO m^{-2} d^{-1}, and the ratio of

production to respiration (P/R) increased from 0.07 to 0.23 over this period. During this time, tadpole density declined from a mean of 10.16 individuals m^{-2} (March 2004) to 0.38 individuals m^{-2} (June 2005).

DISCUSSION

Our results link declining amphibian populations to alterations in stream primary producers over two spatial and temporal scales. Tadpoles reduced algal standing crop and sediments, and altered diatom community composition at both small experimental and larger reach-scales. However, our post-decline studies in the *frog* stream indicated that tadpole-mediated changes that we observed on a short-term, localized scale (i.e., electric exclusion experiments) underestimated changes evident at larger spatial and temporal scales when tadpoles were extirpated from the entire stream. Our data offer insight into expected stream ecosystem changes resulting from the extirpation of amphibians with stream-dwelling larvae.

How do tadpoles affect algal standing crop and community structure on a local scale?

Intact assemblages of grazing tadpoles can reduce algal standing crop and sediments as evidenced by significantly greater chlorophyll *a*, AFDM, and inorganic sediments in exclusion versus control treatments in the *frog* stream. Observed treatment differences in the *frog* stream were not apparent in the *frogless* stream, where tadpoles were absent. This supports our assertion that treatment differences in the *frog* stream were due to tadpole grazing and not an artifact of electrical exclusion.

Treatment differences in response variables were greatest in pools, where tadpoles were not only in greatest abundance, but also tended to be larger in size. Grazing tadpoles can decrease algal standing crop and organic matter accrual by direct ingestion of the benthos, although bioturbation may also alter algal assemblages and sediments (Kiffney and Richardson 2001). As grazing tadpoles move over the substratum, inorganic matter and detritus (e.g., decaying plant matter and fecal accumulations) are displaced in the water column and are subjected to a greater likelihood of being transported away from the grazed area. Effects of tadpole grazing on sediments were more evident in pools, as loose sediments prone to tadpole grazing would have been previously displaced by the higher current shear associated with riffle habitat. With or without tadpoles, amounts of chlorophyll *a* and organic and inorganic mass in both pools and riffles tended to plateau after day 35, likely the result of natural sloughing and regeneration of periphyton.

Indicator species analysis (ISA) of algal community structure suggests that the tadpoles were more efficient at ingesting the less abundant, but possibly easier-to-graze, larger diatom species, which resulted in a higher proportion of smaller diatom species in control versus exclusion treatments. These smaller taxa were almost always present in both treatments, displaying consistent relative *frequency* numbers (based on presence or absence); however, relative *abundances* of these taxa were higher in controls. It is this change in the relative abundance, rather than relative frequency, which is reflected in the ISA. The expectation of grazing-induced changes in the periphyton community is not likely to be reflected in presence/absence of taxa (that might be indicative of changing

nutrient levels, for example), but rather in shifts in abundance as larger taxa are more likely to be removed or displaced from the upper canopy.

While several studies have shown that tadpoles alter algal standing crop (e.g., Dickman 1968; Bronmark and others 1991), their effects on community structure have rarely been addressed (but see Kupferberg 1997; Ranvestel and others 2004). The importance of diatoms as a tadpole food source was shown by Kupferberg (1997), who found that tadpoles metamorphosed earlier, and at a higher weight, when allowed to selectively graze epiphytic diatoms and detritus from the less palatable filamentous green alga *Cladophora glomerata.* While tadpole ingestion may directly decrease overall mean algal size (i.e., biovolume), tadpoles can also locally displace larger, unattached taxa (e.g., *Terpsinöe* and *Amphipleura*) through bioturbation (Ranvestel and others 2004). It is unlikely that tadpoles are truly selective in their grazing, but they probably do have different consumption efficiencies for different algal growth forms (Steinman 1996). Digestibility of algae by tadpoles has been shown to differ among algal taxa. For example, Peterson and Boulton (1999) showed that some diatom taxa, such as the relatively large-celled *Synedra ulna*, are less prone to intact tadpole gut passage, suggesting that benthic algal community structure can be influenced by differential passage of smaller, viable diatoms. These intact diatoms could serve to recolonize the grazed substrata. Although biofilms are not necessarily stratified by growth form, tadpole feeding likely would remove the upper canopy, which is primarily composed of larger taxa with upright growth. This removal of upper canopy taxa may indirectly benefit smaller, more grazer-resistant algal species, as competition for space, light, and nutrients is reduced (McCormick and Stevenson 1989).

How does tadpole grazing affect primary production on local and reach scales?

On a localized scale, we found biomass-specific primary production to be greater in control treatments in our experiments. Hence, although tadpole grazing decreased levels of algal standing crop, the remaining algal community was more productive on a per-unit biomass (AFDM and chlorophyll *a*) basis. It is likely that increased rates of biomass-specific NPP were partly in response to the ingestion or displacement of accumulated sediment by tadpoles, which exposed underlying algal communities to nutrients and light. Not only were feeding trails through sediments often apparent on tiles, we also observed bioturbation of accrued matter from substrate surfaces when tadpoles moved rapidly. We found no difference in areal-specific NPP between treatments, indicating that increased algal biomass, resulting from tadpole exclusion, compensated for declines in biomass-specific NPP in tadpole exclosures.

Grazer density and size have both been linked to beneficial effects of sediment removal on primary production (Power 1990). In the *frog* stream, we found differences in production to be greatest in pools, where *R. warszewitschii* and *Hyla* spp. (the largest of the tadpoles) were most abundant. The less obvious differences in net primary production between control and exclusion treatments in riffles might be attributable to a combination of lower tadpole densities, smaller size of the dominant grazing tadpoles (i.e., *A. zeteki*), and high stream flow that constantly removed sediment.

Previous experiments designed to assess tadpole grazing effects on primary production have shown equivocal results. In an experimental manipulation of two tadpole species, Kupferberg (1997) found that both species reduced areal-specific net primary production. However, one species decreased biomass-specific net productivity,

while the other had no effect. Mechanisms proposed to explain increased biomass-specific net primary production by grazers include the dislodgement of senescent algae through grazing (Lamberti and other 1989), and the stimulation of primary production through the mineralization of nutrients from ingested diatoms and detritus (Seale 1980).

While net production on a per-biomass basis was lower on ungrazed than grazed tiles in the small-scale experiment, mean reach-scale NPP (measured on a *per-unit area* basis rather than on a *per-biomass* basis) increased dramatically from a pre-decline mean of -1587 mg DO m^{-2} d^{-1} to a post-decline mean of -810 mg DO m^{-2} d^{-1}. We found a much stronger effect of tadpole extirpation on whole-stream NPP than we found in the small-scale exclusion experiment. This result was not unexpected, given that the increase in overall stream productivity occurred simultaneously with a considerable increase in algal biomass associated with tadpole losses (Fig. 1.3). Accordingly, the P/R of the *frog* stream shifted toward a higher level of autotrophy as the tadpole decline proceeded (pre-decline P/R = 0.07; post-decline P/R = 0.23).

Do tadpoles account for inter-stream differences in algal standing crop and community structure between our two study streams?

Unlike the *frog* stream, the *frogless* stream lost its historical assemblage of tadpole grazers approximately 8 years prior to our exclusion experiment, and can be viewed as a long-term, whole-stream tadpole exclusion experiment. Most of the same riparian *frog* species found at the *frog* stream were once found at the *frogless* stream, and sufficient time has passed to allow multiple generations of other organisms, such as aquatic invertebrates, to respond to this change. Since aquatic invertebrates were potentially

released from competition for resources (e.g., food and space) when tadpoles were extirpated, it is reasonable to hypothesize that their populations might increase, off-setting initial increases in algal standing crop that we observed post-decline in the *frog* stream. Ecosystem-level responses in algal community structure and function resulting from tadpole declines, therefore, might be ameliorated over time. We found response variables in the *frogless* stream, measured from both the exclusion experiment and from monthly sampling, intermediate in comparison to the ranges of response variables from the *frog* stream. One hypothesis for this pattern is that tadpole losses contributed to long-term changes in the *frogless* stream's algal community, and while other functionally similar consumer communities (i.e., invertebrates) may have responded, they did not completely replace the role of tadpoles. We also observed an increase in algal biomass in the *frogless* stream during late 2004 (many years after tadpoles had been extirpated from the stream), although of lesser magnitude relative to the *frog* stream during the same time period. This reflects the fact that our study streams are not structured entirely by tadpole presence/absence, but rather by a number of complex biotic and abiotic interactions (sensu Mallory and Richardson 2005).

Does algal response to small-scale and short-term experimental tadpole exclusion reflect algal response to whole-stream tadpole extirpation?

Little is known about the ecological consequences of extinction because of difficulties associated with studying species loss in natural settings. Small-scale manipulations, such as removal experiments, can provide insight into the consequences of species loss. However, because the spatial and temporal scales of many experiments are small and

short in comparison to the size and duration of the impacts they attempt to predict, there are concerns about the usefulness of small-scale experiments in predicting whole-stream ecosystem change as a result of widespread species losses (e.g., Kohler and Wiley 1997). For example, Sarnelle (1997) showed short-term enclosure experiments underestimated results found over a longer-term due to long response times of indirect effects. Additionally, small-scale experimental perturbations may underestimate large-scale stream response because of the exchange (of diatoms, for instance) between treatment areas and the surrounding unmanipulated stream community. Ongoing and predictable extinctions of amphibian communities throughout Latin America provide a rare opportunity to compare the results of our experimental study to reach-scale effects of tadpole losses.

Our small-scale exclusion approach to assessing ecosystem alteration accurately predicted the direction of reach-scale responses resulting from the loss of grazing tadpoles in the *frog* stream; each of our three primary response variables (i.e., chlorophyll *a*, AFDM, and inorganic sediments) in control treatments showed significant decreases compared to tadpole-exclusion treatments. Our monthly sampling of natural stream substratum likewise showed that tadpoles reduced algal standing crop and inorganic sediments. However, subsequent to the tadpole decline, algal biomass increased similarly in both pools and riffles in the *frog* stream, despite the lower tadpole densities in riffles prior to extirpation. The extent to which algal biomass in riffles increased when tadpoles were extirpated from the stream was unexpected, given the tadpoles' relatively low pre-decline densities. It is plausible that actual tadpole densities were under-estimated using our riffle sampling techniques. Also, while a number of studies have shown that grazing

herbivores can exert strong control over algal communities (Steinman 1996), much less is known about the extent to which stream grazers can influence algal communities across an environmental gradient, such as stream velocity. Our results suggest that potentially higher energetic costs associated with grazing in riffle habitat, in combination with different tadpole communities in riffle versus pool habitat, resulted in riffle tadpoles exerting a higher *per capita* influence on algal biomass and sediments. Additionally, the increased post-decline algal biomass in pools could have contributed towards the recolonization of riffle algal colonies. Overall, differences in our response variables resulting from whole-stream amphibian extirpations were dramatically more pronounced than predicted by small-scale exclusion.

We also found an altered diatom community that differed significantly in species composition after tadpoles had been extirpated. The effect of localized tadpole exclusion on diatom community structure underestimated differences we observed when tadpoles were extirpated from the entire stream, possibly because available sources of diatoms for the recolonization of grazed areas were restricted by stream-wide tadpole grazing pressure outside of exclusion treatments. However, subsequent to the amphibian decline, diatom communities were released from tadpole grazing pressure, and larger diatom species were more likely to repopulate grazed areas of the stream.

Past experiments have drawn varying conclusions regarding the ability of small-scale experiments to predict the direction of change at larger scales (Peckarsky and others 1997), and few studies have examined whether small-scale experiments can accurately predict the magnitude of response to ecosystem-level alteration (but see Sarnelle 1997; Kohler and Wiley 1997; Greathouse and others 2006). Whole-stream responses to

extensive and permanent tadpole losses might be different than those changes detected with relatively short-term and small-scale manipulations for a number of reasons. A review of benthic grazers by Bronmark and others (1991) showed that periphyton removal rates increased with grazer biomass, so we might predict that algal standing crop would increase when abundant grazers (*i.e.,* tadpoles) are extirpated from an entire stream. However, other studies have shown that trade-offs may occur. For example, while grazing pressure by tadpoles (Osborne and McLachlan 1985) or invertebrates (Gresens 1995) can reduce algal standing crop, algae may benefit also from increased nutrient availability from grazer excretion, especially in nutrient-limited systems. We found that experimental tadpole exclusion predicted the direction of change in algal response that was evident over a reach-scale in the *frog* stream when tadpoles were extirpated; however, the magnitude of change in the algal periphyton community was significantly greater on a reach versus local-scale.

The ecological consequences of consumer losses, such as those resulting from catastrophic amphibian declines, can be difficult to predict, in part due to the rapid loss of many species, potential synergisms, system complexity, and emergent properties of ecosystems (e.g., Polis 1998; Michener and others 2001). Our results show strong linkages between the presence of tadpole grazers and certain stream ecosystem properties. Where abundant, Neotropical tadpoles significantly reduced algal standing crop and inorganic sediment accrual, and they also altered algal community composition. Tadpoles also have the potential to increase biomass-specific primary production but reduce overall reach-scale primary production. These influences on headwater streams are especially relevant in light of on-going, large-scale global declines and extinctions of

amphibian species (Stuart and others 2004). We found that ecosystem-level impacts of amphibian extinctions were more dramatic than results obtained from our small-scale, short-term exclusion experiments, which predicted the direction of change in response variables, but underestimated the magnitude of change. Nonetheless, our experimental results, combined with stream monitoring at the reach scale, indicate that tadpole losses have significant effects on stream ecosystem structure and function.

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Table 1.1. Mean diatom genera biovolume ($mm^3\ m^{-2}$ ± 1 SE) from experimental tiles collected from each stream on day 40 of the experiment. Genera totaling less than 2 $mm^3\ m^{-2}$ are grouped as "other".

	Frog stream (Rio Guabal)				*Frogless stream (Rio Chorro)*			
Taxa	Control Pools	Tadpole exclusion Pools	Control Riffles	Tadpole exclusion Riffles	Control Pools	Tadpole exclusion Pools	Control Riffles	Tadpole exclusion Riffles
Achnanthes	0.72 ± 0.51	22.73 ± 39.52	3.99 ± 4.12	1.66 ± 1.32	81.85 ± 135.36	41.15 ± 42.69	39.78 ± 34.91	19.97 ± 6.05
Achnanthidium	0.56 ± 0.22	1.07 ± 0.53	1.74 ± 2.72	2.64 ± 3.90	0.23 ± 0.17	0.09 ± 0.11	0.14 ± 0.08	0.05 ± 0.04
Amphipleura	2.65 ± 5.92	0.00 ± 0.00	4.36 ± 5.07	2.14 ± 3.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Caloneis	0.01 ± 0.02	0.51 ± 0.96	0.00 ± 0.00	0.00 ± 0.00	1.14 ± 0.90	1.63 ± 1.64	3.07 ± 3.74	1.37 ± 1.14
Cocconeis	3.23 ± 1.29	5.86 ± 5.15	2.16 ± 2.30	3.99 ± 2.85	4.81 ± 2.83	1.42 ± 1.70	1.04 ± 1.38	1.40 ± 1.13
Eunotia	59.47 ± 28.66	145.86 ± 182.65	39.10 ± 34.32	37.69 ± 22.57	17.69 ± 19.13	13.20 ± 11.06	34.82 ± 20.19	39.62 ± 23.59
Frustulia	0.25 ± 0.24	1.38 ± 1.69	0.55 ± 0.63	0.18 ± 0.41	2.16 ± 3.26	5.08 ± 5.79	3.19 ± 2.81	0.87 ± 1.33
Gyrosigma	0.48 ± 0.76	2.30 ± 1.82	0.37 ± 0.45	0.43 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Luticola	0.61 ± 0.62	1.74 ± 2.69	0.65 ± 1.05	2.25 ± 1.99	0.00 ± 0.00	0.14 ± 0.31	0.04 ± 0.08	0.01 ± 0.03
Navicula	6.23 ± 1.82	13.33 ± 5.07	5.14 ± 4.42	10.50 ± 10.81	25.97 ± 33.34	19.65 ± 22.30	7.21 ± 8.17	7.80 ± 7.66
Nupela	14.37 ± 8.30	44.73 ± 30.87	17.33 ± 15.30	39.72 ± 42.22	22.81 ± 12.36	15.86 ± 6.40	17.26 ± 9.22	20.24 ± 12.34
Orthoseira	0.99 ± 2.21	6.33 ± 8.75	2.77 ± 3.85	1.05 ± 1.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.47 ± 3.28
Pinnularia	0.41 ± 0.92	17.17 ± 20.80	3.85 ± 6.62	13.98 ± 19.25	17.09 ± 28.07	3.53 ± 4.05	5.78 ± 4.15	4.31 ± 2.87
Planothidium	0.78 ± 0.68	2.82 ± 2.78	1.20 ± 1.90	3.04 ± 1.99	17.06 ± 11.17	13.31 ± 11.14	9.55 ± 6.40	13.93 ± 7.93
Rhopalodia	2.56 ± 1.70	5.58 ± 4.58	1.64 ± 1.66	2.66 ± 2.74	0.93 ± 2.07	1.11 ± 1.77	0.00 ± 0.00	0.08 ± 0.17
Stenopterobia	0.00 ± 0.00	0.27 ± 0.61	0.00 ± 0.00	0.67 ± 1.51	5.53 ± 5.27	3.03 ± 5.89	0.10 ± 0.22	0.12 ± 0.26
Synedra	2.34 ± 2.49	4.44 ± 6.83	0.70 ± 0.99	3.74 ± 3.67	40.18 ± 72.99	64.95 ± 18.35	26.92 ± 38.18	53.06 ± 22.36
Terpsinoe	47.24 ± 87.09	16.64 ± 18.36	7.23 ± 13.80	29.14 ± 43.48	0.00 ± 0.00	0.00 ± 0.00	9.38 ± 20.98	0.00 ± 0.00
Other	2.44 ± 0.13	4.53 ± 0.18	1.94 ± 0.16	6.05 ± 0.34	3.68 ± 0.31	2.28 ± 0.13	2.14 ± 0.17	1.13 ± 0.09
Total	145.35	297.28	94.71	161.54	241.12	186.44	160.40	165.42

Figure Legends

Figure 1.1. Mean (± 1SE) (A) chlorophyll *a,* (B) AFDM, and (C) inorganic sediments accrued on tiles in pools and riffles in the *frog* stream (tadpoles present) and the *frogless* stream (tadpoles absent) over a 40 day period. Diamonds represent controls (tadpole access) and squares represent tadpole exclusion treatments.

Figure 1.2. Mean (± 1SE) net primary production, as measured by oxygen production, on a per-biomass basis from tiles collected on day 40 of the exclusion experiment. Light bars represent tiles from control treatments (tadpole access), dark bars represent experimental treatments (tadpoles excluded). Asterisks above bars indicate a significant difference between treatments ($p<0.05$).

Figure 1.3. Mean tadpole density (shaded bars) and chlorophyll *a* (black dots) (± 1SE) sampled monthly from pools and riffles of the *frog* stream (A) and the *frogless* stream (B) over 24 months. September 2004 begins adult amphibian decline at the *frog* stream, and transitional period of tadpole decline is shaded.

Figure 1.1.

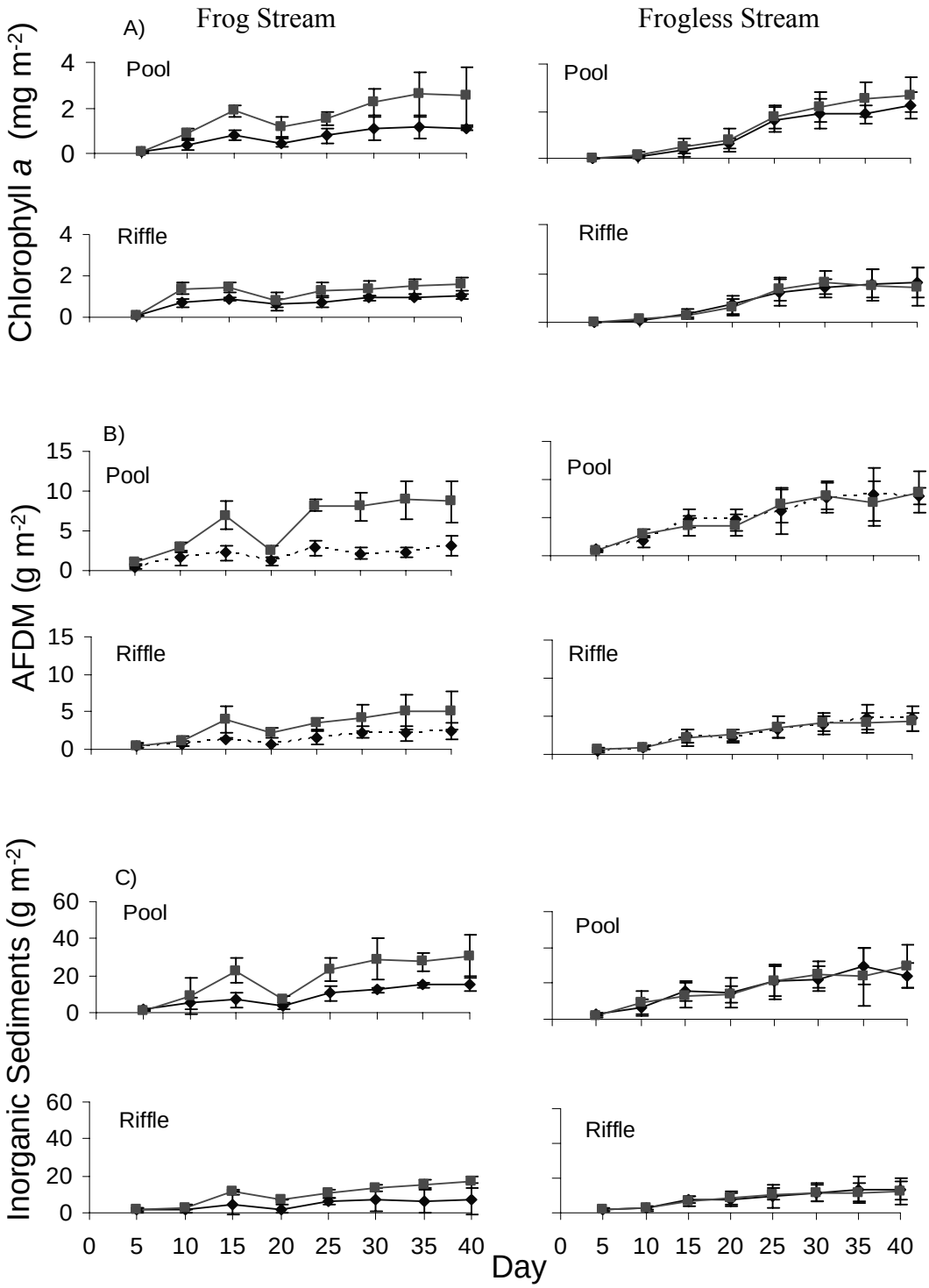

Frog Stream
Frogless Stream
A)
Pool
Riffle
Chlorophyll a (mg m-2)
B)
AFDM (g m-2)
C)
Inorganic Sediments (g m-2)
Day

Figure 1.2.

