EFFECTS OF AMPHIBIAN DECLINES ON TROPHIC INTERACTIONS IN ALGAL-INSECT NEOTROPICAL STREAM COMMUNITIES

by

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(Under the Direction of Catherine M. Pringle)

ABSTRACT

Understanding the effects of biodiversity declines on communities and ecosystems is one of the current grand challenges in ecology. Much research has been devoted to quantifying the effects of species loss from primary producer communities and multi-trophic communities in mesocosms, but less is known about the effects of species loss from food webs from multi-trophic communities. Studies that quantify species loss from multi-trophic communities in the field rely on observational data of populations to infer changes in the interactions between species. However, there is a lack of field data that uses direct observations of species interactions to quantify the effects of species loss on multi-trophic communities. This collection of studies utilizes stable isotopes as well as gut analyses combined with population data to quantify the effects of amphibian declines in highland Panamanian streams on food web structure of an insect-algal community. Results showed that the loss of amphibians can result in changes of resource use by grazing insect genera, but not necessarily lead to changes in their abundance. Furthermore, amphibians had a role in structuring the diatom community that grazing insects could not duplicate, providing insight for why grazing insects did not functionally compensate for grazing tadpoles. Lastly, structure of the whole food web was more resilient to species loss than expected based on models that assume fixed trophic linkages due to a reorganization of the food web, which was driven by shifting resource use and the presence of generalist consumers that immigrated into the community following amphibian declines. These studies showed shifts in resource use within individual populations, but not shifts in the topology of the whole food web, suggesting changes in food web structure maybe more detectable at finer scales, e.g. individual populations, rather than coarser scales, e.g. the whole food web. Additionally, these results highlight the potential immigrant species may have for affecting food web topology following a species loss. Together, these studies provide empirical insight into how species loss can affect food webs, challenging theoretical predictions and providing a framework for future food web research.

INDEX WORDS: amphibian declines, food webs, network, species loss, stable isotopes

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B.A., Elmira College, 2002

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

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December 2014

ACKNOWLEDGEMENTS

I owe a great deal of gratitude to my mentors, colleagues, friends, and family for their support, advice and encouragement during this journey. Special thanks goes to my major advisor Catherine Pringle for her support and patience over the years, particularly in regards to helping me become a better writer and communicator. My committee members, John Drake, Tim Wootton, Amy Rosemond and Jeb Byers, were a consistent source of guidance and inspiration, challenging me intellectually, and improving the quality for each of my dissertation chapters. A special thank you goes to Rebecca Bixby who helped me learn to identify diatoms. I also owe a thank you to those who helped me in the field (Carissa Ganong, Amanda Rugenski, and Therese Frauendorf) and in the lab (Brett Bailey, Melanie Tanner, Jillian Bass, and Rebecca Gordon) as this work could not have been completed without their help. Members, both past and present, of the Pringle, Drake, and Rosemond labs have been particularly helpful, offering encouragement and constructive advice over the years. I am grateful for the financial support, which came from the National Science Foundation (DEB #0717741, DEB #0645875, and DEB #0716680) for the first four years and teaching assistantships from the Odum School of Ecology for the final two years. Additional funding came from the Odum School of Ecology's Small Grants, the UGA Graduate School, the Robert Sheldon Memorial fund, and Sigma Xi.

I also owe a thank you to my parents for fostering (and tolerating) my interest in ecology throughout my life. Finally, and most importantly, thank you to Seyhan for her continuous support, encouragement, understanding and patience during my journey and to Alya who reminded me daily what is most important in life.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

General context – Food webs, which depict who-eats-whom in an ecological community, have a long history in ecological research. The term food chain was coined by Elton (1927) who called all food chains in a community a food cycle (now called a food web). The first food webs were used to describe terrestrial (Summerhayes and Elton 1923, 1928) and marine (Hardy 1924) communities. Lindeman (1942) generated the first food webs that showed the movement of energy through an ecosystem. However, the analysis of food webs for general principles and patterns did not emerge until the late 1970's (Cohen 1978) and was used primarily to examine the complexity-stability debate with empirical data.

The complexity-stability debate figured prominently in ecological research during the middle of the 20th century. Odum (1953), MacArthur (1955), Elton (1958), and Huchinson (1959) argued that more complex ecosystems would be more stable. However, May in a paper (1972) and book (1973) argued that complexity destabilized communities. May did suggest that species rich communities could be stable if C declined with increasing species richness. This would mean that linkage density (L/S, where L = number of links and S = number of species) would remain constant as species richness increased and provided a testable hypothesis to merge theoretical predictions with observed patterns in nature. The first studies to test this relationship used the 13 food webs from Cohen's 1978 book and showed that L/S scaled with increasing species richness (MacDonald 1979, Briand 1983, Briand and Cohen 1984). In 1986, Cohen et al.

(1986) used 113 published food webs to test the relationship between L/S and S, and reported L/S was ~2, or, each species was linked to approximately two other species.

During the late 1980’s and early 1990’s, critics of these studies focused on several key issues in food web ecology and the conclusions of the early food web studies. The first criticism was that the 113 food webs used by Cohen et al. (1986) were not complete representations of ecological communities and that the data were not compiled for food web analyses. The number of species in Cohen’s food webs ranged from 3 to 87, a far cry from the hundreds to thousands of species in many ecological communities. Second, many of the food webs were unevenly resolved, with many higher trophic level species identified to species while lower trophic level species, particularly basal species, were aggregated into a single group. A third criticism was the sampling effort for many of the food webs. Polis et al. (1991) demonstrated the sampling effort required to assemble a complete food web using surveys collected over two decades in the Coachella Valley desert in California, reporting that the number of linkages for many taxa were under sampled after more than a year of sampling. Fourth, new studies specifically compiled to examine food webs showed omnivory was more prevalent than originally found in the earlier food web analyses (Hildrew et al. 1985, Sprules and Bowerman 1988, Warren 1989, Winemiller 1990). Together, these studies undermined many of the conclusions from the early food web studies published in the late 1970’s and early 1980’s.

Food web ecologists tried to address these criticisms with additional studies that used food webs with more species and trophic linkages as well as additional analytical approaches. These higher resolution food webs sought to address many of the criticisms brought up in the 1980’s, particularly in regards to the uneven taxonomic resolution present in early food web studies. These new food webs were then tested for many of the same general principles as the first food webs. One of the first studies to address these criticisms used a food web from Little Rock Lake in Wisconsin using the literature and input from experts to show that L/S does not scale with C (Martinez 1991), undermining a major conclusion from Cohen's food webs (Cohen et al. 1986). Furthermore, a study showed that identifying all trophic linkages was unlikely to significantly change conclusions from food webs that had at least 80% of linkages identified (Martinez et al. 1999). These studies were joined by additional studies that more completely assessed food web structure from ponds (Warren 1989), streams (Thompson and Townsend 1998), lakes (Havens 1992), and a tropical forest (Reagan and Waide 1996) that also failed to support the hypothesis that L/S scales with species richness.

More recently, the complexity-stability debate has morphed into a debate on the effects of species loss on community stability (McCann 2000). Earth is currently in the midst of the sixth mass extinction event and understanding the functional consequences of species loss is one of the eight "grand challenges" identified by the National Academy of Science's National Research Council. There is growing concern that species losses will change species composition and trophic structure, negatively impacting ecosystem function (Thompson et al. 2012). Within this framework, food web studies began to use simulations to make predictions for how species loss might affect food web structure. Using the 16 most evenly resolved food webs in the literature, Dunne et al (2002a,b) showed that increasing food web complexity, measured as connectance ($C= L/S^2$), decreased the community's susceptibility to secondary extinctions. Dunne et al. (2002a,b) showed that communities with a high connectance possessed many species with many linkages and that the loss of these highly connected species would not necessarily lead to the loss of additional species (increased community robustness). However, communities with a low connectance possessed only a few species with many linkages, creating a power-law distribution of the linkage density, and making the community more susceptible to secondary extinctions (decreased community robustness) following the loss of a species with many linkages. However, critics of this approach postulated that natural systems are dynamic, with populations increasing and decreasing in response to a disturbance, such as species loss, and that remaining members of the community may also switch diets in response to a disturbance (Eklof and Ebenman 2006). More recent studies have begun to include these dynamics (population dynamics: Eklof and Ebenman 2006; trophic dynamics: Staniczenko 2010, Theirry et al 2011a,b), but these studies use models with fixed assumptions about prey type, e.g. a predator can only switch to prey that are in the same trophic level as other prey items, omitting the potential for exploitation of novel resources from other trophic levels. The conclusions from Dunne et al. (2002a,b) combined with the more recent critiques leaves a gap within the literature for an empirical study that examines the loss of a species from a natural system.

More recently, food web approaches have begun using trait diversity in models in lieu of species diversity (Reiss et al. 2009). Specifically, biomass has been used as a ‘super trait’, providing information on other traits (Brose et al. 2010) and biomass is used to examine the relationships between trophic position and the effects of consumer diversity on ecosystem function. These studies have shown that biomass can be a useful predictor for trophic relationships, but makes the assumption that organisms of similar size perform similar functions in the ecosystem. Including additional traits, such as feeding mode (e.g. filter feeder, scraper etc.) can better assess mechanisms driving food web structure (Jacob et al 2011). Furthermore, species specific differences among consumers with a similar biomass may also exist, with species specific effects on community structure (O’Connor et al. 2008, Rudolf et al. 2014).

Understanding the consequences of biodiversity loss on food webs is currently an important goal in ecology (Thompson et al. 2012). Much previous research on the effects of species loss on ecosystems has been done in mesocosms or carefully controlled field plots, but ultimately, the effects of species losses need to be studied in natural systems to include the full range of responses by complex, multispecies communities (Reiss et al. 2009). Three questions in food web ecology need to be addressed to improve our understanding of how communities respond to species loss: 1) Do surviving species shift diets? 2) Do surviving species functionally compensate for species loss? 3) How does food web structure respond to species loss? However, many of Earth's ecosystems are degraded, making empirical observations of species losses on food webs from intact, pristine ecosystems difficult. The research presented in this dissertation examines the effects of disease-driven amphibian extirpations on insect-algal food webs from two highland streams in undisturbed catchments in Panama.

Project overview – Amphibians were the most abundant terrestrial vertebrates in many tropical regions (Stebbins and Cohen 1995), but chytrid-driven amphibian declines severely reduced their populations throughout the highlands of Central America (Lips et al. 2006, Whitfield et al. 2007). The Tropical Amphibian Declines in Streams (TADS) Project is an NSF-funded collaborative effort between the University of Georgia, Drexel University, Southern Illinois University, and the University of Maryland that seeks to quantify the consequences of amphibian declines on ecosystem processes in the highlands of Panama. The TADS project has focused on three sites: Fortuna, El Cope, and El Valle. At the start of the TADS project, chytrid-related frog declines had already occurred at Fortuna (Lips 1999), which has since served as a post-reference stream for the project. The two focal sites of my dissertation, El Cope and El Valle, experienced amphibian declines in 2004 and 2006, respectively, which allowed for pre- and post- amphibian extirpation measurements to be collected.

Previous studies from the TADS project characterized the pre- and immediate post-decline communities and quantified several changes to the benthic community. These studies reported that grazing tadpoles reached densities of 50 ind m^{-2} in the focal study streams prior to their extirpation (Connelly et al. 2008, Whiles et al. 2013). Following the extirpation of tadpoles at El Cope, estimates of algal standing stock, measured as chlorophyll *a*, increased more than 4-fold and resulted in a change of diatom community structure on experimental ceramic tiles from small, prostrate taxa to larger and more erect taxa (Connelly et al. 2008). Sediment accrual also increased, more than 2x in riffle habitats and more than 4x in pool habitats, following amphibian extirpations (Connelly et al. 2008). Secondary production of macroinvertebrate functional feeding groups also shifted post-amphibian extirpation as *shredders*, *predators*, and *gatherers* decreased, while secondary production of *scrapers* and *filterers* increased post-extirpation (Colón-Gaud et al. 2010a). Furthermore, an analysis of the food web that grouped macroinvertebrates by functional feeding group revealed that grazing insects in pre-extirpation streams were algal limited (Colón-Gaud et al. 2010a), suggesting the loss of tadpoles could potentially release grazing insects from resource competition and allow them to exploit new resources in the absence of tadpoles. The changes observed in the post-decline stream also suggests that food web structure also changed following amphibian declines, but a detailed assessment of these potential changes has not been done.

These first studies from the TADS project focused on characterizing the macroinvertebrate community at the level of functional feeding groups. One study did quantify the response of genera within the *scraper* functional feeding group to amphibian declines and revealed that, although abundance and biomass of *scrapers* as a group did not change, shifts in the abundance and biomass of genera within the *scrapers* did occur, indicating that changes within the community maybe subtle (Colón-Gaud et al. 2010b). Furthermore, these studies only quantified post-decline changes in the macroinvertebrate and diatom communities in the immediate aftermath of amphibian declines (Connelly et al. 2008, Colón-Gaud et al 2010a). However, during this post-decline sampling period, there could only be 1 or 2 generations of most insect genera which have generation times of >3 months. Therefore, it could be years before the full effect of amphibian declines reverberates through the macroinvertebrate community.

The extirpation of an abundant taxonomic group, such as amphibians, offers a unique opportunity to explore the consequences of biodiversity declines on a food web from a natural system. The goal of my dissertation is to examine longer-term effects of amphibian declines on community structure at the genus level, with a focus on food web structure. My dissertation looks at community structure 2 and 5 years post-amphibian decline and is broken into three research chapters: 1) a stable isotope analysis of four abundant grazing insects to assess the level of functional compensation that may have occurred following tadpole declines, 2) an analysis of the role of grazing tadpoles and insects at regulating the diatom community and to assess potential mechanisms for the lack of functional compensation by grazing insects following amphibian declines and 3) a network analysis to quantify structural changes of an insect-algal food web of a Neotropical stream before and after the loss of its amphibian assemblage.

Chapter 2: Use of stable isotope ratios to characterize potential shifts in the isotopic niches of grazing insects following an amphibian decline in a Neotropical stream

Studies that examine the consequences of species loss tend to focus on community descriptors such as species abundance and community composition. However, the loss of an abundant species may remove a competitor from the community, allowing remaining species to exploit different resources, and potentially altering energy flow and resource use. The purpose of this chapter is to examine the effects of the loss of an abundant grazing tadpole, *Lithobates warszewitschii*, by examining changes in the isotopic signatures and gut contents from a grazing insect community that included four genera, *Thraulodes* spp., *Farrodes* spp., *Petrophila* spp. and *Psephenus* spp. This chapter is an important field-based test on how the loss of an abundant consumer from a diverse community can affect resource use.

Chapter 3: Mechanisms underlying a lack of functional compensation by grazing insects following a disease-driven amphibian decline in a Neotropical stream

The relationship between biodiversity and ecosystem function is not linear, and research efforts have started to turn towards identifying functionally significant components of an ecosystem by focusing on functional trait diversity instead of species diversity (Díaz and Cabido 2001, Petchey et al. 2004, Reiss et al. 2009). This approach assumes that the loss of a species will also result in the loss of functional traits that are important to structuring the community, precluding functional compensation by other species in the community. In this chapter, diversity of traits associated with resource use (gape size) and bioturbation (organism shape) within the grazing community, which includes tadpoles and insects, is measured and the role of tadpoles and insects on structuring the diatom community and accrual of inorganic sediment is assessed.

Additionally, structural equation modeling is used to test alternative hypotheses that examine potential causal pathways that prevented grazing insects from compensating for the top-down effects of tadpoles following amphibian declines.

Chapter 4: Evidence for the persistence of food web structure after amphibian extirpation in a Neotropical stream

Measuring the effects of species loss on food web topology can provide important insight into how species loss may affect community structure and the flow of energy through a community. Network analysis has emerged as a powerful tool for measuring how species loss can affect the food web structure of a species rich community. Theoretical studies that use network analyses assume trophic linkages are fixed, and that a researcher has identified all possible trophic linkages prior to a species loss or other disturbance. However, empirical field studies show a consumer can switch diets in response to the loss of a species, with unstudied consequences on food web structure. The purpose of this chapter is to examine the effects of amphibian declines on the structure of an insect-algal Neotropical stream food web. The importance of this chapter will be to empirically examine the effects of species loss on a food web and test whether predictions made by models that assume fixed trophic linkages can be used to predict the effects of species loss on food web structure.

CHAPTER 2

USE OF STABLE ISOTOPE RATIOS TO CHARACTERIZE POTENTIAL SHIFTS IN THE ISOTOPIC NICHES OF GRAZING INSECTS FOLLOWING AN AMPHIBIAN DECLINE IN A NEOTROPICAL STREAM[1]

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[1] Thomas R. Barnum, P. Verburg, S.S. Kilham, M.R. Whiles, K.R. Lips, C. Colón-Gaud, and C.M. Pringle. *Journal of Tropical Ecology*. 29: 291 – 299. Reprinted here with permission of the publisher.

Abstract

Neotropical streams are losing dominant consumer groups as a result of disease-driven amphibian declines. The herbivorous tadpoles of *Lithobates warszewitschii* were once abundant in the Rio Maria in the Eastern Cordillera Central of Panama, where they consumed algae and organic matter. The decline of this once-abundant grazer has the potential to affect the resources consumed by insect grazers in this system. Stable isotopes were used to characterize changes in the resource use before and after amphibian declines of four abundant insect grazer taxa: *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp. We collected 11 isotope samples of *L. warszewitschii* and 27 isotope samples of these insect taxa in 2006, and then 24 more isotope samples of the same insect taxa again in 2008, 20 mo. after a disease-driven amphibian extirpation. We also tested for potential functional redundancy of insects with tadpoles by comparing the post-decline isotopic niche of each insect taxon to the isotopic niche of *L. warszewitschii.* The isotopic niche of *Psephenus* spp., *Petrophila* spp. and *Stenonema* spp. shifted from 2006 to 2008, but none of the insect taxa in 2008 occupied the same isotopic niche as tadpoles. Our study builds on previous evidence that the ecological roles of tadpoles were not replaced through functional redundancy after amphibian declines.

Introduction

Ecosystems worldwide are facing unprecedented biodiversity declines (Pereira *et al*. 2010). Efforts to understand biodiversity losses generally focus on ecosystem descriptors such as changes in species abundances and community composition. Characterizing changes in energy flow or resource use is more difficult and consequently is usually neglected. The ratio of the stable isotopes of nitrogen (^{15}N) and carbon (^{13}C) can be useful tools for integrating temporal data to characterize the trophic level ($\delta^{15}N$) and energy source ($\delta^{13}C$) of an individual or a population (Newsome *et al.* 2007). The variation in $\delta^{15}N$ and $\delta^{13}C$ of individuals in a population can be used to characterize the δ space (or isotopic niche) of the whole population (Bearhop *et al.* 2004, Bolnick *et al.* 2003). Layman *et al.* (2007) proposed using the convex hull and the mean distance to centroid to describe the size and location of the isotopic niche of a population. The convex hull encompasses the data points of the population and gives an indication of the isotopic niche width of that population while the mean distance to centroid gives information on how similar two populations are in isotopic space. Recently, methods to statistically test for differences in the location in isotopic space of a single population over time, or between two populations, were developed (Jackson *et al.* 2011, Turner *et al.* 2010).

Stable isotopes have been used to examine community responses to species invasion (Nilsson *et al.* 2012, Vander Zanden *et al.* 1999), but not the opposite, species extirpations. Catastrophic amphibian declines occurring in Central America can potentially affect stream ecosystems (Whiles *et al.* 2006). Tadpoles consume algae and detritus and may thus compete with grazing insects (Kupferburg 1997), but they can also facilitate access to these resources for some grazing insects through bioturbation (Ranvestel *et al.* 2004). Therefore, the extirpation of grazing tadpoles could potentially affect access to the resources consumed by grazing insects, with the consequences manifested through changes in the carbon and nitrogen isotopic ratios in the grazing insects.

As part of the Tropical Amphibian Declines in Streams (TADS) project, we used invertebrate samples collected from a Panamanian stream before and after tadpole declines to provide a snapshot of the potential consequences of tadpole declines on the isotopic niches and energy sources of four grazing insect taxa. We also examined the potential for these grazing insects to replace the ecological role of the dominant grazing tadpole *Lithobates warszewitschii*. We measured biomass and examined the diet (using stable isotopes ratios and gut content analysis) of the four grazing insect taxa and *L. warszewitschii* and measured the C and N stable isotope ratios of the epilithon. Post-decline isotopic niches of the grazing insects were compared to the pre-decline isotopic niche of tadpoles to determine if grazing insects occupied a similar isotopic niche to tadpoles after the decline. We predicted that grazing insects would change diets to include more diatoms in the absence of tadpoles, and that at least one grazing insect taxon in 2008 would occupy the same isotopic niche as tadpoles from 2006.

Materials and Methods

Study site

Río Maria is a headwater stream near El Valle de Anton in the eastern Cordillera Central of Panama. Río Maria is a high-gradient stream at ~900 m asl with an average wetted width of 3.5m during the dry season (January to mid-May). Stream flow did not significantly differ between the 2006 and 2008 dry seasons (mean ± 1 SD = 22.4 ± 2.9 L s^{-1} in 2006 and 22.9 ± 2.4 L s^{-1} in 2008). The site remained undisturbed between sampling events, but within 6 mo of our 2008 sampling event a small dam was built and a construction project in the middle of the study reach prevented a second post-decline sampling event.

The tadpoles of more than 20 species were present in the pre-decline amphibian assemblage, including grazing *Hyloscirtus* spp. and *Lithobates warszewitschii.* Amphibian declines occurred at Río Maria during the 2006 wet-season (June – December) and amphibians had declined to 2% of their pre-decline biomass by February 2008 (Whiles *et al.* 2012). The remaining consumer community includes an insectivorous fish (*Brachyraphis roswithae* Meyer and Etzel), the freshwater crab *Pseudothelphusa tristani* Rathburn and ~40 aquatic insect taxa that were common in the headwater streams pre- and post-amphibian decline.

Sampling of insect biomass and resources

Insects were sampled pre-decline (February and March 2006) and post-decline (February and March 2008) along the same 100-m reach. Samples were collected during the dry season because insect biomass and abundance in Neotropical streams are highest during the dry season (Colón-Gaud *et al*. 2010a). Three stove-pipe benthic cores (314 cm^2 sampling area) were collected from depositional habitats (pools) and four Surber samples (930 cm^2 sampling area, 250-μm mesh) were collected from erosional habitats (riffles and runs). All samples were elutriated through a 250-μm sieve and placed in a bag with ~8 % formalin. In the laboratory, all insects from coarse fractions (insects with a body length >1 mm) were removed and identified to genus. Fine fractions (insects with a body length between <1 mm >250 μm) were also examined under a dissecting microscope and were occasionally subsampled (from 1/2 to 1/32) with a Folsom plankton splitter.

Taxa were classified into functional feeding groups (FFG) based on Merritt *et al.* (2008) and previous natural abundance stable isotope data (Verburg *et al.* 2007). Individual insects were measured (total body length) and biomass was estimated using published ash-free dry mass (AFDM) length-mass regressions (Benke *et al.* 1999) or regressions developed from our own specimens using methods of Benke *et al.* (1999). The AFDM was then summed on each sampling date to obtain taxon-specific biomass estimates, which were then habitat weighted according to the proportion of each habitat during base flow conditions (64% riffle/run, 36% pool). Biomass estimates from 2006 (pre-decline) and 2008 (post-decline) of grazing insect taxa were analyzed using a non-parametric bootstrap. Test statistics were the differences between 2006 and 2008 means. The means were compared to permuted means of the pooled 2006 and 2008 data which were resampled 10,000 times. P values were the percentage of permuted differences that lay outside the test statistic with $P < 0.05$ considered significant. Four grazing insect taxa, *Stenonema* spp. (Ephemeroptera: Heptageniidae), *Thraulodes* spp. (Ephemeroptera: Leptophlebiidae), *Psephenus* spp. (Coleoptera: Psephenidae) and *Petrophila* spp. (Lepidoptera: Crambidae), were selected for stable isotope and gut content analysis because they constituted >80% of the grazer biomass. Immature stages of stream insects are not well described to the species level, and thus we used generic level in our study and indicated spp. because we did not know for sure how many species of each genus were present in our study site. Based on morphological characteristics, it appeared that *Stenonema*, *Psephenus*, and *Petrophila,* were represented by one species each and there were no more than three species of *Thraulodes* present.

Epilithon was sampled for biomass and stable isotopes in February-March 2006 (pre-decline) and February-March 2008 (post-decline). A modified Loeb sampler was used to collect a sample from a known area in five to seven riffles and five to seven pools. The samples were filtered onto a glass fibre filter (GFF; particle retention size = 0.7 μm) and dried at 50ºC-60º C for 24 h to obtain dry mass (DM). Samples were habitat weighted according to the proportion of each habitat during base flow conditions (64% riffle/run, 36% pool). A non-parametric bootstrap using the methods to compare insect biomass was used to compare DM means for 2006 and 2008.

Sampling for analysis of natural isotope abundance

The taxa selected for the stable isotope analyses were the four most abundant grazing insect taxa in Río Maria and included *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp., which collectively accounted for 83% of the grazer/scraper biomass in 2006. A minimum of eight individuals of each insect taxon from each sampling date were collected for stable isotope analyses. Individuals were pooled to make sufficient biomass for an isotope sample. For example, if eight individuals were collected, there may only be enough mass for four isotope samples. Because these taxa are < 7 mm, several individuals (two to five) were pooled into a single isotope sample, but a total of 9-22 individuals were sampled per taxon. *Lithobates warszewitschii* tadpoles were also collected for stable isotope analysis in 2006. *Lithobates warszewitschii* was the most abundant grazing tadpole in Río Maria. *Hyloscirtus* spp. were also present but constituted <5% of the grazing tadpole biomass. Tadpoles of other species were not sampled because they occupy separate guilds (e.g. they live in leaf packs or are filter feeders) and we were interested in examining the consequences of the extirpation of a grazing tadpole on grazing insects. Sampling for pre-decline insects and tadpoles for isotope analyses occurred in February 2006. Tadpoles and insects were randomly sampled from the stream by net or hand-picking (picking individuals from rock with forceps) and placed on ice. A Loeb sampler was used to collect epilithon samples which were then filtered onto glass fibre filters (GFF; particle retention size = 0.7 μm). Epilithon samples were not separated into algal and non-algal components because the largest diatom, *Terpsinoe musica* Ehrenberg, would not separate from the non-algal component. Samples were kept on ice or frozen until they could be dried at 50ºC. After drying, samples were ground to a fine powder, weighed, and packed into tin capsules for δ^{15}N and δ^{13}C analysis at the University of Georgia's Odum School of Ecology Analytical

Chemistry Laboratory. Post-decline samples for isotope analysis were collected in February 2008, 20 mo after the amphibian decline began, which ensured that the insects collected for analysis had consumed resources only available well after the amphibians had declined.

Location and size of isotopic niches

Turner *et al.* (2010) used nested linear models to test for changes in the centroid location of a population over time, which could potentially indicate changes in resource use (Zeug *et al.* 2009). The centroid location for each insect grazer was tested for changes from the pre-decline date (2006) to the post-decline date (2008). The Euclidian distance between two centroids was computed and the centroids were considered to occupy different locations if the distance between the two centroids was significantly greater than zero (Turner *et al*. 2010). A parametric Hotelling's T^2 (a multivariate equivalent to the univariate t-test) test statistic was used to compare population mean vectors (Turner *et al*. 2010).

The position of the convex hull in isotopic space was identified using a statistical approach that uses a multivariate ellipse-based metric to generate a standard ellipse area (SEA). The SEA is the bivariate equivalent to the standard deviations in a univariate analysis, reducing the weight of outliers in the population, and allowing for comparisons of populations with different sample sizes (Jackson *et al.* 2011). For small populations (e.g. $N = 3$), a corrected (SEA_C) version of the SEA is an appropriate method for generating the isotopic niche area. SEA_Cs were calculated for each taxon pre- and post-amphibian declines using the methods described in Jackson *et al.* (2011) and the R package SIAR (Parnell *et al.* 2010). An unbalanced one-way ANOVA for each taxon was used to test for changes in energy sources ($\delta^{13}C$) between the 2006 and the 2008 dates.

Analysis of gut contents

Ten individuals (five individuals from 2006 and five individuals from 2008) of each of the four grazer-insect taxa (*Petrophila* spp., *Psephenus* spp., *Stenonema* spp. and *Thraulodes* spp.) and five individuals of *L. warszewitschii* from 2006 were used for gut content analysis. Gut contents were prepared using the methods of Parker & Huryn (2006) with specimens that were collected to estimate total biomass. Guts were removed under a dissecting microscope, placed in a 30-ml syringe and sonicated for 30 s. For *L. warszewitschii* a foregut segment of 8-10 mm was used. Material was then filtered onto a 13 mm, 0.45 μm pore, nitrocellulose fibre filter (Millipore HAPW01300), placed on a microscope slide, and dried at 50°C for 30 min. A drop of Type B immersion oil was used to clear the filter and filters were sealed to the slides with a coverslip and nail polish. Ten fields of view were digitized randomly with a digital camera using brightfield optics at 400× (Olympus BH-2). Pictures were quantified using ImagePro (Media Cybernetics, Inc., Silver Spring, MD, U.S.A.) and particles in each photograph were categorized as animal or plant fragments, filamentous algae, diatoms, fungi, amorphous detritus or non-algal biofilm. Non-algal biofilm is a mixture of autotrophic and heterotrophic micro-organisms in a glycoprotein matrix that is attached to stream substrata. We assumed non-algal biofilm was bacterial and did not make a distinction between the glycoprotein-matrix produced by bacteria from that produced by cyanobacteria. Gut content data from 2006 and 2008 for each insect grazer were transformed to proportions of total contents and a paired one-tailed t-test was used to assess potential changes in the proportion of each food item consumed between years.

Results

Insect biomass and their resources

Insect densities as measured by biomass did not change from 2006 to 2008 (Table 2.1) ($F_{3,54}$ = 1.625, P = 0.19) while epilithon biomass increased from 39.5 ± 6.49 g DM m^{-2} in 2006 to 93.9 ± 31.8 g DM m^{-2} in 2008 ($P < 0.05$).

Isotopic analyses of grazing insects

The standard ellipse areas of the grazing insects from 2006 did not overlap with the ellipse area of tadpoles (Figure 2.1). The total area of the standard ellipses in isotopic space that represents the isotopic niche of the grazing-insect populations did not significantly change on the post-decline date (Figure 2.2). The isotopic niche of *Psephenus* spp., *Petrophila* spp., and *Stenonema* spp. shifted on the post-decline date, but *Thraulodes* spp. did not shift (Figure 2.2). A shift in the mean centroid location for three grazing-insect taxa occurred post decline (Table 2.2) while the mean centroid location of the Loeb samples did not significantly change from 2006 to 2008 (distance = 0.57, Hotteling's T^2 = 4.4, P = 0.14). However, no insects sampled in 2008 occupied the tadpole isotopic niche. The centroid location of *Stenonema* spp. in 2008 was the most similar to the tadpole *Lithobates warszewitschii*, but the mean centroid distance between *L. warszewitschii* and post-decline *Stenonema* spp. were significantly different (distance = 1.83, Hotteling T^2 = 21.4, $P < 0.01$). The $\delta^{13}C$ of only one taxon, *Petrophila* spp. ($F_{1,11}$ = 38.2, $P < 0.001$, Figure 2.3) became significantly more enriched from the first sampling date to the second.

Gut content analysis

Lithobates warszewitschii primarily consumed non-algal biofilm, but 1.5% of its diet was animal material. The relative proportions of food items in the guts of grazing insects changed from 2006 to 2008. The proportion of non-algal biofilm increased from 2006 to 2008 in the guts of *Psephenus* spp. (n = 5, *t* = -3.1, *df* = 7.6, P = 0.007) and *Petrophila* spp. (n = 5, *t* = -2.08, *df* = 6.3, P = 0.04), and the proportion of diatoms decreased in the guts of *Psephenus* spp. (n = 5, *t* = 4.03, *df* = 7.5, P = 0.002) and *Petrophila* spp. (n = 5, *t* = 2.03, *df* = 6.2, P = 0.043) (Figure 2.4). However, the diet of *Stenonema* spp. and *Thraulodes* spp. did not significantly change (Figure 2.4).

Discussion

Our data suggest that grazing insects were not functionally redundant with respect to tadpoles because of increased epilithon, coupled with the lack of response in grazing-insect populations to tadpole declines. Additionally, the observed change in the isotopic niches of three grazing-insect taxa from 2006 to 2008 suggests that tadpoles may have influenced the grazing insect community by reducing sediment accrual on stream substrata through bioturbation (Ranvestel *et al*. 2004). However, the populations of the insect grazers did not increase as the biomass did not change from 2006 to 2008. Together, these results suggest that the response of grazing insect to amphibian declines could be more subtle than can be detected through changes in relatively coarse measurements such as total biomass.

The diets of grazing-insect taxa do not appear to be functionally redundant with grazing tadpoles as none of the four insect taxa occupied the same isotopic niche in 2008 as tadpoles in 2006. The animal material present in the guts of *L. warszewitschii* would enrich the ^{15}N of tadpoles compared to the ^{15}N of grazing insects which could limit the potential of the insects to occupy the same isotopic niche as tadpoles. However, the isotopic niche of all four insects trended towards the isotopic niche of *L. warszewitschii*, and the diets of *Psephenus* spp. and *Petrophila* spp. became more similar to the diets of *L. warszewitschii*, e.g. more non-algal biofilm and fewer diatoms. However, the biomass of *L. warszewitschii* in 2006 was more than five times higher than the grazing insect biomass in 2008, suggesting that the diet shifts of grazing insects alone do not compensate for the loss of tadpoles.

The shift in the isotopic niche of *Psephenus* spp., *Petrophila* spp. and *Stenonema* spp. was unlikely to be caused by changes in isotopic signatures of their resources because the isotopic niche of the epilithon did not change significantly between sampling dates. Gut content analyses of these three taxa revealed that they primarily consumed different proportions of the compartments in the epilithon (e.g. non-algal biofilm, diatoms and filamentous algae) but had little vascular plant material in their guts. The increased percentage of non-algal biofilm in *Psephenus* spp. and *Petrophila* spp. in 2008 may have driven the slight ^{15}N enrichment of their isotopic niches. The slight ^{15}N enrichment could have been the result of an increase in heterotrophic bacteria in their diets. The bacteria decomposing the senescent material would have been δ^{15}N enriched relative to the other epilithon, and the δ^{15}N could consequently have increased the δ^{15}N signature of the grazing insects.

Tadpoles may have also facilitated access to diatoms for small-bodied grazing invertebrates through bioturbation. The grazing insects in this study are small, with late instars 6-7 mm in size and sediment accrual may affect their movement and foraging. Tadpoles can reduce sediment accrual on stream substrata through bioturbation (Connelly *et al.* 2008, Flecker *et al.* 1999), and small-scale manipulation experiments in a similar Panamanian stream showed that baetid mayflies were more abundant on artificial substrates to which tadpoles had access compared to tadpole exclusions (Ranvestel *et al.* 2004). Tadpoles may also have facilitated diatom access by influencing the diatom community structure. When grazing tadpoles were present, the diatom community consisted of adnate diatom taxa, and then shifted to larger-bodied and more erect diatom taxa following amphibian declines (Connelly *et al.* 2008). The shift in diatom taxa may have reduced the abundance of edible diatoms available to the smaller-bodied grazers, particularly *Psephenus* spp. Further studies are needed to determine if grazing insects consume similar diatom taxa as tadpoles and if the diatom taxa consumed by grazing insects differ in the presence and absence of tadpoles.

The lack of population increases by grazing insects is consistent with previous studies in the region. In a similar stream in Panama, the Río Guabal, algal standing stocks increased following amphibian declines (Connelly *et al.* 2008), but the biomass of only one insect taxon (*Farrodes*) out of the twelve examined (including *Stenonema* spp., *Thraulodes* spp., *Psephenus* spp. and *Petrophila* spp.), increased immediately following amphibian declines (Colón-Gaud *et al.* 2010a). The lack of change in biomass of grazing insects in our study stream lends further support to the lack of functional redundancy by grazing insects with respect to tadpoles. Our results also suggest that changes in trophic pathways may occur in the absence of changes in population biomass. Notably, *Petrophila* shifted energy sources from a more algae-based diet to a diet with more non-algal biofilm.

The observed changes in the isotopic niches and diets were unlikely related to annual variation alone. Seasonal and annual changes in insect biomass are well documented in temperate streams, but tropical streams may experience less year-to-year variation because of constant temperature and light availability (Boyero *et al.* 2009). Furthermore, highland

Neotropical streams have unique assemblages compared to lowland Neotropical streams because tadpoles are the most abundant consumers while fish diversity and abundance is low. Long-term data on insect populations are not common from highland Neotropical regions (Boyero *et al.* 2009), but a 2-y study in two mountain streams, the Quebrada Chorro and the Tube stream, in western Panama (~500 km west of our focal study stream) showed no changes in the biomass or abundance of insects in the grazer, shredder, gatherer and predator functional feeding groups from the first to the second study years (Colón-Gaud *et al*. 2010b). Additionally, algal-biofilm standing stocks measured as AFDM also did not significantly change from the first to the second year (Colón-Gaud *et al*. 2010b). Furthermore, in a similar Panamanian stream, Colón-Gaud *et al*. (2010a) showed that biomass of 10 of 12 insect taxa in the scraper functional feeding group did not vary between dry seasons, even with the loss of amphibians. The limited long-term insect population data available from highland Neotropical headwater streams suggests limited inter-annual variability, and, when coupled with our own biomass data, suggest that the stable isotope patterns are not driven by annual variation.

The results of our study are significant from a methodological perspective because detecting changes in centroid locations is a relatively untested analytical method. Turner *et al*. (2010) raised the issue of the number of samples required to detect changes in the centroid location of a population and questioned whether statistically significant changes in centroid location are biologically meaningful (Turner *et al*. 2010). Our study showed significant changes in centroid location occurred in three taxa, with fewer than ten isotope samples for each taxon. The changes in centroid location for two taxa, *Psephenus* spp. and *Petrophila* spp., were also coupled with changes in gut contents, suggesting that the shifts in centroid locations were biologically meaningful. In contrast, the shift in the centroid location for *Stenonema* spp. from

2006 to 2008 was not coupled with changes in gut contents. The results of our study suggest that changes in centroid location should be interpreted with caution and should be coupled with other analyses.

While our study is limited to a single stream and two points in time, our findings are from a field-based survey, rather than mesocosms or small-scale manipulations, and therefore reflect changes in a natural community. Our study also represents a quantitative examination of a stream system at the reach scale in a biologically imperiled region, providing insight into how remaining consumers may respond to the loss of a dominant vertebrate consumer. The ongoing biodiversity crisis necessitates the need for field-based studies that examine changes in biomass and trophic pathways of an assemblage, even when only limited data are available, to fully assess the consequences of biodiversity declines.

Acknowledgements

This research was supported by National Science Foundation grants DEB #0717741 and DEB #0645875. We thank The Smithsonian Tropical Research Institute and Autoridad Nacional del Ambiente (ANAM) for providing logistical support in Panamá. We also thank the Pringle lab and two anonymous reviewers for multiple comments on previous drafts. All research detailed in this manuscript complies with the current laws of the Republic of Panama.

Table 2.1. Densities of the four focal grazing insect taxa from 2006 (pre-decline) and 2008 (post-decline) and the tadpole *Lithobates warszewitschii* from the Rio Maria in the eastern Cordillera Central of Panama. Length is the body length of individuals used for gut content analysis. The data are reported as mean±SE.

	Biomass (mg DM m^{-2})			Length (mm)		
Taxa	2006	2008	P	2006	2008	P
Lithobates warszewitschii	362 ± 210	0		5.52 ± 0.15		
Stenonema spp.	24.0 ± 11.9	11.7 ± 9.95	0.381	6.04 ± 0.86	6.13 ± 1.50	0.95
Thraulodes spp.	66.7 ± 16.7	37.0 ± 7.94	0.006	4.02 ± 0.36	3.48 ± 0.33	0.31
Psephenus spp.	9.18 ± 3.42	3.34 ± 1.65	0.003	2.58 ± 0.24	2.27 ± 0.40	0.53
Petrophila spp.	5.28 ± 1.54	16.9 ± 11.0	0.90	5.96 ± 0.65	7.22 ± 0.83	0.27

Table 2.2. Shifts in the centroid locations of epilithon and the four insect taxa from 2006 (pre-decline) to 2008 (post-decline) from the Rio Maria in the eastern Cordillera Central of Panama. The mean distance is the distance between the centroid of the isotopic niche from the first to the second sampling date for each taxon. No. samples is the number of isotope samples used in the analyses. Numbers in parentheses are the number of individuals in the stable isotope samples. Therefore, there could be one to three individuals in each isotope sample.

	No. samples				
Taxa	2006	2008	Mean distance	Hotteling's T^2	P
Epilithon	17	5	0.57	4.41	0.14
Stenonema spp.	9 (12)	4 (8)	2.72	29.28	< 0.001
Thraulodes spp.	8 (22)	8 (19)	1.05	4.93	0.13
Psephenus spp.	4 (11)	3 (9)	5.13	15.33	0.03
Petrophila spp.	6 (18)	9 (13)	6.36	41.21	<0.001

Figure Legends

Figure 2.1. Biplot of δ^{15}N-δ^{13}C of the insects *Petrophila* spp., *Psephenus* spp., *Stenonema* spp. and *Thraulodes* spp. and the tadpole *Lithobates warszewitschii* from 2006 from the Rio Maria in the eastern Cordillera Central of Panama. Each dot represents and isotope sample that could be composed of one to three individuals. The dotted lines represents the convex hulls as described in Layman *et al*. (2007) and the solid line represents the standard ellipses, the bivariate equivalent of the univariate standard deviations (Jackson *et al*. 2011). Note that there are no insect grazer taxa that overlap the isotopic niche of *L. warszewitschii* tadpoles.

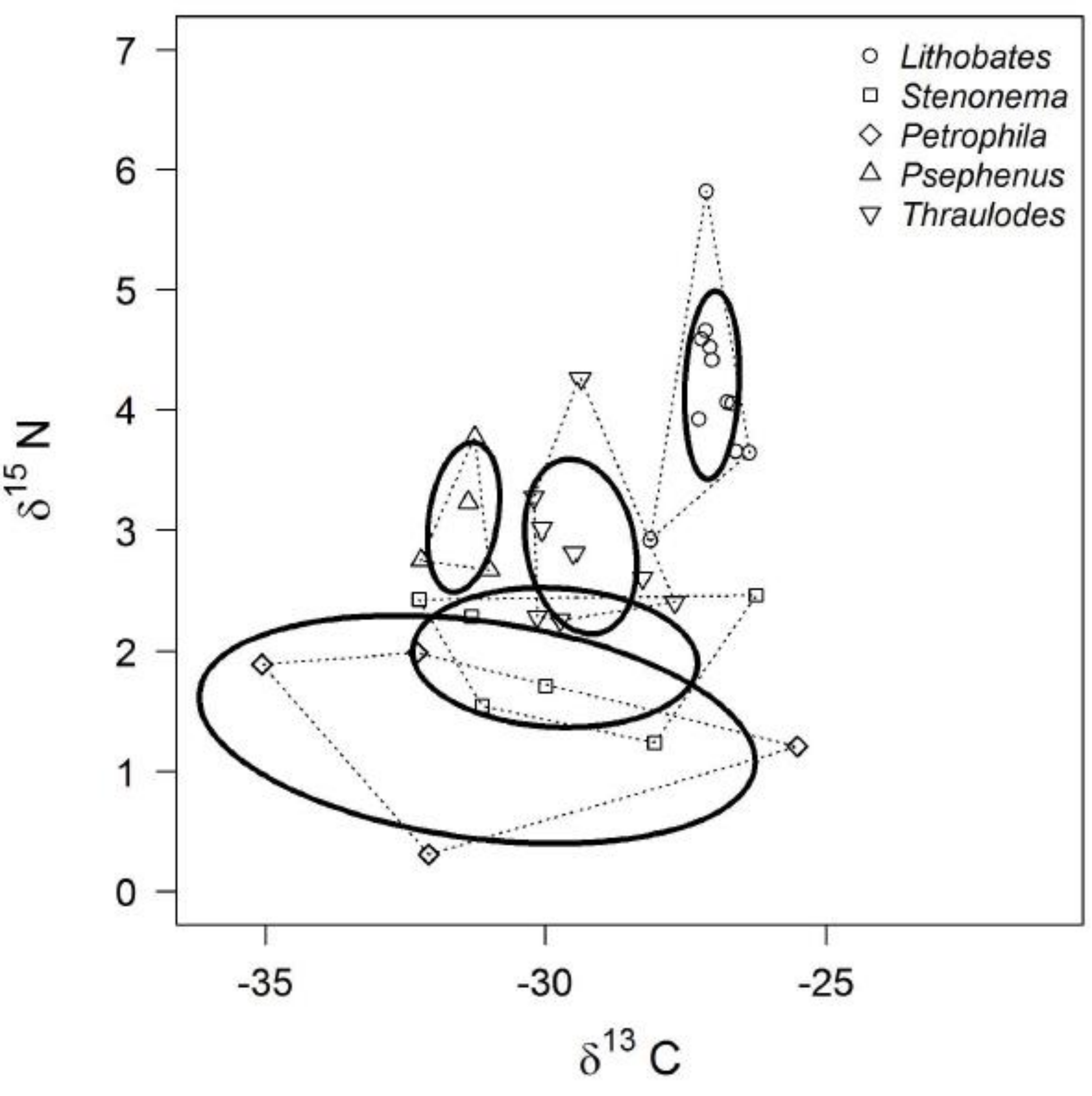
Figure 2.2. The δ^{15}N-δ^{13}C biplot for *Stenonema* spp. (a), *Thraulodes* spp. (b), *Psephenus* spp. (c) and *Petrophila* spp. (d) from the Rio Maria in the eastern Cordillera Central of Panama. Circles are individual Loeb samples and inverted triangles are individual insect sample while open points are pre-decline samples and filled points are post-decline samples. The finely-dotted lines represent the convex hulls as described in Layman *et al*. (2007). The black solid lines are the standard ellipses, the bivariate equivalent of the univariate standard deviations (Jackson *et al*. 2011), for the insect sampled. Dashed black lines are the standard ellipses for the Loeb samples. *Stenonema* spp., *Psephenus* spp. and *Petrophila* spp.occupy a different isotopic niche in 2008 compared to 2006.

Figure 2.3. Stable carbon (δ^{13}C) isotopes of the four most abundant grazing insect taxa sampled from 2006 (pre-amphibian decline, open bars) and 2008 (post-amphibian decline, hashed bars) from the Rio Maria in the eastern Cordillera Central of Panama. Carbon isotopic

ratios represent the carbon sources of an organism. Data are mean and standard deviation. Sample sizes are in Table 2. *** $P < 0.001$

Figure 2.4. Gut content analysis of the grazing insects *Stenonema* spp. (a), *Thraulodes* spp. (b), *Psephenus* spp. (c) and *Petrophila* spp. (d) pre- and post-amphibian declines from the Rio Maria in the e astern Cordillera Central of Panama. *Lithobates warszewitschii* (tadpoles) was the only taxon with animal material in its guts (not included in graph: 1.5% ± 0.6%). Solid bars are *L. warszewitschii*, hashed bars are pre-decline, and open bars are post-decline. Data are means with standard errors. AD = amorphous detritus, NAB = non-algal biofilm, Dia = diatoms, FilAlg = filamentous algae, Fun = fungi, PM = plant material, asterisks denote a significant difference in decline between pre- and post-decline diets of the insect and are not a comparison between the insect and *Lithobates warszewitschii* * $P < 0.05$, ** $P <0.01$

Figure 2.1



7
6
5
4
3
2
1
0
-35
-30
-25
δ^{15} N
δ^{13} C
○ *Lithobates*
□ *Stenonema*
◇ *Petrophila*
△ *Psephenus*
▽ *Thraulodes*