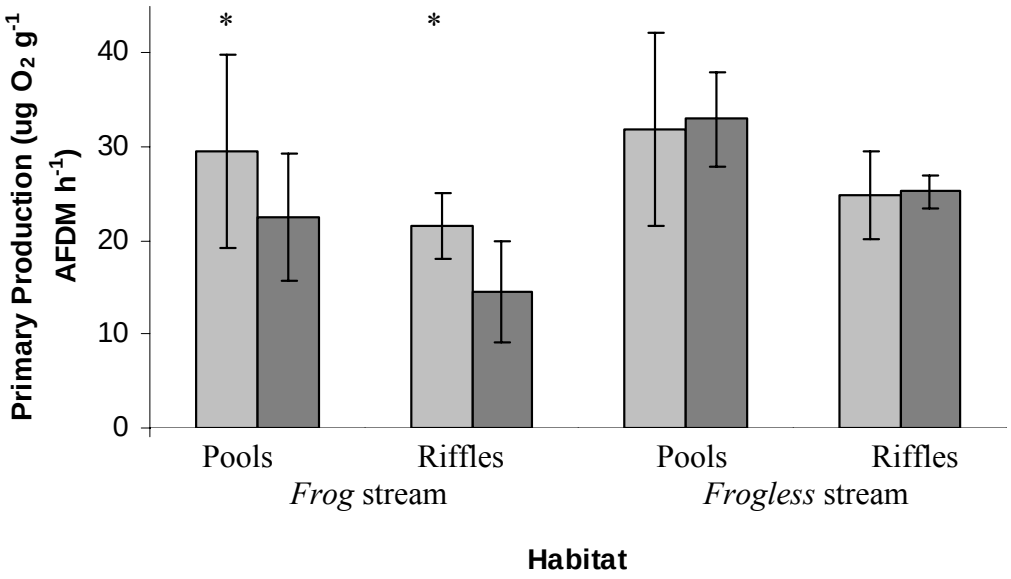

Primary Production (ug O2 g-1 AFDM h-1)
40
30
20
10
0
*
*
Pools
Riffles
Pools
Riffles
Frog stream
Frogless stream
Habitat

Figure 1.3

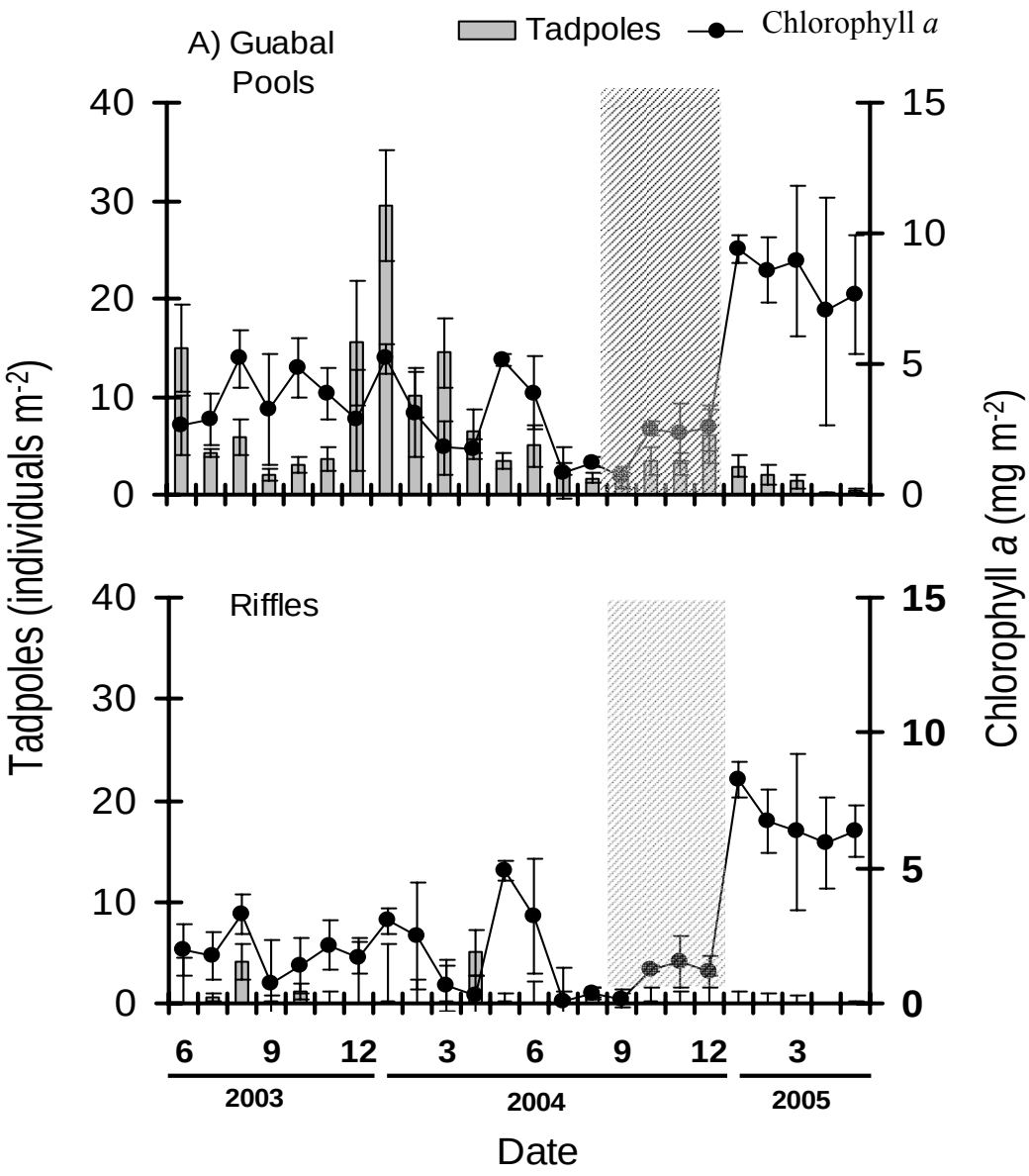
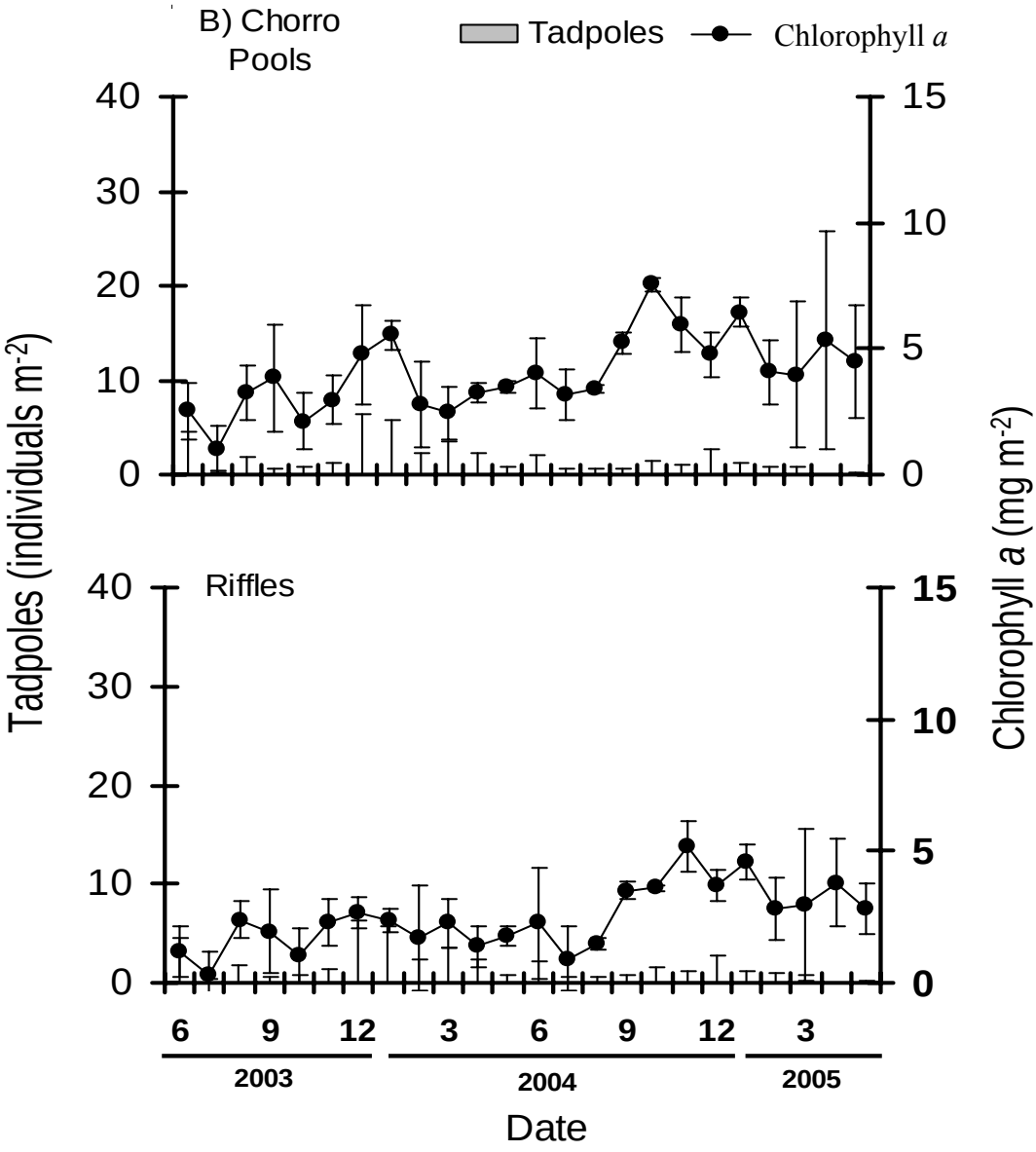

A) Guabal Pools
Tadpoles
Chlorophyll *a*
Riffles
Tadpoles (individuals m-2)
Chlorophyll a (mg m-2)
40
30
20
10
0
15
10
5
0
6
9
12
3
6
9
12
3
2003
2004
2005
Date

Figure 1.3 continued.



CHAPTER 3

INITIAL VERSUS LONG-TERM EFFECTS OF TADPOLE EXTIRPATIONS ON ALGAL RESOURCES AND NITROGEN CYCLING IN A NEOTROPICAL STREAM[2]

[2] Connelly, S.J., C.M. Pringle, M. Hunte-Brown, S Kilham, M.R. Whiles, K.R. Lips, and C.J. Colon-Gaud. To be submitted to Oecologia.

Abstract

Information about temporal patterns in ecological responses to species losses is integral to our understanding of the ultimate effects of declining biodiversity and ecosystem change. As part of the Tropical Amphibian Declines in Streams (TADS) project, we quantified changes in algal basal resources in upland Panamanian streams following larval anuran extirpations. Reach-scale monitoring during and after a catastrophic, disease-driven amphibian decline showed initial 2.8-fold increases in pools ($P < 0.05$) and 6.3-fold increases in riffles ($P < 0.05$) in algal standing crop in the 5 months following extirpation. Over the longer-term (3 years), the magnitude of this initial change dampened to increases of 1.9-fold in pools ($P < 0.05$) and 3.5-fold in riffles ($P < 0.05$) over pre-extirpation levels. Similarly, total organic content (AFDM) of benthic biolfilms increased significantly by 2.2 fold in pools ($P < 0.05$) and 2.3 fold in riffles ($P < 0.05$) in the initial 5-month post-extirpation period, with the magnitude of these changes dampening slightly to a 2-fold increase in pools and 1.9-fold increase in riffles (over pre-extirpation levels) over 3 years. There were initial significant increases ($P < 0.05$) in chl *a*:AFDM ratios 5 months post-extirpation, but ratios returned to pre-extirpation levels over 3 years. Algal food quality (as C:N) increased slightly, but not significantly, during the initial 5-month post-extirpation period and remained constant over 3-years. Mean $\delta^{15}N$ in biofilms (measured over the reach scale) was significantly ($P < 0.01$) depleted initially following tadpole extirpation and remained significantly depleted 3-years post-extirpation (3.55 ‰ pre- versus 2.69‰ post-), suggesting that the loss of tadpoles reduced N-availability for uptake by algae and other microbes in benthic biofilms. Observed increases in abundance and production of some grazing macroinvertebrate taxa

probably contributed to the gradual reduction in the difference between initial and longer-term post-decline algal biomass over the long-term. However, algal biomass was still 2-fold greater than pre-extirpation levels after 3 years, indicating that grazing macroinvertebrates did not completely compensate for the loss of tadpoles.

KEYWORDS

Amphibian declines, time scale, Panama, algal periphyton, stable isotopes

INTRODUCTION

Ecosystems across the planet are experiencing accelerating losses of biota, with largely unknown long-term consequences. In response to these losses, there has been increasing effort to better characterize and predict ecosystem-level consequences. However, predicting effects of species loss, and interpreting results of experiments that quantify effects of these losses, can be difficult (Chapin et al. 1992, Petchey et al. 2004). In part, this is a result of the inherent challenge in conducting research at ecologically relevant spatial and temporal scales. Ecosystems are dynamic, and responses to a perturbation, such as the loss of a dominant taxon, could become apparent across various levels of biological organization of remaining species, including physiological, behavioral, and population levels. The extent to which ecological experiments and observations, performed over relatively short temporal scales, are indicative of ecosystem change over more ecologically meaningful time scales is largely unknown (Kohler and Wiley 1997; Symstad et al. 2003). For logistical reasons, the results of short-term studies have often been generalized to longer temporal scales, even though the limitations of this approach (e.g., time-lags in responses of indirect effects) are recognized (Sarnelle 1997).

Evaluating potential ecosystem changes over appropriate timescales following extinction events is particularly relevant in light of ongoing amphibian declines. Amphibians can be a ubiquitous component of both terrestrial and aquatic ecosystems, and dramatic and apparently permanent declines in amphibian communities across the globe are increasingly well-documented (e.g., Stuart et al. 2004; Lips et al. 2006). Tadpoles are functionally dominant consumers in some Neotropical streams, and a number of short-term studies have shown that tadpole grazing can significantly increase primary productivity, reduce algal standing crop, and reduce the quality and quantity of fine particulate organic matter (e.g., Kupferberg 1997; Ranvestel et al. 2004; Connelly et al. 2008). However, few studies have examined the influence of tadpoles on stream ecosystem structure at more ecologically relevant spatial and temporal scales (i.e., stream reach scale over a period of years).

In a previous study (Connelly et al. 2008), we documented changes in algal biomass and composition at the reach-scale in an upland Panamanian stream over 2 years, inclusive of a 5-month period following a dramatic, disease-driven decline of amphibians. We found significant increases, relative to a "control" stream, in algal standing crop and biofilm AFDM in the 5 months following tadpole declines. During this period, the macroinvertebrate community composition also shifted. Shredder production declined, and some grazing mayfly taxa responded positively to the increased algal standing crop, suggesting the potential for functional redundancy between grazing tadpoles and grazing macroinvertebrates (Colon-Gaud 2008, Colon-Gaud et al. 2009). Here, we extend our post-extirpation sampling from 5 to 36 months. We also provide data on longer-term changes in biofilm quality (stoichiometry) and nitrogen cycling (as

indicated by stable isotopes in algae). This catastrophic event also provided the rare opportunity to document short- and longer-term stream ecosystem-level responses following a massive decline of a dominant grazer assemblage. To our knowledge, no studies have documented relatively "long-term" (i.e., up to 3 years) changes in basal food resources, or compared short-term trends (i.e., on the scale of months) to longer-term patterns, following the loss of an entire group of consumers in a freshwater system.

Our objective was to test the following hypotheses: (1) initial high levels of algal standing crop, observed 5-months post tadpole extirpation, will not be sustained over 3 years; (2) algal food quality (as measured by biofilm C:N ratios) will decrease post-extirpation in both the short- (5 months) and longer- (3 years) term; and, (3) nutrient cycling will be altered post-extirpation, as indicated by decreased fractionation of nitrogen stable isotopes in biofilm.

Materials and Methods

Study Sites

Rió Guabal (focal stream)

Our focal stream, Rió Guabal, is located in the Parque Nacional G. D. Omar Torrijos Herrera, El Copé, Coclé, Panama ($8^{o}40'$N, $80^{o}35'$W). Our study reach is part of a high-gradient stream characterized by distinct pool-run-riffle sequences, with substrates consisting of pebbles and gravel, with frequent cobbles, boulders, and depositional sandy areas. At the onset of our study, the stream drainage supported 74 species of amphibians,

40 of which were found in the riparian zone. Of these, 23 had stream-dwelling larvae (Lips et al. 2003). Tadpoles occurred in all stream habitats. Biofilm-grazing tadpoles of *Rana warszewitschii, Hyloscirtus palmeri*, and *H. colymba* were numerous and prominent, achieving densities of up to 50 m^{-2} in pools; *Atelopus varius* grazed biofilms in riffles, and tadpoles were the only vertebrate biofilm grazers in this stream at this elevation (Ranvestel et al. 2004).

Dead and dying frogs infected with the pathogenic fungus *Batrachochytrium dendrobatidis* were first observed at the focal stream during September 2004. Subsequent frog mortality was high through January 2005, resulting in greatly reduced riparian amphibian abundances (Lips et al. 2006). A dramatic reduction in tadpole density, compared with previously documented numbers, occurred concurrently. Between September 2004 and January 2005, overall tadpole densities declined from a mean of 8.2 tadpoles m^{-2} to 1.3 tadpoles m^{-2} ($P = 0.042$, RIA, Connelly et al. 2008). We characterize the periods of June 2003 – September 2004 as 'pre-extirpation', February 2005 – June 2005 as 'initial post-extirpation', and July 2005 – February 2008 as 'longer-term post-extirpation'. The transitional period during tadpole extirpation (October 2004 – January 2005) was not used in our analyses.

Quebrada Chorro (frogless reference stream)

We compared algal standing crops, stoichiometry, and natural abundance isotopic signatures in our focal stream to Quebrada Chorro (frogless stream), which has been essentially devoid of amphibians since 1996 due to a catastrophic extirpation event associated with the fungal pathogen *Batrachochytrium dendrobatidis* (Lips 1999). This

stream, approximately 200 km west of the focal stream, drains the Reserva Forestal Fortuna, Chiriquí, Panama (8°42'N, 82°14'W) and our past studies (Colon-Gaud et al. 2009, Connelly et al. 2008) have treated it as a reference stream in terms of order, discharge, geology, temperature, canopy cover, and nutrient concentrations. Amphibian populations have been monitored at the frogless stream since 1993 (Lips 1999; Lips unpublished data). The amphibian assemblage once included the biofilm-grazing tadpole species that were found at the focal stream at the onset of our study. Lips (1999) documented a mass mortality event and subsequent population decline of the amphibians at this stream in 1996. Stream tadpole densities in the frogless stream were documented as high as 50 m^{-2} in 1993 (Lips 1999); however, virtually none (average during our study < 0.01 tadpoles

m^{-2}) have been seen since 2000.

Data collection and analyses

To characterize algal standing crop, biofilm AFDM, $\delta^{13}C$, $\delta^{15}N$ and percent change of C, N, and P in biofilm over time, rocks from both streams were sampled monthly beginning June 2003. The frogless stream was sampled through June 2005 and sampling at the focal stream continued through February 2008, with the exception of a 12-month period between August 2006 and September 2007 when our project experienced a hiatus in funding. A benthic sampler, modified after Loeb (1981), was used to quantitatively sample biofilm from a known area of these natural substrata. Five sub-samples of benthic biofilm were collected from rock surfaces, during base flow, in each of five pools and riffles along a 200 m reach of both streams. The five sub-samples were pooled

resulting in 5 riffle and 5 pool samples on each sampling date (n = 10 samples per date per reach). Samples were placed in a cooler and transported to the laboratory where the homogenate was transferred to a beaker and diluted to a known volume. While stirring, three 40-100 ml subsamples were removed and filtered through Whatman glass fiber filters (0.7 μm). Filters were frozen and transported to either Drexel University (for isotope analysis preparation) or the University of Georgia. The first filter of each sample was dried at 60°C for 24 h, weighed to the nearest 0.0001 g, ashed at 500°C for 2 h, and reweighed to determine ash-free dry mass (AFDM) and inorganic sediments. The second filter was analyzed for chlorophyll *a* with a Turner Designs model 10-AU fluorometer (Turner Designs, Inc., Sunnyvale, CA, USA) using standard methods (APHA 1998). A subset of the third filters (n = 4 per month, from 2 pools and 2 riffles) were placed in a drying oven at 65°C for 24 h. The dried biofilm homogenate was processed either by scraping the material from the filter and weighing into 5x9 mm pressed tin capsules (when possible because of sufficient amount of dried material); or by removing 2 punches of 0.9 cm diameter from the filters and loading into 5x9 mm tin capsules (when material was of insufficient amount to be separated from the filter). Tin capsules were placed in 96-well plates, with duplicate samples included for every fifth sample. The samples were analyzed for carbon, nitrogen, and C and N stable isotope content at the Odum School of Ecology Analytical Chemistry Laboratory, University of Georgia, Athens, GA, using a Carlo Erba NA 1500 CHN analyzer coupled to a Finnigan Delta C mass spectrometer. Poplar and bovine standards were inserted after 12 samples. Isotope ratios are expressed as $\delta^{13}C$ ‰ or $\delta^{15}N$ ‰ according to the equation:

$$\delta^{13}C \text{ ‰ or } \delta^{15}N \text{ ‰} = [(R_{sample}/R_{standard}) - 1] \times 1000 \; \delta‰$$

where R_{sample} is the ^{13}C:^{12}C or ^{15}N:^{14}N ratio of the sample and $R_{standard}$ is the ^{13}C:^{12}C or ^{15}N:^{14}N ratio of the standard (PeeDee belemnite carbonate for $\delta^{13}C$ and atmospheric N for $\delta^{15}N$).

A portion of this third filter was also used to determine P-content of the biofilm for the months of February-June 2004 (pre-extirpation) and February-June 2005 (post-extirpation). Samples were weighed into acid-washed and pre-weighed ceramic crucibles, ashed at 500^{o}C for 4 h, acid digested, and analyzed spectrophototomically at the Analytical Chemistry Laboratory, University of Georgia (APHA 1998). Ground pine needles (US National Institute of Standards and Technology 1575a) were used as a standard, with a recovery rate of >95%. All C, N and P data are presented as either percent of dry mass or as molar ratios.

Monthly tadpole abundance surveys began in June 2003 and continued through June 2005. After June 2005, tadpole surveys were conducted quarterly. Tadpoles in three randomly chosen pools were quantified with a stove pipe benthic corer (22 cm diameter) that was modified with external rubber flaps at the base, which helped seal the bottom of the sampler when substrata were irregular. The core sampler was pushed approximately 3 cm into the substrata, and tadpoles were removed with a dip net (15 x 10 x 10 cm), counted, identified, and released. Kick net sampling was used to quantify tadpoles in three randomly chosen riffles, according to methods described by Heyer et al. (1994) and Hauer and Resh (1996).

Water samples were taken monthly (2 replicates/sampling date) from the focal stream between August 2003 and May 2006 and analyzed for NO_3-N, NH_4-N and SRP. Samples were filtered through 0.45 um Millipore filters, frozen, and transported to the

Analytical Chemistry Laboratory at the University of Georgia. SRP was measured spectrophotometrically using the Ascorbic Acid method (APHA 1998). Nitrate and NH_4-N were measured using the cadmium reduction and phenate methods respectively (APHA 1998).