Figure 2.2

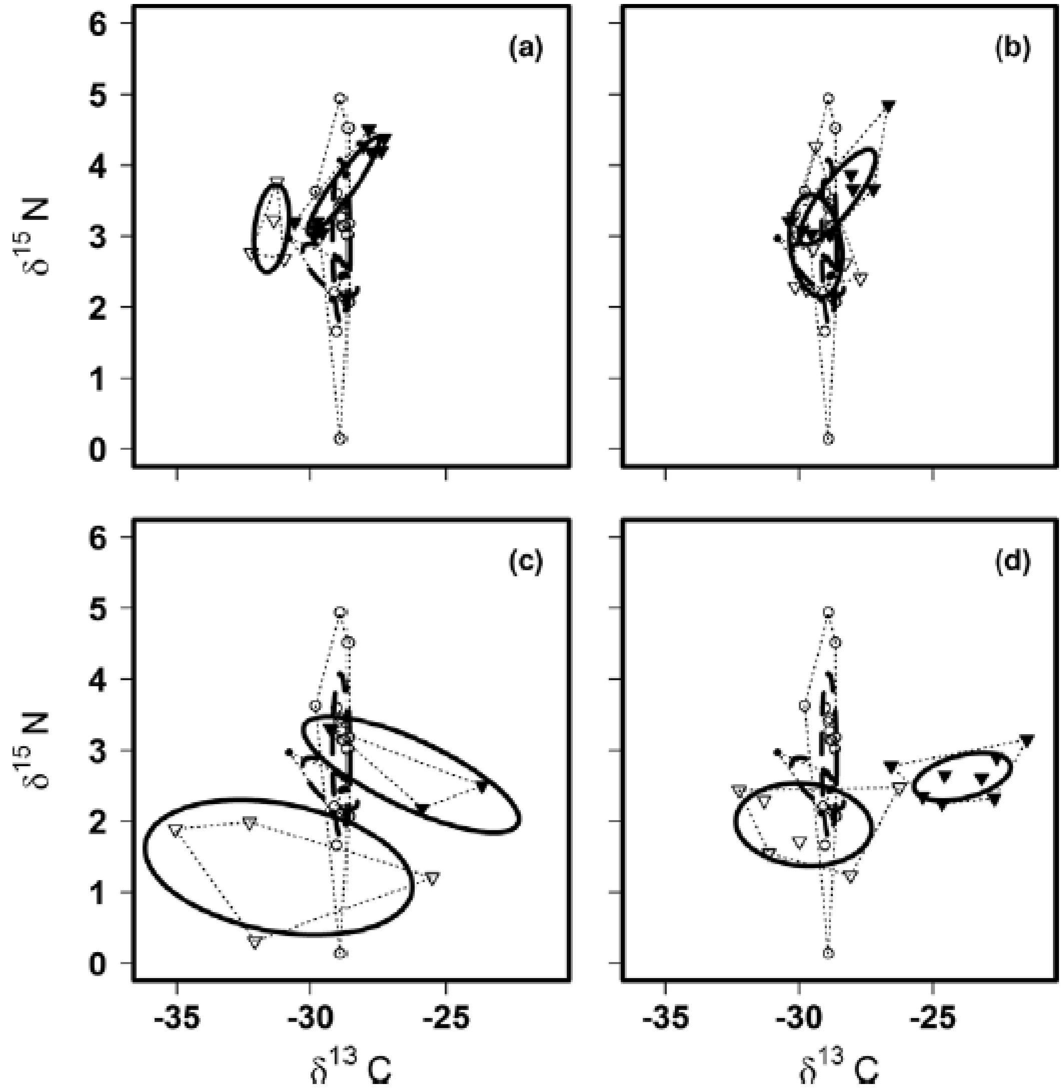


Figure 2.3

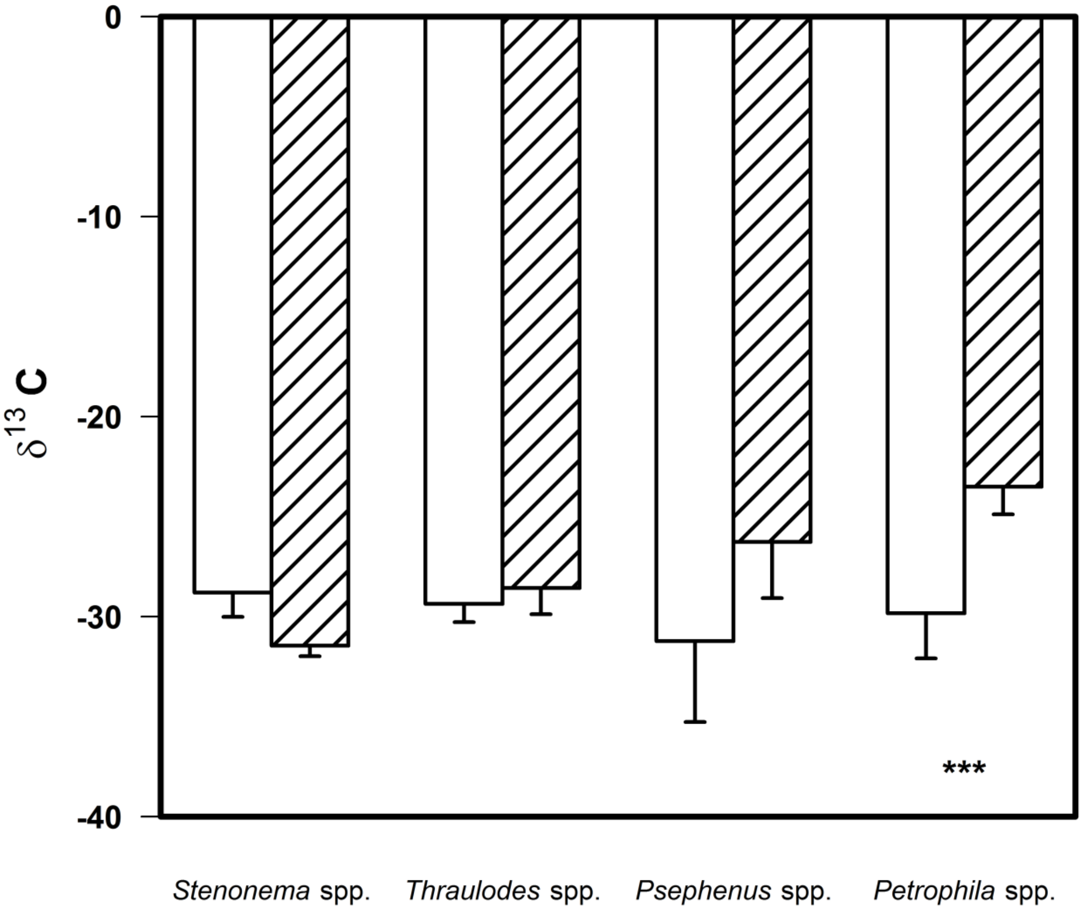
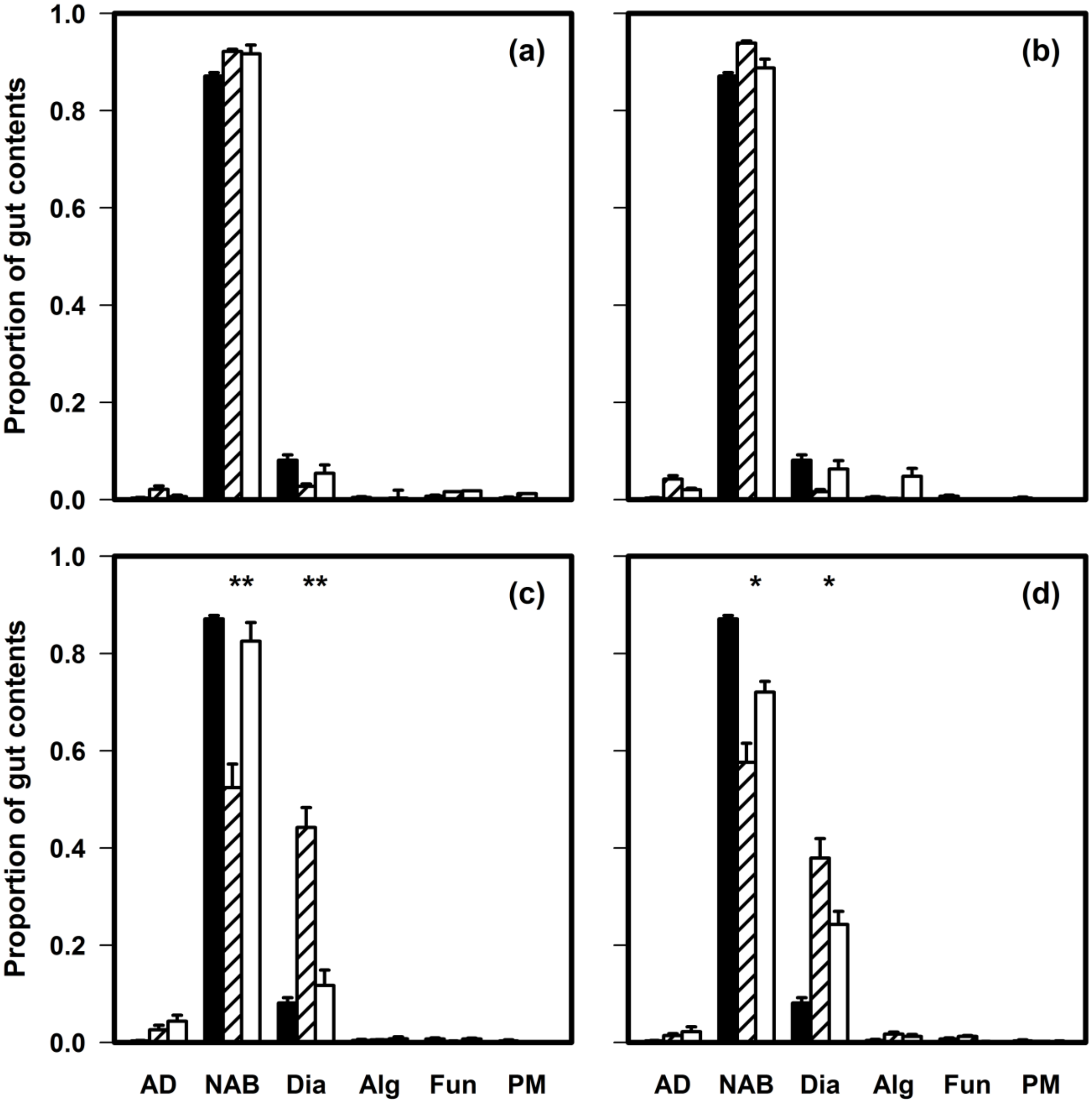

0
-10
-20
-30
-40
δ^{13}C
***
Stenonema spp.
Thraulodes spp.
Psephenus spp.
Petrophila spp.

Figure 2.4



(a)
(b)
(c)
(d)
Proportion of gut contents
1.0
0.8
0.6
0.4
0.2
0.0
**
**
*
*
AD
NAB
Dia
Alg
Fun
PM

CHAPTER 3

MECHANISMS UNDERLYING A LACK OF FUNCTIONAL COMPENSATION FOLLOWING A DISEASE-DRIVEN AMPHIBIAN DECLINE IN A NEOTROPICAL STREAM[1]

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[1] Thomas R. Barnum, J.T. Wootton, R. Bixby, J.M. Drake, D. Stoker, C. Colón-Gaud, A.T. Rugenski, T.C. Frauendorf, S. Connelly, S.S. Kilham, M.R. Whiles, K.R. Lips, and C.M. Pringle. To be submitted to *Functional Ecology*

Abstract

1. Understanding the roles of different consumers in structuring a community is essential to determining effects of declining consumer diversity on community structure. Here, we examine the role of grazing tadpoles and insects on structuring a diatom community as well as the accrual of inorganic sediment and the subsequent effects of disease-driven tadpole losses in a Panamanian stream.

2. Previous studies showed that tadpole declines led to increased algal-standing stocks, which included increases in large-sized diatoms and inorganic sediment on stream substrata. Increased algal-standing stock was predicted to have a positive effect on the abundance of grazing insects, but their abundance decreased following amphibian extirpations.

3. In this study, we examine potential mechanisms that prevented the abundance of grazing insects from increasing following the loss of grazing tadpoles. We compared grazing insect abundances and diatom community composition by collecting monthly samples over three months in 2004 (pre-extirpation) and in 2009 (post-extirpation). We measured traits of tadpoles and insects associated with grazing (gape size) and bioturbation (body shape), quantifying changes in trait diversity from pre- to post-decline. Structural equation modelling (SEM) was used to test alternative hypotheses regarding potential pathways that limited the response of grazing insects to tadpole declines. An electivity index (E*) was used to quantify changes in the selectivity for diatoms of insect grazers from pre-decline to post-decline. Tadpoles were predicted to selectively consume large-sized diatoms while insects were predicted to selectively consume small-sized diatoms.

4. The abundance of small- bodied grazing insects was lower in 2009 compared to 2004. The abundance of small-, medium-, and large-sized diatom taxa was higher in 2009 than in 2004. Trait diversity was also lower post-decline than pre-decline, driven by the loss of tadpole’s large

gape size and large body shape from the community. The SEM showed that tadpoles had stronger effects on the size structure of the diatom community than did insects. Tadpoles selectively consumed medium-sized diatoms but avoided large-sized diatoms. In contrast, insects selectively consumed the small-sized diatoms but switched to medium-sized diatoms after amphibian declines.

5. Our results showed that tadpoles' role in structuring the community could not be duplicated by insects. Surprisingly, gape size (and consequently resource use) did not play a critical role in structuring the diatom community as tadpoles did not selectively consume the diatom size classes that were most affected by grazers. Instead, tadpoles' strongest effects on diatoms appeared to be through bioturbation, an effect that insects could not replicate.

Introduction

Biodiversity loss often has a negative effect on community stability, function and productivity, but mechanisms underlying such effects remain controversial (Hooper et al. 2005, Cardinale et al. 2006, 2012). One potential explanation is that decreasing species richness can correspond to increasing the chances that the loss of any given species will disproportionately impact community structure (i.e., the sampling effect) (Loreau and Hector 2001). Species also exhibit different functional traits, morphological characteristics that can correspond to a species' role in the community. For example, gape size affects resource use, which can consequently affect community dynamics (Persson et al. 1996) and some species in a community may possess traits that have a greater effect on community structure (Burkepile and Hay 2008, Jousset et al. 2011). Quantifying functional trait diversity within a community, rather than species diversity, may allow for the detection of species that have a significant impact on community organization and provide an avenue for linking effects of biodiversity loss with declines in ecosystem function (Duffy 2009).

It is also important to understand how species loss at higher trophic levels can affect community structure and ecosystem function, given the susceptibility of higher trophic levels to extinction (Duffy 2002, Balvanera et al. 2006). Most studies linking functional trait diversity to community structure and ecosystem function have focused on species at the basal trophic level, such as primary producers (Balvanera et al. 2006, Duffy et al. 2007). However, consumers at higher trophic levels also possess traits which may have a significant impact on community structure (Rasher et al. 2013). In particular, a consumer within a grazing assemblage may have traits that can strongly influence the structure of the plant community (Oliff and Ritchie 1998, Knapp et al. 1999).

Larval amphibians (tadpoles) in the headwater streams of Central America are part of the grazing community, which also includes invertebrates (e.g., insects), and may possess traits that can significantly influence the structure of the diatom community. Prior to their extirpation by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), tadpoles represented the most abundant, large-bodied benthic consumers in this community, consuming biofilm consisting of diatoms, algae, bacteria, and detritus (Ranvestal et al. 2004, Barnum et al. 2013). Grazing insects had diets generally similar to those of tadpoles (Barnum et al. 2013, Frauendorf et al. 2013), but insect grazers were much smaller than tadpoles, with most late instar larvae < 7 mm in length. Grazing tadpoles may also affect insect grazers by facilitating access to resources, through sediment removal (bioturbation), for small-bodied insects (<3.5 mm) and competing for resources with large-bodied insects (>3.51 mm) (Ranvestal et al. 2004, Colón-Gaud et al. 2010).

Previous studies from the Tropical Amphibian Declines in Streams (TADS) project showed that amphibian declines in the highlands of Panama caused long-term increases in algal-standing stocks and sediments over a 4 year period (Connelly et al. 2008, Connelly et al. 2014). In response to increased algal resource availability, populations of insect grazers, particularly mayflies (Ephemeroptera) were predicted to increase, and at least partially compensate for the loss of amphibians (Colón-Gaud et al. 2010). However, the abundance of most grazing insect taxa did not increase following tadpole declines (Colón-Gaud et al. 2010), and either declined or remained similar five years post-decline (Barnum et al. *submitted*).

We addressed three objectives in this study to determine why insect grazers did not functionally compensate for the loss of tadpoles. Objective 1 examined if the loss of grazing tadpoles resulted in a loss of trait diversity from the grazing community. We hypothesized that tadpole extirpation would cause a decrease in trait diversity within the grazing community, with the decrease driven by a decline in gape and shape size. Objective 2 compared diets of grazing tadpoles and insects on three different size classes of diatoms both pre- and post-decline. We hypothesized that small-bodied grazing insects were gape-limited, preventing them from consuming the large-bodied diatoms, whose populations increased following amphibian declines (Connelly et al. 2008). We predicted that large-bodied grazing insects and tadpoles would selectively consume the largest diatoms, but that small-bodied grazing insects would avoid the largest diatoms. Objective 3 used structural equation models (SEM) to evaluate alternative causal hypotheses for why grazing insects did not compensate for the loss of grazing tadpoles. The results from our study provide empirical insight, from a natural system, into how the loss of trait diversity from a higher trophic level (grazing consumers) can impact community structure.

Methods

Study system

The Rio Guabal is located at ~750 m a.s.l. in the Parque Nacional G.D. Omar Torrijos Herrera, El Copé, Coclé, Panama (8º40’N, 80º35’W). Sampling occurred along a 400 m transect characterized by riffle/pool sequences with a boulder/cobble substrate and canopy coverage >80% (Connelly et al. 2008). Prior to the arrival of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), the stream-dwelling amphibian assemblage was characterized by more than 23 species, with *Hyloscirtus colymba, Hyloscirtus palmeri,* and *Lithobates warszewitschii* as the most common grazing tadpole species. *Brachyraphus roswithae* (pelagic fish consumer) and *Trichomycterus striatusan* (an uncommon benthic fish consumer), *Pseudothelphusa* sp. (crab), and *Macrobrachium* spp. (shrimp) were also present in the stream, but had no significant effect on the benthic algal-community (Connelly et al. 2008) and were therefore excluded from this study.

Diatom/insect/tadpole communities

Diatom, insect and tadpole communities were sampled monthly in the Rio Guabal over 3-mo periods in the dry season both pre- (Jan-Mar 2004) and post- (Feb-Apr 2009) amphibian decline. Diatom abundance and inorganic sediment samples were collected each month using a Loeb (4.25 cm^2) sampler from five pools and five riffles along a 400 m reach. A subsample (100 mL) was used to measure inorganic sediment and a second subsample (20 mL) was preserved for diatom identification and enumeration using the methods in Connelly et al. (2008). Diatoms were prepared for identification by placing 2.5 mL of the preserved sample in 2.5 mL of 30% H_2O_2 for 24 h, then rinsing with deionized water five times to remove oxidation byproducts. The processed samples were then evaporated onto coverslips and mounted to microscope slides with Meltmount (Cargille-Sacher Laboratories, Inc.). Samples were examined under oil immersion at 1000x along transects until 600 valves were enumerated or until ten transects were examined. Valves were identified to genus based on the taxonomic literature (Bourrelly and Manguin 1952, Foged 1984, Silva-Benavides 1996). Cell biovolume for each genus was calculated by measuring 10 valves and applying them to published geometric equations (Hillebrand et al. 1999). The abundance of diatoms on substrata was then estimated from the subsample's volume using a standardized surface area (cm^2). Diatom genera were placed into one of three size classes: small-sized (1-1000 μm^3), medium-sized (1001-10,000 μm^3), and large-sized (<10,000 μm^3).

Twelve genera of grazing insects were sampled from one 200 m reach within the 400 m reach where diatoms were sampled. Six pools were sampled with a core sampler (314 cm^2 sampling area) and eight riffles were sampled with a Surber sampler (930 cm^2, 250-µm mesh). Macroinvertebrates were identified to genus and total body length was measured to the nearest 0.1 mm. Genera were separated into two size classes based on body length: <3.5mm and >3.51 mm. Size class criteria were based on previous studies that suggest tadpoles are resource facilitators for small-bodied insects but resource competitors for large-bodied insects (Ranvestel et al. 2004, Colón-Gaud et al. 2010). Haphazard sampling was done to collect additional insect specimens for gut content analysis in April 2009 to augment the low number of individuals collected during the post-decline sampling period.

Grazing tadpoles (*Hyloscirtus* and *Lithobates*) were sampled using methods from Heyer et al. (1994). Each month, six random samples were collected, three from each habitat type (pools and riffles) in the 400 m stream reach where diatom samples were collected. Riffles were sampled by manually disturbing substrata and holding a 250-µm mesh D-net (22 x 46 cm) immediately downstream of the sampled area. Pools were sampled with a stove-pipe benthic corer (22 cm diameter). Numbers of tadpoles collected were corrected by sample area to estimate tadpole densities.

Trait and diet assessment

For the twelve insect genera and two tadpole genera, we measured four morphological traits (gape size, head capsule width, body length, and body biomass) of at least six individuals based on the assumption that these traits were the most important in terms of mediating community structure, specifically, diatom community composition and the accrual of inorganic sediment. Gape size was selected because it relates to resources consumed (e.g., mean cell size of diatoms consumed) (Persson et al. 1996, Carnicer et al. 2009, Rudolf and Lafferty 2011) and we predicted that the small gape size of small-bodied insect grazers would limit their consumption of the largest-sized diatom genera. The product of the head capsule width and biomass (HCxBio) relates to an organisms' shape and was hypothesized to relate to bioturbation (e.g., reduced sediment accrual). The HCxBio was selected as a bioturbation trait because it allowed us to account for the mass and width of an organism – particularly as insect taxa varied in shape, i.e. cylindrical or flat-bodied. Locomotion can also decrease sediment accrual, but we chose to use traits that could be quantified to take advantage of recently developed multi-dimensional methods for estimating functional diversity (Villeger et al. 2008; Laliberte and Legendre 2010).

Three traits, body length, head capsule width, and gape size, were directly measured to the nearest 0.01mm using a dissecting scope at 20× with an ocular micrometer eyepiece (scale length 1mm with line width 0.01 mm). Body length of insects included the length from the labrum to the most distal tergite, while body length of tadpoles was measured as the snout to vent length (SVL) (McDiarmid and Altig 1999). Body length was then used to estimate the biomass (measured as AFDM [ash-free dry mass]) for each individual using published length-mass regressions (Benke et al. 1999) and length-mass regressions developed with our own specimens (Colón-Gaud et al. 2010). Head capsule width for macroinvertebrates and tadpoles was determined by measuring the distance between the outer parts of the eyes. The gape of the tadpole was obtained by measuring the tadpole's jaw sheath transversely (McDiarmid and Altig 1999). Macroinvertebrate gapes were measured transversely after removing the mandibles, labium, and hypopharynx, which exposed the opening of the mouth.

After traits were measured for each specimen, the gut was removed, suspended in 5 mL of deionized H_2O and sonicated for two minutes. The sample was then filtered onto a 13 mm nitrocellulose membrane, which was then placed onto a microscope slide, dried at 40°C for twenty minutes, cleared with a drop of type B immersion oil and sealed with a coverslip and nail polish. For tadpoles, only the first 5 – 10 mm of the foregut were filtered and analyzed. Gut contents were viewed at 1000× using bright field optics on measured transects until 300 diatoms were identified to genus or ten transects were completed. Diatom valves are often broken when consumed; therefore, for each diatom, the percentage of the valve relative to the whole valve was estimated (e.g., 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%). These estimates were then extrapolated to whole-valve units to estimate the number of whole diatom cells in each gut.

Statistical analysis

The abundance for each group of tadpoles, insects, and diatoms and the weight of inorganic sediment were compared between sampling years with a randomization test. For each group, the difference between the mean pre- and post-extirpation abundance was compared to its null distribution, which was built from the difference between the means of three pre- and three post-extirpation values obtained from resampling with replacement the pooled pre- and post-extirpation monthly abundance data. The null distribution was generated from resampling the data 10,000 times. P-values were the percentage of permuted differences that lay outside the test statistic (observed difference between pre- and post-decline sampling dates) with $P < 0.05$ considered significant.

Objective 1: Trait diversity (gape size and HCxBio) indices in the grazing community were calculated from the abundance data for grazing insects and tadpoles that were collected three months pre-decline (2004) and three months post-decline (2009). The mean abundance for each size class (small: <3.5 mm; large: >3.51 mm) for each genus for the pre- and post-decline sampling periods were calculated. Additionally, the mean body length for each size class for each genus was calculated. This mean body length was then used to estimate the gape size and head capsule width for each size class for each genus using regressions developed from the data collected from the individuals used for gut content analysis while the biomass was estimated using length-mass regressions from Benke et al. (1999) and our own data. The estimated biomass and head capsule width were then multiplied to get the value for the HCxBio trait. Collinearity among traits was tested using a variance inflation factor (vif), which is the factor that the standard errors (the variance) are inflated, with 5 as a cutoff (O’Brien 2007).

The trait diversity of the grazing community was measured using four indices: (1) functional richness; (2) functional divergence; (3) functional evenness; and (4) functional dispersion. Functional richness (FRic) is the portion of trait space occupied by the community, while functional divergence (FDiv) is a measure of the proportion of species abundances that are supported by extreme functional traits (Villéger et al. 2008). Functional evenness (FEve) measures changes in the distribution of species abundances in functional space by measuring the minimum spanning tree that links all species. Functional dispersion (FDis) is the weighted mean distance of individual species (or groups) in the community to the centroid of all species in multidimensional trait space, and simultaneously measures trait dissimilarity and evenness within the community (Botta-Dukát 2005, Laliberté and Legendre 2010). FDis is weighted by species abundances, shifting the centroid towards species that are more abundant. Communities with high FDis are composed of evenly distributed, dissimilar traits while communities with low FDis are composed of unevenly distributed, similar traits.

A null model was used to test if the changes observed in trait diversity were greater than expected by chance. We randomized the observed abundances ($n = 30$ for pools, $n = 48$ for riffles) from pre-decline and post-decline for groups (small-and large-bodied insects as well as tadpoles) to test if differences in trait diversity between pre- and post-decline were greater than expected by chance. The observed monthly abundances for each group were randomized, with replacement, and reassigned to groups so that a new pre- and post-decline mean for each group was generated. The four trait measures, FRic, FEve, FDiv, and FDis, for the new pre- and post-decline means were calculated and the randomization procedure repeated 9999 times, with the difference of the four trait metrics saved after each iteration. The probability of rejection was the number of random values smaller or equal to the observed value, plus 1 (making the observed value part of the null distribution) divided by 10,000. A value of $P < 0.05$ indicated that the differences between pre- and post-decline were greater than expected by chance.

Objective 3: A relativized electivity index (E*) was used to compare the proportion of prey in consumer guts to the proportion of prey in the environment (Vanderploeg and Scavia 1979, Lechowicz 1982). Values for E* range from -1 to 1, where a positive number indicates a prey item is selectively consumed while a negative number indicates that the prey item is avoided. A value near 0 indicates the prey item is consumed in proportion to the environment. The E* index is less reliable when large numbers of prey taxa are available or when prey taxa are rare in the consumer's gut (Lechowicz 1982). Therefore, prey taxa were only included in the analysis if they accounted for ≥1% of either the consumer's diet or the prey population (Parker 1994). The value of E* was estimated for each of the three diatom size classes for small- and large-bodied grazing insects and tadpoles to assess insect and tadpole selectivity for diatom size classes pre-decline and potential shifts by insects post-decline. Grazing insects were separated into a group based on their body length (either >3.51 mm or <3.5 mm), which consequently removed taxonomic classifiers. By separating grazing insects into size classes, we could detect potential changes in the size of diatoms selectively consumed from pre- to post-decline sampling dates. The gut contents for a total of 346 insects and 18 tadpoles were analyzed and an E* for each individual was calculated; the mean ± SE for each size class in pools and riffles for pre- and post-decline sampling periods was then reported.

Objective 3: Structural equation modeling (SEM) was used to examine alternative hypotheses for why grazing insects did not respond to tadpole declines. SEM uses variance-covariance matrices between predictor and response variables to examine causal hypotheses between members of a community or network (Grace 2006). The robustness of the SEM is based on the model fit (i.e., a chi-square) to the data, rather than on individual significant tests within the model (Grace 2006). Several alternative models are built with the data to describe plausible causal pathways between compartments to test which structure might best describe the data. Alternative models maybe very similar in structure, including many of the same pathways, but have subtle differences, such as reversing the direction of a pathway between two compartments. Models are then compared using their AIC_c and the model with the lowest AIC_c is selected as the most parsimonious model of the system.

For our study stream, we constructed SEMs separately for pools and riffles that tested three potential hypotheses that explained a lack of compensation by grazing insects to amphibian declines. First (hypothesis 1), insect abundances may have been negatively affected by increased inorganic sediment on stream substrata that accrued after amphibian declines, which can inhibit grazing insect foraging and mobility. Second (hypothesis 2), gape-limitation prevented grazing insects from exploiting the increased abundance of large-bodied diatoms. The increased abundance of large-bodied diatoms was hypothesized to have a negative effect on small-bodied diatoms, further reducing the availability of resources available to insects and thereby having an overall negative effect on insect abundance. Third (hypothesis 3), tadpoles have a top-down effect on the abundance of diatoms and inorganic sediment, while grazing insects have no effect on the abundance of diatoms and inorganic sediment. Hypotheses 1 and 2 tests for indirect pathways by which the loss of amphibians may have negatively affected the abundance of grazing insects, while Hypothesis 3 tests the direct effects of different groups (tadpoles and small- as well as large-bodied grazing insects) to structure the diatom community.

The models were constructed using the abundance of tadpoles, insects, diatoms, and inorganic sediment. Since the distribution of tadpoles in the stream was heterogeneous, with many samples yielding no specimens, tadpoles were treated as either present or absent in the SEM. For insects, the mean abundance for each size class (<3.5 and >3.51) for each sample was used, with 36 samples (3 cores × 2 stream reaches × 3 months × 2 years [2004: pre-decline, 2009: post-decline]) and 48 samples (4 Surbers × 2 stream reaches × 3 months × 2 years [2004:

pre-decline, 2009: post-decline]) for pools and riffles respectively. For diatoms and sediment, there were 30 samples (5 Loebs × 3 months × 2 years [2004: pre-decline, 2009: post-decline]) for each riffle and pool. Within each model, tadpoles were the exogenous variable (an independent variable that influences other variables but is itself not influenced by other variables) while large-bodied insects (>3.51 mm total body length), small-bodied insects (<3.5 mm total body length), sediment, small-bodied diatoms (<1000 μm^3), medium-sized diatoms (1001 – 10,000 μm^3) and large-bodied diatoms (>10,000 μm^3) were the six endogenous variables (variables influenced by the exogenous variables and other endogenous variables). All SEM's were run in the R package *lavaan* (Rosseel 2012) using Full Information Maximum Likelihood (FIML) as an estimator, which allows for uneven sample sizes of the variables (i.e. there were 48 insect and 30 diatom samples in riffles). In each model, the relationship between two variables was considered significant at the 5% level. Results of the SEM are presented as standardized path coefficients, which show the strength of the relationship (a causal linkage) between variables (compartments) in the model (Grace 2006). For example, the model tests for specified relationships between variables. If the relationship between two variables is significant, a line is drawn connecting the two variables in the path diagram and the standardized path coefficient is reported next to this line. In our path diagrams, causal linkages that are significant are reported using a solid line but use a dashed line if the causal linkage is not significant.

Results

Abundance of small-bodied insects (<3.5 mm) was lower in pools during the post-decline versus pre-decline sampling period, while the abundance of large-bodied insects (>3.5 mm) did not significantly change between sampling periods in pools or riffles (Fig 1) (Appendix A).

Conversely, populations of small-, medium-, and large-sized diatoms were greater in the post-amphibian decline sampling period compared to the pre-decline sampling period (Appendix A) (mean and SE for individual taxa reported in Appendix C). The pattern of higher abundance of diatom groups differed between pools and riffles, with small-sized diatoms increasing the most in riffles and large-sized diatoms increasing the most in pools (Fig 1).

Indices of functional trait diversity were lower in the post- (2009) versus pre- (2004) decline sampling period. Under the null model, post-amphibian decline (1) functional richness was lower than expected in pools (Fig 2.)(2004: 100%, 2009: 0.019%, $P = 0.005$) and riffles (2004: 100.0%, 2009: 2.2%, $P = 0.001$); (2) functional divergence was not lower than expected in pools (2004: 76.6%, 2009: 69.1%, $P = 0.23$) or riffles (2004: 71.1%, 2009, 95.8%, $P = 0.99$); (3) functional evenness was not lower than expected in pools (2004: 10.2%; 2009: 20.5%, $P = 0.95$) or riffles (2004: 5.7%, 2009: 13.4%, $P = 0.96$) and (4) functional dispersion (FDis) was significantly lower than expected in pools (2004:27.9%, 2009:5.2%, $P = 0.04$), but not in riffles (2004:14.7%, 2009: 3.0%, $P = 0.33$).

Gut content analyses revealed differences in the size classes of diatoms consumed by grazing insects from the pre-decline sampling date to our post-decline sampling date. Gut content analyses also showed that *Hyloscirtus colymba* tadpoles did not consume as many diatoms as did many insect genera (Tables 1 and 2). Gut contents of insects showed that several genera from small- and large- size classes, particularly in riffles, consumed more medium-sized diatoms in 2009 compared to 2004 (Table 1 and 2). The electivity index (E*) showed that tadpoles preferentially consumed medium-sized diatoms (1001 -10,000 μm^3) but not large-sized diatoms (Fig. 3). During our sampling period in 2004, when tadpoles were present, grazing insects selected small-sized diatoms ($<1000\mu m^3$) (Fig. 4). However, in our sampling period in 2009, grazing insects selectively consumed medium-sized diatoms (1001 -10,000 μm^3) (Fig. 4).

The SEM that hypothesized that tadpoles had an effect on the abundance of diatoms and inorganic sediment while insects had none (Hypothesis 3), fit the available data better than the model that hypothesized that inorganic sediment accrual limited grazing insect population recruitment (Hypothesis 1: Path diagram in Appendix B) (Riffles: $\Delta AIC_c = 21.05$, Pools: $\Delta AIC_c = 30.56$), while the model that tested resource limitation (Hypothesis 2) failed to fit the data ($\chi^2 = 0.000$, path diagram in Appendix B). Tadpoles negatively affected sediment accrual as well as the abundance of small- and large-sized diatoms in pools (Pools: $X^2 = 1.21$, $AIC_c = 1335.4$, df = 3, p = 0.75), and small-sized diatoms in riffles (Riffles: $X^2 = 0.667$, $AIC_c = 1796.8$, df = 4, p = 0.95) (Fig 5). In contrast, grazing insects had no effect on inorganic sediment accrual or on the abundance of any diatom size classes in pools or riffles (Fig 5).

Discussion

Our results suggest that grazing insects were unable to duplicate grazing tadpoles' role in structuring the benthic community and thus could not functionally compensate for the loss of tadpoles. Results supported our hypothesis that trait diversity in the grazing community would be lower after amphibian declines. Furthermore, grazing tadpoles had stronger top-down effects on the diatom community than insects (Fig. 5). Together, these lines of evidence strongly suggest that tadpoles possessed functional traits that were absent in insects, and that these traits likely had strong effects on structuring the diatom community, precluding functional compensation by insects following amphibian declines.

Results supported our prediction that small-bodied insect grazers would selectively consume small- and medium-sized diatoms, but would not consume large-sized diatoms (Fig 4). However, grazing tadpoles and large-bodied insect grazers did not selectively consume large-sized diatoms either (Fig. 3 and 4), indicating gape limitation was not the mechanism that limited the top-down effects of insect grazers on large-sized diatoms. The most abundant large-sized diatom was the chain-forming *Terpsinoe musica*. These diatoms have thick frustules made from silica, and their high silica content may have made this species resistant to grazing from all consumers. However, we do not have estimates of the silica content for *Terpsinoe* or other diatoms in the community that would be required to further test this hypothesis. Selective foraging is documented in other systems (Knapp et al. 1999) and may have a significant role in structuring a community (Pastor and Naiman 1992). Our results suggest that identifying a consumer's preferred prey may be more than a function of prey size.

In pools, tadpole effects on diatom community structure and inorganic sediment appeared to be primarily due to bioturbation rather than direct consumption. Tadpoles had a significant negative effect on the abundance of large-sized diatoms (Fig. 5) but the prediction that tadpoles would selectively consume large-sized diatoms was not supported (Fig 3). The negative effect of tadpoles on large-sized diatoms is likely to be caused by dislodgement of large diatoms while feeding which is consistent with effects of other benthic herbivores that have rasping or scraping mouthparts (Rosemond 2000). In addition, inorganic sediment constituted a small fraction of the gut contents of these tadpoles (Barnum et al. 2013, Frauendorf et al. 2013). Together, these lines of evidence suggest that bioturbation, and not resource use, was the primary mechanism by which tadpoles regulated the diatom community and the accrual of inorganic sediment.

In riffles, tadpoles only had a negative effect on small-sized diatoms, possibly through the combined effects of consumption and bioturbation. The reduced effects of tadpoles in riffles may have been driven by two factors: lower densities and stream flow. Water flow varies between pools and riffles, with pools experiencing slow flow and riffles experiencing faster flow. The faster flow found in riffles could impact the diatom community in a similar manner as grazers, reducing the abundance of large-sized diatoms and sediment accrual, while exposing adnate diatoms to light and nutrients (Passy 2007). The lower tadpole abundance in riffles compared to pools may also have an important role in reducing the effects that tadpoles had on the abundance of diatoms. In our system, the abundance of tadpoles in riffles was 75% lower compared to pools and may have been a major contributor to the reduced effects of tadpoles on diatoms. However, flow data specific to riffle habitats would be required to partition the exact roles of tadpoles and stream flow on diatom communities.

Including species abundances, rather than just the presence or absence of species traits, may be a key factor to understanding the ultimate consequences of consumer losses on ecosystems. Functional richness (FRic) in riffles and pools was lower post-decline verses the pre-decline sampling dates. However, FRic does not use abundance and can be prone to outliers (Laliberte and Legendre 2010). Functional dispersion (FDis) includes species abundances and trait richness, but only declined in pools, where tadpoles also exerted stronger top-down effects on the abundance of diatoms and inorganic sediment (Fig 3a). FDis did not decline in riffles, but riffles had lower densities of tadpoles than pools (Appendix A) where tadpoles also had weaker effects on the diatom community and inorganic sediment (Fig 3b).

The presence of tadpoles may also have led to niche complementarity, where the presence of one species allows for greater uptake of resources by the community (Fonara and

Tilman 2008, Harpole and Tilman 2007). Previous research showed that when tadpoles were present, the uptake of algal-production by consumers was nearly twice of what was available, but resource uptake by consumers declined to only 35% of what was available immediately following tadpole declines (Colón-Gaud et al. 2010a). The electivity index suggests that tadpoles and algal-grazers may have partitioned diatom size classes in 2004; grazing insects selectively consumed the smallest diatoms when tadpoles were present (Fig. 5) while tadpoles selectively consumed mostly medium-sized diatoms (Fig. 4). In the absence of tadpoles, grazing insects switched diets and preferred to consume medium-sized diatoms, particularly in riffles (Fig 5). The switch in selectivity to medium-sized diatoms was partly driven by an increase in the populations of some medium-sized diatom taxa, such as *Pinnularia* and *Synedra*, which were also consumed more often in the absence of amphibians (Table 1 and 2).

Our results show the utility of using SEMs to examine interactions between groups in a community that are difficult to observe directly. Previous manipulative experiments showed that tadpoles have strong, negative effects on the abundance of diatoms and the accrual of inorganic sediment (Ranvestal et al. 2004, Connelly et al. 2008), but these experiments were not designed to partition the effects of insects and tadpoles on these groups. Using SEMs allowed us to compare the effects of tadpoles and insects on the abundance of diatoms and the accrual of inorganic sediment, showing that insects have no effects on either group (Fig 3). However, lack of effect by grazing insects on sediment and diatoms in the SEM was not experimentally tested in our study stream, which is encouraged to make strong causal inferences (Wootton 1994). The results are supported by studies from other regions. For example, in Puerto Rico, mayflies in the same families as those in our study stream did not have a significant impact on sediment accrual (Cross et al. 2008).

Other factors that were unaccounted for in our analyses may have impacted the algal community and accrual of inorganic sediment. For example, traits, such as an organism's mobility, may have reduced the accrual of inorganic sediment. However, the impact of mobility would also have been related to body size (Queirós et al. 2013), e.g., small, fast moving organisms, such as the mayfly *Baetodes*, would have a reduced effect on sediment accrual compared to a large, fast moving organism. Mouthpart structure may also be another important trait. Algal-grazers with rasping or scraping mouthparts often have the strongest negative effects on algal biomass and community structure (Kohler and Wiley 1997, Gelwick and Matthews 1992, Holomuzki et al. 2006). The tadpoles, which had the strongest effects on the diatom community, had rasping mouthparts. However, some mayfly taxa in our study stream, such as *Farrodes* and *Thraulodes*, also had scraping mouthparts but did not have a negative impact on the abundance of diatoms (Fig 3). This suggests that mouthpart structure alone is not enough to determine if a consumer will have a strong impact on the diatom community, and other traits such as gape size, which is known to have a significant effect on structuring communities (Persson et al. 1996), may need to be considered. We did perform the analysis with these two additional traits but the results were similar (Appendix C). Annual variation in stream discharge could also affect the accrual of inorganic sediment or diatom community structure (Passy 2007), but stream discharge was relatively constant during our two sampling periods (58 l s^{-1} in 2004, 60 l s^{-1} in 2009). The relatively constant rate of stream discharge between sampling years would make the observed shifts in the diatom community that occurred in pools and riffles unlikely to be solely attributed to changing discharge conditions.

Results suggest that large-bodied benthic consumers in our study stream have an important role of reducing the accrual of both algae and sediment in streams, a role that cannot be replaced by smaller-bodied species. These results provide important mechanistic insight into stream functioning as the extirpation of large-bodied consumers from streams is common, including the loss of the caddisfly *Glossosoma* in Michigan (Kohler and Wiley 1997), shrimps in Puerto Rico (Pringle and Blake 1994, Greathouse 2006), and armored catfish in Venezuela (Taylor et al. 2008, Hall et al. 2011). Following the decline of these groups from their respective streams, algal-standing stocks and sediment accrual also increased, suggesting that these large-bodied consumers served similar functional roles as the tadpoles in our study. Furthermore, some of these streams experienced increased production of smaller-bodied insects, which did not functionally compensate in terms of resource consumption for the loss of the large-bodied fish (Hall et al. 2011), and supporting our conclusion that smaller-bodied consumers cannot duplicate the functional role of larger-bodied consumers.

Examining the roles of species within a trophic guild in structuring the community is critical to understanding the impacts of continuing species losses. Our results showed that tadpoles had stronger effects on structuring the primary producer community than insects. Tadpoles also possessed functional traits that were absent in insects, (e.g. larger gape size and larger body shape) resulting in an overall decline of trait diversity from the grazing community when tadpoles were extirpated and provide a mechanistic link for why insects did not functionally compensate for tadpole declines. Together, these results suggest that identifying species with traits that significantly contribute to the trait diversity of a trophic guild maybe also be an avenue for identifying a species that has strong effects on structuring the community.

Acknowledgements

Research was supported by National Science Foundation grants DEB #0717741, DEB #0645875, and DEB #0716680. We thank the Smithsonian Tropical Research Institute and Autoridad Nacional del Ambiente (ANAM) for providing logistical support in Panamá. All research detailed in this manuscript complies with the current laws of the Republic of Panamá.

Table 3.1. Number of diatoms (mean ± SE) in three different size classes in the guts of tadpoles and grazing insects collected from pools where Sm = small sized diatoms (<1000 μm^3), Med = medium sized diatoms (1001 – 10,000 μm^3), Lg = large sized diatoms (>10,000 μm^3). The number of specimens sampled is reported in the N column, with sample size from 2004 reported first and the sample size from 2009 reported second (2004,2009). The numeral following the genus name denotes body size: '1' = <3.5 and '2' = >3.51. Tadpole taxa are *Hyloscirtus* and *Lithobates* and were only present in 2004. A '0' signifies no diatoms were found. NA denotes that either no individuals of that size class were sampled in that year, or, if the SE is NA, indicates only one individual had diatoms of that size class in their gut.

	N	2004		2009		2004		2009		2004		2009	
Taxon		Sm	± SE	Sm	± SE	Med	± SE	Med	± SE	Lg	± SE	Lg	± SE
Tadpoles													
Hyloscirtus	5,0	39.9	16.5	NA	NA	14.0	6.9	NA	NA	4.3	1.4	0	0
Lithobates	4,0	397.5	77.1	NA	NA	70.4	20.6	NA	NA	15.9	12.2	0	0
Insects													
*Baetodes*1	0,2	0	0	553.58	249.98	0	0	23.82	8.04	0	0	0	0
*Baetodes*2	0,3	0	0	15.73	NA	0	0	84.96	30.85	0	31.4	0	0
*Farrodes*1	7,12	54.07	66.02	36.34	20.22	12.55	16.07	16.22	8.99	0.61	0.02	7.34	NA
*Farrodes*2	5,8	139.58	247.69	0	0	34.84	56.64	0	0	0	9.14	0	0
*Hagenulopsis*1	2,2	19.29	16.85	33.81	21.40	16.82	11.75	13.97	2.14	0	0	0	0
*Haplohyphes*1	1,2	4.22	0.29	24.10	15.01	3.84	2.16	34.18	15.43	0.81	0.00	4.84	NA
*Haplohyphes*2	0,2	0	0	8.86	6.65	0	0	20.43	11.74	0	0	0	0
*Leptohyphes*1	1,7	17.35	11.53	14.85	8.69	3.37	1.99	12.56	8.21	1.57	NA	2.75	1.37
*Tricorythodes*1	4,15	27.84	25.88	34.96	25.21	8.57	6.78	18.29	13.79	4.68	3.63	3.16	1.76
*Psephenus*1	3,6	62.06	33.61	111.58	50.92	0.22	0	3.69	1.42	0	0	0	0
*Psephenus*2	0,1	299.29	181.55	330.41	255.12	9.31	NA	7.59	3.25	0	0	0	0

Table 3.2. Number of diatoms (mean ± SE) in three different size classes in the guts of tadpoles and grazing insects collected from riffles where SM = small sized diatoms (<1000 μm^3), Med = medium sized diatoms (1001 – 10,000 μm^3), LG = large sized diatoms (>10,000 μm^3). The number of specimens sampled is reported in the N column, with sample size from 2004 reported first and the sample size from 2009 reported second (2004,2009). The numeral following the genus name denotes body size: ‘1’ = <3.5 and ‘2’ = >3.51. Tadpole taxa are *Hyloscirtus* and *Lithobates* and were only present in 2004. A ‘0’ signifies that no diatoms were found. NA denotes that either no individuals of that size class were sampled in that year, or, if the SE is NA, indicates only one individual had diatoms of that size class in their gut.

	N	2004		2009		2004		2009		2004		2009	
Taxon		Sm	± SE	Sm	± SE	Med	± SE	Med	± SE	Lg	± SE	Lg	± SE
Tadpoles													
Hyloscirtus	4,0	24.7	9.2	NA	NA	5.4	1.5	NA	NA	3.2	0.4	NA	NA
Lithobates	4,0	192.3	32.4	NA	NA	42.5	11.5	NA	NA	3.3	0.4	NA	NA
Insects													
*Baetis*1	3,7	82.5	80.9	44.3	40.2	12.7	15.9	5.1	2.6	0	0	0	0
*Baetis*2	1,7	0	0	343.4	413.3	0	0	21.8	15.1	0	0	0.4	0.1
*Baetodes*1	11,8	45.2	46.6	111.4	97.2	16.2	14.9	49.7	34.4	0	0	23.4	12.7
*Baetodes*2	0,10	NA	NA	75.6	40.1	NA	NA	13.1	2.2	NA	NA	3.9	NA
*Farrodes*1	4,10	9.8	8.8	31.4	28.8	2.7	1.1	16.2	7.2	0	0	3.8	NA
*Farrodes*2	2,7	12.3	9.5	33.0	18.7	4.9	2.4	17.4	10.8	0	0	0	0
*Hagenulopsis*1	0,2	0	0	176.3	123.9	0	0	75.7	49.2	0	0	0	0
*Haplohyphes*1	8,10	19.8	17.7	31.6	18.9	7.9	6.8	18.5	6.6	0.8	0.3	6.9	6.8
*Leptohyphes*1	9,3	11.5	5.1	41.8	48.2	6.4	3.4	18.2	10.1	1.0	0.5	0	0
*Stenonema*1	0,1	NA	NA	19.3	6.8	NA	NA	0	0	NA	NA	0	0
*Stenonama*2	5,6	62.1	18.6	256.7	62.7	9.5	2.7	126.7	17.5	25.3	NA	0	0
*Thraulodes*1	4,8	17.8	11.2	69.9	59.1	5.4	3.6	23.3	11.9	1.2	NA	0	0

*Thraulodes*2	6,13	71.6	69.0	222.0	142.7	25.4	25.4	75.2	49.6	1.2	0.3	114.3	NA
*Tricorythodes*1	10,11	89.0	175.0	49.8	35.9	12.4	15.3	48.3	29.9	0	0	6.4	3.0
*Tricorythodes*2	2,0	13.9	6.6	0	0	4.0	1.1	0	0	0.9	0.3	0	0
*Psephenus*1	6,2	127.6	43.8	150.9	49.4	0.8	0.2	7.9	7.1	9.1	NA	0	0
*Psephenus*2	3,10	624.7	309.1	606.2	208.4	4.7	2.8	8.2	4.3	0	0	0.8	0.6
*Petrophila*1	4,0	84.1	56.0	NA	NA	0.9	0.2	NA	NA	0	0	NA	NA
*Petrophila*2	7,9	935.1	359.8	2080.4	743.5	23.6	8.6	60.9	45.9	0	0	0	0

Figure 3.1. Proportional change and the standard error (SE) for different sampling years (2004 and 2009) for the abundance for diatoms, insects, and tadpoles and weight for inorganic sediment in (a) pools and (b) riffles of the Rio Guabal prior to amphibian declines (n= 3 samples for tadpoles, n=6 samples for insects, and n=5 samples for diatoms and sediment in each of 3 mo. in 2004) and five years post amphibian declines (n= 3 samples for tadpoles, n=6 samples for insects, and n=5 samples for diatoms and sediment in each of 3 mo. in 2009). P values are from a permutation test that compares means from 2004 to 2009.

Figure 3.2. Change in two-dimensional trait space of functional trait richness from 2004 to 2009 in (a) pools and (b) riffles. Axes are quantitative traits (gape size and the product of the head capsule width and biomass) that were extracted from a principal coordinate analysis (PCoA). The red shaded area is trait space in 2004 only and the purple shaded area is trait space that overlaps in 2004 and 2009. Note that the purple shaded area is not visible in 2004. P values are from a permutation test that compares means from 2004 to 2009.

Figure 3.3. The mean ± SE of the electivity indices of 3 diatom size classes (Sm = <1000 μm^3, Med = 1000 – 10,000 μm^3, Lg = >10,000 μm^3) for tadpoles from pools and riffles. Electivity indices indicate a consumer's preference for a prey item based on the proportion of the prey in the gut relative to the proportion of the prey in the environment. A value >>0 indicates a highly preferred prey item while a value <<0 indicates high aversion to that prey item. Values near '0' indicate no selection by the consumer for that prey item. The number of guts analyzed (sample size) are reported in the legend.

Figure 3.4. The mean ± SE of the electivity indices of 3 diatom size classes (Sm = <1000 μm^3, Med = 1000 – 10,000 μm^3, Lg = >10,000 μm^3) for two size classes of insects (body sizes are

<3.5 mm and >3.51 mm) for pools (a and c) and riffles (b and d). Grazing insects were pooled based on their body length, which consequently removed taxonomic classifiers. This was done because tadpoles were hypothesized to be resource facilitators for small bodied grazing insects but resource competitors for larger grazing insects. By separating grazing insects into size classes, we could detect potential changes in the size of diatoms preferred by grazing insects from pre- to post-decline sampling dates. In 2004, insects preferentially consumed the smallest diatoms in the community. In 2009, when tadpoles were absent, insect diets shifted to medium sized diatoms. The number of guts analyzed (sample size) for each group are reported in the legend.

Figure 3.5. Path diagrams showing the effects of (clockwise from top) tadpoles, large-bodied insects (Lg INSECTS), large-sized diatoms (Lg DIATOMS), medium-sized diatoms (Med DIATOMS), small-sized diatoms (Sm DIATOMS), inorganic sediment (SEDIMENT) and small-bodied insects (Sm Insects) in (a) pools and (b) riffles. The first row of figures test the hypothesis that increased sediment accrual following amphibian declines reduced the recruitment of early-instar insects to later-instar insects in pools (a) and riffles (b). Both models fit the data ($\chi^2 > 0.05$) The second row of figures test the hypothesis that insects were resource limited, specifically, that increasing populations of large-bodied diatoms limited resource availability for mayflies in pools (c) and riffles (d), but these models did not fit the data ($\chi^2 < 0.05$ for both). The third row of figures test the hypothesis that tadpoles have a top-down effect on the abundance of diatoms and inorganic sediment, while grazing insects have no effect on the abundance of diatoms and inorganic sediment in pools (e) and riffles (f). The models represented in figures (e) and (f) were the models that best fit the data. Lines connecting compartments represent pathways included in

the path analysis with the standardized coefficients of a statistically significant interaction ($P < 0.05$) between two groups reported with solid lines while dashed lines represent an interaction that was tested within the model but was not statistically significant. The abundance of small- and large-bodied insect grazers, tadpoles, small-, medium-, and large-bodied diatoms and sediment accrual from pre- and post-decline were used in the model.

Figure 3.6. A scanning electron micrograph of the diatom *Terpsinoe*, the largest diatom in the community, and the mouth opening for four size classes of *Farrodes* (Ephemeroptera). The body length of each *Farrodes*, starting at the top and going clockwise, is 7.6 mm, 3.1 mm, 1.2 mm, and 4.7 mm. The white line at the base of the head capsule shows the width of the gape by spanning the esophagus, the structure immediately after the mouth opening. The image shows the scale of *Terpsinoe*, the largest diatom in the diatom community, to the mouth opening of four different size classes of *Farrodes* (Ephemeroptera).

Figure 3.1.

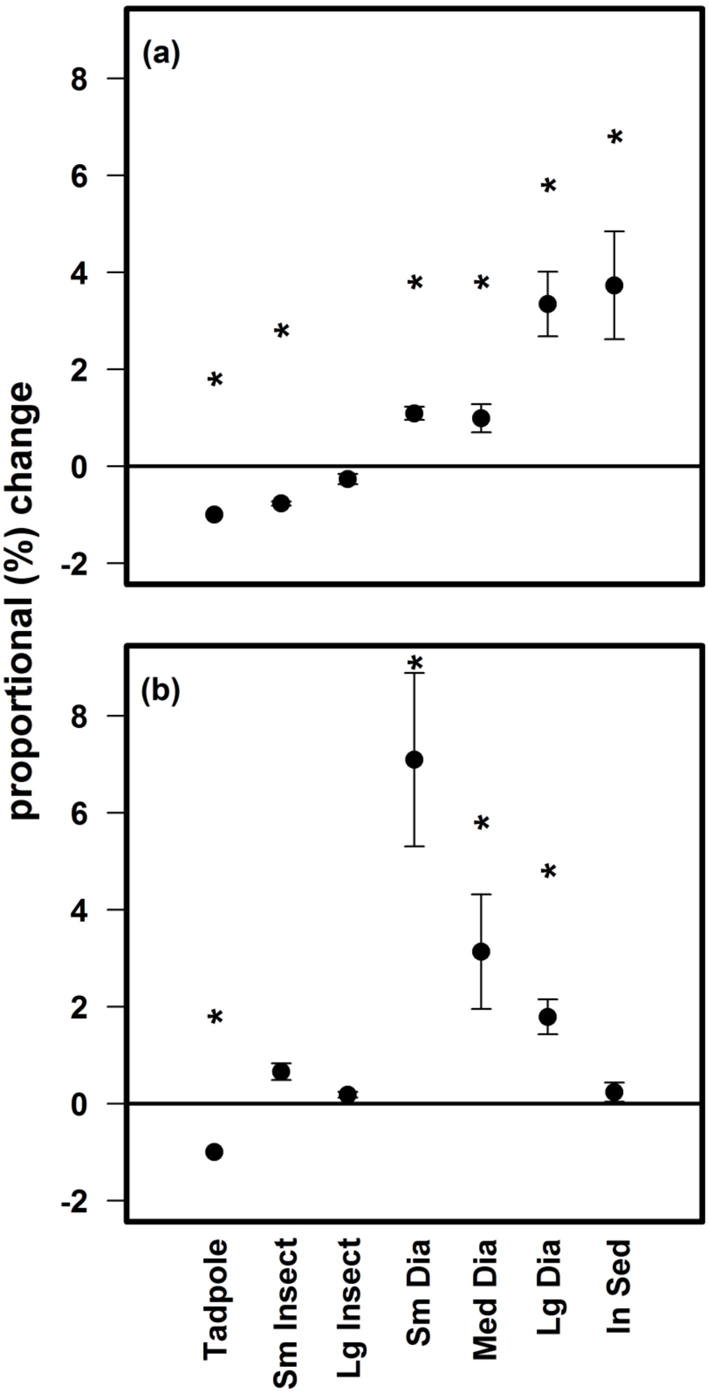

(a)
(b)
proportional (%) change
8
6
4
2
0
-2
*
Tadpole
Sm Insect
Lg Insect
Sm Dia
Med Dia
Lg Dia
In Sed

Figure 3.2.