Instream substrate composition was estimated quarterly. At the focal stream, substrata consisted of 21% silt, 9% sand, 20% gravel, 24% pebble, and 26% cobble. At the frogless stream, substrata consisted of 8% silt, 32% sand, 15% gravel, 22% pebble, and 23% cobble.

One-way ANOVA was used to compare biofilm $\delta^{13}C$, $\delta^{15}N$, %C, %N, %P, C:N, C:P, and N:P in riffles and pools within both sites as well as between each stream. Values were log-transformed where necessary to satisfy the assumption of normality of variances. To test for differences between pre- and post-extirpation monthly means of chlorophyll *a*, AFDM, Chl *a*:AFDM, and C:N in the focal stream, we constructed confidence intervals. Values with non-overlapping confidence intervals were considered significantly different at $\alpha = 0.05$.

Results

Pre- and post-extirpation comparisons of chlorophyll *a*, AFDM, and Chl a:AFDM in biofilms

Levels of chlorophyll *a* measured in pools over a 200 reach in the focal stream increased significantly ($P < 0.05$) by 2.8 fold from a pre-extirpation monthly mean of 3.00 ± 1.31 mg m^{-2} ($n = 16$) to 8.31 ± 0.63 mg m^{-2} ($n = 5$) during the initial post-extirpation period (through 5-months), followed by a significant ($P < 0.05$) decrease to 5.70 ± 1.63 mg m^{-2}

(n = 20) during the longer-term post-extirpation period (i.e., 3 years) (Fig. 2.1). Chlorophyll *a* in riffles followed a similar trend, increasing significantly ($P < 0.05$) by 6.3 fold from a pre-extirpation mean of 1.07 ± 1.27 mg m^{-2} (n = 16) to 6.74 ± 0.62 mg m^{-2} (n = 5) initial post-extirpation, but decreasing significantly ($P < 0.05$) to 3.74 ± 1.73 mg m^{-2} (n = 20) during the longer-term post-extirpation period (Fig.1). Biofilm AFDM in pools increased significantly ($P < 0.05$) by 2.2 fold from a pre-extirpation mean of 19.14 ± 5.04 g m^{-2} (n = 16) to 41.95 ± 4.98 g m^{-2} (n = 5) during the initial post-extirpation, followed by a significant ($P < 0.05$) decrease to 37.90 ± 2.27 g m^{-2} (n = 20) during the longer-term post-extirpation period. Biofilm AFDM in riffles increased significantly ($P = 0.05$) by 2.3 fold from a pre-extirpation mean of 10.74 ± 7.32 g m^{-2} (n = 16) to 24.25 ± 2.14 g m^{-2} (n = 5) initial post-extirpation, but decreased significantly ($P = 0.05$) to 20.84 ± 4.24 g m^{-2} (n = 20) during the longer-term post-extirpation period (Fig. 2.2).

The ratio of Chl *a*:AFDM of pool biofilm increased, though not significantly, from 0.16 ± 0.02 to 0.19 ± 0.01, but returned to pre-extirpation levels of 0.15 ± 0.01 over the longer-term post-extirpation period (Fig. 2.3). Chl *a*:AFDM of riffle biofilm increased significantly ($P = 0.05$) from 0.16 ± 0.01 to 0.28 ± 0.01 initially, but then decreased to near pre-extirpation levels of 0.18 ± 0.01 over the 3-year post-extirpation period (Fig. 2.3).

Changes in biofilm $\delta^{13}C$ and $\delta^{15}N$

The $\delta^{15}N$ biofilm signature in combined pool and riffle habitat sampled over a 200 m reach decreased significantly ($P < 0.01$) from 3.55 ± 1.10‰ pre-extirpation to 2.67 ±

0.51‰ and 2.69 ± 0.68‰ during initial (5 months) and longer-term (3 years) post-extirpation periods (Table 2.1). There were no significant changes in the $\delta^{13}C$ biofilm signature within the focal stream (pool and riffle habitat combined) during the periods following tadpole extirpation. Values remained nearly constant, at -29.46 ± 3.94‰ pre-extirpation (n = 16), -28.90 ± 0.52‰, initial post-extirpation (n = 5), and -30.51 ± 1.23‰ during the longer-term post-extirpation (n = 20; Table 2.1). There were no significant differences between pre- and initial post-extirpation in mean $\delta^{13}C$ and $\delta^{15}N$ values in the frogless stream (Table 2.1).

Effects of stream and habitat type on biofilm $\delta^{13}C$ and $\delta^{15}N$

The pre-extirpation biofilm $\delta^{15}N$ signature was significantly ($P < 0.01$) higher in the focal stream relative to the frogless stream for riffle and pool habitats combined (3.55 ± 1.10‰ vs. 1.25 ± 0.98‰; Table 2.1) over a 200 m reach. However, immediately following tadpole-extirpation, the $\delta^{15}N$ signature in the focal stream decreased to 2.67 ± 0.51‰, and the $\delta^{15}N$ signatures were not significantly different between streams. There were no significant differences in biofilm $\delta^{15}N$ signatures between riffles and pools in either the focal or frogless stream (Table 2.1).

The pre-extirpation $\delta^{13}C$ signatures in the focal stream were significantly more depleted than in the frogless stream (-29.53 ± 1.09‰ vs. -25.92 ± 3.01‰; $P < 0.0001$ in riffles and -29.34 ± 1.13‰ vs. -25.96 ± 1.17‰; $P < 0.001$ in pools). Initial post-extirpation $\delta^{13}C$ values were more depleted, although not significantly, in the focal stream relative to the frogless stream for both habitats combined (-28.90 ± 0.52‰ vs. -

25.90 ± 1.85‰). There were no significant differences in biofilm $\delta^{13}C$ signatures between riffles and pools in either the focal or frogless stream (Table 2.1).

Changes in C, N, and P in biofilms

There were no significant changes in biofilm %C, %N, or %P between pre- and post-extirpation periods. Percent C increased from 6.04 ± 2.73 to 8.27 ± 2.07 between the pre-extirpation and the initial post-extirpation period, although returned to near pre-extirpation levels of 6.78 ± 2.73 during the longer-term post-extirpation (Fig. 2.4). Percent N remained nearly constant between the pre- and initial post-extirpation periods, (0.60 ± 0.25 vs. 0.61 ± 0.11) and in the longer-term decreased slightly to 0.55 ± 0.22. Biofilm %P decreased from 0.28 ± 0.13 to 0.22 ± 0.14 between the pre-extirpation and the initial post-extirpation periods (Fig. 2.4).

There were no significant changes in biofilm C:N, C:P, or N:P ratios between periods. C:N initially increased slightly from 13.66 ± 2.35 to 15.98 ± 1.37 five months following tadpole extirpation, and remained at 15.07 ± 2.25 longer-term (3 years). C:P initially increased from 59.10 ± 43.78 to 121.42 ± 68.03 between the initial and post-extirpation periods. N:P increased from1.00 ± 0.74 to 1.61 ± 0.75 between pre- and initial post-extirpation (Fig. 2.4).

There were no significant differences in stream water nutrient concentrations between periods. Stream water NH_4-N increased from 5.79 ± 1.60 to 21.76 ± 4.41 ug/L, NO_3-N increased from 238.20 ± 29.05 to 250.60 ± 27.26 ug/L and SRP

decreased from 9.23 ± 2.56 to 7.69 ± 1.06 ug/L between pre- and post-extirpation periods (14 months pre-decline, 18 months post-decline; Fig. 2.5).

Discussion

As hypothesized, we did not observe sustained elevated algal standing crop over 3 years. The 3-fold increase in algal standing crop that we documented during the initial post-extirpation period (5 months) was reduced to an increase of 1.8 fold in the longer-term (3 years). We hypothesize that this pattern is related to increased abundance of some functionally similar grazing invertebrate taxa such as mayflies, which was observed in the focal stream over the 5 months immediately following tadpole extirpation (Colon-Gaud 2008). However, increases in grazing macroinvertebrates post-extirpation clearly did not replace the top-down effect of tadpoles in terms of overall effects on algal standing crop, which was still almost 2-fold greater than pre-extirpation levels 3 years later.

Predicting the long-term impacts of tadpole extinctions is challenging, partly because little is known about the degree to which other aquatic organisms, such as grazing insects, may eventually compensate for the loss of grazing tadpoles. In a study assessing differences in macroinvertebrate communities between our focal (pre- tadpole extirpation) and frogless "control" (tadpoles extirpated in 1996) streams, Colon-Gaud et al. (2009) found higher scraper production in the frogless stream and higher shredder production in the focal stream. In the initial period following tadpole extirpation in the focal stream, there was a shift in the functional structure of the invertebrate community; shredder production declined and there was a concomitant increase in some grazer

species (Colon-Gaud 2008). The grazer community shifted away from smaller-bodied grazers, such as *Psephenus* (Psephenidae) and towards larger-bodied taxa, such as *Petrophila* (Pyralidae) (Colon-Gaud 2008).

Our previous small-scale tadpole-exclusion experiment, and algal monitoring 5 months post-extirpation, in the focal stream showed a shift in the algal community towards larger-sized diatom cells, with more upright growth forms, in response to absence of tadpoles. These changes in mean diatom size and growth form of the algal community in the absence of tadpoles could be responsible for differences in the macroinvertebrate communities between streams, and the post-extirpation increase in macroinvertebrate size in the focal stream. In contrast to grazing tadpoles, smaller macroinvertebrate grazers may be gape-limited, and unable to ingest the larger, more upright diatom taxa (e.g., *Amphipleura* and *Terpsinoe*), which increased in abundance post-extirpation (Connelly et al. 2008). Previous work in our study stream indicates that algal periphyton was a limiting resource, particularly when tadpoles were present, potentially resulting in a more generalist feeding strategy by invertebrate scrapers (Colon-Gaud et al. 2009). Initial increases in algal standing crop, during the months immediately following tadpole extirpation, could have resulted in more selective feeding on algae by macroinvertebrates, as algae previously consumed by tadpoles became available to invertebrate grazers.

Biologically relevant time scales must be considered when assessing the potential for functional compensation by grazing macroinvertebrates following tadpole declines. The speed at which these remaining functionally similar groups respond may be driving some of the differences in responses of algal basal resources that we found between the

initial and longer-term post decline periods. The importance of allowing for delayed compensation and using appropriate time scales for assessing effects of the loss of a keystone species was demonstrated by the experimental removal of kangaroo rats from a desert ecosystem; for 20 years remaining local species were unable to fully utilize the newly available resources, at which point an alien species of mouse colonized and compensated almost entirely for the removed species (Ernest and Brown 2001). The positive response of grazing macroinvertebrate communities in our focal stream may have become more pronounced in the longer-term (relative to their initial, 5 month response) due to reproductive delays. Developmental times for invertebrates in neotropical streams like our study sites vary from as fast as 17 days for some Chironomidae to several months for many Trichoptera (Jackson & Sweeney 1995, Colon-Gaud 2008). The longer-term amelioration of initial dramatic increases in biofilm in our focal stream could reflect the time necessary for shifts in abundance and community structure of grazing macroinvertebrates over multiple generations in response to a newly available food resource.

We found a more dramatic increase in chlorophyll *a* in riffles (6.3 fold) than we did in pools (2.8 fold) during the initial post-extirpation (5 month) period. However, the difference between the relative increases was ameliorated in the longer-term (over 3 years), to a 3.5 fold increase in riffles and a 1.9 fold increase in pools relative to pre-extirpation values. This decrease in differences may be explained by the habitat preferences of some grazing invertebrates in these streams. Colon-Gaud (2008) found significant increases in initial post-extirpation (5 month) production and abundance in the mayfly families Baetidae and Leptophebiidae, which were much more abundant in riffle

versus pool habitats. A stronger response to the increase in algal food by grazing mayflies in riffle habitats, relative to the weaker response of macroinvertebrate grazers in pool habitats, may be responsible for ameliorating the difference in chlorophyll *a* increases between habitat types over the longer-term.

How does tadpole loss influence biofilm food quality and stream nutrients?

The slight decrease in biofilm quality (i.e., increased C:nutrients; Fig. 2.4) that we documented following tadpole extirpation may be due to direct effects of the loss of the dominant tadpole grazers. One possibility is that the thickness of epilithic algal mats increased following tadpole losses, which could increase nutrient diffusion limitation (sensu Bothwell 1988), thereby limiting nutrients availability to underlying biofilm and decreasing overall nutritional quality of the biofilm. Also, Connelly et al. (2008) showed that tadpole grazing not only significantly reduced the amount of algal standing crop, but also altered algal species community composition and physiognomy. The change in biofilm quality could be due to the higher percentage of larger algal cells (i.e., lower surface to area ratio than smaller cells) that was found following tadpole extirpation.

The increase in Chl *a*:AFDM that we initially observed after 5 months post-extirpation returned to pre-extirpation levels after 3 years. Although biofilm is often assumed to be largely comprised of algae (Lock 1993), it is a complex mix of organic matter of which algae is one component (Frost et al. 2005). The initial increase in proportion of algae, relative to other organic matter, in the biofilm was likely a reflection of the dramatic increase in algal standing crop resulting from the loss of tadpole grazing and the lack of immediate functional response by macroinvertebrate grazers. However,

the proportion of other organic matter, relative to algae, increased in the biofilm over the longer-term (3 years). This relative increase in accrual of non-algal organic matter may be an indication of the loss of sediment bioturbation by tadpoles; prior to extirpation, tadpoles in this same stream played an important role in recycling depositional particulate matter into the water column (Ranvestel et al. 2004, Whiles et al. 2006, Connelly et al. 2008). The longer-term increased accumulation of sediments likely offset potential increases of algal growth because of light and nutrient blocking. Additionally, the slight increases in C:N and C:P ratios that we found initially following tadpole extirpation could be due in part to the decrease in relative amounts of detritus in biofilm. Algae-fed tadpoles have been shown to decrease the C:N ratio of associated detrital particles, presumably through the regeneration of nutrients in the water, which stimulate conditioning activity of leaf microbes (Iwai and Kagaya 2007).

Because of differences in physiology between anuran larvae and macroinvertebrates, it is possible that the subtle changes that we found in biofilm stoichiometry are attributable to not only the direct effects of the missing tadpole community, but also to the subsequent shift in community composition, and body stoichiometry, of the remaining aquatic insects. It is known that not only top-down (e.g., grazing) but also bottom-up (e.g., nutrient availability) factors influence primary producer communities. Ecological stoichiometry provides a framework for understanding how organisms differ in their nutrient recycling roles. Because elemental composition differs among taxa of organisms, different groups of organisms, e.g., tadpoles and macroinvertebrates, are predicted to recycle nutrients at dissimilar rates (sensu Evans-White and Lamberti 2006). The loss of grazing tadpoles, followed by the

initial shift in the community structure of grazing invertebrates (Colon-Gaud 2008), could result in altered nutrient availability to the biofilm if the organisms in the new grazing community differed (relative to the tadpoles) in their body and excretion stoichiometry (sensu Sterner et al. 2002).

Stream nutrient levels are similar pre- vs. post-extirpation and appear to vary with discharge. Wide monthly fluctuations in ambient nutrient levels are likely driven by seasonal stream flow, leading to the greater dilution and transport of NO_3, NH_4, and SRP, particularly during the wet season (September through January) when mean discharge tends to be highest.

How does tadpole extirpation alter nutrient cycling, as indicated by changes in biofilm $\delta^{13}C$ and $\delta^{15}N$?

Findings strongly support our hypothesis that tadpoles played a key role as nitrogen recyclers in the focal stream. Mean monthly $\delta^{15}N$ in biofilm decreased by 0.88‰ between the pre-extirpation and initial post-extirpation period, and remained relatively depleted over 3 years. Stable isotopes of nitrogen are useful tools for identifying linkages within food webs and providing information about trophic nutrient transfers (Peterson and Fry, 1987; O'Reilly et al. 2002; Woodward and Hildrew, 2002), with comparatively higher $\delta^{15}N$ signals indicative of nitrogen that has been biologically processed.

We hypothesize that tadpole-processed nitrogen is made available to algae and other microbes through egestion. One line of evidence supporting this hypothesis is that the rate of tadpole egestion in our focal stream during the dry season was considerable, at approximately 10 mg $m^{-2}hr^{-1}$ AFDM, and organic seston, partially comprised of tadpole

feces, transported in the focal stream has been shown to be significantly higher in N than in the frogless stream (Whiles et al. 2006, Colon-Gaud et al. 2008). Biofilm-grazing tadpoles (*Rana warszewitschii*) in a Panamanian stream were also found to have feces that is N-depleted relative to the tadpoles (5.01 and 5.50, respectively; Piet Verburg, unpublished). Therefore, as tadpole feces enter the stream, nitrogen (depleted in ^{15}N relative to tadpoles, but enriched relative to biofilm) becomes biologically available to algae and other microbes. Our findings suggest that microbial uptake of this tadpole-processed enriched nitrogen is reflected in the enriched $\delta^{15}N$ biofilm value we found pre-extirpation.

In addition to egestion, tadpole excretion may also contribute significant amounts of biologically processed nitrogen. Whiles et al. (2006) quantified size-dependant tadpole ammonium excretion rates in the focal stream, and found that excretion by tadpoles provided up to 7% of stream ammonium for uptake by biofilm. The decreased levels of %N in biofilm, which we documented longer-term post-extirpation (Fig. 2.4), also suggest that tadpole excretion was important in pre-extirpation stream nutrient dynamics

Further supporting our hypothesis that tadpoles play a key role in nitrogen recycling are the significantly different isotopic N-signals observed *between* study streams, with the $\delta^{15}N$ signal of the biofilm higher in the pre-extirpation focal stream than in the frogless stream. Stream substrate composition in our focal stream consisted of more silt relative to the frogless stream (21% vs. 8%). We hypothesize that tadpole feces and excretion in our focal stream could be responsible for the different isotopic N-signals between streams, with the lower $\delta^{15}N$ biofilm signal in the frogless stream reflecting the

lack of N-processing by tadpoles. The more enriched N in biofilm, both in the focal stream pre- versus post-extirpation and in our focal stream relative to the frogless stream, suggests that the source of N available for uptake by biofilm changed once tadpoles were lost from the system.

Changes in the Chl *a*:AFDM ratios among the pre-extirpation, initial post-extirpation, and longer-term post-extirpation periods may also be driving the response in ^{15}N values in our focal stream, as there is likely a difference in the N-signature between the algal and detrital components of the biofilm (sensu Frost et al. 2002). We suggest that the aforementioned lines of evidence, resulting from the loss of the tadpole community, may be driving the depleted values of $\delta^{15}N$ we found following tadpole extirpation.

The biofilm $\delta^{13}C$ signal in our focal stream was significantly more depleted than in the frogless stream and this may result from differences between biofilm composition (i.e., proportion of algae, other microbes, and detritus comprising the biofilm) in the streams. The fecal component of the biofilm contributed by tadpoles could result in high rates of CO_2 production because of feces-associated bacterial and fungal decomposer communities. This carbon will be depleted in heavier isotopes, and reflected in the more negative $\delta^{13}C$ signal of the biofilm.

We found no significant differences between the $\delta^{13}C$ and $\delta^{15}N$ signatures of the biofilm in riffle or pool habitat in either stream. A number of studies have shown that abiotic factors, such as water velocity, can affect biofilm $\delta^{13}C$ (Finlay 2004; Finlay et al. 1999; Trudeau and Rasmussen 2003). However, similar to our findings, studies by France and Cattaneo (1998) and MacLeod and Barton (1998) have shown that $\delta^{13}C$

signatures did not change inversely with current velocity. These inconsistent findings could be related to different rates of photosynthesis and respiration taking place in the different habitats. In habitats that are relatively high in respiration (e.g., where there are high levels of decomposition), the CO_2 supply rate is increased, and the favored lighter $^{12}CO_2$ is available for uptake by biofilm. Therefore, decomposition in detrital pool habitats counters the effect of current velocity in riffles (which mixes lighter atmospheric C into the water column), with the end result being no appreciable difference between isotopic signals in riffles versus pools.

In conclusion, despite widespread declines of biodiversity, little is known about how ecosystems will respond to biotic losses over ecologically meaningful time scales. This study is the first to characterize relatively "long-term" changes in basal food resources, and to compare initial trends in changes of basal resources to longer-term patterns, following the loss of an entire larval amphibian community. Our 5-year study, which documented changes occurring in a natural field setting, indicates that the loss of tadpole grazers significantly influenced algal basal resources; however, the magnitude of their effects changed through time, and 3 years following tadpole losses macroinvertebrate grazers have not fully replaced the role of grazing tadpoles. Given that there is no indication of amphibian communities recovering at sites where they have previously been decimated (Lips 1999), our continuing study will allow us to more fully assess the eventual consequences of tadpole extirpation on algal basal resources.

Acknowledgements

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Table 2.1 . Carbon and nitogen isotope composition of biofilms in the focal and frogless streams (mean ± SD) . Values were collected monthly from pool (n=2) and riffle (n=2) habitats over 45 months (focal stream) and 24 months (frogless stream). Units are per mil (‰)

Pre-Extirpation		Initial Post-Extirpation		Long-term Post-Extirpation	
Focal Stream	Frogless Stream	Focal Stream	Frogless Stream	Focal Stream	Frogless Stream
$\delta^{13}C$					
Pool Habitats					
-29.34 (± 1.13)	-25.96 (± 1.17)	-28.84 (± 0.57)	-27.90 (± 1.16)	-30.32 (± 1.30)	n/a
Riffle Habitats					
-29.53 (± 1.09)	-25.92 (± 3.01)	-29.00 (± 0.39)	-25.05 (± 2.29)	-30.62 (± 1.16)	n/a
Combined Pools and Riffles					
-29.46 (± 3.94)	-25.94 (± 2.25)	-28.90 (± 0.52)	-25.90 (± 1.85)	-30.51 (± 1.23)	n/a
$\delta^{15}N$					
Pool Habitats					
4.34 (± 1.14)	1.31 (± 0.86)	3.12 (± 0.68)	1.65 (± 0.87)	3.24 (± 0.77)	n/a
Riffle Habitats					
3.02 (± 0.55)	1.22 (± 1.06)	2.48 (± 0.21)	1.08 (± 0.53)	2.49 (± 0.71)	n/a
Combined Pools and Riffles					
3.55 (± 1.10)	1.25 (± 0.98)	2.67 (± 0.51)	1.25 (± 1.01)	2.69 (± 0.68)	n/a

Figure Legends

Figure 2.1. Mean tadpole density (shaded bars) and chlorophyll *a* (black dots) (± 1SE) sampled monthly from: (A) pools; and (B) riffles of the focal stream over 45 months. Adult amphibian decline began in September 2004. The transitional period of tadpole extirpation is shaded. PE indicates the pre-extirpation period, TR indicates the transitional period, IPE indicates the initial post-extirpation period, and LTPE indicates the longer-term post-extirpation period.