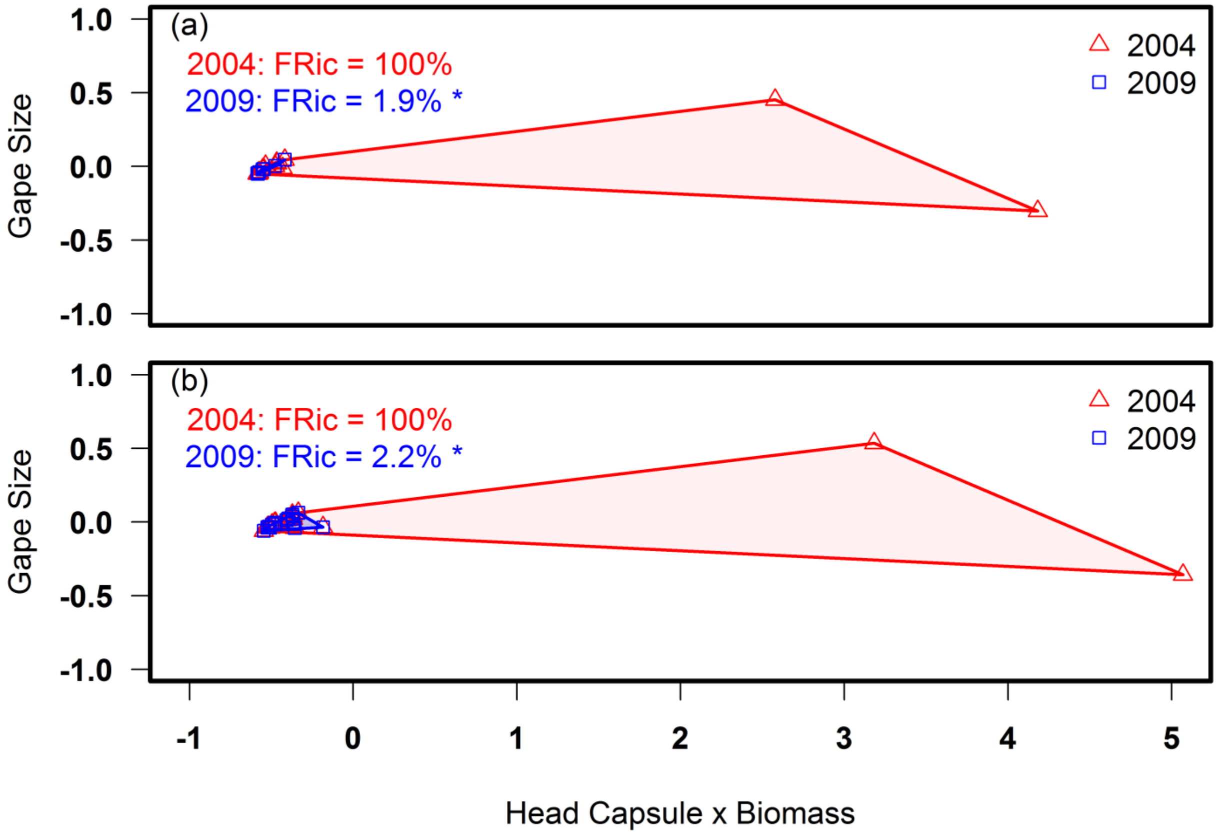

(a)
2004: FRic = 100%
2009: FRic = 1.9% *
△ 2004
□ 2009
(b)
2004: FRic = 100%
2009: FRic = 2.2% *
△ 2004
□ 2009
Gape Size
1.0
0.5
0.0
-0.5
-1.0
-1
0
1
2
3
4
5
Head Capsule x Biomass

Figure 3.3.

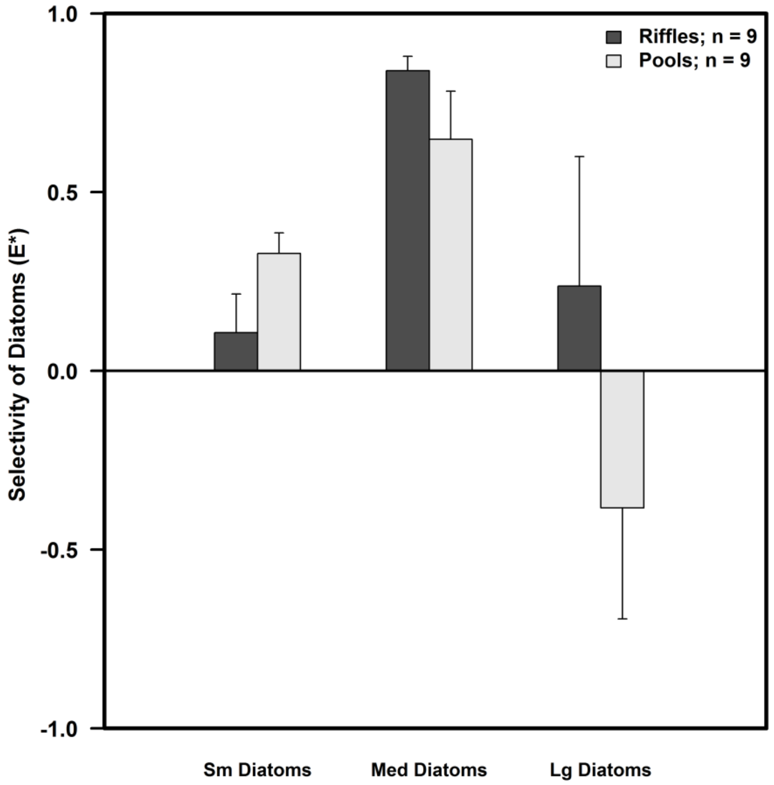

1.0
0.5
0.0
-0.5
-1.0
Selectivity of Diatoms (E*)
Riffles; n = 9
Pools; n = 9
Sm Diatoms
Med Diatoms
Lg Diatoms

Figure 3.4.

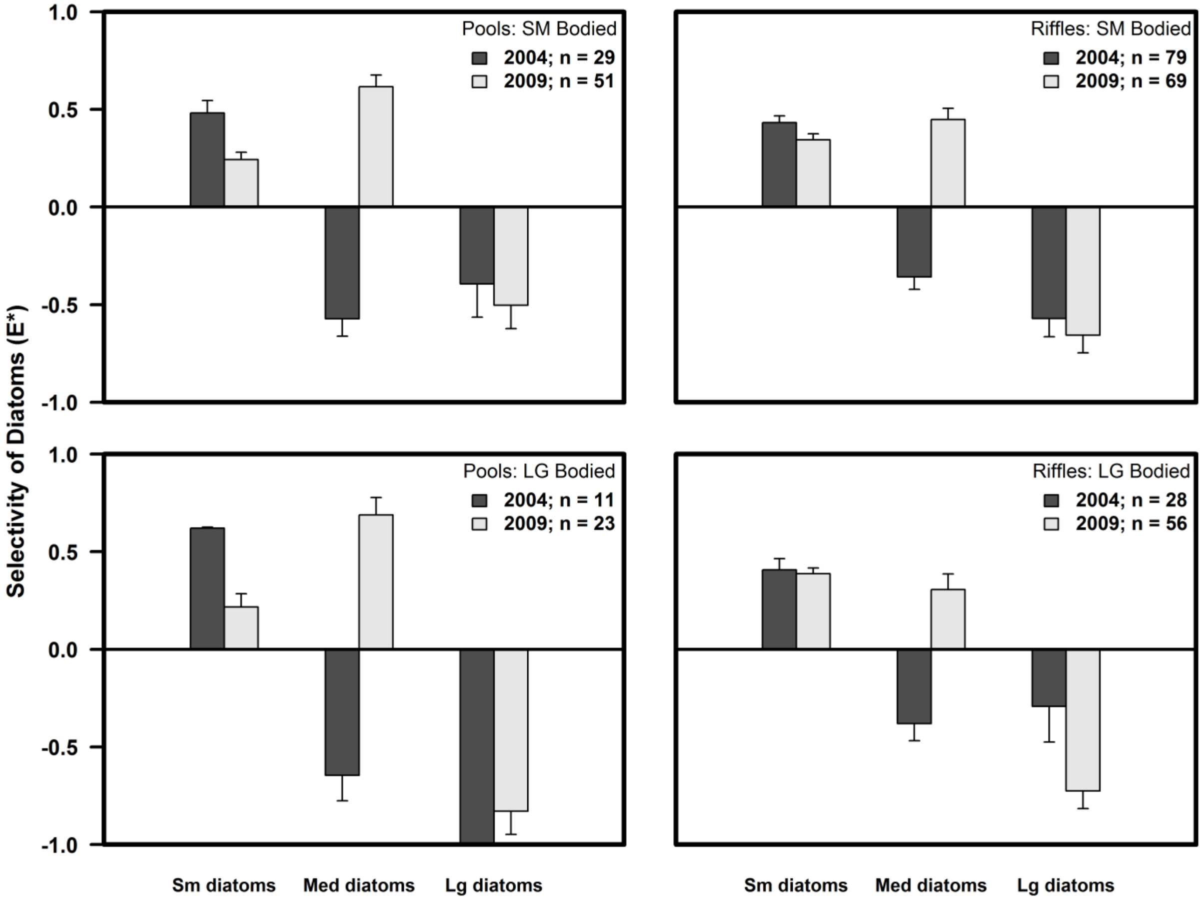

Selectivity of Diatoms (E*)
1.0
0.5
0.0
-0.5
-1.0
Pools: SM Bodied
2004; n = 29
2009; n = 51
Riffles: SM Bodied
2004; n = 79
2009; n = 69
Pools: LG Bodied
2004; n = 11
2009; n = 23
Riffles: LG Bodied
2004; n = 28
2009; n = 56
Sm diatoms
Med diatoms
Lg diatoms

Figure 3.5

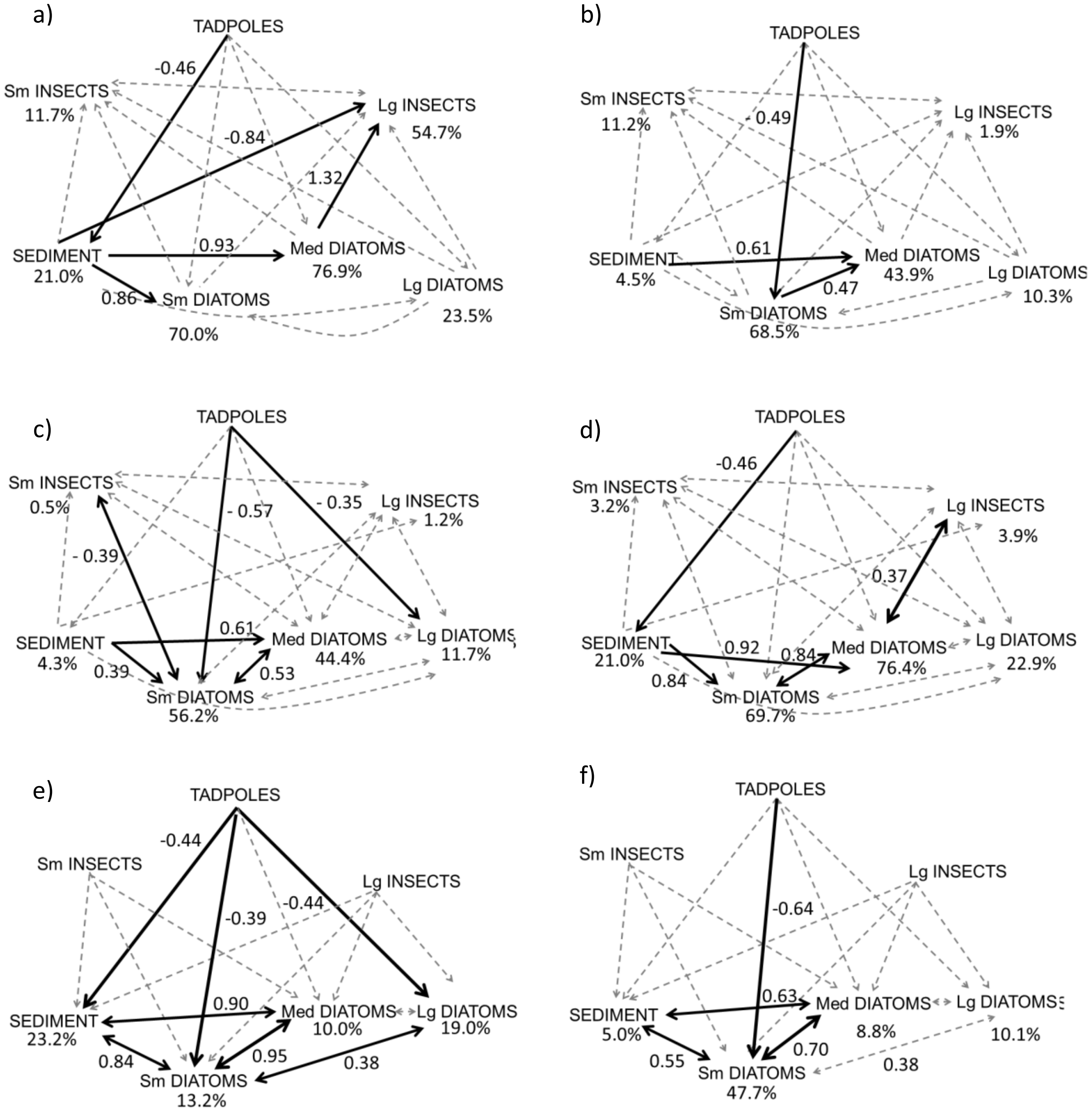
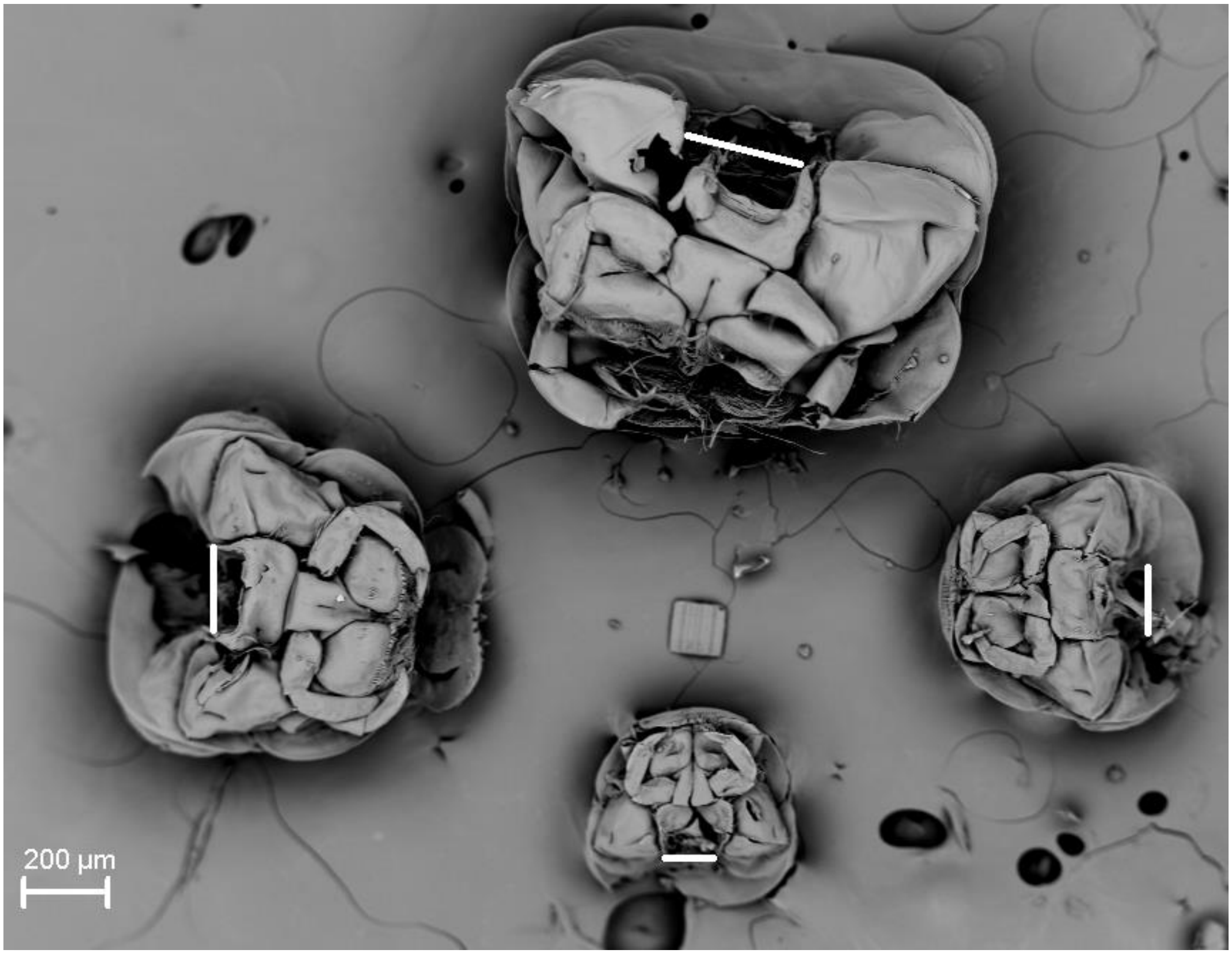

a)
TADPOLES
-0.46
Sm INSECTS
11.7%
Lg INSECTS
54.7%
-0.84
1.32
SEDIMENT
21.0%
0.93
Med DIATOMS
76.9%
Lg DIATOMS
23.5%
0.86
Sm DIATOMS
70.0%
b)
TADPOLES
Sm INSECTS
11.2%
Lg INSECTS
1.9%
- 0.49
SEDIMENT
4.5%
0.61
Med DIATOMS
43.9%
Lg DIATOMS
10.3%
0.47
Sm DIATOMS
68.5%
c)
TADPOLES
Sm INSECTS
0.5%
- 0.57
- 0.35
Lg INSECTS
1.2%
- 0.39
SEDIMENT
4.3%
0.61
Med DIATOMS
44.4%
Lg DIATOMS
11.7%
0.39
0.53
Sm DIATOMS
56.2%
d)
TADPOLES
-0.46
Sm INSECTS
3.2%
Lg INSECTS
3.9%
0.37
SEDIMENT
21.0%
0.92
0.84
Med DIATOMS
76.4%
Lg DIATOMS
22.9%
0.84
Sm DIATOMS
69.7%
e)
TADPOLES
-0.44
Sm INSECTS
Lg INSECTS
-0.39
-0.44
SEDIMENT
23.2%
0.90
Med DIATOMS
10.0%
Lg DIATOMS
19.0%
0.84
0.95
0.38
Sm DIATOMS
13.2%
f)
TADPOLES
Sm INSECTS
Lg INSECTS
-0.64
SEDIMENT
5.0%
0.63
Med DIATOMS
8.8%
Lg DIATOMS
10.1%
0.55
0.70
0.38
Sm DIATOMS
47.7%

Figure 3.6.



CHAPTER 4

EFFECTS OF SPECIES LOSS ON FOOD WEB STRUCTURE: A NETWORK ANALYSIS REVEALS EVIDENCE FOR STRUCTURAL PERSISTENCE AFTER AMPHIBIAN EXTIRPATION IN A NEOTROPICAL STREAM[1]

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Abstract

Species losses are predicted to simplify food web structure, and disease driven amphibian declines in Central America offer an opportunity to test this prediction. Assessment of insect community composition combined with gut content analyses was used to generate periphyton-insect food webs for a Panamanian stream, both pre- and post-amphibian decline. We then used network analysis to assess the effects of amphibian declines on food web structure. Although 48% of consumer taxa, including many insect taxa, were lost between pre- and post-amphibian decline sampling dates, connectance declined by less than 3%. We then quantified the resilience of food web structure by simulating the loss of tadpoles and cascading extirpations from the pre-decline food web. Simulations overestimated effects of species loss on connectance by more than 60% and linkage density by nearly 40%. New trophic linkages in the post-decline food web reorganized the food web topology, changing the identity of 'hub' taxa, and consequently reducing the effects of amphibian declines on many food web attributes. Resilience of food web attributes was driven by a combination of changes in consumer diets, particularly those of insect predators, as well as the appearance of generalist insect consumers, suggesting that food web structure is maintained by factors independent of the original trophic linkages.

Keywords: amphibian declines, connectance, food web structure, network analysis, species loss

Introduction

Understanding effects of species losses on food web structure can help to address significant gaps in our knowledge of how declining biodiversity affects ecosystems (Thompson *et al.* 2012). Food webs depict *who-eats-whom* and can be used to characterize species interactions across multiple trophic levels. Previous food web research has predicted that, as highly-connected species are extirpated, secondary extinctions (extinction cascades) will co-occur as remaining species lose resources (Dunne et al. 2002, Dunne and Williams 2009). More recently, models have examined effects of anthropogenic disturbances on food web structure, showing a simplification of food web structure with environmental degradation (Coll et al. 2008, 2011). Trophic linkages in these models are static since the food webs studied either represent a snapshot in time (Thompson and Townsend 2005) or are aggregated over unspecified time scales (Martinez 1991). Such models are implicitly “brittle” as they assume trophic relationships are fixed and unable to compensate for species loss. However, in nature, trophic linkages are often dynamic, with consumers switching to different prey in response to the loss of a competitor or changing abiotic conditions. Consequently, surviving species may mitigate the loss of a species on food web structure through adaptive foraging, such as diet expansion (Staniczenko et al. 2010). Food web structure may also be influenced through indirect effects, such as facilitation, which can result in unpredictable feedbacks, altering community composition and dynamics (Wootton 1994, Montoya et al. 2009). Trophic dynamics and indirect effects could mean that effects of species loss on food web structure cannot be adequately represented with static food web models, and that food web structure may not be coupled to, but rather independent of, the trophic interactions of which they are comprised.

The lack of pre- and post-extirpation food web data is a major challenge to quantifying effects of biodiversity loss on food web structure. Analyzing snapshots of a food web over time, rather than analyzing an aggregated food web, can provide insight into how an ecological network responds to a disturbance (Cohen et al. 2009) and may aid in differentiating the roles of individual species within the community. The Tropical Amphibian Declines in Streams (TADS) project allowed us to analyze snapshots of a benthic stream food web before and after sudden catastrophic amphibian declines driven by the chytrid fungus *Batrachochytrium dendrobatidis* (Lips et al. 2006). This epizootic has been moving from Costa Rica through Panama, creating a "*natural experiment*," allowing for pre- and post-decline comparisons (Whiles et al. 2006, 2012). Previous TADS studies illustrated how the extirpation of larval amphibians (tadpoles) affected ecosystem properties in the Rio Guabal, a Panamanian headwater mountain stream, showing increased algal standing stock (Connelly et al. 2008) and decreased production of predator and shredder macroinvertebrates (Colón-Gaud et al. 2010a). Tadpoles consumed algae and diatoms (Ranvestel et al. 2004) and were likely consumed by insect predators, making them connected to the benthic food web through multiple trophic linkages. Consequently, tadpole extirpation may have affected food web structure.

We quantify differences in the structure of periphyton-insect stream food webs, with and without amphibians, in the Rio Guabal, a highland Panamanian stream. First, we quantify differences in the insect community and food web structure with and without amphibians. We predict that the structure of food webs without amphibians would be simplified (e.g. reduced complexity) compared to food webs with amphibians. Second, we determine the effects of extinctions on a static food web through computer simulations. We reasoned that if the attributes of the post-decline food webs were similar to simulated food webs, then static food webs can be effective tools for assessing effects of species loss. However, if the attributes of the post-decline food web were similar to the pre-decline food web, then indirect effects may have a significant role in structuring food web topology.

Methods

Study system

Rio Guabal is a headwater stream at ~750 m a.s.l. in the Parque Nacional G.D. Omar Torrijos Herrera, El Copé, Coclé, Panama (8º40'N, 80º35'W). The stream is characterized by distinct pool-run/riffle sequences with a gravel and boulder substrate. Canopy cover is >80%, with occasional tree-fall gaps (Connelly et al. 2008). The stream had twenty-three species of stream-dwelling tadpoles occurring in all stream habitats, including riffles, runs, pools, detrital leaf packs, and marginal pools (Lips et al. 2003). The most abundant tadpoles were *Atelopus varius* tadpoles in riffle habitats while *Lithobates warszewitschii*, *Hyloscirtus colymba*, and *Hyloscirtus palmeri* occurred in pool habitats (Ranvestel et al. 2004). Amphibian declines in this region began in September 2004 and continued through January 2005 (Lips et al. 2006). Two species of fish (*Brachyraphis roswithae* and *Trichomycterus striatus*), along with shrimp (*Macrobrachium* spp.) and crabs (*Pseudothelphusa* sp.) also occurred within the stream, but were not included in the food web analysis. The most abundant fish, *B. roswithae*, are surface feeders (Ranvestel et al. 2004) and *T. striatus* were extremely uncommon, with only one individual observed on 14 consecutive days of sampling a 500 m reach in April 2009. Crabs and shrimps were also excluded from the food web because of their low abundance and they were not observed in the riffles and pools where macroinvertebrates and diatoms were sampled (Ranvestal et al. 2004, Connelly et al 2008). Furthermore, an N^{15} tracer study showed that *B. roswithae* and *Pseudothelphusa* derived little of their nitrogen from the benthic community (Whiles et al. 2013). The insect community that was included in the food web was constrained by the logistics of invertebrate sampling, which means free-swimming *Limnocoris* were excluded from the food web. However, including any of these taxa would not change the general conclusions of the study as we quantified the deletion and formation of new linkages from the taxa present.

Food web construction

Rio Guabal's insect community was assessed, over a 500 m reach during the dry season, three times pre-decline (February 4th, March 5th, and April 7th 2004) and three times post-decline (February 18th, March 23rd, and April 7th 2009). Five years separated sampling events to allow time for populations to respond to amphibian declines. Each month, six stove-pipe benthic core samples (314 cm^2 sampling area) were collected from pool habitats and eight Surber samples (930 cm^2 sampling area) were collected from riffle/run habitats. Invertebrates were identified to genus (except Chironomidae) and then measured to estimate biomass using methods described in Colón-Gaud *et al.* (2010a). Biomass estimates for each taxon are reported in Appendix A.

The diatom and algae community on natural substrata were sampled from five riffles and pools on the same days and from the same reach as the macroinvertebrate community with a benthic sampler (modified after Loeb 1981) during base flow. Diatom community composition was assessed using the methods in Connelly *et al.* (2008), with a minimum of 600 diatom frustules identified to genus from each sample. Diatom densities for each genus are reported in Appendix B.

Tadpoles were collected from the stream in 2004 following methods described in Colón-Gaud *et al.* (2010a). For permitting reasons, the tadpoles of *A. varius* were collected from a stream a few kilometers to the east, in the Altos del Piedra, Panama in February 2004. From April 7th-22nd 2009, additional, haphazard sampling for insects was done to increase the sample size for gut content analyses of rarer taxa. Sampling was performed with a D-net to collect insects, which were then sorted from other material in the field and preserved in 8% formalin.

Food web linkages were identified through gut content analyses, using individuals collected for biomass estimates during the latter part of the dry season in March and April 2004 and April 2009. These months were selected due to the presence of the largest dragonfly instars which emerge during the transition between the dry and wet season (Pritchard 1996). The number of prey items for these predators' increases with body size (Woodward and Hildrew 2002), maximizing the number of predator-prey linkages we could identify. Insect taxa with a biomass of >2 mg ash-free dry mass (AFDM) m^{-2} mo^{-1} were selected for the food web, and consequently gut content analysis, as taxa with a smaller biomass generally had less than one individual collected each month and were excluded from the analysis. This criteria means that rare and small taxa (riffle beetle larva and uncommon mayfly genera) were excluded from the food web, while taxa that were rare but big, such as the odonate *Argia*, were included in the food web. The riffle beetle and mayfly genera that were excluded had sister genera that were included, making the exclusion of these rarer taxa unlikely to change our main results.

Guts of algal-grazing tadpoles and non-predator macroinvertebrates were removed and suspended in water, filtered onto a nitrocellulose membrane, slide mounted, then sealed with a coverslip and nail polish. The gut contents were then examined for diatoms and animal fragments through oil immersion brightfield optics at 1000× on an Olympus CH30. For each gut, a maximum of three hundred diatoms were identified to genus in up to ten measured transects. Plant material and filamentous algal cells were noted and included in the food web. Gut contents of macroinvertebrate predators were removed and the head capsules of prey items were used to identify prey to genus. Guts were then prepared using the same technique as tadpoles and non-predator macroinvertebrates. The filter was scanned at 100× for prey fragments such as cerci and tarsi and then examined at 1000× for additional prey items and diatoms until a maximum of three hundred diatoms were identified to genus in up to ten measured transects. The number of individuals sampled per taxon depended on the availability of specimens and ranged from 4 to 21 individuals. Rarefaction curves for each taxon were created to assess sampling effort. Additional linkages that would be discovered with further sampling was estimated using the Chao2 species estimator using EstimateS (Colwell et al. 2012) (Appendix C).

Four food webs of the benthic community were constructed for: pools pre-amphibian decline (2004); riffles pre-amphibian decline (2004); pools post-amphibian decline (2009); and riffles post-amphibian decline (2009). Separate food webs with nodes ('species') and linkages (trophic interactions) were created for riffles (faster water flow/shallower depth) and pools (slower water flow/deeper depth) because these two habitats contained distinct tadpole assemblages. 'Species' within the food web were identified to genus as this was the lowest taxonomic level we could use while keeping taxa in functionally similar groups across time. Trophic interactions between species were included when a prey item was identified at least once in the gut of the consumer. Taxa with a biomass >2 mg AFDM $m^{-2}mo^{-1}$ were included in the pre- and post-decline food webs. Diatom genera present in gut contents in 2004 but absent in 2009 were assumed to be secondarily extinct.

Network analyses

For each of the four food webs, we examined characteristics of the whole network and individual nodes. A node's *degree* is the number of incoming and outgoing linkages, and the frequency of occurrence of degrees within the network defines the *degree distribution*.

Betweenness centrality is a measure of centrality in the food web literature and is an indication of central position in the food web which, for each species *i*, is defined as the fraction of species pairs *j* and *k*, such that species *i* falls on the shortest path from *j* to *k* (Estrada 2007, Jordan et al. 2007). Species with a high betweenness centrality are important because they mediate many indirect interactions between species (Estrada 2007) and are topological 'hubs' in a food web (Lai et al. 2012). For the whole food web network, we examined fifteen coarse-grained attributes that, based on a search of the literature, are predicted to change as a consequence of species loss (Dunne et al. 2002, Coll et al. 2008) (Table 1). Several network-level statistics are summaries of the degree distribution including linkage density, connectance, and skewness and variance of the degree distribution. While linkage density and connectance are commonly measured food web characteristics, skewness and variance of the degree distribution are less commonly calculated. We included them in our analysis because they summarize the tendency of the network to be structured by only a few key taxa versus diffuse interactions distributed over many taxa. Particularly, a left-skewed degree distribution indicates relatively few taxa with many linkages, while a right-skewed degree distribution indicates a large number of taxa with many linkages. The variance of the degree distribution is important for showing the dispersion around the mean. Other coarse-grained food web attributes were size of the network (number of species): average path length; maximum trophic level; generality; vulnerability; and fraction of species in the following categories: basal taxa; herbivores; intermediate consumers; top predators; omnivores; and cannibals. Food web attributes, including *betweenness centrality*, were calculated in R (R Core Team) using the igraph (Csárdi and Nepusz 2006), e1071, and Foodweb packages and our own R scripts.

Simulated tadpole extirpations

We also simulated the effects of tadpole extirpation on food web structure. For the first simulation (simulation 1), we started with the adjacency matrix for 2004, which depicts those nodes that are connected to each other, encoding the extant food web for both pools and riffles and removed tadpole and macroinvertebrate taxa with a biomass of <2.0 mg AFDM m^{-2} mo^{-1} in 2009. Next, any taxa that were no longer connected to the food web were removed. The second simulation (simulation 2) started with the adjacency matrix for 2009 and then removed species that were only present in 2009 to test if observed differences in food web structure from 2004 to 2009 were the result of new species or newly-formed connections among the original species. Following these extinctions, the fifteen network attributes and betweenness centrality were re-calculated for the simulated food webs. These simulations provided a numerical prediction of the change in food web structure given the "brittleness" assumption that food web structure depends on its constituent species and does not adapt to perturbations.

The effect of extirpation on each food web attribute was calculated by comparing the observed pre-decline and post-decline food webs to the simulated food webs that excluded tadpoles and macroinvertebrates [z=(post-decline – simulation 1)/(pre-decline – simulation 1)] and new species [z=(simulation 2 – simulation 1)/(pre-decline – simulation 1)]. For any given food web attribute, z=0 indicates that the post-decline food web is identical to that predicted by simulations and is consistent with the hypothesis that food web characteristics derive from the constituent trophic linkages. Values of $z<0$ indicate that a food web attribute has changed even more than the simulated loss would predict. At the other extreme, values of $z>1$ indicate the pre-decline attribute is intermediate between post-decline and the simulation. Cases where $0<z<1$ indicate that the post-decline food web is intermediate between simulated and pre-decline and that the food web exhibits some degree of structural resilience to species loss. Of these, attributes for which z>0.5 are more similar to pre-decline, while those for which z<0.5 are more similar to the simulated species loss.

Results

Empirical network analysis

The total number of taxa in the food web (including tadpoles, macroinvertebrates, and diatoms) declined from 60 to 44 (25%) in pools and from 65 to 55 (15%) in riffles from Feb-April 2004 to Feb-April 2009 (Fig. 1). In pool habitats, 9 of the 17 macroinvertebrate taxa present in 2004 were present in 2009, while there was 1 new macroinvertebrate taxon present in 2009 (Appendix A). Riffle habitats experienced similar declines in macroinvertebrate taxa, with 15 of the 28 macroinvertebrate taxa present in 2004 remaining in 2009, while there were 5 new macroinvertebrate taxa in 2009 (Appendix A).

A total of 891 tadpole and macroinvertebrate guts were analyzed and 1793 linkages were identified from the four food webs. Gut content analysis yielded 18,128 observations for pools in 2004, 17,187 observations for pools in 2009, 54,308 observations for riffles in 2004, and 38,214 observations for riffles in 2009. The means from the species estimator curves indicate that 85% to 90% of all trophic linkages in the each of the four food webs were identified (Appendix C).

In pool habitats, 303 of the 412 linkages (73%) in 2004 were absent in 2009 while 98 of the 207 linkages (45%) in 2009 were absent in 2004 (Fig 1a and 1b). Four predator taxa accounted for 57 of 98 new linkages (58%), with 51 of the 57 linkages occurring between predators and diatoms. The number of diatom valves and algal cells in the guts of these four predators increased from 141 ± 47 (mean ± SE) in 2004 to 429 ± 75 in 2009 (p = 0.001)

(Appendix D). Nineteen (19%) of the new linkages were associated with *Macrelmis*, a taxon absent from the pre-decline food web. The remaining 22 (22%) linkages were between herbivores and diatoms. Connectance declined by 7.1% from 0.114 in 2004 to 0.107 in 2009 while linkage density declined by 30.6% from 6.86 in 2004 to 4.76 in 2009 (Table 2).

In riffle habitats, 390 of the 658 linkages (59%) present in 2004 were absent in 2009 while 222 of the 489 linkages (47%) in 2009 were absent in 2004 (Fig 1c and 1d). The five insect taxa that were only found post-decline accounted for 140 (63%) of these 222 new linkages, with 130 linkages occurring between diatoms and insects. These five new insect taxa were connected to a significantly larger number of species than surviving consumers from the pre-decline food web (Wilcoxon Rank Sum Test: W = 20.5, p = 0.002). Nineteen (8%) of the new linkages were extant insects consuming other insects. Eighteen (8%) of the remaining 63 linkages were between diatoms and predators, with 13 of these linkages occurring between odonates and diatoms. The odonates, *Philogenia* and *Heteragrion*, also had increased densities of diatoms and filamentous algae (*Audouinella chantransia*) in their guts post-amphibian decline (110±67 in 2004 to 2365 ± 756 in 2009, p = 0.02, and 50 ± 30 in 2004 to 443 ± 79 in 2009, p=0.001 respectively) (Appendix D). The remaining 45 (20%) linkages were between herbivores and diatoms. Connectance increased by 5.8%, from 0.153 in 2004 to 0.162 in 2009, while linkage density declined by 11.9% from 10.1 in 2004 to 8.89 in 2009 (Table 2). In post-decline food webs from riffles and pools, fewer taxa had lots of linkages as the degree distributions became more left-skewed.

The identity of those taxa that were central to the food web also changed from 2004 to 2009, as taxa with the highest *betweenness centrality* indices changed (Table 3). In 2004, the tadpoles (*Lithobates* and *Hyloscirtus* in pools and *Atelopus* in riffles) had the highest centrality values. In 2009, 7 of the 10 taxa in pools and 8 of the 10 taxa in riffles with the highest centrality values were not ranked in 2004.

Simulated extirpations

The z-scores for several food web attributes post-decline, that are associated with food web complexity (e.g. connectance, maximum trophic level, and omnivory), were either close to 1 or exceeded 1, indicating that the attributes from post-decline food webs were more similar to pre-decline attributes than to attributes from food webs that simulated species loss (Fig 2). The second simulation tested the effect of new taxa on food web structure by excluding taxa present only in 2009 from the 2009 food webs. In riffles, the z-score was near 0 for many of the attributes from the second simulation, indicating that the attributes were more similar to the attributes from the first simulation. In pools the z-score was near 1 for many of the attributes from the second simulation, indicating the attributes were more similar to the attributes from the pre-decline food web (Fig 2).

Discussion

Our findings show smaller effects of species loss on food web structure than we might predict through simulated species deletions, challenging predictions of species loss from food webs that assume fixed trophic linkages. In our study stream, food web structure was resilient, despite a 48% decline in consumer diversity (macroinvertebrates and tadpoles) from 2004 to 2009, as coarse-grained measures changed less than we predicted by simulating species deletions from the network. The resilience of food web structure was driven primarily through two pathways - the appearance of new generalist consumers in riffles and diet expansion by predators in pools. Each pathway in each habitat accounted for ~60% of the new linkages. Although the attributes that describe food web topology changed little from pre- to post-decline, many new linkages and genera massively reconfigured the food web, changing the identity of ‘hub’ taxa in the periphyton-insect food web. Consequently, in the post-decline food web, many attributes associated with food web complexity remained similar to pre-decline food webs. The resilience of food web structure to species loss suggests that factors independent of the original trophic linkages, such as indirect effects and diet expansion, may have played a significant role in maintaining food web structure.

The lack of change to coarse-grained attributes of food web structure is unlikely the result of intra- or inter-annual variation. Some coarse-grained attributes, such as connectance, can exhibit intra-annual variability, but this variability is driven by the availability of resources (Woodward et al. 2005). In our study stream, macroinvertebrates, with the exception of odonates, are multivoltine, with overlapping generations, resulting in a range of size classes present in the stream year round (Colón-Gaud et al. 2010a) and suggesting that resource availability for insect predators is not temporally variable. Populations of diatom genera also fluctuate little from the wet season to the dry season and only underwent major changes in community composition after amphibian declines (Connelly et al. 2008), suggesting that resource availability for grazing macroinvertebrates is not temporally variable. Furthermore, an analysis of the trophic basis of production prior to amphibian declines showed that the diets of macroinvertebrates do not shift with seasons, indicating that macroinvertebrates are not shifting diets as environmental conditions fluctuate (Frauendorf et al. 2013). Some diet changes observed in our current study (Appendix D) were corroborated with changes in the stoichiometric homeostasis of insect consumers. For example, *Anacroneuria* showed high deviance in body stoichiometry, including decreased P-concentrations 7 years following amphibian declines. This decreased P could be the result of a dietary shift with *Anacroneuria* consuming less animal- and more plant-material (e.g. diatoms and other algae) in the absence of tadpoles (Appendix D). Furthermore, insect taxa that did not show high plasticity of body stoichiometry, such as *Farrodes, Thraulodes,* and *Anchytarsus,* did not show variation in the number of diatoms consumed from pre- to post-decline, a result corroborated for *Thraulodes* at a second stream (Barnum et al. 2013). These lines of evidence suggest that the availability of resources is not seasonally or annually variable, and consequently unlikely to drive the changes we observed in the food web.

Inter-annual variation of environmental conditions could also cause changes to the stream community. For example, sediment accrual can reduce macroinvertebrate populations (Connolly and Pearson 2007), but sediment accrual in the Rio Guabal was attributed to amphibian declines, which increased immediately after their decline in 2004 and remained elevated 4 years post-decline (Connelly et al. 2014). Anthropogenic inputs, such as agricultural runoff or silt from road crossings, are not a factor since our study stream is located in a national park at the top of a mountain, limiting upstream inputs. Furthermore, drought, which can negatively affect macorinvertebrate diversity in streams (Woodward et al 2012, Ledger et al. 2013), was an unlikely driver of our results as discharge between sampling in 2004 and 2009 did not vary, averaging 58 L s^{-1} and 60 L s^{-1} respectively during our sampling periods, and at no time during our sampling between 2004 and 2009 was the stream dry (Connelly et al. 2014). These factors make environmental variability an unlikely driver of the results reported in this study.

Observed differences in the macroinvertebrate community between 2004 and 2009 were also unlikely to have been driven by inter-annual variation. Previous TADS studies show the benthic macroinvertebrate community changed in the 6 months immediately following tadpole extirpations, including declines in the biomass of insect filter-feeders (e.g., *Leptonema*, *Macronema*, *Chimarra*, *Wormaldia*, and *Simulium*) and grazers (*Petrophila* and *Psephenus)*(Colón-Gaud et al. 2010a, Colón-Gaud et al. 2010b). The biomass of these taxa, except for *Simulium*, remained low in 2009 relative to 2004 (Appendix A). Furthermore, declines in insect filter-feeders and grazers also decreased 6 years post-amphibian extirpation in Río Maria, a highland stream ~ 30 km to the east of Rio Guabal (Rantala et al. in review). Macroinvertebrate diversity in Rio Maria declined by 42%, similar to the 48% diversity decline observed in the Rio Guabal after 4 years. Studies from other streams have also shown decreases to insect richness with amphibian declines over 7 and 14 years post-decline, including declines in algal-grazer, filter-feeder, and predator diversity (Rugenski 2013). In contrast, a two year study in two streams in western Panama that lack amphibians showed little annual variation in the macroinvertebrate community (Colón-Gaud et al. 2010a). These lines of evidence suggest strongly that changes to the macroinvertebrate community between 2004 and 2009 were linked to tadpole extirpations.

Although our results of a binary food web suggest that coarse-grained measures of food web structure are resilient to amphibian declines, changes in the weighted linkages between genera may have occurred. Assessing changes to a network with weighted linkages could reveal changes in the dietary preferences of algal-grazing insects, such as mayflies. In the 6 months following amphibian declines, insect grazer richness and abundance, including algal-grazing mayflies, declined (Colón-Gaud et al. 2010a). This was accompanied by increased diatom densities, a primary resource for algal-grazing insects and mayflies, and a shift in the diatom community from smaller-bodied diatoms (<5000 μm3) to larger-bodied diatoms (>5,000 μm^3) (Connelly et al. 2008). This pattern in the insect-grazer and diatom communities continued 4 years post-decline, as 3 mayfly genera in riffles and 4 mayfly genera in pools declined to < 2mg AFDM m^{-2} mo^{-1} (Appendix A and B). Using the population changes in the insect-grazer and diatom community as evidence, amphibian declines may have changed the food web by altering the linkage weights within the food web but is beyond the scope of this investigation.

Changes in consumer feeding behavior, most notably in the Odonata and other insect predators, played a major role in re-structuring the food web (Appendix D). Increased numbers of diatom genera in the guts of odonates occurred in individuals from both riffle and pool habitats. Additionally, there were significant increases in the numbers of diatom valves and filamentous algae (*A. chantransia*) in the guts of several odonate taxa, indicating that the increased prevalence of diatom genera in odonate guts is unlikely to be due to incidental ingestion or from the gut contents of prey (Appendix D). Moreover, the availability of preferred prey for odonates, such as tadpoles and macroinvertebrates, declined following amphibian declines (Colon-Gaud et al. 2010b, Appendix A). Previous studies have documented that predatory aquatic insects will consume diatoms when the abundance of preferred prey decreases (Lancaster et al. 2005). Combined, our data suggest that a major change in the consumer feeding behavior of odonates occurred from 2004 to 2009.

Our *betweenness centrality* results indicate the difficulty of identifying which taxa will occupy a central position in a food web following species loss. These findings support a conservation strategy that advocates protecting the whole-ecosystem (Ives and Cardinale 2004) versus a strategy that focuses efforts on specific species that occupy central positions in the food web (Tyliankis et al. 2010). *Betweenness centrality* is an indicator of the importance of a species within a community (Estrada 2007) and the potential role of that species as a mediator of indirect effects between other species (Lai et al. 2012). The *betweenness centrality* values of species prior to amphibian declines did not predict which species would become important to the topology of the food web after amphibian declines. Species central to post-decline food web topology were not central to pre-decline food web topology, as taxa exhibiting the highest *betweenness centrality* values post-decline were unranked pre-decline. Our results indicate that a strategy protecting taxa with high *betweenness centrality* values would not be effective because it could exclude those taxa that ultimately became central to the food web.

Our findings are consistent with the prediction that food web robustness to species loss, as measured by the number of secondary extinctions following a primary extinction, increases with connectance (Dunne et al. 2002, Eklöf and Ebenman 2006). However, food web studies that use static trophic linkages and do not consider compensation through endogenous change in population size or behavioral adaptation require a species to lose all resources before a secondary extinction occurs (Eklöf and Ebenman 2006). This implies that secondary extinctions are constrained to species at trophic levels higher than the trophic level of the primary extinction. In our study, taxa that went secondarily extinct did not lose all of their resources and many occupied the same trophic level as tadpoles. Food web studies that use static trophic linkages, but include population dynamics, predict more secondary extinctions than models that do not include population dynamics and also predict secondary extinctions to occur at all trophic levels (Kupferberg 1997, Eklöf and Ebenman 2006). Our results agree more with these predictions, since the majority of taxa lost from the community occupied basal and intermediate trophic levels.

Our findings are also consistent with a less common prediction that suggests food web structure may be determined by basic rules that are independent of community composition. These studies use snapshots of the food web to make year-to-year comparisons, showing little annual variability in food web structure despite >50% species turnover (Cohen et al. 2009, Kaartinen and Roslin 2012). A similar pattern occurred in stream channels that were subjected to drought conditions: changes to the organization of these stream food webs occurred following drought, but connectance did not change, despite overall declines to species richness (Woodward et al. 2012, Ledger et al. 2013). Our results corroborate the conclusion from these studies that basic rules may structure food webs and is a result likely to be missed by analyzing aggregated food webs. However, our results are also unique as we suggest mechanistic causes of food web resilience. Furthermore, we also used a null model to compare our observed results against our predicted results, bolstering our conclusion that species loss may have a smaller effect on food web structure than models with static linkages would predict.

Our results also suggest that trophic interactions and indirect effects should be incorporated into the analyses of ecological networks to yield better insight into the consequences of changing species composition (Bascompte 2009, Ings et al. 2009, Melián et al. 2009). Most of the macroinvertebrates that declined by 2009 were not trophically linked to tadpoles. For example, tadpoles facilitate access to food resources for some taxa through bioturbation, reducing inorganic sediment accrual on stream substrates (Kupferberg 1997, Ranvestel et al. 2004). Increased sediment accrual may reduce macroinvertebrate populations by negatively affecting macroinvertebrate mobility and foraging (Connolly and Pearson 2007). Tadpoles also increase the nutritional quality of biofilm through excretion, by reducing biofilm C:N and C:P (Connelly et al. 2008, Rugenski et al. 2012). These examples demonstrate how a species can mediate community composition, even when species are not trophically linked, directly underscoring the importance of indirect effects in structuring ecological communities.

Although apex consumers are most susceptible to extinctions (Estes et al. 2011), tadpole extirpations represented the loss of a group from an intermediate trophic level. The loss of species at intermediate trophic levels is common and consequences can cascade both upward and downward through an ecological community via trophic and non-trophic pathways (Estes et al. 2011). Moreover, species at intermediate trophic levels can affect community composition in a wide range of ecosystems and are frequently extirpated by several mechanisms (Estes et al. 2011). For example, grazing and omnivorous shrimps have been extirpated from many highland streams in Puerto Rico by dam construction (Greathouse et al. 2006), and grazing bison were extirpated from the North American plains by overhunting (Knapp et al. 1999). Both of these extirpations resulted in increased primary producer biomass and changes to the consumer community, patterns also observed at the Rio Guabal (Connelly et al. 2008, Colón-Gaud et al. 2010a). Effects of these extirpations on community structure and ecosystem function highlight the importance of including species from intermediate trophic levels when considering effects of species loss.

In our study stream, several important food web structural attributes were more resilient to species loss than predicted by a simulation that assumed fixed trophic linkages. The resilience of food web structure appears to have been a function of new linkages that formed after the loss of a dominant taxonomic group, changes in consumer diets, and appearance of “new” generalist taxa that appeared post-decline. Many studies that examine effects of species loss on food web structure assume that food web structure is only shaped by the original trophic linkages. However, our study suggests that dynamic trophic interactions and indirect effects may serve an important role in structuring food webs after species loss, providing support for the hypothesis that food web structure is shaped by factors independent of their original trophic linkages.

Acknowledgements

Research was supported by National Science Foundation grants DEB #0717741, DEB #0645875, and DEB #0716680. We thank the Smithsonian Tropical Research Institute and Autoridad Nacional del Ambiente (ANAM) for providing logistical support in Panamá. We thank Becky Bixby for help with diatom identification and Robert Verb for help with the red algae identification. We thank Ford Ballantyne, Richard Shefferson, Tad Dallas, William G. McDowell, and Daniel Stouffer for stimulating conversations while analyzing the data and writing the manuscript. Maximum trophic level was calculated using R script provided by Owen Petchey. We also thank Cori Richards-Zawacki for providing the *Atelopus* tadpoles for gut content analysis and thank Timothy Wootton for comments on a previous draft. All research detailed in this manuscript complies with the current laws of the Republic of Panamá.

Table 4.1. Food web attributes used to characterize food web structure and that were predicted to change following tadpole declines based on a review of the literature. The prediction for skewness is that the degree distribution will become more left skewed following a species loss. Variance is not predicted (NP) to change following species loss.

abbreviation	attribute	Description	prediction	reference
$S^{\dagger}$	Species	Number of trophic species	↓	2
L/S	Linkage density	Number of linkages per species	↓	2
C	Connectance	The fraction of realized links (L/S^2)	↓	2
Skew*	Skewness	The asymmetry of the degree distribution	←	2
Var*	Variance	The variance of the degree distribution	NP	NP
Path	Average path length	The average shortest path length between all species pairs	↓	1
MaxTL	Maximum trophic level	Maximum trophic level of the top predator	↓	3
Gen	Generality	The standard deviation for the number of prey per species	↑	1
Vul	Vulnerability	The standard deviation for the number of predators per species	↓	1
% Bas	Fraction of basal taxa	Fraction of taxa with no prey	↑	1
% Herb	Fraction of herbivores	Fraction of taxa that feed on basal taxa	↑	2
% Int	Fraction of intermediate consumers	Fraction of consumers with predators and prey	↑	2
% Top	Fraction of top predators	Fraction of consumers with prey, but no predators	↓	2, 4
% Omn	Fraction of omnivores	Fraction of species that feed at more than one trophic level	↓	1
% Cann	Fraction of cannibalism	Fraction of species that feed on their own species	↓	1

*Attributes uncommon in food web literature

†For richness, *S*, species with the same prey and predators are usually lumped into trophic groups. However, we needed to account for changes in diet in response to species loss and wanted to maintain the criteria for *S* to be consistent across time. Therefore, we used the number of genera (except for Chironomidae which were classified as non-Tanypodinae and Tanypodinae) for *S* because genus was the lowest taxonomic level we could use while keeping taxa in functionally similar groups.