Figure 2.2. Mean tadpole density (shaded bars) and AFDM (black dots) (± 1SE) sampled monthly from: (A) pools; and (B) riffles of the focal stream over 45 months. Adult amphibian decline began in September 2004. The transitional period of tadpole extirpation is shaded. PE indicates the pre-extirpation period, TR indicates the transitional period, IPE indicates the initial post-extirpation period, and LTPE indicates the longer-term post-extirpation period.

Figure 2.3. Mean tadpole density (shaded bars) and Chl *a*:AFDM (black dots) sampled monthly from: (A) pools; and (B) riffles of the focal stream over 45 months. Adult amphibian decline began in September 2004. The transitional period of tadpole extirpation is shaded. PE indicates the pre-extirpation period, TR indicates the transitional period, IPE indicates the initial post-extirpation period, and LTPE indicates the longer-term post-extirpation period.

Figure 2.4. Mean concentrations of %C, %N, and %P, and ratios of C:N, N:P and C:P from biofilm in focal and frogless streams. All values are ± 1SE. Values of C and N were collected over 45 months (n = 4; combined from 2 pools and 2 riffles) in the focal stream (shaded bars), and 24 months in the frogless stream (solid bars). P was sampled 5 months during 2004 (pre-extirpation) and 5 months in 2005 (initial post-extirpation).

Figure 2.5. Mean concentrations of (A) nitrate (NO_3-N), (B) ammonium (NH_4-N), and (C) SRP (dark line) and mean monthly stream discharge (dotted line) in focal stream. Water nutrient samples were collected over 32 months (n = 2). The shaded bar indicates the frog decline at our focal stream.

Figure 2.1

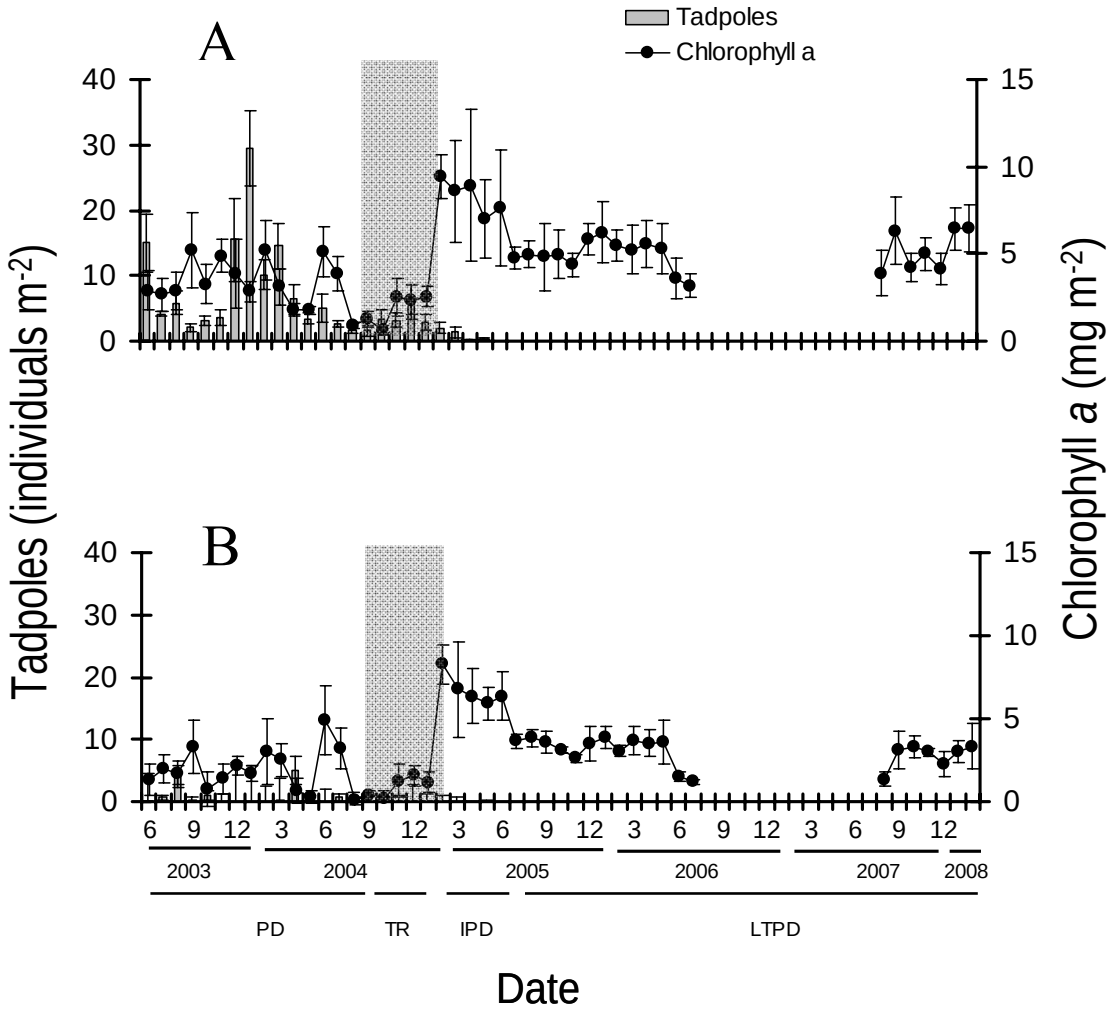

Tadpoles
Chlorophyll a
A
B
Tadpoles (individuals m-2)
Chlorophyll a (mg m-2)
40
30
20
10
0
15
10
5
0
6 9 12 3 6 9 12 3 6 9 12 3 6 9 12 3 6 9 12
2003
2004
2005
2006
2007
2008
PD
TR
IPD
LTPD
Date

Figure 2.2

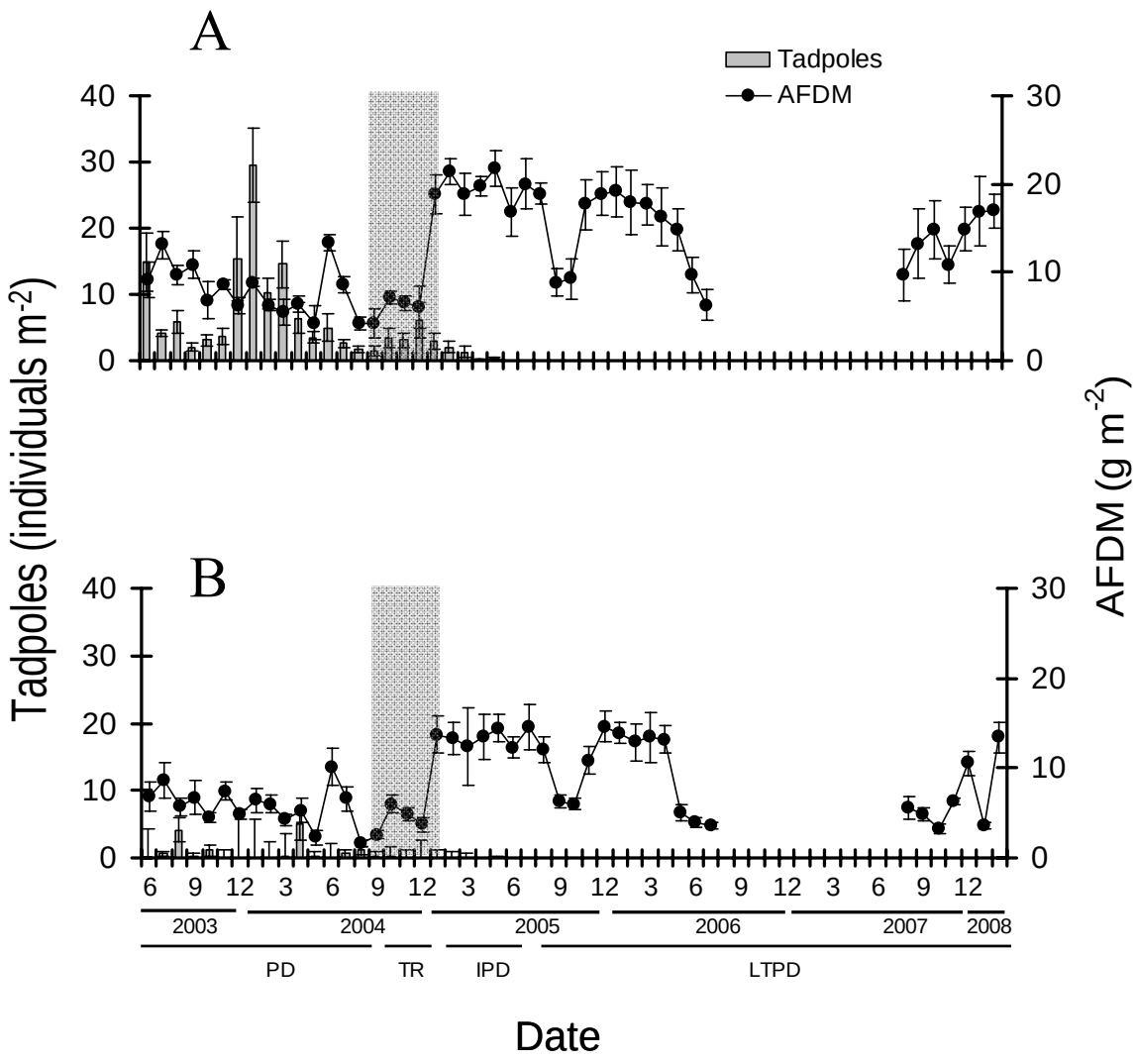

A
B
Tadpoles
AFDM
Tadpoles (individuals m-2)
AFDM (g m-2)
0
10
20
30
40
6
9
12
3
2003
2004
2005
2006
2007
2008
PD
TR
IPD
LTPD
Date

Figure 2.3.

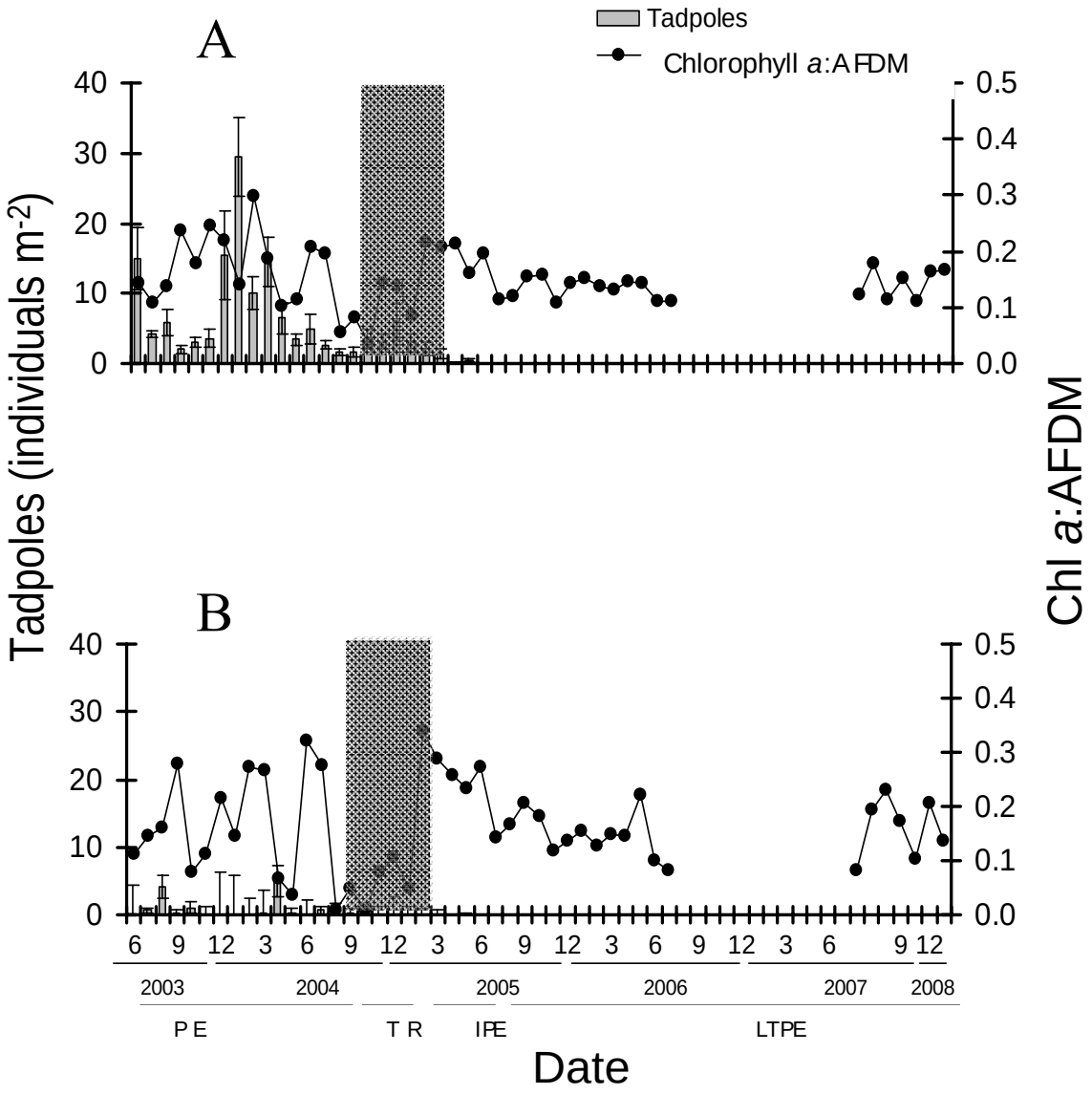

A
B
Tadpoles
Chlorophyll a:AFDM
Tadpoles (individuals m-2)
Chl a:AFDM
40
30
20
10
0
0.5
0.4
0.3
0.2
0.1
0.0
6 9 12 3 6 9 12 3 6 9 12 3 6 9 12 3 6 9 12
2003
2004
2005
2006
2007
2008
PE
TR
IPE
LTPE
Date

Figure 2.4.

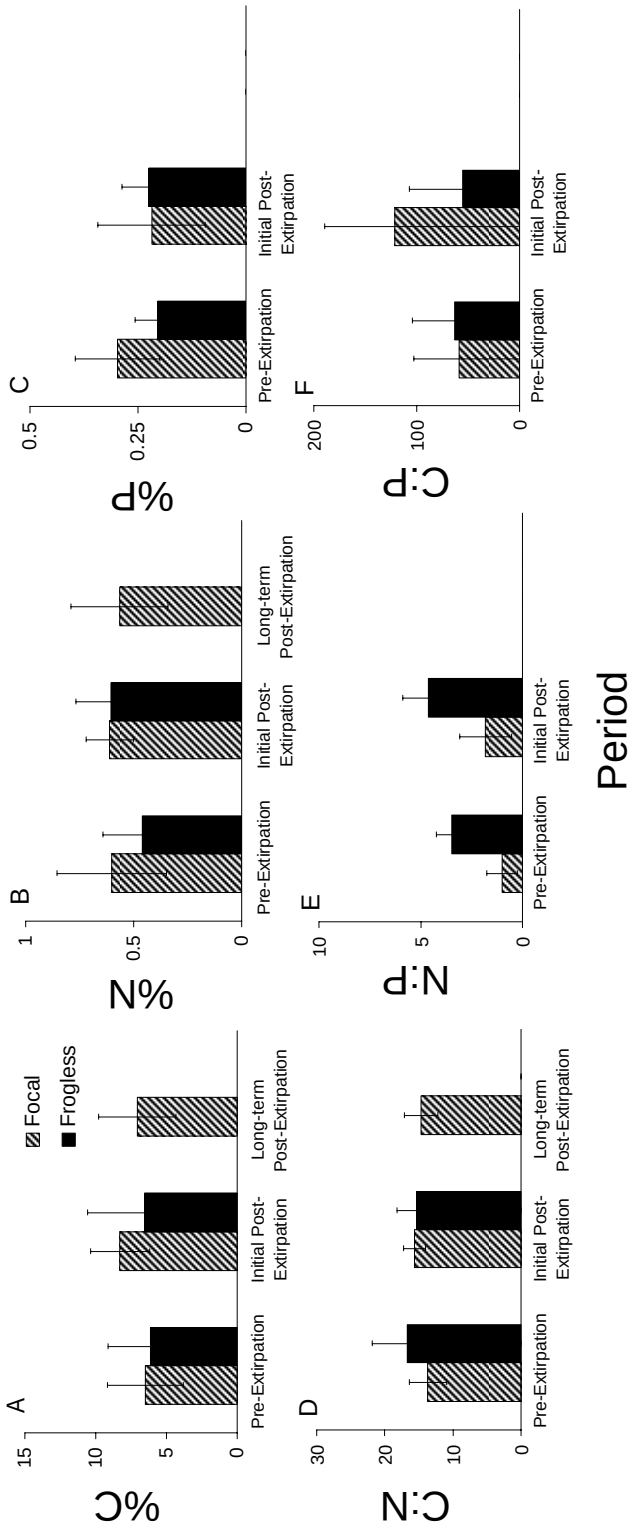
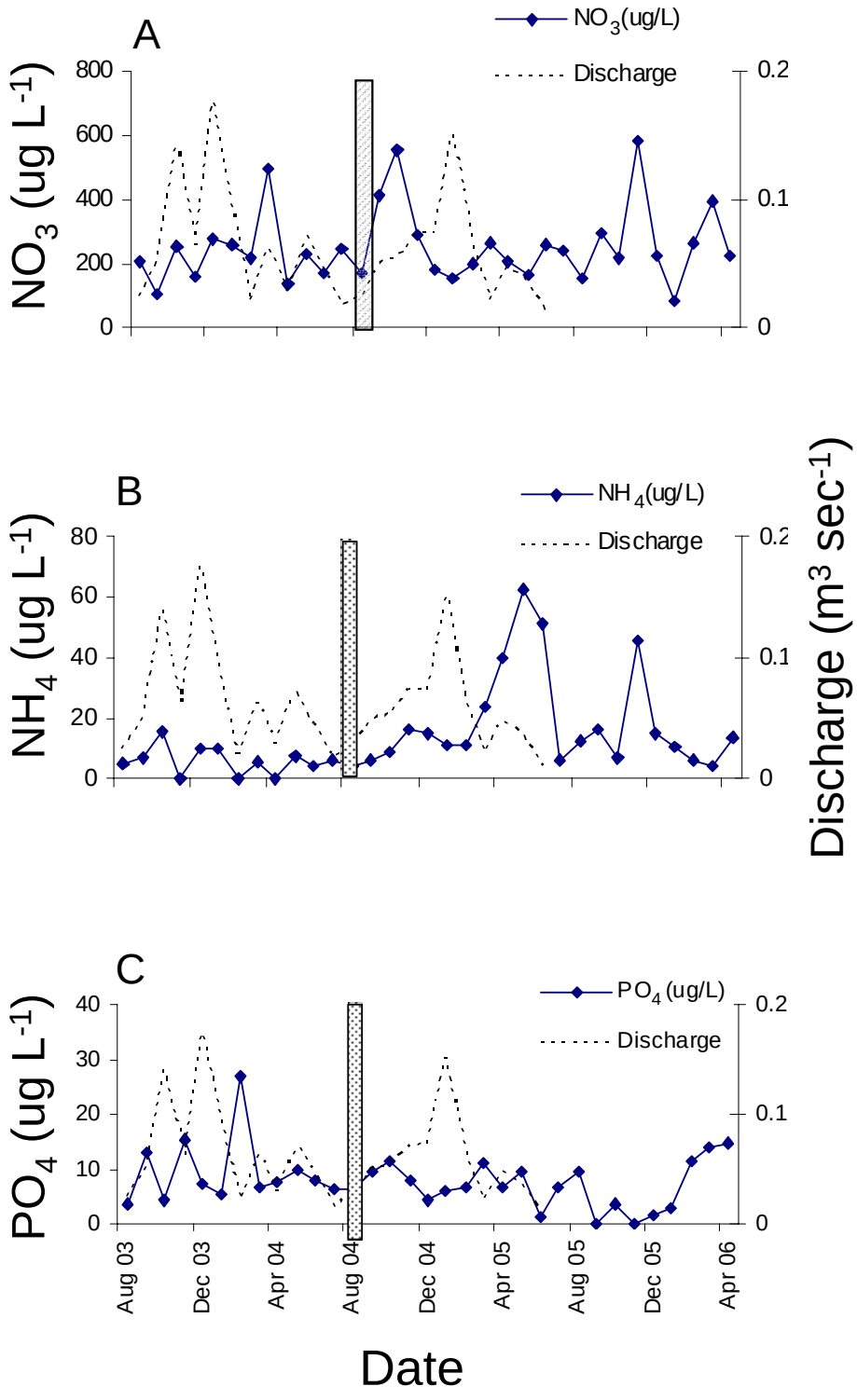

A
B
C
D
E
F
%C
%N
%P
C:N
N:P
C:P
Focal
Frogless
Pre-Extirpation
Initial Post-Extirpation
Long-term Post-Extirpation
Period

Figure 2.5.


A
NO_3(ug/L)
Discharge
NO_3 (ug L^{-1})
800
600
400
200
0
0.2
0.1
0
B
NH_4(ug/L)
Discharge
NH_4 (ug L^{-1})
80
60
40
20
0
0.2
0.1
0
Discharge (m^3 sec^{-1})
C
PO_4(ug/L)
Discharge
PO_4 (ug L^{-1})
40
30
20
10
0
0.2
0.1
0
Aug 03
Dec 03
Apr 04
Aug 04
Dec 04
Apr 05
Aug 05
Dec 05
Apr 06
Date

CHAPTER 4

EXPERIMENTAL COMPARISON OF LEAF LITTER DECOMPOSITION DYNAMICS IN TWO STREAMS- WITH AND WITHOUT TADPOLES: EVIDENCE FOR LOCALIZED EFFECTS OF GLASS FROG (CENTROLENIDAE) TADPOLES[3]

[3] Connelly, S.J., C.M. Pringle, M.R. Whiles, K.R. Lips, S. Kilham and R. Brenes. To be submitted to Freshwater Biology.

SUMMARY

1. Of the relatively few studies that have examined the consequences of amphibian declines on stream systems, virtually all have focused on changes in algal-based food webs or algal communities that resulted from the decline of algal-grazing tadpoles. As part of the Tropical Amphibian Declines in Streams (TADS) project, we compared leaf litter decomposition dynamics between two Neotropical streams in Panama: a stream with an intact community of tadpoles *(with frogs)* and one where tadpoles were extirpated *(frogless)*. Our approach involved experimental exclusion of glass frog (Centrolenidae) tadpoles to assess their effects.

2. The *frog* stream contained a complete assemblage of larval native anurans (23 species) and we identified five species of glass frog (Centrolenidae) larvae that were commonly associated with leaf detritus and associated sediments in pools. Our study provides some of the first published information on densities of Centrolenid tadpoles (0-318 tadpoles m^{-2}) and biomass-specific excretion rates (0.063 + 0.018 ug N mg $tadpole^{-1}$ h^{-1}).

3. We experimentally excluded tadpoles from single-species leaf packs incubated over a 40-day period in both the *frog* and f*rogless* study streams. *Centrolene prosoblepon* and *Cochranella albomaculata* were the most common taxa colonizing leaf packs in control treatments and they were very patchily distributed (0.0 – 33.3 m^{-2}).

4. In control treatments occupied by tadpoles, we found significantly higher mean levels of fungal biomass than in tadpole exclusion treatments ($P<0.10$; 56.9 vs 42.7 mg C g^{-1}

AFDM). There were no significant differences in leaf mass loss or temperature-corrected leaf decomposition rates between control treatments in the stream *with frogs* versus the *frogless* stream or between control and tadpole exclusion treatments within each stream. Likewise, there were no significant differences in leaf pack microbial respiration rates or macroinvertebrate abundance between treatments or streams. Invertebrate community composition on leaf packs was similar between treatments and streams and fauna was dominated by larval Chironomidae, Simuliidae (Diptera), and *Anchytarsus sp.* (Coleoptera).

5. Significantly higher fungal biomass in leaf packs containing tadpoles in control treatments suggests that Centrolenid tadpoles play a role in stimulating growth of stream fungal communities. We suggest that this is due to fungal uptake of nutrients excreted by tadpoles.

6. The stream *with frogs* did not exhibit higher decomposition rates than the *frogless* stream. Effects of Centrolenid tadpoles on leaf decomposition dynamics is much more subtle than the dramatic effects of grazing tadpoles on algal primary producers that we report in previous studies. Observed seasonal differences in Centrolenid densities and their patchy colonization of experimental leaf packs suggest that their effects on detrital dynamics may be most important across relatively small spatial scales.

Key words: tadpoles, Neotropical streams, decomposition, fungal biomass, amphibian declines

INTRODUCTION

Little is known about the natural history and functional roles of many larval anuran amphibians (Altig *et al*., 2007), and changes to ecosystem processes resulting from their losses are poorly understood (but see Colon-Gaud *et al*., 2008; Connelly *et al.,* 2008). Much of what is known is the result of studies focusing on the assumption that tadpoles function predominately as herbivores, and a number of studies have looked at interactions among tadpoles, primary producers, and functionally-similar aquatic macroinvertebrate grazers. However, tadpoles are found in a wide variety of freshwater systems, they occupy a high diversity of microhabitats within these systems, and they are known to consume a diet much more diverse than primary producers (Altig *et al*., 2007).

Few data are available that document natural stream densities, feeding behavior and food requirement of stream-dwelling tadpoles. Moreover, the degree to which the presence or absence of tadpoles may affect leaf litter decomposition dynamics and microbial and macroinvertebrate communities is relatively unknown. This is especially true of tadpoles within the glass frog (Centrolenidae) family. Species of this fossorial group have characteristic long tails and fusiform bodies, are generally adapted to burrowing into sediments (Villa & Valerio, 1982), and are commonly found in dense leaf packs and accumulated decaying organic matter of many Neotropical streams (McDiarmid & Altig, 1999). Gut contents analysis indicates glass frog tadpoles ingest a high proportion of fine benthic detritus, although recent stuidies suggest the tadpoles assimilate energy primarily from microbes associated with the particles and not from the detritus itself (Hunte-Brown, 2006; Whiles *et al*., 2006) The tadpoles of many glass frog species have yet to be formally described, however, and species-specific natural history

characteristics are generally unknown. While numerous experiments have shown that various aquatic macroinvertebrates can influence stream leaf litter decomposition dynamics, the role of locally abundant anuran larvae, with respect to litter decomposition communities and dynamics, is unknown.

The objectives of our study were to: (1) to compare leaf litter decomposition dynamics between two neotropical streams in Panama: a stream with an intact tadpole community *(with frogs)* and one where tadpoles were extirpated *(frogless)*; and (2) characterize the Centrolenid tadpole assemblage associated with decaying leaves and to experimentally assess how tadpole presence and absence affects leaf decomposition rates and fungal, bacterial and macroinvertebrate communities associated with decaying leaves.

We hypothesized that: (1) the stream *with frogs* would have significantly higher decomposition rates than the *frogless* stream; and (2) experimental exclusion of Centrolenid tadpoles from leaf packs in the *frog* stream would result in (a) reduced decomposition rates (i.e. reduced loss of leaf mass over time); (b) increased fungal and bacterial biomass on leaves; and (c) increased abundance of shredder and filter-feeding macroinvertebrates.

METHODS

Study Sites

This study was conducted in two upland Panamanian streams, the Rio Guabal and the Quebrada Chorro. Rio Guabal (hereafter referred to as the *frog* stream), is a second order stream located in the Parque Nacional G. D. Omar Torrijos Herrera, El Copé, Coclé,

Panama ($8^{o}40'$N, $80^{o}35'$W). Our study reach is part of a heavily forested, high-gradient stream characterized by distinct pool-run-riffle sequences, with substrates consisting of pebbles and gravel, with frequent cobbles, boulders, and depositional sandy areas. Forty species of riparian frogs were documented at this stream, 23 of which had stream-dwelling larvae (Lips *et al*., 2003). Tadpoles occurred in all stream habitats, including detrital accumulations in pools where six species of glass frog (Centrolenidae) tadpoles are found (Lips *et al*., 2003).

The second stream, Quebrada Chorro (*frogless* stream) is approximately 200 km from the *frog* stream. The *frogless* stream drains the Reserva Forestal Fortuna, Chiriquí, Panama ($8^{o}42'$N, $82^{o}14'$W). The *frogless* stream is similar to the *frog* stream in terms of order, geology, canopy cover, substrates, and nutrient concentrations, and is characterized by riffle and run sequences with isolated pools (Colón-Gaud et al. 2008). Past studies have treated the *frogless* stream as a control relative to the *frog* stream (Whiles et al., 2006; Connelly et al., 2008; Colon-Gaud et al., 2009). There are two distinct seasons that characterize these watersheds, a drier season (~January to April) and a pronounced wet season (~May to December). The *frogless* stream watershed suffered a catastrophic extirpation event associated with the fungal pathogen *Batrachochytrium dendrobatidis* in 1996 (Lips, 1999). Fifty-seven anuran species were previously documented at the site, and once included the Centrolenid anurans found at the *frog* stream (Lips, 1999). Virtually no tadpoles (average during our study = < 0.01 tadpoles m^{-2}) have been seen since 2000. More detailed descriptions of these two study sites are found in Connelly *et al*., 2008 and Colon-Gaud *et al*., 2009.

Ambient Centrolenid tadpole desities and ammonium excretion rates

To estimate natural background Centrolenidae tadpole density at the *frog* stream, surveys were conducted monthly during July, August and September 2003 (wet season); February, March and April 2004 (dry season); and May, June, July and August 2004 (wet season). Tadpoles in three randomly chosen depositional habitats were quantified with a stove pipe benthic corer (22 cm diameter) that was modified with external rubber flaps at the base to help seal the bottom of the sampler when substrate was irregular. The core sampler was pushed approximately 3 cm into the substrate, and tadpoles were removed with a dip net (15 x 10 x 10 cm), counted, identified to species, and released (following methods described in Connelly *et al*., 2008).

Mass-specific ammonium excretion rates were estimated from seven Centrolenid tadpoles ranging in mass from 1.4 to 78.5g collected from the *frog* stream. Tadpoles were placed in 60 mL centrifuge vials filled with stream water and incubated streamside for 1 hour. After removal of tadpoles, NH_4^+ concentration in the water in the centrifuge vials was immediately measured following method of Holmes *et al.* (1999), as modified by Taylor *et al.* (2007). Ammonium concentrations after 1 hour were corrected for background concentrations from tubes without tadpoles and then standardized for mass of each tadpole. All fluorometric measurements were made within 5 hrs of the termination of each incubation trial using a Turner Designs 10-AU or Trilogy fluorometer (Turner Designs, Inc., Sunnyvale, CA). Tadpole biomass was estimated using length-mass relationships we developed following methods of Benke *et al.* (1999). Tadpole body lengths were measured at the termination of excretion incubations.

Tadpole exclusion experiment

Tadpole exclusion experiments were run consecutively in each of our two study streams from May 26 to July 4, 2004 (in the *frog* stream) and from July 6 to August 15, 2004 (in the *frogless* stream). Electric exclusion devices (0.5 m^2 frames constructed of PVC tubing and concentric copper wire loops), modified from Pringle & Hamazaki (1997), were used to exclude tadpoles from experimental leaf packs placed on the stream bottom. Similar devices have been used effectively to exclude tadpoles from grazing artificial substrata at this study site (Ranvestel *et al*., 2004; Connelly *et al*., 2008), and the exclusion devices were tested prior to this experiment by placing several individual glass frog tadpoles within each replicate and confirming that the tadpoles promptly exited each electrified frame. At each stream, 10 treatment pairs (one electrified device and one control device, i.e., without electricity) were placed under naturally accumulated leaf litter in pools and runs of comparable 400 m reaches. Locations chosen were based on similar accumulations of organic sediments, current velocities (near zero at base flow), depths (19-29 cm), and percent canopy cover (75%-85%). Current velocity was measured with a Marsh McBirney current meter and canopy cover was measured with a spherical densiometer.

Freshly fallen leaves were collected from several individual trees of a common riparian species, *Trichospermum galeotti* (Turcz.) Kosterm., that were growing within 5 m of each stream bank. Leaves were air-dried for 2 days, and six g of leaves were placed in course mesh (1 x 1 cm) plastic bags (15 x 20 cm). The large mesh size was used to allow access to the largest Centrolenid tadpoles in the stream. Eight leaf packs were fastened horizontally with plastic cable ties within each of the 20 PVC frames, for a total

of 80 bags per treatment per stream. Replicates of each pair were situated at least 0.5 m apart and placed entirely under, to the extent possible, detrital accumulations in pools and runs, and anchored with tent stakes to ensure leaf pack contact with the stream bed. Each replicate was visited for 3 min on each of 20 days and 20 nights (for a total of 40 hours for all treatments in each stream) during the experiment to ensure that the electric exclusion devices were functioning properly and to verify that other potential macroconsumers, such as fishes and shrimps, were not entering the replicates.

On day 0, one leaf pack was taken to each stream site and returned to the lab to control for handling losses. Thereafter, every 5 days over the 40-day experiment one randomly selected leaf pack was removed from each replicate (20 leaf packs each sampling date; 10 control and 10 tadpole exclusion). The leaf pack was cut from each PVC frames, placed within a fine mesh dip net to prevent the potential escape of tadpoles and invertebrates, removed from the stream and placed in a Ziploc® bag along with the contents of the dip net. Leaf packs were placed in a cooler and transported to the laboratory where the leaves were rinsed, along with the contents of the bag, over a 250 um mesh sieve to remove tadpoles, invertebrates and sediments. Tadpoles were measured and identified to species, and were returned to the stream. Macroinvertebrates were identified and preserved in 8% formalin solution. Tadpole dry mass was estimated using mass-length regression according to the equation tadpole mass = $(6.61 \times 10^{-5}) \times (\text{length}^{3.6132})$. Forty leaf disks (8 mm) were punched from randomly selected leaves from each pack. Twenty disks were dried and ashed to determine mean disk biomass (AFDM), and the remaining 20 were used to determine microbial respiration, and fungal and bacterial biomass. Remaining leaf material was dried for 24 h at 40 ^{0}C, weighed, and a

~1 g subsample was ashed at 500 ^{0}C and reweighed to determine AFDM. Day 0 leaf packs were used to estimate initial AFDM.

Fungi

Fungal biomass associated with leaf packs was estimated by measuring ergosterol concentration, following methods described by Suberkropp & Weyers (1996). On each sampling date, ten leaf disks from each leaf pack were placed in 5 mL methanol and transported to the laboratory. Ergosterol was extracted from leaf disks in alkaline methanol by refluxing for 30 min, partitioning into pentane, drying, and re-dissolving in methanol. Ergosterol concentration was determined by comparing absorbance at 282 nm after separation from other lipids by high-performance liquid chromatography (HPLC; Shimadzu Co.) with a standard concentration of ergosterol (Fluka). To convert ergosterol to fungal dry mass, we used an ergosterol concentration of 5.5 μg mg^{-1} of mycelial dry mass, following Gessner & Chauvet (1993).

Bacteria

Bacteria were counted using epifluorescence microscopy after staining cells with DAPI (Velji & Albright, 1993; Porter & Fieg, 1980). Five disks from each leaf pack were preserved in a 0.2-um filtered solution of 5% formaldehyde and stored at 4^{o}C. In the laboratory, bacterial cells were separated from leaf material by sonication (HT 150 Sonicator, VWR Scientific Inc., West Chester, PA, USA) (Weyers & Suberkropp, 1996) and 2 mL subsamples were placed in a Millipore vacuum filter manifold and stained with 10 ug/mL 4',6-diamidino-2-phenylindole (DAPI) solution for 10 minutes in the dark. Samples were filtered through black polycarbonate membrane filters (0.22 um, Poretics) backed with a 0.45 um Millipore cellulose nitrate filter and mounted on glass microscope

slides. Slides were refrigerated in the dark until counted (Velji & Albright, 1986). Ten random fields per slide were counted using 1000x epifluorescent microscopy. Cell biovolumes were estimated using geometric shapes (Psenner, 1993; Wetzel & Likens, 2000) and bacterial carbon was calculated by multiplying biovolumes by 5.6×10^{-13} g C /um^3 (Bratback, 1985).

Respiration

Microbial respiration was measured as oxygen uptake by decomposing leaves at stream water temperature. On each collection date, 5 leaf disks from each leaf pack were placed in 30 mL glass respiration chambers containing unfiltered stream water and initial oxygen concentration was obtained using a YSI Model 58 Dissolved Oxygen Meter (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio, USA) with a self-stirring probe. Water was re-circulated over a period of 20 min in darkness and a final oxygen reading was obtained. Four additional chambers containing only stream water were used as controls. Oxygen consumption was expressed as O_2 uptake per leaf AFDM per hour.

Water chemistry

As part of our regular TADS monthly sampling, water samples were taken during base flow from the *frog* stream once during June 2004 and from the *frogless* stream once during July 2004 and analyzed for NO_3-N, NH_4-N and SRP. Samples (2 replicates/stream) were filtered through 0.45 um Millipore filters, frozen, and transported to the Analytical Chemistry Laboratory at the University of Georgia. SRP was measured spectrophotometrically using the Ascorbic Acid method (APHA 1998). Nitrate and NH_4-N were measured using the cadmium reduction and phenate methods respectively (APHA 1998).

Statistical Methods

We calculated leaf decay rate (k_{day}) for each leaf pack by regressing the natural log of % leaf mass remaining against days. To correct for temperature differences between the *frog* and *frogless* streams, processing coefficients were computed using temperature ($k_{degree\ day}$). Daily water temperature was recorded over the course of each 40-day experiment for determination of accumulated degree days above 0 ^{o}C. We used repeated measures ANOVA to test for potential differences in temperature corrected leaf pack breakdown rates, fungal biomass, bacterial biomass, respiration, and invertebrate abundance through time. Leaf pack mass loss, fungal biomass, bacterial biomass, and respiration values were log-transformed prior to analysis to correct for non-normality of variances. Analyses were performed on SAS System for Windows, Version 8.0 (SAS Institute, Cary, NC, USA) using $\alpha = 0.05$. We conducted the analyses using all control and tadpole exclusion replicates (10 control sites and 10 paired tadpole exclusion sites). Since not all leaf packs in control treatments were colonized by tadpoles, we additionally tested for tadpole effects using only pairs of quadrats in which control (n=4) leaf packs were colonized by more than one tadpole. Since results of both types of analyses were similar (i.e., non-significant P-values; $P > 0.05$), we present only the results including all 10 sites. To test for overall differences in fungal biomass in control treatments where tadpoles were present versus absent, and in tadpole exclusion treatments, we constructed 90% confidence intervals around mean fungal biomass values. These results are significant at alpha < 0.10.

RESULTS

Mean stream discharge during the 40 day study at the *frog* stream was 48.93 ± 2.25 L s^{-1}, mean rainfall was 6.0 mm d^{-1}, mean water temperature was 21.07 ± 0.04 ^{o}C, and pH was 8.2. NO_3-N was 169 ug, NH_4-N was 4 ug L^{-1}, PO_4-P was 8 ug L^{-1}. At the *frogless* stream, there was higher and more variable mean discharge (93.29 ± 5.40 L s^{-1}), higher mean daily rainfall (12.5 mm d^{-1}), lower mean water temperature (18.20 ± 0.04 ^{o}C). Stream pH was 8.1. Levels of nutrients were similar; NO_3-N was 153 mg L^{-1}, NH_4-N was 5 ug L^{-1}, and PO_4-P was 20 mg L^{-1}

Background tadpole densities: We found wide variation in the ambient densities of Centrolenid tadpoles during periodic sampling of detrital pools in the *frog* stream (sampled between July 2003 and August 2004), ranging from 0.0 – 318.5 tadpoles m^{-2}. Five species of Centrolenid tadpoles were found during dry season (February - April). *Centrolene proseblepon* were the most abundant, with densities of 18.96 ± 6.87 m^{-2}. *Centrolenidae ilex*, *C. albomaculata*, *Hyalinobatrachium colybiphyllum*, and a Centrolenidae sp. were also found at much lower densities (Table 3.1). Overall mean glass frog tadpole density during this period was 31.09 ± 11.01 m^{-2}. Only two species of glass frog tadpoles were found during the wet season (May – September) and densities were greatly reduced (Table 3.1).

The mean mass-specific tadpole excretion rate was 0.063 ± 0.018 ug N mg tadpole^{-1} h^{-1}.

Experimental tadpole colonization and densities: We found patchy distribution of tadpoles in our experimental exclusion study, with densities in tadpole access leaf packs ranging widely, from 0.00 – 33.33 tadpoles m^{-2}. Thirty-one tadpole individuals

colonized the leaf packs over the course of the experiment in the *frog* stream, of which 29 were in the control treatments and 2 were in electric exclusion treatments (Table 3.2). All species of tadpoles colonizing leaf packs were glass frogs (Centrolenidae). *Centrolene prosoblepon* and *Cochranella albomacula* were encountered most frequently (22 and 7 individuals, respectively) and there was one individual each of *Hyalinobatrachium colymbiphyllum* and an unknown Centrolenidae.

Of the ten control replicates, four were uncolonized by tadpoles during the 40-day experimental period. Those six replicates that were colonized had 1 (2 replicates), 5, 6, 6, and 10 tadpoles over the course of the experiment. Mean Centrolenid tadpole densities in the control treatment was 9.99 ± 13.00 individuals m^{-2}. In control replicates colonized by more than 1 tadpole during the 40 days, densities ranged from 16.65 – 33.33 individuals m^{-2} (mean = 23.31 ± 8.75).

Tadpole biomass in the control treatments ranged from 0 – 55.92 g m^{-2} (mean = 15.24 ± 22.58) in the *frog* stream. In control replicates of the *frog* stream that were colonized by more than 1 tadpole during the experiment, tadpole biomass ranged from 13.50 – 55.92 g m^{-2} (mean = 34.97 ± 23.01). The electric exclusion devices were effective in deterring tadpoles. Only two small tadpoles were found in the exclusion treatments, and mean tadpole density and biomass in the exclusion treatments were more than an order of magnitude smaller than in access treatments, ranging from 0.00 – 3.33 individuals m^{-2} (mean = 0.67 ± 1.47) and 0 – 0.70 g m^{-2} (mean = 0.02 ± 0.10), respectively. No tadpoles were observed in control or tadpole exclusion replicates in the *frogless* stream.

Leaf pack mass loss: There were no differences in mass loss between control and tadpole exclusion treatments ($F_{1,8} = 0.00$, $P = .99$) (Fig. 3.1) in the *frog* stream. On the final day of the experiment (day 40) in the *frog* stream, 58.99% of leaf mass remained in control treatments ($k_{day} = 0.028\ d^{-1}$) and 58.12% remained in the tadpole exclusion treatment ($k_{day} = 0.028\ d^{-1}$). Similarly, there were no differences in mass loss between treatments in the *frogless* stream ($F_{1,8} = 1.2$, $P = 0.20$), with 58.19% ($k_{day} = 0.027\ d^{-1}$) and 60.43% remaining ($k_{day} = 0.027\ d^{-1}$), respectively. After correcting for temperature differences between streams, there were no statistical differences in rates of mass loss in either control ($k_{degree\ day} = 0.0013$ vs $k_{degree\ day} = 0.0015\ d^{-1}$) or tadpole exclusion ($k_{degree\ day} = 0.0014$ vs $k_{degree\ day} = 0.0015\ d^{-1}$) treatments. Leaf mass loss was generally constant (linear) over the course of the experiment.

Respiration: Microbial respiration did not differ between tadpole access and exclusion treatments in the *frog* stream ($F_{1,8} = 0.30$, $P = .35$) or in the *frogless* stream ($F_{1,8} = 0.65$, $P = .60$) (Fig. 3.2). On day 40 of the experiment, mean respiration was 2.55 $\pm$ 0.61 mg O_2 $AFDM^{-1}$ h^{-1} (control) and 2.61$\pm$ 1.01 (tadpole exclusion) in the *frog* stream. Respiration was lower, although not significantly so, in the frogless stream, with mean respiration of 2.08 $\pm$ 0.86 mg O_2 $AFDM^{-1}$ h^{-1} in control treatments and 2.19 $\pm$ 0.95 in tadpole exclusion exclusion treatments.

Fungal biomass: Fungal biomass, as indicated by ergosterol concentrations, were similar between control and tadpole exclusion treatments in the *frog* stream ($P > 0.05$) and between treatments in the *frogless* stream (Fig. 3.3). Fungal biomass was highest on day 40 of the experiment, when levels reached 82.65 $\pm$ 13.87 mg C g^{-1} AFDM (control) and 77.11 $\pm$ 11.34 (tadpole exclusion) in the *frog* stream, and 71.71 $\pm$ 15.97 mg C g^{-1}

AFDM (control) and 77.62 ± 10.03 (tadpole exclusion) in the *frogless* stream. There were no statistical differences in fungal biomass between streams. When replicates were grouped by presence or absence of tadpoles (i.e., un-colonized control leaf packs and tadpole exclusion leaf packs compared to tadpole-colonized control leaf packs), we found increased levels of fungal biomass, averaged over the 40-day experiment, on tadpole-colonized control leaf packs (56.9 vs. 42.7 mg C g^{-1} AFDM) (Fig. 3.4)

Bacterial biomass: Levels of bacterial biomass did not differ between control and tadpole exclusion treatments in the *frog* stream ($F_{1,8} = 0.20$, P = .40) or in the *frogless* stream ($F_{1,8} = 0.15$, P = .35) (Fig. 3.5). Bacterial biomass tended to level by day 30 of the experiment, at which time there was 0.23 ± 0.06 mg C g^{-1} AFDM in control treatments and 0.18 ± 0.05 in tadpole exclusion treatments in the *frog* stream, and 0.21 ± 0.05 (control treaments) and 0.21 ± 0.04 (tadpole exclusion treatments) in the *frogless* stream. There were no statistical differences in bacterial biomass between streams.

Macroinvertebrates: The most common macroinvertebrate taxa to colonize leaf packs in the *frog* and *frogless* streams were dipterans in the families Chironomidae and Simuliidae (*Simulium* sp.) and the coleopteran *Anchytarsus* sp (Table 3.3; Fig. 3.6). Leaf packs were colonized much less frequently by the Coleopterans *Phanocerus* sp., the Ephemeropterans *Baetis* sp. and *Tricorythodes* sp., and Oligochaetes (Table 3.3). There were no statistical differences between control and exclusion treatments within streams or between streams.