Reference: (1) (Coll et al. 2008), (2) (Dunne et al. 2002), (3) (McHugh et al. 2010), (4) (Odum 1985)

Table 4.2. Topological attributes for pre-amphibian decline (2004), post- amphibian decline (2009) and the simulated removal (Sim1 and Sim2) of tadpoles and macroinvertebrates from pool and riffle food webs. (Sim1 assumes trophic linkages are static by using the 2004 food web, but with taxa absent in 2009 removed. Sim2 tests if food web resilience is driven by new species or the formation of new linkages by using the 2009 food web and removing taxa present only in 2009. Abbreviations for food web attributes are defined in Table 1.)

	Pools				Riffles			
attribute	2004	2009	Sim1	Sim2	2004	2009	Sim1	Sim2
S	60	44	41	42	65	55	48	49
C	0.11	0.10	0.079	0.10	0.15	0.16	0.15	0.14
L/S	6.8	4.7	3.4	4.47	10.1	8.9	7.3	7.1
Skew	0.63	1.08	2.03	1.25	-0.61	0.088	0.71	-0.24
Var	77	60	51	61	81	74	73	71
Path	1.10	1.17	1.23	1.18	1.16	1.13	1.10	1.10
MaxTL	2.47	2.29	2.26	2.29	2.77	2.30	2.30	2.30
GenSD	1.57	1.92	2.26	1.99	1.25	1.38	1.60	1.58
VulSD	0.98	0.78	0.78	0.72	1.01	0.86	0.82	0.80
% Basal	0.65	0.77	0.78	0.79	0.57	0.64	0.69	0.69
% Herb	0.23	0.13	0.14	0.09	0.29	0.20	0.21	0.18
% Int	0.33	0.11	0.15	0.17	0.42	0.33	0.27	0.29
% Top	0.016	0.044	0.073	0.048	0.015	0.033	0.041	0.020
% Omn	0.11	0.12	0.06	0.07	0.17	0.16	0.15	0.12
% Cann	0.067	0.00	0.00	0.00	0.031	0.00	0.041	0.00

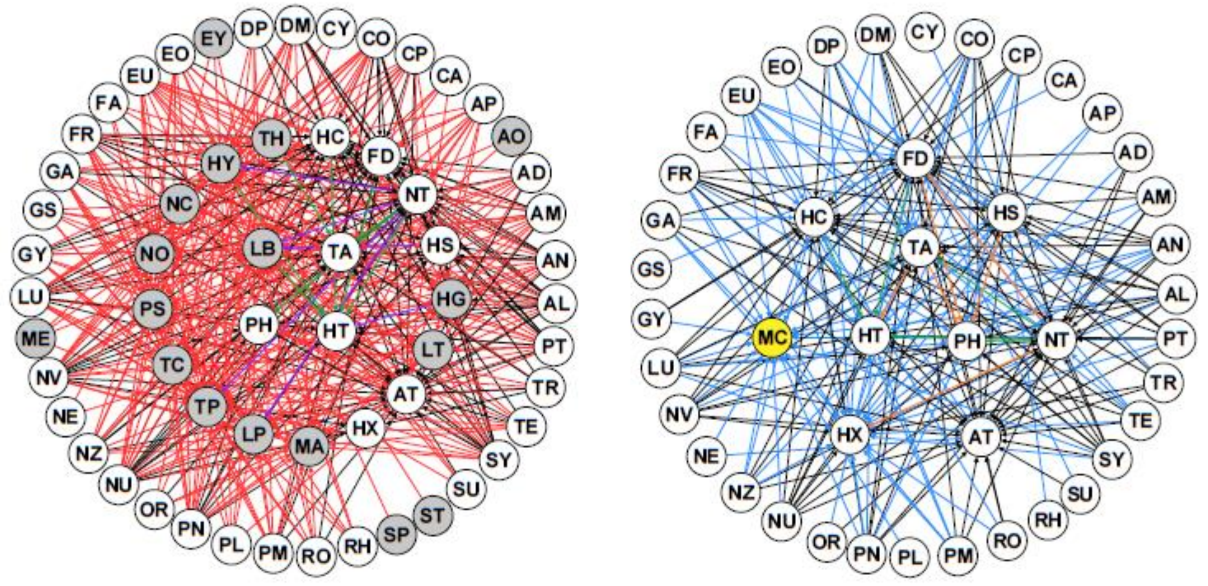
Table 4.3. *Betweenness centrality* (Centrality) indices for taxa, including macroinvertebrates (M), diatoms (D), and tadpoles (T), with the ten highest values for pre-decline (2004), post-decline (2009) from pools and riffles. (Betweenness centrality is a measure of a species importance to the food web's topology, with higher values indicating a central, or 'hub', role for those taxa in the food web.)

Pools				Riffles			
pre-decline (2004)		post-decline (2009)		pre-decline (2004)		post-decline (2009)	
Taxon	centrality	taxon	centrality	Taxon	centrality	taxon	centrality
Lithobates (T)	316.9	*Farrodes* (M)	224.9	*Atelopus* (T)	124.3	*Anacroneuria* (M)	129.3
Hyloscirtus (T)	158.1	*Heterelmis* (M)	101.6	*Simulium* (M)	118.6	*Cora* (M)	112.5
Tipula (M)	131.7	*Anchytarsus* (M)	97.1	*Thraulodes* (M)	91.8	*Philogenia* (M)	85.3
Anchytarsus (M)	101.4	*Macrelmis* (M)	82.2	Non-tanypodinae (M)	88.3	*Hexatoma* (M)	58.5
Non-tanypodinae (M)	92.6	*Hexatoma* (M)	78.9	*Chimarra* (M)	68.9	*Thraulodes* (M)	58.1
Nupela (D)	61.7	Non-tanypodinae (M)	57.9	*Stenonema* (M)	64.5	*Anchytarsus* (M)	57.9
Psephenus (M)	60.6	*Hexacylloepus* (M)	52.2	Filamentous algae (D)	64.0	*Hagenulopsis* (M)	52.6
Navicula (D)	57.3	*Heteragrion* (M)	34.8	*Leptonema* (M)	59.9	*Smicridea* (M)	45.2
Neoelmis (M)	56.9	Tanypodinae (M)	26.0	*Anchytarsus* (M)	54.0	*Leptonema* (M)	41.2
Tricorythodes (M)	53.8	*Philogenia* (M)	11.3	*Wormaldia* (M)	50.5	*Baetis* (M)	37.5

Figure 4.1. A schematic diagram of pre- (a and c) and post- (b and d) amphibian decline food webs from pools (a and b) and riffles (c and d) with lines representing trophic linkages and circles representing species. Basal taxa (primary producers and plant detritus) are located on the outer circle, primary consumers (tadpoles and insect grazers) on the middle circle, and intermediate consumers and top predators on the inner-most circle. Taxa abbreviations: AC=*Anacroneuria,* AD=*Adlafia*, AL=*Audouinella*, AM=*Achnanthidium*, AN=*Achnanthes,* AR=*Argia,* AO =*Amphora*, AP=*Amphipleura*, AR=*Argia,* AS=*Atelopus,* AT=*Anchytarsus*, BA=*Baetodes,* BE=*Baetis,* CA=*Caloneis,* CH=*Chimarra,* CO=*Cocconeis,* CP=*Chaempinnularia*, CR=*Cora*, CY=*Cyclotella*, DM=*Diadesmis*, DP=*Diploneis*, EO=*Eolimna,* EU=*Eunotia*, EY=*Encyonema,* FA=*Fallacia*, FD=*Farrodes,* FR=*Frustulia,* GA=*Gomphonema,* GS=*Gomphosenia*, GY=*Gyrosigma,* HA=*Haplohyphes,* HC=*Hexacylloepus,* HE=*Heterelmis,* HG=*Hagenulopsis,* HT=*Heteragrion,* HX=*Hexatoma*, HY=*Hyloscirtus,* LB=*Lithobates,* LP=*Leptohyphes,* LT=*Leptonema,* LU=*Luticola*, MA=*Macronema,* MC=*Macrelmis,* ME=*Melosira*, NC=*Nectopsyche,* ND=*Neurocordulia,* NE=*Neidium,* NO=*Neoelmis,* NT=Non-tanypodinae, NU=*Nupela,* NV=*Navicula,* NZ=*Nitzschia,* OR=*Orthosiera,* PC=*Phanocerus*, PH=*Philogenia*, PL=*Placoneis*, PM=*Planothidium,* PN=*Pinnularia*, PR=*Petrophila,* PS=*Psephenus,* PT=Plant, RH=*Rhoicosphenia,* RO=*Rhopalodia*, SL=*Simulium,* SM =*Smicridea,* SO=*Stenonema*, SP=*Stephanodiscus,* ST=*Stenoterobia,* SU=*Surirella,* SY=*Synedra*, TA=Tanypodinae, TC=*Tricorythodes,* TE=*Terpsinoe*, TH=*Thraulodes*, TP=*Tipula*, TR=*Tryblionella*, WO=*Wormaldia*

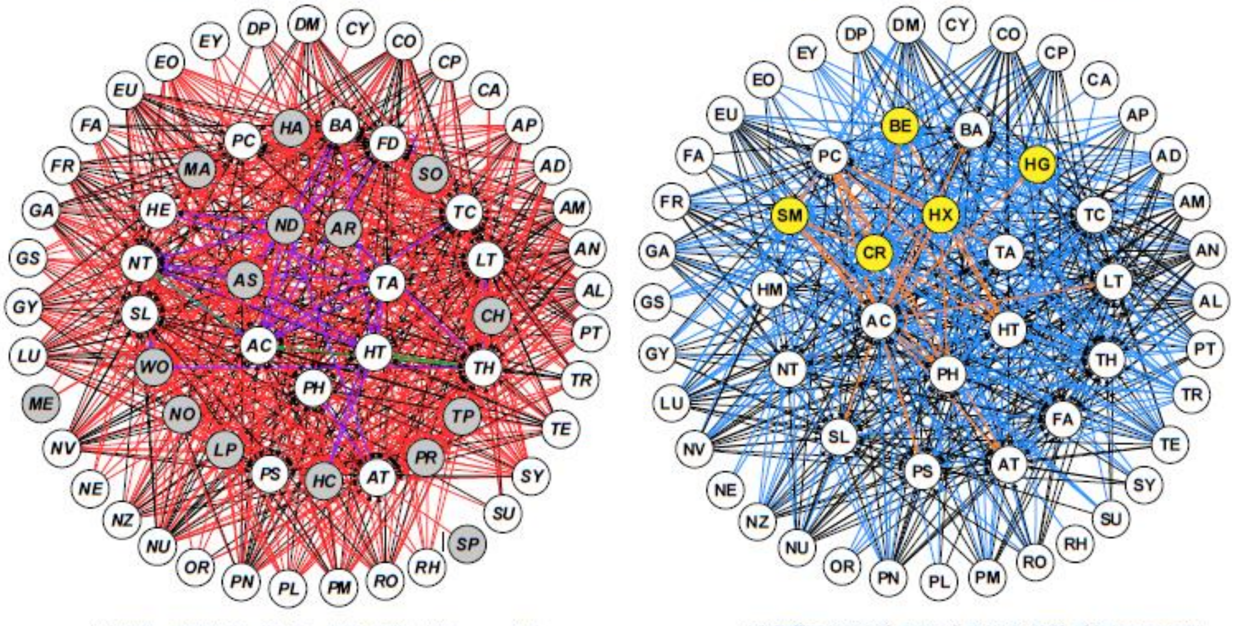
Figure 4.2. The effect of amphibian declines on food web attributes for (a) riffles and (b) pools. A z-score above “1” (dashed line) signifies that the attribute from the pre-decline food web is intermediate to the post-decline and simulated food web. A number between “0” (solid line) and “0.5” (dotted line) signifies that the attribute from the post-decline food web is more similar to the simulated food web while a number between “0.5” and “1” signifies the attribute from the post-decline food web is more similar to the pre-decline food web. Values <“0” indicate the attribute changed more than the simulation predicted. Connectance, linkage density, and maximum trophic level are important indicators of food web complexity. Sim1 reflects the simulated removal of tadpoles and macroinvertebrates from the pre-decline food web and is compared to the intact 2009 food web. Sim2 reflects the 2009 food web with only 2004 taxa, where taxa present only in 2009 were excluded, and is compared to the 2009 food web. Abbreviations for food web attributes are defined in Table 1.

Figure 4.1



(a) Pools 2004: S = 60, C = 0.11, 66% of linkages lost

(b) Pools 2009: S = 44, C = 0.10, 45% of linkages new



(c) Riffles 2004: S = 65, C = 0.15, 60% of linkages lost

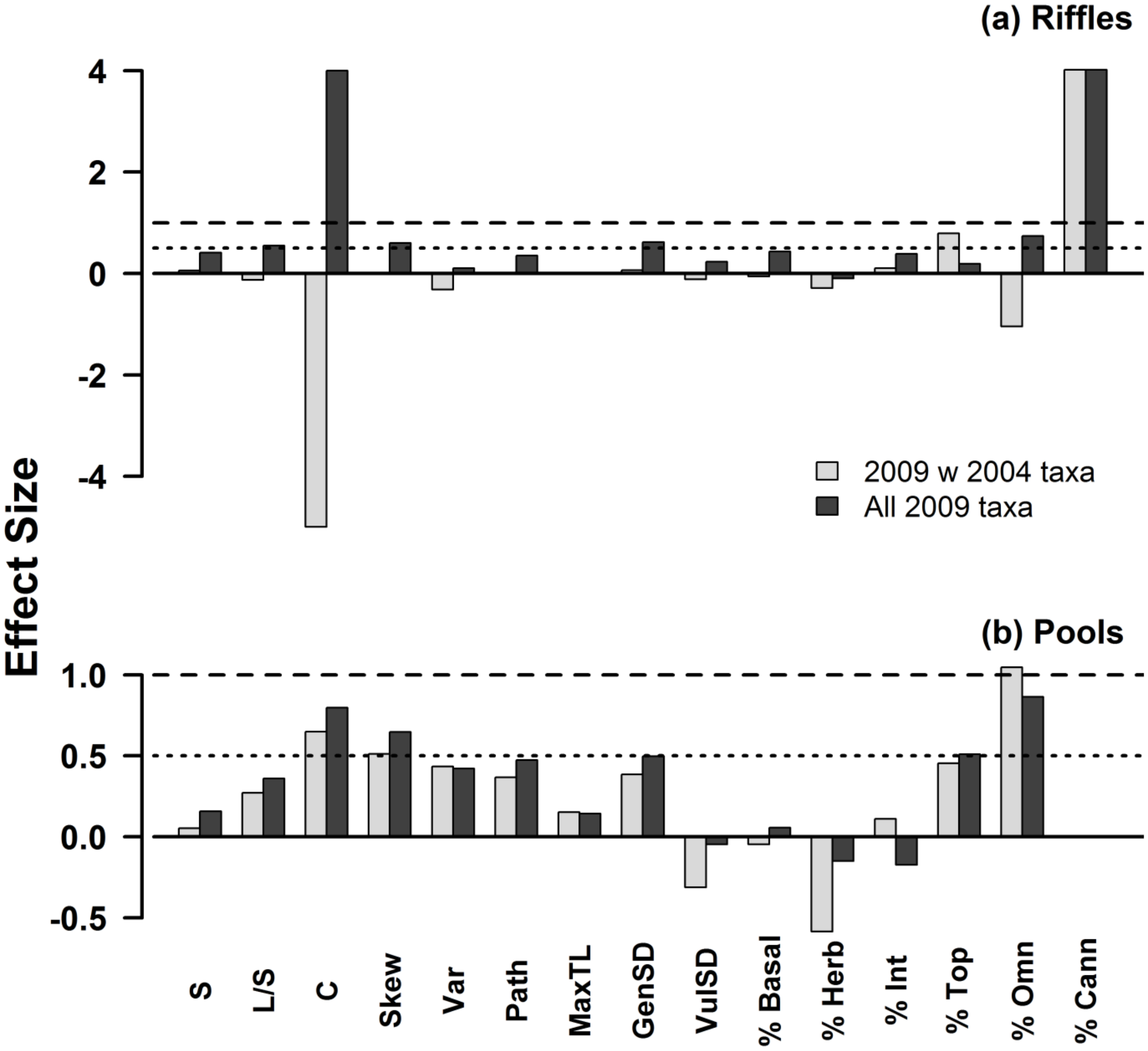
(d) Riffles 2009: S = 55, C = 0.16, 47% of linkages new

Nodes
○ Present Pre- and Post-decline
● Present Pre-decline only
● Present Post-decline only

Basal to Primary Consumer Trophic Linkages
← Present Pre- and Post-decline
← Present Pre-decline only
← Present Post-decline only

Consumer to Consumer Trophic Linkages
← Present Pre- and Post-decline
← Present Pre-decline only
← Present Post-decline only

Figure 4.2



(a) Riffles
4
2
0
-2
-4
2009 w 2004 taxa
All 2009 taxa
Effect Size
(b) Pools
1.0
0.5
0.0
-0.5
S
L/S
C
Skew
Var
Path
MaxTL
GenSD
VulSD
% Basal
% Herb
% Int
% Top
% Omn
% Cann

CHAPTER 5

CONCLUSIONS

Summary of dissertation objectives – The research presented in this dissertation examined the effects of amphibian declines on an insect-algal benthic community. Chapter 2 explored the effect of amphibian declines on resource use by 4 genera of grazing insects. Chapter 3 examined potential mechanisms for why, despite an increase in algal-standing stock following amphibian declines, the abundance of grazing insects did not increase following amphibian declines and at least partially compensate for amphibian declines. Chapter 4 examined the effects of amphibian declines on food web structure. Previous research has focused on using simulations to predict the effects of species loss on food web structure (Dunne et al. 2002a,b, Coll et al. 2008), but this chapter used empirical data to directly quantify the effects of species loss on food web structure. Together, these studies provide empirical insight from a natural system into how species loss from higher trophic levels (e.g. consumers) can impact food web structure and dynamics.

Chapter 2 summary – Prior studies have examined the effects of species introductions on resource use (Vander Zanden and Casselman 1999, Nilsson et al. 2012) but the loss of a species may result in the loss of a resource competitor, allowing surviving species to exploit different resources. The purpose of this chapter was to examine the effects of tadpole declines on the resource use of 4 grazing insect genera. A combination of stable isotope analyses and gut content analyses were used to quantify changes in resource use for 4 insect genera in the presence and absence of tadpoles, and to compare their resource use post-decline with tadpoles to test for potential functional redundancy in the stream community. The results showed changes in resource use by 3 of the 4 insect genera, but none of the genera occupied the same isotopic niche as tadpoles, suggesting that there was no functional compensation by grazing insects after amphibian declines. Importantly, two of the genera that shifted resource use following amphibian declines showed no changes in abundance, suggesting that measuring ecosystem descriptors, such as abundance, may not be enough to detect changes in a community following a species extirpation.

Chapter 3 summary – The focus of this chapter was to examine potential mechanisms for why grazing insects did not functionally compensate for the role of tadpoles in reducing algal-standing stock. Several analytical approaches were used to quantify the role of tadpoles and insects on structuring the diatom community and accrual of inorganic sediment. The results supported our hypothesis that the loss of tadpoles would also result in lower trait diversity in the grazing community. Furthermore, grazing tadpoles had stronger top-down effects on the diatom community than grazing insects. Together, these lines of evidence suggest tadpoles had a role in structuring the benthic community that grazing insects could not duplicate which precluded functional compensation by grazing insects following amphibian declines.

Chapter 4 summary – The objective of this chapter was to assess changes to food web structure in the presence and absence of amphibians. Field sampling and gut content analyses were used to construct pre- and post-decline food webs for pools and riffles and the structure of each food web was analyzed using network analysis. The results of the network analyses were then compared to a null model that assumed fixed trophic linkages to test for the resiliency of food web structure to species loss. The results showed that food webs were structurally resilient to species loss, with smaller changes than predicted by the null model. Resiliency of the food web structure in pools was driven by diet expansion by predators and in riffles by the presence of consumers that were absent in the pre-decline food web. The results of the study suggest food web structure may be more robust to species loss than previously predicted and that food web structure may be maintained by factors independent of the original trophic linkages.

General conclusions –Biodiversity declines are occurring worldwide with poorly understood effects on food webs. Taking advantage of a natural experiment that resulted in the loss of an abundant and functionally important group my dissertation focused on three conceptual questions to test the effects of species loss on food webs.

Question 1: Do surviving species shift diets? The results showed that surviving grazing insects shifted diets in response to tadpole declines, including a shift from selectively consuming small-sized diatoms to medium-sized diatoms. Some predator taxa also shifted diets to include more diatoms and filamentous algae following amphibian declines.

Question 2: Do surviving species functionally compensate for species loss? There was no evidence that grazing insects functionally compensated for the loss of grazing tadpoles. Post-amphibian decline, grazing insects did not occupy the same isotopic niche as grazing tadpoles suggesting that, although they shifted resources, they were not consuming the same resources as grazing tadpoles. Furthermore, grazing tadpoles had stronger top-down effects on the diatom community and sediment accrual than grazing insects, suggesting grazing tadpoles had a role in structuring the benthic community that grazing insects could not duplicate.

Question 3: How does food web structure respond to species loss? The effects of amphibian declines on food web topology were less than we might expect from models that use static trophic linkages and showed resiliency of the whole food web structure.

The resiliency of food web structure was partly driven by the arrival of generalist consumers that were absent from the pre-decline food web. This is an important result because it suggests regional diversity may serve a significant role in structuring a community following species loss. Most food web studies focus on local diversity, such as a stream reach, and exclude species at broader regional scales, such as watersheds, that may move into a localized community following a species loss. Examining characteristics of species, such as dispersal distance or generalist feeding habits, at a regional scale may provide higher predictive power into how regional species pools can affect the food web structure of a local community following a perturbation, such as habitat degradation or species loss.

The resiliency of food web structure was also partly driven by shifting diets following amphibian declines. Notably, diets of some predators shifted to include more diatoms and filamentous algae following amphibian declines, suggesting that consumers can change feeding habits following a perturbation. However, some predator taxa were also lost from the community and may have been unable to shift diets following amphibian declines. Identifying species that can exploit novel resources, particularly if resources from other trophic levels, may provide an avenue for identifying species in communities that are resistant to perturbations.

The loss of amphibians affected community organization as insect diversity declined and the structure of the diatom community shifted to larger-sized diatoms. Interestingly, the effects of amphibian declines on community organization were detected in the diatom community within months of amphibian declines (Connelly et al. 2008) but took years for the effects to reverberate through the insect community, highlighting a need for long-term monitoring to fully quantify long-term impacts of a disturbance on a community. The faster response of diatom populations to amphibian declines likely occurred because of the diatoms faster generation times compared to insects, making diatoms a potential sentinel group for assessing changes in community organization following a disturbance (Dixit et al. 1992).

The results from the individual studies appear to be diametrically opposed, with the first two chapters suggesting high variability in diets at the genus level while the third chapter suggests low variability to the structure of the whole food web. However, these findings are congruent with studies that examine biomass of populations and communities, which show high variability of individual populations but low variability of community biomass (Tilman 1996). My research suggests that the pattern of higher variability of populations but lower community variability may also occur in food webs, the first evidence for this phenomenon. This is an important contribution to the food web literature as the results challenge theoretical assumptions that whole food web structure is inextricably linked to individual taxa and their trophic linkages.

The studies presented in this dissertation used direct observations to assess the effects of tadpole declines on the food web of an insect-algal benthic community. The studies are limited in spatial and temporal scale, but they provide important empirical insight into the effects of species loss on food webs, particularly as the data come from a natural system that experienced a 'natural' loss rather than from mesocosm experiments. Consequently, the broader contribution of these studies to the food web literature is the strength of this dissertation, particularly as the results challenge many assumptions in the theoretical literature. The research presented here will provide an important empirical baseline for future food web studies as ecologists continue to elucidate the impacts of ongoing species losses on ecological communities.

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APPENDICES CHAPTER 3

Appendix A

Table 1. Values are means and standard error (SE) for different sampling years (abundance for diatoms, insects, and tadpoles or weight for inorganic sediment) in pools and riffles of the Rio Guabal prior to amphibian declines (n= 3 samples for tadpoles, n=6 samples for insects, and n=5 samples for diatoms and sediment in each of 3 mo. in 2004) and five years post amphibian declines (n= 3 samples for tadpoles, n=6 samples for insects, and n=5 samples for diatoms and sediment in each of 3 mo. in 2009). P values are from a permutation test that compares means from 2004 to 2009.

Table 1.

	Pools					Riffles				
Variable	2004 with tadpoles		2009 without tadpoles			2004 with tadpoles		2009 without tadpoles		
	Mean	SE	Mean	SE	*P*	Mean	SE	Mean	SE	*P*
Tadpoles (m^{-2})	31.85	6.12	0.00	0.00	<0.01	8.57	7.15	0.00	0.00	0.04
Small insects (< 3.5 mm m^{-2})	16.64	6.47	3.59	1.01	0.03	17.42	3.07	10.93	1.93	0.08
Large insects (> 3.51 mm m^{-2})	0.59	0.22	0.66	0.22	0.49	18.63	3.94	26.81	3.89	0.07
Small diatoms (cells cm^{-2})	1841.43	364.82	3978.74	1031.83	0.03	657.29	202.87	2400.39	329.66	<0.01
Medium diatoms (cells cm^{-2})	809.16	180.79	1967.36	693.64	0.05	242.76	78.34	451.57	100.39	0.05
Large diatoms (cells cm^{-2})	16.35	5.33	65.25	18.32	0.01	9.46	2.93	17.94	3.97	0.05
Sediment (g m^{-2})	53.61	2.57	278.74	81.46	<0.01	10.27	0.53	14.37	3.24	0.09

Appendix B

Table 1 and 2. Mean number of diatom valves m^{-2} in riffles (Table 1) and pools (Table 2) pre- (2004) and post- (2009) amphibian declines.