DISCUSSION

Effects of Centrolenid tadpoles on leaf decomposition dynamics are much more subtle than the dramatic effects of grazing tadpoles on algal primary producers that we report in previous TADS studies conducted in our same study streams (e.g., Connelly *et al.* 2008). The stream *with frogs* exhibited the same decomposition rates and similar decomposition dynamics as the *frogless* stream and findings clearly did not support our initial hypothesis that significantly higher decomposition rates would occur in the study stream *with frogs* versus the *frogless* stream. Accordingly, experimental exclusion of Centrolenid tadpoles from leaf packs in the *frog* stream had no significant effect on decomposition rates. We attribute these findings, in part, to the relatively low densities and patchy distribution of Centrolenid tadpoles associated with leaf packs (Tables 3.1, 3.2). Moreover, in our study Centrolenid tadpoles did not directly shred decomposing leaf material, and observations suggest that they grazed the surfaces of leaves for microbes and used decomposing leaf packs as habitat and/or refugia.

Our findings of significantly higher fungal biomass in leaf packs containing Centrolenid tadpoles in control treatments suggest that fungal growth may be stimulated by tadpole excretion. Even though Centrolenid ammonium excretion rates reported here (0.063 ± 0.018 ug N mg tadpole^{-1} h^{-1}) are lower than those reported for other larval frog taxa in our stream (Whiles et al. 2006), ammonium may become more concentrated due to limited water exchange associated with leaf packs that have accumulated in areas of low stream flow.

Tadpole distribution

Habitat heterogeneity within our study stream likely played a role in the patchy distribution of our tadpole community. Although stream structure is often broadly characterized, such as pool, run, or riffle habitat, for example, these generalizations may overly simplify the complex physical, chemical, and biological processes that tadpoles are likely to respond to. Lotic systems are recognized as being variable and physically complex, and subtle differences in flow direction, deposition of sediments, dissolved oxygen, and chemical concentrations can result in a mosaic of microhabitats (Groffman & Bohlen, 1999).

Tadpoles have been categorized as stream ecosystem engineers, and can significantly modify their immediate habitat structure during foraging, particularly by displacing accrued sediments (Flecker *et al*., 1999), thereby increasing habitat quality and stimulating colonization by additional tadpoles. Additionally, tadpoles can alter the spatial heterogeneity of their habitat through other mechanisms. For example, concentrating waste deposition (i.e., excretion and egestion) in certain areas can significantly influence localized nutrient cycling (McClain *et al*., 2003). Whiles *et al*. (2006) indicated that a significant portion of nutrient rich sediments in our study stream is comprised of tadpole feces. This fecal resource is quickly colonized by the microbial community and becomes a potential resource for re-ingestion by tadpoles. Because the easily suspended and transported feces accumulate only in areas with little or no current, fecal accumulations become most concentrated in detrital pools. The attraction by Centrolenid tadpoles to this patchy accumulated fecal matter within pool microhabitats may be at least partially driving the patterns of tadpole habitat utilization we found.

We do not attribute the patchy colonization of control treatments by Centrolenid tadpoles in our study to experimental artifacts; mean colonization of the packs was higher than mean wet season background Centrolenid tadpole densities (Tables 3.1, 3.2), indicating that the leaf packs were suitable habitat for glass frog tadpole communities.

Leaf litter breakdown

Although the *frogless* stream mean daily temperature was approximately 2 ^{o}C cooler than the *frog* stream, we found no differences in leaf litter daily breakdown rates (k_{day}) or in temperature corrected breakdown rates ($k_{\ degree\ day}$) between the streams,. The lower temperature in the *frogless* stream likely slowed decomposition rates, although the *frogless* stream had discharge approximately twice that of the *frog* stream, and discharge due to storm events was more flashy relative to the *frog* stream (Fig. 3.1). We suggest that the similarity in breakdown rates between streams may be due in part to the net effect of combining higher and more flashy discharge with lower temperature in the *frogless* stream. Overall, leaf pack breakdown rates in our study streams (k_{day} = 0.027 to 0.028) broadly overlap other published rates, across different plant species, in upland tropical systems of Brazil k_{day} (= 0.014; Goncalves & Callisto, 2006), Costa Rica (k_{day} = 0.037 to 0.039; Wright & Covich, 2005) and Colombia (k_{day} = 0.024 to 0.065 to; Mathuria & Chauvet, 2002)

Our study found similar leaf mass loss over 40 days between the tadpole access and exclusion leaf packs, and we saw no physical evidence of leaf pack degradation due to tadpole presence. Previous studies analyzing gut content have shown that larval centrolenids do ingest a high proportion of detritus (McDiarmid & Altig, 1999), of which

leaf litter is often a large component. However, the nutritional content of stream detritus can be largely driven by the colonizing microbial community rather than the leaf material itself (e.g., Arsuffi & Suberkropp, 1986). Tadpoles are unable to break down ingested cellulose (Savage, 1952), and the rasping mouthpart morphology of Centrolenid larvae suggests that they are not well-adapted to shred leaf litter. Rather, the tadpoles likely scrape the biofilm matrix from leaf surfaces into suspension and ingest the suspended biofilm, along with incidental fine particulate leaf material which had been previously broken down through other biotic and abiotic mechanisms. A previous study in our focal stream, using carbon and nitrogen stable isotopes, indicated that centrolenid tadpoles assimilated primarily the associated microbes rather than the particles of detritus that they ingested (Hunte-Brown, 2006; Whiles *et al*., 2006). Leaf litter breakdown rates of our study and results of Hunte-Brown (2006) suggest that centrolenid tadpoles do not functionally behave as leaf shredders, but instead ingest leaf biofilms and microbe-rich fine benthic particles which had settled in detrital leaf packs.

Microbial communities

Where centrolenid tadpoles were abundant, we found significantly higher levels of fungal biomass (Fig. 3.2). There are several possible explanations for this association. Foraging Centrolenid tadpoles may be responding to microbes as an energy-rich food resource; that is, they may be attracted to the leaf packs which have higher levels of fungal biomass. Conversely, the presence of tadpoles may be directly responsible for increased fungal biomass. Nitrogen is recognized as limiting the biomass and activity of aquatic microbes on leaves (Suberkropp, 1998), and several studies have indicated that the growth of

microbes in aquatic systems responds positively to nutrient additions (Stelzer *et al*., 2003; Graneli *et al*., 2004). The positive relationship we found between fungal biomass and tadpole presence may be the result of fungi responding to tadpole-driven nutrient regeneration (i.e., through tadpole egestion and excretion), as was similarly documented by Iwai & Kagaya (2007), who found that tadpole-mediated nutrient regeneration by tadpole feeding activity increased stream leaf litter nutritional quality.

Water nutrient levels (NO_3-N, NH_4-N, and PO_4-P) measured during our study were similar between streams. Likewise, we found similar mean monthly nutrient levels over time (July 2003 – June 2005; unpublished data), suggesting that water column nutrient availability would not likely drive differences in microbial biomass between streams. However, the localized effects of the mass-specific glass frog excretion rates we measured (0.063 ± 0.018 ug N mg tadpole^{-1} h^{-1}) may be responsible for increased fungal biomass in tadpole-colonized leaf packs in the stream *with frogs*. Mass-specific rates of nutrient excretion by tadpoles are higher than that of other stream-dwelling vertebrates, such as fish (Vanni, 2002), and based on tadpole densities measured during the 2004 dry season, overall tadpole community excretion represented ~7% of the dry season bulk uptake estimates of ammonium within the *frog* stream (Whiles et al., 2006). This finding and results of our study suggest that Centrolenid tadpoles likely play an important role in nutrient cycling, particularly during the dry season when their densities are highest, and when reduced stream flow provides less dilution of their excreted ammonium. We did not expect significant increases in fungal biomass (resulting from increased nutrient availability) to be offset by decreases in fungi biomass due to tadpole grazing on microbes, as most fungal biomass associated with submerged leaves is not on the leaf

surface, but instead in the interior of the leaf, and thereby protected from browsing. The Centrolenid excretion rates we measured were somewhat lower than we had expected, based on previous work in our study stream which indicated that ammonium excretion rates for other tadpole taxa (e.g., Rana *warszewitschii* and *Hyloscirtus* sp.) ranged from 0.15 μg hr^{-1} to 3.6 μg hr^{-1} (Whiles et al., 2006). This difference may be due in part to the lower activity level of Centrolenid tadpoles (compared to other stream-dwelling tadpole species associated with more lotic habitats) and potentially different temperatures associated with the benthic leaf pack accumulations where glass frog tadpoles are found versus more open water habitats of *Rana* and *Hyloscritus* tadpoles (Hauer & Lamberti, 1996).

Our hypothesis that tadpole exclusion in the *frog* stream would result in increased leaf pack bacterial biomass was unsupported. The tadpoles in the stream *with frogs* most likely ingested bacterial communities on leaf packs, and the tadpoles could have also physically disrupted and displaced leaf microbe communities through bioturbation associated with feeding. However, the bacterial communities may have also responded positively to tadpole presence, as tadpoles may have reduced the microbial boundary layer. It is also possible that tadpole grazing may have removed the upper layer of dead or less productive bacterial cells, exposing underlying bacteria to oxygen and nutrients. Similarities in levels of bacterial biomass between control and tadpole exclusion treatments may be the net result of negative effects on bacterial biomass through grazing, combined with positive effects on bacterial biomass due boundary layer removal and increased N-availability to bacteria through nutrient recycling by tadpoles.

Macroinvertebrate communities

We did not find increased abundances of shredder or filter-feeding macroinvertebrates in response to tadpole exclusion, as we had predicted. Many species of the most abundant taxa colonizing the leaf packs, Chironomid larvae, feed on particulate organic material; however, their populations were not reduced in the presence of tadpoles, suggesting that they are not competing with tadpoles for food resources or refugia. Because of their small size, relative to tadpoles, they may be ingesting detrital particles smaller than Centrolenids consume. Additionally, they may be feeding from areas of the leaf packs uncolonized by tadpoles, for example on the outside of leaf packs, in contrast to the tadpoles which tended to burrow into the experimental leaf packs in the stream *with frogs* (personal observation). We also found a high number, although patchy colonization, of black fly larvae. Generally recognized as filter feeders, Simuliidae, like the Chironomidae larvae, did not appear to compete with tadpoles for food or space. However, Simuliidae are often associated with at least some degree of moving water, suggesting that our leaf pack treatments were not entirely within homogenous habitat, and portions of the relicates may have experienced greater stream flow than other areas, particularly during periods of relatively high discharge. Within both streams we found several individuals of the beetle larvae, *Anchytarsus*, throughout the experiment, although there were no significant differences in their abundance between control and exclusion treatments or between streams. Because of their very low densities, they likely did not affect our decomposer community response variables or result in any significant competition with glass frog tadpoles. Of the other macroinvertebrate groups associated with our leaf packs (i.e., *Baetis* and *Phanocerus* mayflies, and oligochaetes), more than

one individual from any taxa was found on only one sampling date, and these groups did not appear to influence or be influenced by Centrolenid tadpole presence or absence.

In conclusion, our results suggest that the functional importance of Centrolenid tadpoles may be important within localized microhabitats, particularly with respect to fungal biomass. The stream *with frogs* did not exhibit higher decomposition rates than the *frogless* stream, as we hypothesized. Overall, the effects of Centrolenid tadpoles on leaf decomposition dynamics are much more subtle than the dramatic effects of grazing tadpoles on algal primary producers that we found in previous studies in our experimental streams. Results of our experimental work, and reach-scale population density monitoring, indicate that glass frog tadpoles can be locally and seasonally abundant, although their populations are relatively patchy. To fully characterize effects of glass frog losses on leaf litter decomposition dynamics, it should be recognized that they may be most important across relatively small spatial scales.

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Table 3.1 Mean tadpole density (± 1SE) by season of Centrolenid tadpoles sampled in depositional habitat of frog stream. Wet season estimates were sampled monthly May - September, dry season was sampled February-March.

		C. proseblepon	C. ilex	C. albomaculata	H. colymbiphyllum	Centrolenidae sp.	Combined species
Wet Season	Total tadpoles	0	0	0	1	2	3
	Tadpoles m^{-2}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.54	1.08 ± 1.07	1.62 ± 1.19
	Habitat-weighted tadpoles m^{-2}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.34	0.68 ± 0.68	1.02 ± 0.76
Dry Season	Total tadpoles	25	0	8	6	2	41
	Tadpoles m^{-2}	18.96 ± 6.87	0.00 ± 0.00	6.07 ± 5.99	4.55 ± 3.14	1.52 ± 1.05	31.09 ± 11.01
	Habitat-weighted tadpoles m^{-2}	5.54 ± 1.96	0.00 ± 0.00	1.84 ± 1.84	1.81 ± 1.41	0.53 ± 0.39	9.72 ± 3.76

Table 3.2 Total tadpoles and mean tadpole density (± 1SE) colonizing experimental leaf packs over 40-day experiment in the *frog* and *frogless* stream.

		C. proseblepon	*C. albomaculata*	*H. colymbiphyllum*	*Centrolenidae sp.*	*Combined species*
Frog stream						
Tadpole access	Total tadpoles	20	7	1	1	29
	Tadpoles m^2	8.33 ± 0.00	2.91 ± 0.00	0.41 ± 0.54	0.41 ± 1.07	9.99 ± 13.00
Tadpole exclusion	Total tadpoles	2	0	0	0	2
	Tadpoles m^2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.83 ± 0.02
Frogless stream						
Tadpole access	Total tadpoles	0	0	0	0	0
	Tadpoles m^2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tadpole exclusion	Total tadpoles	0	0	0	0	0
	Tadpoles m^2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.3. Mean abundance (individuals m-2) of macroinvertebrates colonizing experimental and control leaf packs over 40 days in the frog and frogless streams. Means are ± 1SE.

	Frog Stream		Frogless Stream	
Taxa	Experimental	Control	Experimental	Control
Anchytarsus	22.06 ± 0.40	24.98 ± 0.63	18.32 ± 0.49	24.14 ± 0.63
Baetis	0.83 ± 0.17	0.83 ± 0.17	1.25 ± 0.19	0.42 ± 0.13
Chironomidae	255.58 ± 3.65	292.62 ± 6.60	304.28 ± 4.83	273.48 ± 6.80
Oligochaeta	2.48 ± 0.28	1.54 ± 0.17	1.18 ± 0.13	1.78 ± 0.20
Phanocerus	1.67 ± 0.20	0.83 ± 0.17	0.83 ± 0.17	0.42 ± 0.13
Simulium	160.26 ± 3.99	146.10 ± 2.99	187.73 ± 3.73	191.89 ± 4.60
Tricorythodes	2.50 ± 0.26	3.75 ± 0.24	1.25 ± 0.19	0.83 ± 0.17

Figure Legends

Figure 3.1. Mean daily discharge and temperature over 40 days in the frog (solid line) and frogless (dashed line) stream. Discharge is represented by heavy lines and temperature is represented by light lines.

Figure 3.2. Mean leaf mass remaining (% AFDM; ± 1SE) over 40 days in the (a) frog and (b) *frogless* stream in tadpole access (n=10 per date) and tadpole exclusion (n=10 per date) treatments.

Figure 3.3. Mean leaf pack respiration (±1SE) in the *frog* and *frogless* stream on day 40 of the experiment. Shaded bars represent leaf pack from control treatments (tadpole access), dark bars represent tadpole exclusion treatments (tadpoles excluded).

Figure 3.4. Fungal biomass (± 1SE) on submerged leaf packs in (a) *frog* stream and (b) *frogless* stream. Diamonds represent tadpole access (n=10 per date) and squares represent tadpole exclusion (n=10 per date) treatments.

Figure 3.5. Mean fungal biomass (± 1SE) on submerged leaf packs in *frog* stream (tadpoles present). Bars represent 90% confidence intervals. Asterisk denotes a significant difference between leaf packs with tadpole grazing and leaf packs without tadpole grazing (due to electric exclusion or no colonization by tadpoles).

Figure 3.6. Bacterial biomass (± 1SE) on submerged leaf packs in (a) *frog* stream and (b) *frogless* stream. Diamonds represent tadpole access and squares represent tadpole exclusion treatments.

Figure 3.7. Mean densities (± 1SE) of most abundant macroinvertebrate taxa colonizing submerged leaf packs in the *frog* stream and *frogless* stream over 40 days. Squares represent tadpole access (n=10 per date) and diamonds represent tadpole exclusion (n=10 per date) treatments.

Figure 3.1

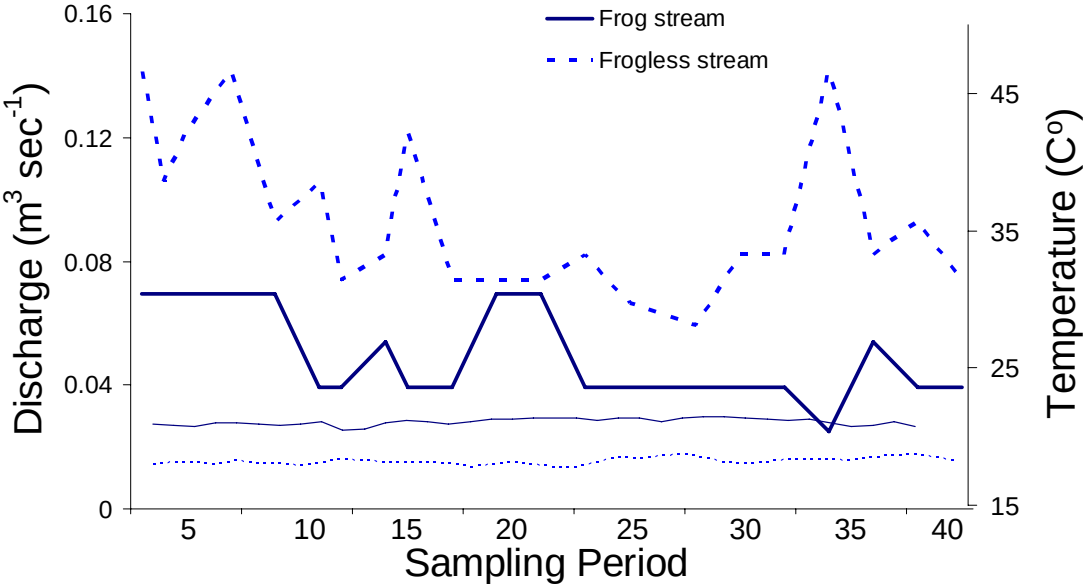

Frog stream
Frogless stream
Discharge (m3 sec-1)
Temperature (Co)
Sampling Period
0.16
0.12
0.08
0.04
0
45
35
25
15
5
10
15
20
25
30
35
40

Figure 3.2

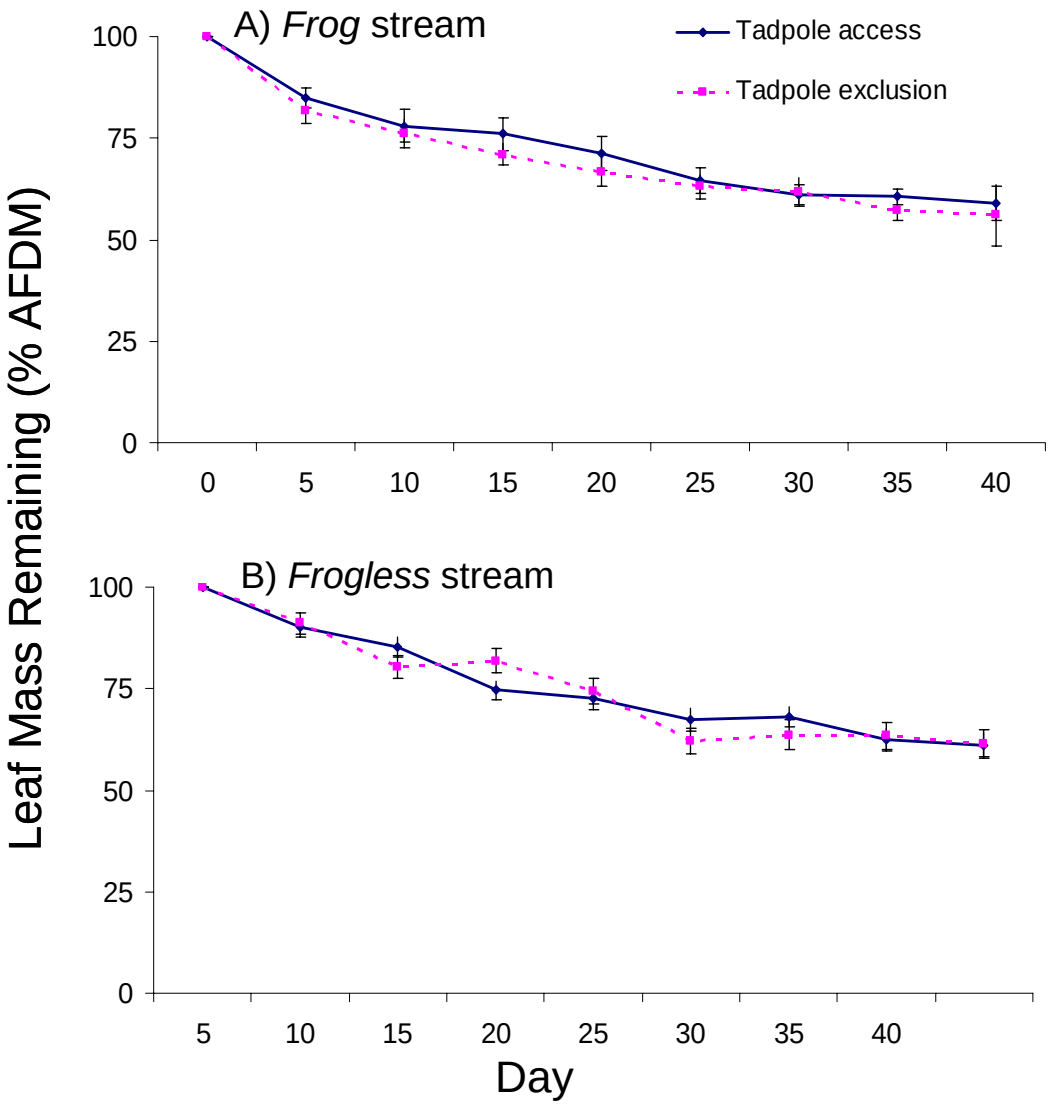

A) *Frog* stream
Tadpole access
Tadpole exclusion
100
75
50
25
0
0
5
10
15
20
25
30
35
40
B) *Frogless* stream
100
75
50
25
0
5
10
15
20
25
30
35
40
Day
Leaf Mass Remaining (% AFDM)

Figure 3.3

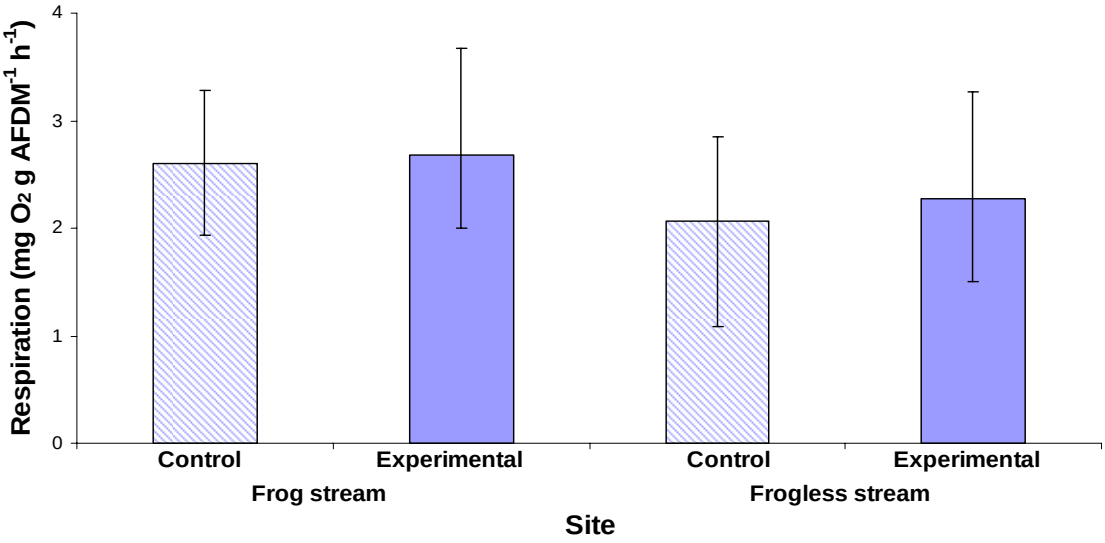

Respiration (mg O2 g AFDM-1 h-1)
4
3
2
1
0
Control
Experimental
Control
Experimental
Frog stream
Frogless stream
Site

Figure 3.4

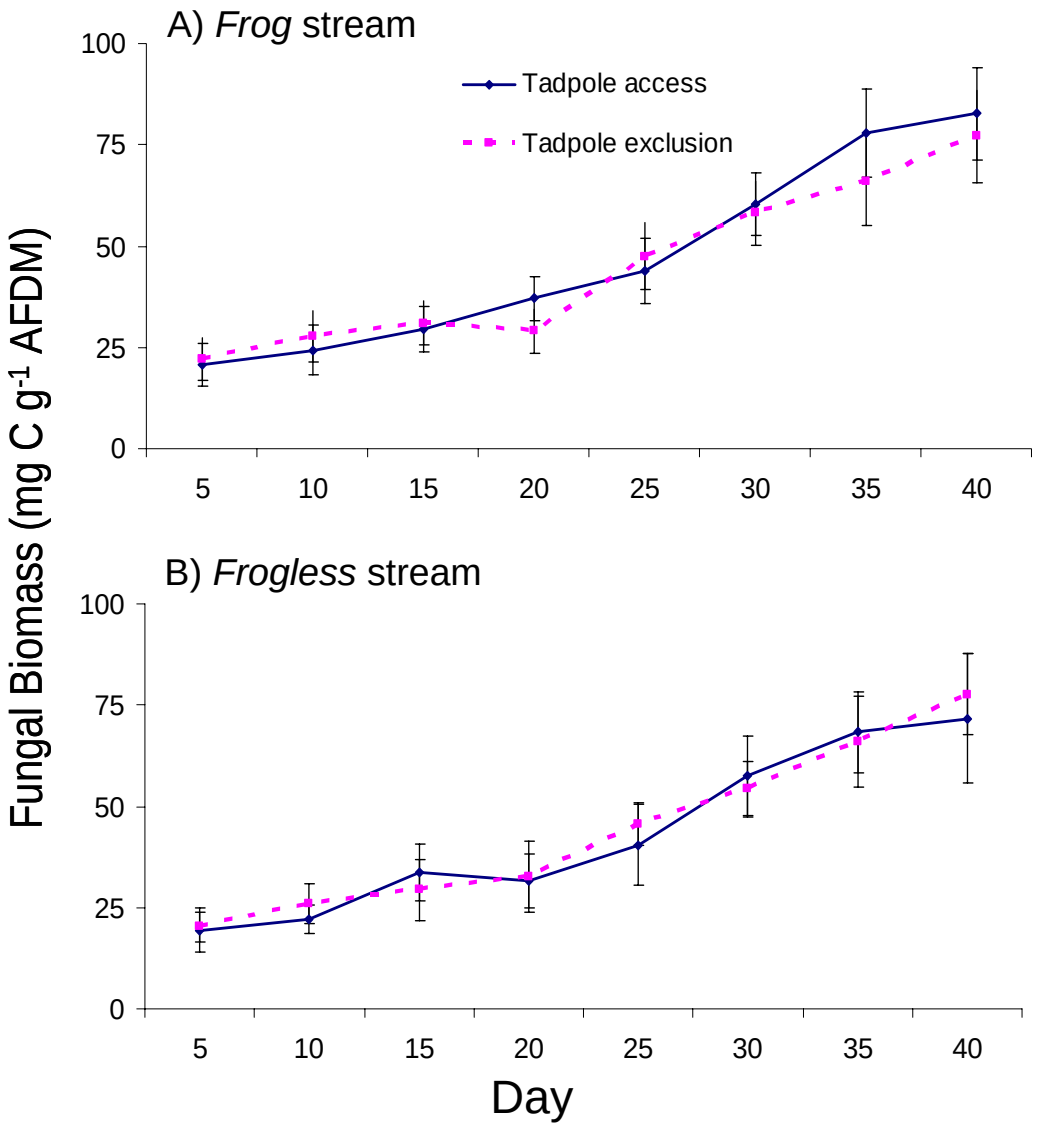

A) *Frog* stream
Tadpole access
Tadpole exclusion
B) *Frogless* stream
Fungal Biomass (mg C g-1 AFDM)
Day
0
25
50
75
100
5
10
15
20
25
30
35
40

Figure 3.5

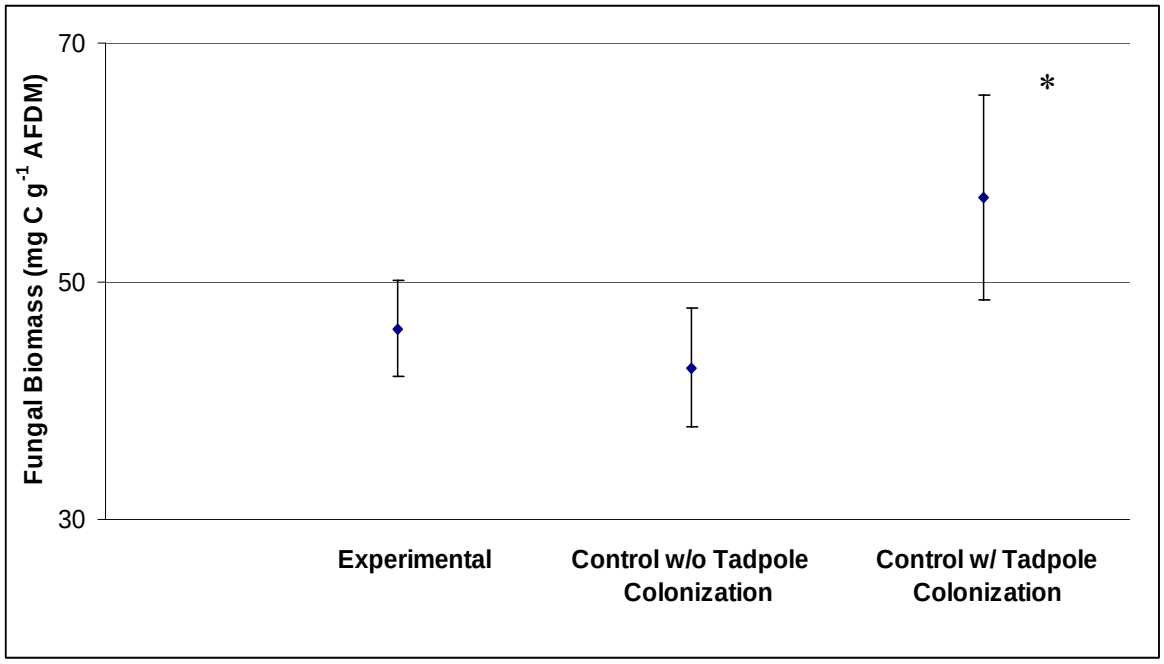

70
50
30
Fungal Biomass (mg C g-1 AFDM)
*
Experimental
Control w/o Tadpole Colonization
Control w/ Tadpole Colonization

Figure 3.6

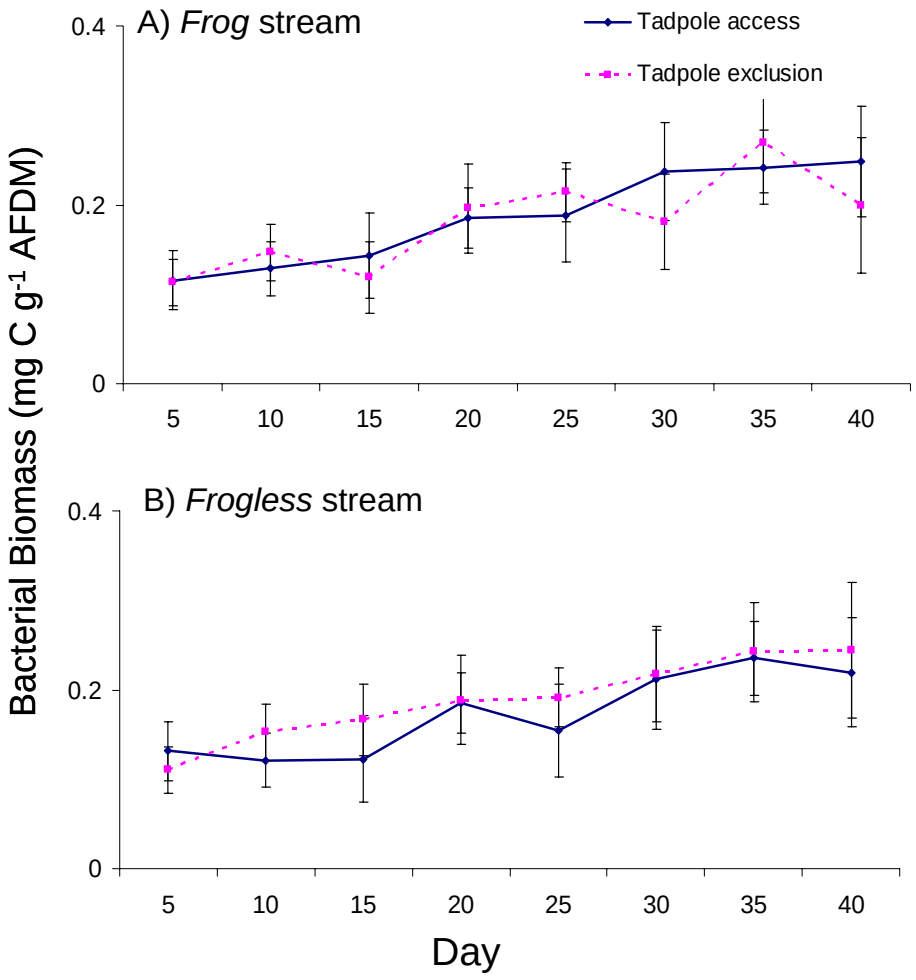
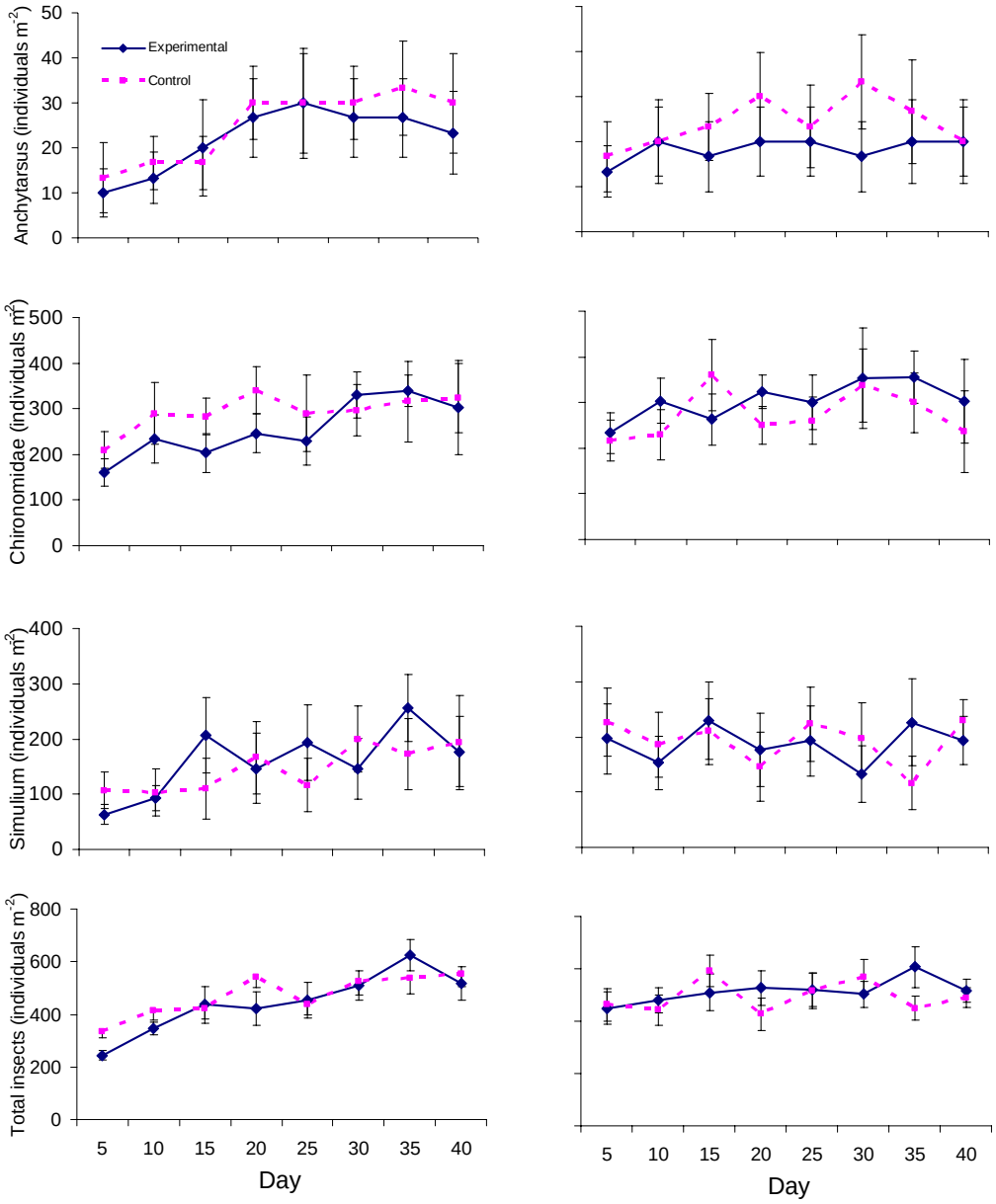

A) *Frog* stream
Tadpole access
Tadpole exclusion
B) *Frogless* stream
Bacterial Biomass (mg C g-1 AFDM)
0.4
0.2
0
5
10
15
20
25
30
35
40
Day

Figure 3.7


Experimental
Control
Anchytarsus (individuals m-2)
Chironomidae (individuals m-2)
Simullium (individuals m-2)
Total insects (individuals m-2)
Day
Day

CHAPTER 5

CONCLUSIONS

Dissertation summary

We tested the hypotheses that tadpole-exclusion experiments in a stream with an intact amphibian community would: (1) result in algal standing crop and community composition similar to that found in a stream where tadpoles had been extirpated; and (2) predict the *direction*, but under-estimate the *magnitude*, of changes in algal standing crop and community composition resulting from whole-stream tadpole extirpation. We combined experimental manipulation with reach-scale monitoring through a 5-month period following a catastrophic amphibian decline (Chapter 2). Next, we tested the hypotheses that: (1) the initial high levels of algal standing crop that we observed 5-months post tadpole extirpation would not be sustained over 3 years; (2) algal food quality (as measured by biofilm C:N ratios) would decrease post-extirpation in both the short- (5 months) and longer- (3 years) term post-extirpation periods; and (3) nutrient cycling would be altered post tadpole extirpation, as indicated by decreased fractionation of nitrogen stable isotopes in biofilm (Chapter 3). Lastly, we examined the ecosystem role of glass frog tadpoles, and hypothesized that: (1) the stream *with frogs* would have significantly higher decomposition rates than the *frogless* stream; and (2) experimental exclusion of centrolenid tadpoles from leaf packs in the stream *with frogs* would result in: (a) reduced decomposition rates; (b) increased fungal and bacterial biomass on leaves; and (c) increased abundance of shredder and filter-feeding macroinvertebrates.

Although global amphibian declines are well-documented, there has been a lack of quantitative information on the ecological significance of losses of larval stream-dwelling amphibians. Moreover, the extent to which results from small-scale manipulations are predictive of larger-scale ecosystem responses to amphibian declines has generally remained untested. In Chapter 2, our results link declining amphibian populations to alterations in stream primary producers over two spatial and temporal scales. Tadpoles reduced algal standing crop and sediments, and altered diatom community composition at both the small experimental and the larger reach scales. On a localized scale, we found biomass-specific primary production to be greater in control treatments (tadpole access) in our experiments, indicating that the algal community was more productive on a per-unit biomass (AFDM and chlorophyll *a*) basis. The study also found that the tadpoles were more efficient at ingesting the less abundant, but possibly easier-to-graze, larger diatom species, which resulted in a higher proportion of smaller diatom species where tadpoles had grazing access.

Reach-scale post-decline studies in the stream *with frogs* indicated that the tadpole-mediated changes we observed in the short-term and over a localized scale (i.e., electric exclusion experiments) predicted the direction of change; however, the experimental study underestimated the magnitude of changes that were evident at larger spatial and temporal scales when tadpoles were extirpated from the entire stream. Overall, our experimental results, combined with stream monitoring at the reach scale, indicated that larval amphibian losses have significant effects on stream ecosystem structure and function.

In Chapter 3, we examined whether the short-term trends we observed initially following tadpole losses would persist over the longer-term. We found that the 2.8-fold increases in pools and 6.3-fold increases in riffles in levels of chlorophyll *a* that we recorded 5-moth post-extirpation were reduced to increases of 1.9-fold in pools and 3.5-fold in riffles over pre-extirpation levels over the 3 year post-extirpation period. Similarly, the initial increases in total organic content (AFDM) of benthic biofilms of 2.2 fold in pools and 2.3 fold in riffles were reduced to a 2-fold increase in pools and 1.9-fold increase in riffles over 3 years. Algal food quality increased slightly, but not significantly, during the initial 5-month post-extirpation period and remained constant over 3-years. Mean $\delta^{15}N$ in biofilms (measured over the reach scale) was significantly depleted initially following tadpole extirpation and remained depleted 3-years post-extirpation (3.55 ‰ pre- versus 2.69‰ 3 years post-), suggesting that the loss of tadpoles reduced N-availability for uptake by algae and other microbes. Increases in abundance and production of some grazing macroinvertebrate taxa probably contributed to the gradual reduction in the difference between initial and longer-term post-decline algal biomass over the long-term. However, algal biomass was still 2-fold greater than pre-extirpation levels after 3 years, indicating that grazing macroinvertebrates did not completely compensate for the loss of tadpoles.

In Chapter 4 we experimentally investigated the ecological role of detrital-dwelling glass frog tadpoles, and quantified decomposition dynamics of our two study streams. Experimental results from the stream *with frogs* indicated that the effects of centrolenid tadpoles on leaf decomposition dynamics are much more subtle than the dramatic effects of grazing tadpoles on algal primary producers. Contrary to what we

predicted, experimental exclusion of centrolenid tadpoles from leaf packs in the stream *with frogs* had no significant effect on decomposition rates, bacterial biomass, leaf pack respiration, or associated macroinvertebrate communities. Furthermore, the stream *with frogs* exhibited similar decomposition rates as the *frogless* stream. However, we found significantly higher fungal biomass in leaf packs containing centrolenid tadpoles in control treatments, and we suggest that fungal growth may be stimulated by tadpole excretion. This study provides some of the first data on ambient centrolenid tadpole abundance and distribution, and documents patchy distribution of our tadpole community, which we attributed to habitat heterogeneity within our study stream. Our results suggest that the functional importance of centrolenid tadpoles may be important within localized microhabitats, particularly with respect to fungal biomass.

Suggestions for future research

Results indicate that amphibian losses clearly influenced many aspects of stream ecosystem structure and function. However, further studies are needed to better assess the ultimate ecological consequences of amphibian declines, especially since there are no published reports of amphibian population recovery at locations where they have been decimated (Lips *et al.*, 2006).

Some aspects of stream structure and processes that we quantified (e.g., algal food quality and leaf breakdown rates) showed no or only subtle response to tadpole losses. This may be because ecosystem-level consequences in response to change can be slow (Slavik *et al*., 2004). Little is known about how ecosystems change over ecologically meaningful time scales in response to biotic losses; therefore, a logical next step for the

TADS project is to build on our current data set, which currently spans nearly six years, through continued stream reach-scale monthly monitoring at our 2 study sites. This would include sampling, using the modified Loeb technique, of the stream substrates (characterizing periphyton biomass and stoichiometry, and inorganic sediment accrual) to assess longer-term stream responses and to detect potential ecosystem changes that may become apparent over more meaningful time scales.

We suggested that the shift away from smaller-bodied taxa towards larger-bodied taxa in the macroinvertebrate grazer community, immediately following tadpole losses, may be due, in part, to the shift in the algal community towards larger, more upright algal species found in the absence of tadpoles. The extent to which macroinvertebrates may eventually compensate for the loss of grazing tadpoles is unknown, although it may depend on how the macroinvertebrates respond not only to changes in periphyton biomass, but also how they ultimately respond to changes in the physiognomy of the algal community. Continued monitoring of algal community composition would be useful, as these potential changes in algae may be driving the initial responses we are observing in the macroinvertebrate community.

Our data were collected from relatively remote and poorly-studied headwater tropical streams; however, disease-driven amphibian declines are occurring on all continents and in many habitats where amphibians are found. Studies similar to ours, conducted across other ecosystems, are needed to more fully understand the ecological roles of amphibians, and to determine whether the trends that we documented in our Panamanian study reaches are indicative of ecosystem-changes occurring globally. The fungal pathogen, *Batrachochytrium dendrobatidis*, continues to move at a predictable rate

across Panama, towards Colombia. There are a number of currently uninfected amphibian populations located ahead off this disease front, although these populations are expected to be extirpated within several years as a result of the disease arriving. Pre-decline data should be collected from these additional sites, following the stream monitoring regime used at our two study streams, which would allow for potential changes in periphyton biomass, stoichiometry, and community composition, in response to the predicted amphibian population declines, to be characterized.

Much basic natural history information, such as life cycles, diets, energy assimilation, and habitat use is unknown for the larval and adult stages of many tropical amphibians. This is true not only for anurans with stream-dwelling tadpoles and adult frogs associated with riparian habitats, but also for hundreds of other species found in tropical systems, which have varied life history characteristics and habitat requirements. For example, our research focused primarily on four conspicuous algal-grazing tadpole species and five species of tadpoles associated with detrital leaf packs; however, the study stream drainage *with frogs* once supported 74 amphibian species, 40 of were found in the riparian zone, and 23 of which had stream-dwelling larvae (Lips *et al*., 2003). Other groups of larval frogs, such as *Colostethsus* sp. (Family Dendrobatidae), with tadpoles that tend to congregate in stream marginal pools, and *Bufo* sp. (Family Bufonidae), with tadpoles that are often found in seeps, need to be studied to more completely characterize the consequences of amphibian declines. Unfortunately, however, basic natural history information will never be known for dozens of amphibian species. In fact, some species have gone extinct at our TADS study sites before their formal species descriptions were published (e.g., Mendelson *et al*., 2008), and new

species continue to be discovered and described from tropical habitats that are likely to soon be infected with *Batrachochytrium dendrobatidis* (e.g., Lehr & Catenazzi, 2009). To more fully quantify the effects of amphibian losses on stream ecosystems, there is an urgent need to characterize the ecological roles of more species within this incredibly diverse group.

Although there are no published reports of amphibian populations recovering after a chytrid-related decline, recent personal observations at the *frogless* study stream indicate that some anuran species are persisting, and abundances of grazing tadpole species may be increasing in some stream reaches. Additionally, recent studies have suggested that some amphibian species may be developing resistance to the fungal disease. For example, Brucker *et al*. (2008) discovered that mutualistic bacteria on amphibian skin can prevent infection by the chytrid fungus, and suggest that the bacteria may play a role in long-term resistance to *Batrachochytrium dendrobatidis* by vulnerable amphibians. Furthermore, successful breeding programs (e.g., Gagliardo *et al*. 2008), which have focused on maintaining captive populations of dozens of rare tropical amphibian species, have opened the door to the possibility of reintroductions of species that have been extirpated from their native habitat. The possibilities of both future population recoveries and reintroductions of amphibians to sites in Panama where they had been previously extirpated (e.g., our study site at El Cope) would be valuable from a restoration ecology perspective, by allowing us to determine the extent to which our study stream may return to its previous, amphibian dominated state. Perhaps, a new equilibrium state may have been reached at our study stream during the years without amphibians, which will not allow for successful recolonization by the extirpated species.

A study addressing the relative importance of biotic (e.g., tadpoles) vs abiotic factors (e.g., discharge) in structuring periphyton assemblages would be a useful addition to our understanding of the role that tadpoles play in stream ecosystems. Is discharge a stronger driver of algal periphyton biomass and community composition than tadpole grazing? Does the relative importance of these factors change seasonally with fluctuating tadpole abundance (which are higher during dry season) and discharge (which is lower during dry season)? Also, by obtaining baseline data from additional healthy streams (but which are expected to be devoid of amphibians soon with the arrival of chytrid), before and after assessments of stability and resilience to disturbance can be made. This would enable us to address the question whether streams are more resilient to abiotic disturbance with an intact amphibian community, or whether streams become more susceptible to abiotic disturbances as they become biotically impoverished.

Further studies are needed to better understand the trophic status and food web interactions of larval and adult amphibians. Continued research should build on our stable isotope results, which characterized some amphibian feeding relationships in our study streams, and more fully describe the riparian food web interactions in habitats where intact amphibian communities exists. For example, the species-specific food items for most of the ~70 adult amphibians at our study stream in El Cope will remain unknown, and how these unidentified prey populations are responding to the amphibian declines is also unknown. Stable isotope analysis has proven to be a useful tool in indicating some of these trophic relationships, and could prove useful in predicting changes in food web structure where tadpoles are lost.

A potentially important relationship not addressed with our study is the amphibian-mediated energy flow across habitat types. Some temperate amphibian species are known to be significant energy links between terrestrial and aquatic systems (e.g., Regester *et al.,* 2006). Quantifying energy sources entering (e.g., amphibian egg masses) and leaving (e.g., metamorphic frogs) healthy neotropical streams would increase our understanding of the importance stream-breeding amphibians to stream and riparian food web structure, and help predict ecosystem-level consequences of on-going amphibian losses.

In conclusion, little is known about how ecosystems will respond to biotic losses over ecologically meaningful time scales. This study is the first to characterize relatively long-term changes in basal food resources, to compare initial trends in changes of basal resources to longer-term patterns following the loss of a larval amphibian community, and to document ambient population densities of glass frog tadpoles and quantify their role in leaf decomposition processing. Our 5-year study, which documented changes occurring in a natural field setting, indicates that the loss of tadpoles significantly influenced stream ecosystems; however, the magnitude of their effects changed through time, and 3 years following tadpole losses other consumers have not fully replaced the role of tadpoles. Given that there is no indication of amphibian communities recovering at sites where they have previously been decimated (Lips 1999), our continuing study will allow us to more fully assess the ultimate consequences of amphibian declines on neotropical stream ecosystems.

REFERENCES

Altig R, Whiles MR, Taylor CL. 2007. What do tadpoles really eat? Assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. Freshwater Biology 52: 386-395.

APHA. 1985. Standard methods for the examination of water and wastewater, 16. American Public Health Association, Washington, D.C.

APHA (1998) Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association, Washington, D.C.

Arsuffi TL, Suberkropp K. 1986. Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. Journal of the North American Benthological Society 5:297-305.

Benke, AC, Huryn AD, Smock LA, Wallace JB. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18:308-343.

Bothwell ML (1988) Growth rate response of lotic periphyton diatoms to experimental phosphorus enrichment: the influence of temperature and light. Canadian Journal of Fisheries and Aquatic Sciences 45:261-270

Bourrelly P, Manguin E. 1952. Algues d'eau douce de la Guadeloupe et dépendances. Centre National de la Recherche Sientifique, Société d'Edition d Enseignement Supérieur, Paris, France.

Bratback G. 1985. Bacterial biovolume and biomass enumerations. Applied and Environmental Microbiology 49:1488-1493.

Bronmark C, Rundle SD, Erlandsson A. 1991. Interactions between freshwater snails and tadpoles: competition and facilitation. Oecologia 87:8-18.

Brucker RM, Harris RN, Schwantes CR, Gallaher TN, Flaherty DC, Lam BA, Minbiole KP (2008) Amphibian chemical defense: antifungal metabolites of the microsymbiont Janthinobacterium lividum on the salamander Plethodon cinereus. Journal of Chemical Ecology 34:422-1429.

Carpenter SR, Frost TM, Heisey D, Kratz TK. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70:1142-1152.

Chapin FS, Schulze ED, Mooney HA (1992) Biodiversity and ecosystem processes. Trends in Ecology and Evolution 7:107-108

Chapin FS, III, Sala OS, Burke IC, Grime JP, Hooper DU, Lauenroth WK, Lombard A, Mooney HA, Mosier AR, Naeem S, Pacala SW, Roy J, Steffen WL, Tilman D. 1998. Ecosystem Consequences of Changing Biodiversity. Science 48:45-52.

Colón-Gaud C (2008) Macroinvertebrate community response to catastrophic amphibian declines in neotropical streams (dissertation). University of Southern Illinois, Carbondale.

Colón-Gaud C, Peterson SD, Whiles MR, Kilham SS, Lips KR, Pringle CM (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: 301-312.

Colón-Gaud C, Whiles MR, Kilham SS, Lips KR, Pringle CM, Connelly S, Peterson SD

(2009) Assessing ecological responses to catastrophic amphibian declines: Patterns of macroinvertebrate production and food web structure in upland Panamanian streams. Limnology and Oceanography 54:331-343

Connelly S, Pringle CM, Bixby RJ, Brenes R, Whiles MR, Lips KR, Kilham S, Huryn AD (2008) Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small scale experiments predict effects of tadpole loss? Ecosystems 11:1262-1276

de Sousa F. 1999. Fortuna Reserva Biologica. Editorial Universitaria, Panama.

Dickman M. 1968. The effect of grazing by tadpoles on the structure of a periphyton community. Ecology 49:1188-1190.

Dufrêne M, Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

Ernest SKM, Brown, JH (2001) Delayed compensation for missing keystone species by colonization. Science 292:101-104

Evans-White M, Lamberti GA (2006) Stoichiometry of consumer-driven nutrient recycling across nutrient regimes in streams. Ecology Letters 9:1186-1197.

Finlay J, Power M, Cabana G (1999) Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. Limnol Oceanogr 44:1198-1203.

Finlay JC (2004) Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography 49:850-861.

Flecker AS. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77:1845-1854.

Flecker AS, Feifarek BP, Taylor BW. 1999. Ecosystem engineering by a tropical

tadpole: Density-dependent effects on habitat structure and larval growth rates. Copeia 22:495-500.

Foged N. 1984. Freshwater and littoral diatoms from Cuba. Bibliotheca Diatomologica, 5, 1-243.

France R, Cattaneo A (1998) $\delta^{13}C$ variability of benthic algae: effects of water color via modulation by stream current. Freshwater Biology 39:617-622.

Frost PC, Elser JJ, Turner MA (2002) Effects of caddisfly grazers on the elemental composition of epilithon in a boreal lake. Journal of the North American Benthological Society 21:54-63

Frost PC, Cross WF, Benstead JP (2005) Ecological stoichiometry in freshwater benthic ecosystems: an introduction. Freshwater Biology 50:1781-1785.

Gagliardo, R, Crump P, Griffith E, Mendelson J, Ross H, Zippel K. (2008) The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. International Zoo Yearbook 42:125-135.

Gessner M.O. & Chauvet E. (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Applied and Environmental Microbiology 59, 502-507.

Goncalves JF Jr, Callisto M. 2006. Leaf-litter breakdown in 3 streams in temperate, Mediterranean, and tropical Cerrado climates. Journal of the North American Benthlogical Society 25: 344-355.

Graneli W, Bertilsson S, Philibert A. 2004. Phosphorus limitation of bacterial growth in high Artic lakes and ponds. Journal of Aquatic Sciences 66:430-439.

Greathouse EA, Pringle CM, McDowell WH. 2006. Do small-scale enclosure-exclosure

experiments predict effects of large-scale extirpation of freshwater migratory fauna? Oecologia 149:709-717.

Gresens SE. 1995. Grazer diversity, competition and the response of the periphyton community. Oikos 73:336–346.

Groffman PM, Bohlen PJ. 1999. Soil and sediment biodiversity. BioScience 49:139-148.

Hauer FR, Lamberti GA. 1996. Methods in Stream Ecology. Acedemic Press, San Diego, Ca.

Holmes RM, Aminot A, Kerouel R, Hooker BA, Peterson BJ. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 56: 1801-1808.

Hunte-Brown ME. 2006. The effects of extirpation of frogs on the trophic structure in tropical montane streams in Panama. PhD Dissertation, Drexel University, Philadelphia, Pennsylvania.

Iwai N, Kagaya T (2007) Positive indirect effect of tadpoles on a detritivore through nutrient recycling. Oecologia 152:685-694

Jackson JK, Sweeney BW. 1995. Egg and larval developmental times for 35 species of tropical stream insects from Costa Rica. Journal of the North American Benthological Society 14:115-130.

Hauer FR, Resh VH. 1996. Benthic macroinvertebrates. Pages 339-369 *In* FR Hauer and GA Lamberti, eds. Methods in stream ecology. Academic Press, Inc., New York.

Hector A, Bagchi R. 2007. Biodiversity and ecosystem multifunctionality. Nature 448:188-191.

Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, and Foster MS. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institute Press. Washington D.C.

Hillebrand H, Durselen CD, Kirschtel D, Pollingher U, Zohany T. 1999. Biovolume Calculation for pelagic and benthic microalgae. Journal of Phycology 35:403-424.

Holomuzki JR, Collins JP, Brunkow PE. 1994. Trophic cascades of fishless ponds by tiger salamander larvae. Oikos 71:55-64.

Hooper DU, Chapin FS, III, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge D, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3-35

Kiffney PM, Richardson JS. 2001. Interactions among nutrients, periphyton, and invertebrate and vertebrate (*Ascaphus truei*) grazers in experimental channels. Copeia 2001:422-429.

Kohler SL, Wiley MJ. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. Ecology 78:2164-2176.

Krammer K, Lange-Bertalot H. 1986. Bacillariophyceae 1. Teil: Naviculaceae. In: Süsswasserflora von Mitteleuropas*, 2/1* (Eds H Ettl, J Gerloff, H Heynig & D Mollenhauer), pp. 1-876. Gustav Fischer Verlag, Jena.

Krammer K, Lange-Bertalot H. 1988. Bacillariophyceae 2. Teil: Bacillariaceae,

Epithemiaceae, Surirellaceae. In: Süsswasserflora von Mitteleuropas*, 2/2* (Eds H Ettl, J Gerloff, H Heynig & D Mollenhauer), pp. 1–596. Gustav Fischer Verlag, Jena.

Krammer K, Lange-Bertalot H. 1991. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In*:* Süsswasserflora von Mitteleuropas*, 2/3* (Eds H Ettl, J Gerloff, H,Heynig & D Mollenhauer), pp. 1–576. Gustav Fischer Verlag, Jena.

Kupferberg S. 1997. Facilitation of periphyton production by tadpole grazing: functional differences between species. Freshwater Biology 37:427-439.

Lamberti GA, Gregory SV, Hawkins CP, Wildman RC, Ashkenas LR, Denicola DM. 1992. Plant-herbivore interactions in streams near Mount St. Helens. Freshwater Biology 27:237-247.

Lamberti GA, Gregory SV, Ashkenas LR, Steinman AD, McIntire CD. 1989. Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. Ecology 70:1840-1856.

Lehr E, Catenazzi A. (2009) A new species of minute *Noblella* (Anura: Strabomantidae) from southern Peru: the smallest frog of the Andes. Copeia 2009:148-156.

Lips KR. 1999. Mass mortality and population declines of anurans at an upland site in Western Panama. Conservation Biology 13:117-125.

Lips KR, Reeve J, Witters L. 2003. Ecological traits predicting amphibian population Declines in Central America. Conservation Biology 17:1078-1088.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier

AP, Collins JP. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences USA 103:3165-3170.

Lock MA. 1993. Attached microbial communities in rivers. In: Aquatic Microbiology: An Ecological Approach (Ed. T.E. Ford), pp. 113-138. Cambridge University Press, Cambridge.

Loeb SL. 1981. An *in situ* method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. Limnology and Oceanography 26:394-399.

Mallory MA, Richardson JS. 2005. Complex interactions of light, nutrients and Consumer density in a stream periphyton-grazer (tailed frog tadpoles) system. Journal of Animal Ecology 74:1020-1028.

March JG, Pringle CM, Townsend MJ, Wilson AI. 2002. Effects of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island stream. Freshwater Biology 47:377-390.

Marzolf ER, Mulholland PJ, Steinman AD. 1994. Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences 51:1591–1599.

Mathuriau C, Chauvet M. 2002. Breakdown of leaf litter in a neotropical stream. Journal of the North American Benthlogical Society 21: 384-396.

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC,

Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312.

McCormick PV, Stevenson RJ. 1989. Effects of snail grazing on benthic algal community structure in different nutrient environments. Journal of the North American Benthological Society 8:162-172.

McCune B, Mefford MJ. 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 4.37 MjM Software, Gleneden Beach, Oregon, U.S.A.

McDiarmid RW, Altig R. 1999. Tadpoles: The biology of anuran larvae. University of Chicago press, Chicago, Ill.

McIntyre PB, Jones LE, Flecker AS, Vanni MJ. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. Proceedings of the National Academy of Sciences104:4461-4466.

MacLeod NA, Barton DR (1998) Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. Canadian Journal of Fisheries and Aquatic Sciences 55:1919-1925.

Mendelson JR III, Savage JM, Griffith E, Ross H, Kubicki B, Gagliardo R. (2008) Spectacular New Gliding Species of *Ecnomiohyla* (Anura: Hylidae) from Central Panama. Journal of Herpetology 42:750-759.

Methvin BR, Suberkropp K. 2003. Annual production of leaf-decaying fungi in 2 streams. Journal of the North American Benthological Society 22:554-564.

Michener WK, Baerwald TJ, Firth P, Palmer MA, Rosenberger JL, Sandlin EA,

Zimmerman H. 2001. Defining and unraveling biocomplexity. Bioscience 51:1018-1024.

O'Reilly CM, Hecky HE, Cohen AS, Plisnier PD (2002) Interpreting stable isotope food webs: Recognizing the role of time averaging at different trophic levels. Limnology and Oceanography 47:306-309

Osborne PL, McLachlan AJ. 1985. The effect of tadpoles on algal growth in temporary, rain-filled rock pools. Freshwater Biology 15:77–87.

Owens M. 1974. Measurements on non-isolated natural communities in running waters. Pages 111–119 *in* R. A Vollenweider, editor. A manual on methods for measuring primary production in aquatic environments. Blackwell Scientific, Oxford, UK.

Patrick R, Reimer CW. 1966. The diatoms of North America exclusive of Alaska and Hawaii.Volume I. Monographs of the Academy of the National Sciences of Philadelphia 13:1–688.

Peckarsky BL, Cooper SD, McIntosh AR. 1997. Extrapolating from individual behavior populations and communities in streams. Journal of the North American Benthological Society16:375-390.

Petchey OL, Downing AL, Mittelbach GG, Persson L, Steiner CF, Warren PH, Woodward G (2004) Species loss and the structure and functioning of multitrophic aquatic systems. Oikos 104:467-478.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:193-320.

Peterson CG, Boulton AJ. 1999. Stream permanence influences microalgal food

availability to grazing tadpoles in arid-zone springs. Oecologia 118:340-352.

Polis GA. 1998. Stability is woven by complex webs. Nature 395:744-745.

Porter KG, Feig YS. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25:943-948.

Power ME. 1990. Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. Ecology 71:897-904.

Pringle CM, Blake GA. 1994. Quantitative effects of Atyid shrimp (Decapoda), (Atyidae) on the depositional environment in a tropical stream – Use of electricity for experimental exclusion. Canadian Journal of Fisheries and Aquatic Sciences 51:1443-1450.

Pringle CM, Hamazaki T. 1997. Effects of fishes on algal response to storms in a tropical stream. Ecology 79:2432-2442.

Psenner R. 1993. Determination of size and morphology of aquatic bacteria by Automated image analysis. Pages 339-346 *in* PF Kemp, BF Sherr, EE Sherr and JJ Cole, editors. Handbook of methods in aquatic microbiology. Lewis, Boca Raton, Florida, USA.

Ranvestel AW. 2002. The Influence of Grazing Tadpoles on Algal Biovolume, Sediment Dynamics, and Aquatic Insects in a Tropical Stream. MS thesis, Southern Illinois University.

Ranvestel AW, Lips KR, Pringle CM, Whiles MR, Bixby RJ. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biology 49:274-285.

Regester KJ, Lips KR, Whiles MR. 2006. Energy flow through early life history stages of

ambystomatid salamanders in midwestern forest ponds. Oecologia 147:303-314.

Ricciardi A, Rasmussen JB. 1999. Extinction rates of North American Freshwater Fauna. Conservation Biology 13:1220-1222.

Rosemond AD, Mulholland PJ, Elwood JW. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264-1280.

Sarnelle OJ. 1997. *Daphnia* effects on microzooplankton: comparisons of enclosure and whole-lake responses. Ecology 78:913-928.

SAS, Version 8.0 SAS Institute, Cary, NC, USA.

Savage RM. 1952. Ecological, physiological, and anatomical observations of some species of anuran tadpoles. Proceedings of the Zoological Society of London 122:467-514.

Schofield KA, Pringle CM, Meyer JL, Sutherland AB. 2001. The importance of crayfish in the breakdown of rhododendron leaf litter. Freshwater Biology 46:1-14.

Seale DB. 1980. The influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. Ecology 61:1531-1550.

Silva-Benavides AM. 1996. The epilithic diatom flora of a pristine and polluted river in Costa Rica, Central America. Diatom Research 11:105-142.

Slavik K, Peterson BJ, Deegan LA, Bowqen WB, Hershey AE, Hobbie JE. (2004) Long-term responses of the Kuparuk river ecosystem to phosphorus fertilization. Ecology 85:939-54.

Stebbins RC, Cohen NW. 1995. A natural history of amphibians. Princeton University Press. Princeton, New Jersey.

Steinman AD. 1996. Effects of grazers on freshwater benthic algae. Pages 341-373 *in* RJ

Stevenson, MLBothwell, and RL Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York.

Stelzer RS, Heffernan J, Likens GE. 2003. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. Freshwater Biology 48:1925-1937.

Sterner PC, Elser JJ, Turner, MA (2002) Effects of caddisfly grazers on the elemental composition of epilithon in a boreal lake. JNABS 21:54-63

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Waller RW. 2004. Status And trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

Suberkropp K. & Weyers H. (1996) Application of fungal and bacterial production methodologies to decomposing leaves in streams. Applied and Environmental Microbiology, 62, 1610-1615.

Suberkropp K. 1998. Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. Mycological Research 102:998-1002.

Symstad AJ, Chapin FS III, Wall DH, Gross KL, Huenneke LF, Mittelbach GG, Peters DPC, Tilman D. (2003) Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. BioScience 53:89-98.

Taylor BW, Keep CF, Hall RO Jr., Koch BJ, Tronstad LM, Flecker AS, and Ulseth AJ. 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society 26:167–177.

Tilman D, Downing J. 1994. Biodiversity and stability in grasslands. Nature 367:363-365.

Trudeau V, Rasmussen JB (2003) The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. Limnol Oceanogr 48:2194-2199.

Vanni MJ (2002) Nutrient cycling by animals in freshwater ecosystems. Annual Review of Ecology and Systematics 33:341-370.

Velji MI & Albright LJ. 1986. Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter, and kelp blade samples following pyrophosphate and ultrasound treatments. Canadian Journal of fisheries and Aquatic Sciences 32:121-126.

Velji MI & Albright LJ. 1993. Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. In: *Handbook of Methods in Aquatic Microbial Ecology* (Eds P.F. Kemp, B.F. Sherr, E.B. Sherr & J.J. Cole), pp. 139-142. Lewis Publishers, Boca Raton, FL, USA.

Villa J & Valerio CE. 1982. Red, white, and brown. Preliminary observations on the color of the centrolenid tadpole (Amphibia: Anura: Centrolenidae). Brenesia 19/20: 577-589.

Wetzel RG & Likens GE. 2000. Limnological analyses. Third edition. Springer Verlag, Berlin, Germany.

Weyers HS & Suberkropp K. 1996. Fungal and bacterial production during the breakdown of yellow poplar leaves in 2 streams. Journal of the North American Benthological Society 15:408-420.

Whiles MR, Lips KR, Pringle CM, Kilham SS, Bixby RJ, Brenes R, Connelly S, Colon-

Gaud JC, Hunte-Brown M, Huryn AD, Montgomery C, Peterson S. 2006. The effects of amphibian population declines on the structure and function of Neotropical stream ecosystems. Frontiers in Ecology and the Environment 4:27-34.

Wissinger SA, Whiteman HH, Sparks GB, Rouse GL, Brown WS. 1999. Foraging trade-Offs along a predator-permanence gradient in subalpine wetlands. Ecology 80:2102-2116.

Woodward G, Hildrew A (2002) Food web structure in riverine landscapes. Freshwater Biology 47:777-798.

Wright MS, Covich AP. 2005. The effects of macroinvertebrate exclusion on leaf breakdown rates in a tropical headwater stream. Biotropica 37:403-408.

Young RG, Huryn AD. 1998. Comment: Improvements to the diurnal upstream downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences 55:1784–1785.