Table 1. Riffles

Taxa	2004 Mean (± SE)	2009 Mean (± SE)
Nupela	1922.80 (685.34)	6661.67 (1010.72)
Synedra	32.32 (17.67)	141.74 (40.53)
Nitzschia	89.96 (34.76)	258.78 (65.25)
Eolimna	120.94 (54.59)	341.35 (96.59)
Orthosiera	0.50 (0.50)	4.31 (2.04)
Achnanthes	211.30 (88.29)	401.37 (110.97)
Melosira	8.51 (7.43)	37.80 (20.92)
Luticola	177.18 (52.49)	330.93 (98.61)
Amphipleura	5.01 (5.01)	17.35 (7.89)
Adlafia	24.25 (12.74)	119.50 (76.97)
Rhoicosphenia	3.00 (2.50)	8.89 (4.35)
Planothidium	20.40 (10.19)	35.05 (10.84)
Frustulia	55.46 (22.78)	92.71 (31.28)
Tryblionella	42.01 (26.62)	67.23 (22.62)
Gomphonema	33.57 (13.68)	53.83 (24.28)
Placoneis	6.74 (6.20)	12.35 (5.69)
Gomphosenia	6.50 (3.25)	11.82 (6.74)
Diploneis	2.17 (1.69)	3.67 (1.91)
Rhopalodia	0.50 (0.50)	1.67 (1.67)
Navicula	530.03 (235.68)	668.41 (175.42)
Surirella	0.00 (0.00)	1.67 (1.67)
Stenoterobia	0.00 (0.00)	0.42 (0.42)
Terpsinoe	40.50 (11.87)	44.66 (13.68)
Chaempinnularia	2.50 (2.50)	3.34 (2.27)
Neidium	3.00 (2.50)	2.67 (1.89)
Achnanthidium	55.53 (28.92)	48.53 (13.84)
Pinnularia	117.54 (51.40)	99.91 (25.39)
Planothidium	18.14 (10.33)	12.77 (9.10)
Diadesmis	53.46 (31.70)	41.96 (13.09)
Fallacia	13.59 (8.18)	8.58 (3.66)
Cyclotella	6.01 (2.56)	3.83 (2.11)
Gyrosigma	45.53 (13.70)	28.87 (10.72)
Eunotia	113.74 (52.82)	55.44 (20.66)
Cocconeis	88.95 (21.98)	57.97 (13.43)
Amphora	21.29 (10.28)	2.15 (1.70)

Table 2. Pools

Taxa	2004 Mean (± SE)	2009 Mean (± SE)
Terpsinoe	367.04 (112.80)	1906.07 (574.21)
Synedra	679.03 (209.78)	4858.91 (2197.04)
Nupela	52720.89 (7697.34)	110429.40 (27803.97)
Eolimna	4395.32 (941.30)	9076.62 (2257.56)
Cyclotella	0.00 (0.00)	60.93 (30.21)
Melosira	0.00 (0.00)	1731.65 (1060.39)
Frustulia	7157.30 (2682.06)	17403.08 (5994.27)
Rhoicosphenia	0.00 (0.00)	53.32 (37.28)
Surirella	27.53 (27.53)	196.43 (121.11)
Pinnularia	3106.09 (1166.24)	9682.25 (5483.23)
Navicula	19700.94 (3894.36)	31726.43 (9919.58)
Luticola	7345.41 (1500.19)	11236.54 (3515.37)
Tryblionella	972.66 (419.43)	1757.17 (735.63)
Planothidium	467.98 (96.19)	677.32 (211.09)
Diadesmis	1426.87 (384.74)	1929.27 (657.95)
Planothidium	0.00 (0.00)	8.93 (8.93)
Diploneis	169.76 (70.43)	204.36 (70.77)
Gomphosenia	385.39 (143.33)	477.34 (245.90)
Achnanthes	8607.12 (1796.71)	9603.47 (3702.97)
Amphipleura	527.62 (220.91)	620.49 (471.28)
Caloneis	128.46 (72.64)	155.99 (113.08)
Nitzschia	10639.61 (4185.86)	11405.43 (4345.11)
Neidium	128.46 (109.60)	137.02 (47.08)
Stenoterobia	2349.06 (2134.21)	2388.15 (1401.98)
Adlafia	1312.17 (492.83)	1318.05 (373.96)
Orthosiera	27.53 (27.53)	27.28 (19.83)
Chaempinnularia	233.99 (113.31)	220.89 (65.37)
Rhopalodia	59.64 (32.12)	36.46 (21.01)
Amphora	165.17 (117.51)	49.85 (31.47)
Gyrosigma	935.96 (415.08)	420.62 (129.85)
Encyonema	275.28 (220.99)	0.00 (0.00)
Fallacia	467.98 (224.95)	128.46 (57.72)
Achnanthidium	3275.84 (825.28)	1973.45 (280.38)
Eunotia	3005.15 (711.67)	1310.90 (351.15)
Placoneis	1495.69 (570.60)	239.36 (104.38)
Gomphonema	1876.50 (488.45)	517.23 (176.14)
Cocconeis	3252.90 (1030.29)	512.76 (181.26)

Appendix C

Trait diversity indices that included four traits: 1) gape size, 2) head capsule width x biomass, 3) locomotion (free swimmer, crawler or web spinner [*Petrophila*]), and 4) mouthpart morphology (collector-gatherer or scraper) for pool and riffle habitats. P-values were calculated from a randomization that permuted the observations and then calculated the difference between randomized pre- and post-decline values 9999. P values < 0.05 were considered significant.

Variable	Pool			Riffle		
	Pre-decline	Post-decline	p-value	Pre-decline	Post-decline	p-value
FRic	0.14	4.52 x 10-19	< 0.01	2.11	0.01	< 0.01
FEve	0.20	0.06	< 0.01	0.13	0.15	0.35
FDiv	0.82	0.90	0.71	0.74	0.82	0.72
FDis	1.78	0.89	0.05	1.11	0.88	0.29

Appendix D

Number of diatoms (mean ± SE) classified by genus in the guts of tadpoles and grazing insects collected from pools and riffles. The number of specimens sampled for gut content analysis is reported in the N column. The numeral following the genus name denotes body size: ‘1’ = <3.5 and ‘2’ = >3.51. Tadpole taxa are *Hyloscirtus* and *Lithobates* and were only present in 2004. A ‘0’ signifies that no diatoms were found. NA denotes that either no individuals of that size class were sampled in that year, or, if the SE is NA, indicates only one individual had diatoms of that size class in their gut.

Taxon	N	Habitat	Year	*Achnanthes* Mean	*Ach* SE	*Acnanthidium* Mean	*Achnanthidium* SE
Hyloscirtus	5	Pool	2004	52.24	25.21	256.61	220.24
Lithobates	4	Pool	2004	421.61	195.85	102.43	44.11
Farrodes1	7	Pool	2004	14.21	NA	4.46	NA
Farrodes2	5	Pool	2004	99.73	15.55	242.66	198.30
Hagenulopsis1	2	Pool	2004	3.93	NA	7.86	NA
Hagenulopsis2	1	Pool	2004	4.00	NA	0	0
Leptohyphes1	1	Pool	2004	0	0	15.71	NA
Psephenus1	3	Pool	2004	37.47	11.52	22.42	6.97
Psephenus2	0	Pool	2004	93.11	NA	93.11	NA
Tricorythodes1	4	Pool	2004	24.18	NA	8.10	NA

Taxon		Habitat	Year	*Achnanthes* Mean	*Achnanthes* SE	*Acnanthidium* Mean	*Achnanthidium* SE
Baetodes1	2	Pool	2009	864.57	NA	403.70	NA
Baetodes2	3	Pool	2009	74.16	NA	37.08	NA
Farrodes1	12	Pool	2009	4.04	0.34	8.76	NA
Farrodes2	8	Pool	2009	89.98	55.77	27.97	13.11
Hagenulopsis2	2	Pool	2009	0	0	19.43	NA
Haplohyphes2	2	Pool	2009	8.15	NA	10.19	NA
Leptohyphes1	7	Pool	2009	14.86	NA	0	0
Psephenus1	6	Pool	2009	58.26	45.36	732.81	574.88
Psephenus2	1	Pool	2009	83.55	72.34	130.97	7.81
Tricorythodes1	15	Pool	2009	28.80	2.21	9.73	1.89

Taxon	Habitat	Year	*Adlafia* Mean	*Adlafia* SE	*Amphipleura* Mean	*Amphipleura* SE
Hyloscirtus	Pool	2004	0	0	0	0
Lithobates	Pool	2004	0	0	3.74	NA
Farrodes1	Pool	2004	4.74	NA	0	0
Farrodes2	Pool	2004	38.49	19.15	0.57	NA
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	4.00	NA	0	0
Leptohyphes2	Pool	2004	0	0	1.57	NA
Psephenus1	Pool	2004	8.32	0.59	0	0
Psephenus2	Pool	2004	8.73	NA	0	0
Tricorythodes1	Pool	2004	4.27	0.24	1.33	0.88

Taxon	Habitat	Year	*Adlafia* Mean	*Adlafia* SE	*Amphipleura* Mean	*Amphipleura* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	18.54	NA	0	0
Farrodes1	Pool	2009	4.38	NA	0	0
Farrodes2	Pool	2009	30.62	NA	0	0
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	0	0	4.84	NA
Leptohyphes1	Pool	2009	0	0	0	0
Psephenus1	Pool	2009	8.49	4.44	0	0
Psephenus2	Pool	2009	0	0	0	0
Tricorythodes1	Pool	2009	8.64	NA	1.21	0.18

Taxon	Habitat	Year	*Chaempinnularia* Mean	*Chaempinnularia* SE	*Cocconeis* Mean	*Cocconeis* SE
Hyloscirtus	Pool	2004	4.26	NA	5.15	2.65
Lithobates	Pool	2004	28.69	16.38	54.58	21.03
Farrodes1	Pool	2004	0	0	3.73	1.95
Farrodes2	Pool	2004	22.94	5.88	45.95	43.39
Hagenulopsis1	Pool	2004	3.93	NA	79.36	NA
Hagenulopsis2	Pool	2004	0	0	29.61	NA
Leptohyphes2	Pool	2004	0	0	3.93	NA
Psephenus1	Pool	2004	0	0	184.13	177.00
Psephenus2	Pool	2004	4.36	NA	12.07	4.22
Tricorythodes1	Pool	2004	0	0	26.83	24.18

Taxon	Habitat	Year	*Chaempinnularia* Mean	*Chaempinnularia* SE	*Cocconeis* Mean	*Cocconeis* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	0	0
Farrodes1	Pool	2009	0	0	3.07	NA
Farrodes2	Pool	2009	2.79	NA	6.53	4.07
Hagenulopsis2	Pool	2009	9.72	NA	0	0
Haplohyphes2	Pool	2009	0	0	0	0
Leptohyphes1	Pool	2009	0	0	2.01	1.59
Psephenus1	Pool	2009	0	0	1.52	1.31
Psephenus2	Pool	2009	0	0	9.87	1.30
Tricorythodes1	Pool	2009	0	0	0	0

Taxon	Habitat	Year	*Diploneis* Mean	*Diploneis* SE	*Encyonema* Mean	*Encyonema* SE
Hyloscirtus	Pool	2004	4.26	NA	8.52	NA
Lithobates	Pool	2004	30.35	NA	151.76	NA
Farrodes1	Pool	2004	4.01	NA	0	0
Farrodes2	Pool	2004	0	0	0	0
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	0	0	0	0
Psephenus1	Pool	2004	0	0	1.78	NA
Psephenus2	Pool	2004	0	0	0	0
Tricorythodes1	Pool	2004	0	0	0	0

Taxon	Habitat	Year	*Diploneis* Mean	*Diploneis* SE	*Encyonema* Mean	*Encyonema* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	0	0
Farrodes1	Pool	2009	2.19	NA	0	0
Farrodes2	Pool	2009	9.46	1.99	0	0
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	0	0	0	0
Leptohyphes1	Pool	2009	4.54	NA	0	0
Psephenus1	Pool	2009	0	0	0	0
Psephenus2	Pool	2009	0	0	0	0
Tricorythodes1	Pool	2009	5.30	2.46	0	0

Taxon	Habitat	Year	*Eolimna* Mean	*Eolimna* SE	*Eunotia* Mean	*Eunotia* SE
Hyloscirtus	Pool	2004	0	0	29.56	14.43
Lithobates	Pool	2004	633.55	402.82	130.49	38.43
Farrodes1	Pool	2004	0	0	19.69	9.74
Farrodes2	Pool	2004	302.82	161.20	71.88	46.29
Hagenulopsis1	Pool	2004	0	0	21.21	NA
Hagenulopsis2	Pool	2004	12.01	NA	1.60	NA
Leptohyphes2	Pool	2004	3.93	NA	0	0
Psephenus1	Pool	2004	48.85	41.12	51.87	12.71
Psephenus2	Pool	2004	294.86	NA	160.28	145.44
Tricorythodes1	Pool	2004	0	0	14.55	9.31

Taxon	Habitat	Year	*Eolimna* Mean	*Eolimna* SE	*Eunotia* Mean	*Eunotia* SE
Baetodes1	Pool	2009	0	0	85.74	NA
Baetodes2	Pool	2009	0	0	40.79	NA
Farrodes1	Pool	2009	4.38	NA	49.06	NA
Farrodes2	Pool	2009	0	0	17.21	5.05
Hagenulopsis2	Pool	2009	0	0	13.60	NA
Haplohyphes2	Pool	2009	0	0	2.34	NA
Leptohyphes1	Pool	2009	0	0	0.83	NA
Psephenus1	Pool	2009	32.01	7.72	15.10	6.74
Psephenus2	Pool	2009	235.92	NA	14.59	5.24
Tricorythodes1	Pool	2009	0	0	14.00	11.05

Taxon	Habitat	Year	*Fallacia* Mean	*Fallacia* SE	*Frustulia* Mean	*Frustulia* SE
Hyloscirtus	Pool	2004	0	0	7.78	1.87
Lithobates	Pool	2004	0	0	15.38	NA
Farrodes1	Pool	2004	0	0	3.12	NA
Farrodes2	Pool	2004	0	0	0	0
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0.80	NA
Leptohyphes2	Pool	2004	0	0	0	0
Psephenus1	Pool	2004	4.72	NA	0	0
Psephenus2	Pool	2004	0	0	9.31	NA
Tricorythodes1	Pool	2004	7.66	NA	1.08	0.27

Taxon	Habitat	Year	*Fallacia* Mean	*Fallacia* SE	*Frustulia* Mean	*Frustulia* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	0	0
Farrodes1	Pool	2009	0	0	2.75	1.64
Farrodes2	Pool	2009	24.50	NA	1.86	0.66
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	3.90	NA	77.91	9.24
Leptohyphes1	Pool	2009	0	0	0	0
Psephenus1	Pool	2009	3.72	NA	13.24	NA
Psephenus2	Pool	2009	0	0	13.88	NA
Tricorythodes1	Pool	2009	0	0	5.40	NA

Taxon	Habitat	Year	*Gomphonema* Mean	*Gomphonema* SE	*Gomphosenia* Mean	*Gomphosenia* SE
Hyloscirtus	Pool	2004	12.02	5.47	0	0
Lithobates	Pool	2004	97.22	32.71	19.74	NA
Farrodes1	Pool	2004	0	0	0	0
Farrodes2	Pool	2004	0	0	0	0
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	0	0	0	0
Psephenus1	Pool	2004	4.45	NA	0	0
Psephenus2	Pool	2004	135.02	NA	0	0
Tricorythodes1	Pool	2004	6.75	NA	4.50	NA

Taxon	Habitat	Year	*Gomphonema* Mean	*Gomphonema* SE	*Gomphosenia* Mean	*Gomphosenia* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	0	0
Farrodes1	Pool	2009	2.63	NA	0	0
Farrodes2	Pool	2009	14.98	NA	8.32	NA
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	3.72	1.38	0	0
Leptohyphes1	Pool	2009	0	0	0	0
Psephenus1	Pool	2009	12.77	NA	0	0
Psephenus2	Pool	2009	0	0	0	0
Tricorythodes1	Pool	2009	3.85	NA	0	0

Taxon	Habitat	Year	*Gyrosigma* Mean	*Gyrosigma* SE	*Luticola* Mean	*Luticola* SE
Hyloscirtus	Pool	2004	11.73	NA	11.03	5.94
Lithobates	Pool	2004	0	0	63.86	31.87
Farrodes1	Pool	2004	1.85	0.52	15.63	NA
Farrodes2	Pool	2004	0	0	5.12	NA
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	1.18	NA	0	0
Psephenus1	Pool	2004	0	0	4.17	NA
Psephenus2	Pool	2004	0	0	4.08	NA
Tricorythodes1	Pool	2004	1.20	0.60	15.15	10.65

Taxon	Habitat	Year	*Gyrosigma* Mean	*Gyrosigma* SE	*Luticola* Mean	*Luticola* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	31.52	NA
Farrodes1	Pool	2009	0	0	4.04	0.34
Farrodes2	Pool	2009	0	0	26.73	16.60
Hagenulopsis2	Pool	2009	0	0	17.97	NA
Haplohyphes2	Pool	2009	0.70	0.31	5.10	NA
Leptohyphes1	Pool	2009	0	0	13.79	12.14
Psephenus1	Pool	2009	0	0	5.52	3.09
Psephenus2	Pool	2009	0	0	9.35	NA
Tricorythodes1	Pool	2009	0	0	9.49	5.44

Taxon	Habitat	Year	*Navicula* Mean	*Navicula* SE	*Nitzschia* Mean	*Nitzschia* SE
Hyloscirtus	Pool	2004	14.52	5.01	67.91	12.46
Lithobates	Pool	2004	1724.92	632.98	24.18	1.74
Farrodes1	Pool	2004	6.62	6.17	0	0
Farrodes2	Pool	2004	13.14	4.49	0	0
Hagenulopsis1	Pool	2004	0.79	NA	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	3.14	NA	0	0
Psephenus1	Pool	2004	4.82	0.37	0	0
Psephenus2	Pool	2004	15.52	NA	0	0
Tricorythodes1	Pool	2004	25.08	11.38	9.90	NA

Taxon	Habitat	Year	*Navicula* Mean	*Navicula* SE	*Nitzschia* Mean	*Nitzschia* SE
Baetodes1	Pool	2009	42.87	NA	0	0
Baetodes2	Pool	2009	79.72	NA	0	0
Farrodes1	Pool	2009	10.95	6.07	0	0
Farrodes2	Pool	2009	60.92	27.11	26.73	18.40
Hagenulopsis2	Pool	2009	17.49	NA	0	0
Haplohyphes2	Pool	2009	39.30	3.62	0	0
Leptohyphes1	Pool	2009	31.51	17.48	0	0
Psephenus1	Pool	2009	5.91	2.81	0	0
Psephenus2	Pool	2009	20.07	11.11	0	0
Tricorythodes1	Pool	2009	15.52	4.94	0	0

Taxon	Habitat	Year	*Nupela* Mean	*Nupela* SE	*Orthoseira* Mean	*Orthoseira* SE
Hyloscirtus	Pool	2004	43.43	16.56	8.52	NA
Lithobates	Pool	2004	757.44	228.84	0	0
Farrodes1	Pool	2004	59.56	35.33	0	0
Farrodes2	Pool	2004	2346.71	1547.01	0	0
Hagenulopsis1	Pool	2004	142.61	NA	0	0
Hagenulopsis2	Pool	2004	22.01	NA	0	0
Leptohyphes2	Pool	2004	69.14	NA	0	0
Psephenus1	Pool	2004	297.91	169.41	0	0
Psephenus2	Pool	2004	927.68	848.49	0	0
Tricorythodes1	Pool	2004	84.36	48.54	0	0

Taxon	Habitat	Year	*Nupela* Mean	*Nupela* SE	*Orthoseira* Mean	*Orthoseira* SE
Baetodes1	Pool	2009	1368.31	NA	0	0
Baetodes2	Pool	2009	153.87	NA	0	0
Farrodes1	Pool	2009	74.76	59.20	0	0
Farrodes2	Pool	2009	136.04	38.17	0	0
Hagenulopsis2	Pool	2009	119.02	NA	0	0
Haplohyphes2	Pool	2009	70.95	28.42	0	0
Leptohyphes1	Pool	2009	57.01	27.29	0	0
Psephenus1	Pool	2009	298.80	49.74	0	0
Psephenus2	Pool	2009	1717.38	1210.37	0	0
Tricorythodes1	Pool	2009	48.62	8.41	0	0

Taxon	Habitat	Year	*Pinnularia* Mean	*Pinnularia* SE	*Placoneis* Mean	*Placoneis* SE
Hyloscirtus	Pool	2004	37.91	32.15	0	0
Lithobates	Pool	2004	66.78	18.35	0	0
Farrodes1	Pool	2004	1.42	NA	0	0
Farrodes2	Pool	2004	2.84	NA	0	0
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0.60	NA	0	0
Leptohyphes2	Pool	2004	1.77	NA	0	0
Psephenus1	Pool	2004	0	0	0	0
Psephenus2	Pool	2004	0.22	NA	15.52	NA
Tricorythodes1	Pool	2004	4.34	2.31	0	0

Taxon	Habitat	Year	*Pinnularia* Mean	*Pinnularia* SE	*Placoneis* Mean	*Placoneis* SE
Baetodes1	Pool	2009	21.44	NA	0	0
Baetodes2	Pool	2009	35.22	NA	0	0
Farrodes1	Pool	2009	10.46	9.99	0	0
Farrodes2	Pool	2009	16.55	5.88	0	0
Hagenulopsis2	Pool	2009	7.77	NA	0	0
Haplohyphes2	Pool	2009	49.59	28.13	0	0
Leptohyphes1	Pool	2009	37.52	35.25	0	0
Psephenus1	Pool	2009	1.37	0.65	0	0
Psephenus2	Pool	2009	3.83	0.85	0	0
Tricorythodes1	Pool	2009	13.47	4.17	0	0

Taxon	Habitat	Year	*Planothidium* Mean	*Planothidium* SE	*Rhoicosphenia* Mean	*Rhoicosphenia* SE
Hyloscirtus	Pool	2004	33.95	NA	0	0
Lithobates	Pool	2004	13.26	NA	29.77	16.08
Farrodes1	Pool	2004	1.86	NA	0	0
Farrodes2	Pool	2004	54.76	NA	0	0
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	0	0	0	0
Psephenus1	Pool	2004	8.49	NA	0	0
Psephenus2	Pool	2004	0	0	15.52	NA
Tricorythodes1	Pool	2004	23.78	NA	0	0

Taxon	Habitat	Year	*Planothidium* Mean	*Planothidium* SE	*Rhoicosphenia* Mean	*Rhoicosphenia* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	7.42	NA	0	0
Farrodes1	Pool	2009	4.38	NA	0	0
Farrodes2	Pool	2009	0	0	0	0
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	0	0	0	0
Leptohyphes1	Pool	2009	0	0	0	0
Psephenus1	Pool	2009	2.65	NA	0	0
Psephenus2	Pool	2009	19.82	NA	0	0
Tricorythodes1	Pool	2009	0	0	0	0

Taxon	Habitat	Year	*Rhopalodia* Mean	*Rhopalodia* SE	*Synedra* Mean	*Synedra* SE
Hyloscirtus	Pool	2004	18.05	NA	4.18	2.44
Lithobates	Pool	2004	0	0	50.82	29.59
Farrodes1	Pool	2004	0.47	NA	1.82	0.63
Farrodes2	Pool	2004	0.57	NA	12.53	11.96
Hagenulopsis1	Pool	2004	0	0	1.96	NA
Hagenulopsis2	Pool	2004	0	0	1.60	NA
Leptohyphes2	Pool	2004	0.39	NA	0.20	NA
Psephenus1	Pool	2004	0	0	0.24	NA
Psephenus2	Pool	2004	0	0	0.22	NA
Tricorythodes1	Pool	2004	0	0	1.23	1.02

Taxon	Habitat	Year	*Rhopalodia* Mean	*Rhopalodia* SE	*Synedra* Mean	*Synedra* SE
Baetodes1	Pool	2009	0	0	7.15	NA
Baetodes2	Pool	2009	0	0	4.63	NA
Farrodes1	Pool	2009	0.44	NA	4.91	4.45
Farrodes2	Pool	2009	1.65	NA	25.38	15.68
Hagenulopsis2	Pool	2009	0	0	12.63	NA
Haplohyphes2	Pool	2009	3.22	0.68	9.12	4.64
Leptohyphes1	Pool	2009	0	0	4.79	3.14
Psephenus1	Pool	2009	0	0	1.18	0.48
Psephenus2	Pool	2009	0	0	9.21	3.26
Tricorythodes1	Pool	2009	0	0	2.23	0.67

Taxon	Habitat	Year	*Terpsinoe* Mean	*Terpsinoe* SE	*Tryblionella* Mean	*Tryblionella* SE
Hyloscirtus	Pool	2004	4.26	NA	4.55	NA
Lithobates	Pool	2004	28.15	NA	0	0
Farrodes1	Pool	2004	0	0	8.05	NA
Farrodes2	Pool	2004	0	0	8.65	NA
Hagenulopsis1	Pool	2004	0	0	0	0
Hagenulopsis2	Pool	2004	0	0	0	0
Leptohyphes2	Pool	2004	0	0	0	0
Psephenus1	Pool	2004	0	0	4.17	NA
Psephenus2	Pool	2004	0	0	0	0
Tricorythodes1	Pool	2004	10.68	10.48	4.03	NA

Taxon	Habitat	Year	*Terpsinoe* Mean	*Terpsinoe* SE	*Tryblionella* Mean	*Tryblionella* SE
Baetodes1	Pool	2009	0	0	0	0
Baetodes2	Pool	2009	0	0	0	0
Farrodes1	Pool	2009	0	0	1.31	NA
Farrodes2	Pool	2009	7.34	NA	0	0
Hagenulopsis2	Pool	2009	0	0	0	0
Haplohyphes2	Pool	2009	0	0	50.45	NA
Leptohyphes1	Pool	2009	0.62	NA	14.41	NA
Psephenus1	Pool	2009	0	0	3.91	1.38
Psephenus2	Pool	2009	0	0	0	0
Tricorythodes1	Pool	2009	0.66	0.42	0	0

Taxon	N	Habitat	Year	*Achnanthes* Mean	*Ach.* SE	*Acnanthidium* Mean	*Achnanthidium* SE
Hyloscirtus	4	Riffle	2004	23.26	7.73	15.26	0.91
Lithobates	4	Riffle	2004	305.94	107.61	80.54	24.75
Baetis1	3	Riffle	2004	12.22	NA	0	0
Baetis2	1	Riffle	2004	10.64	NA	0	0
Baetodes1	11	Riffle	2004	59.38	39.93	5.40	0.56
Baetodes2	1	Riffle	2004	41.17	NA	15.23	NA
Farrodes1	4	Riffle	2004	16.90	NA	10.89	NA
Farrodes2	2	Riffle	2004	4.17	0.05	6.67	2.86
Haplohyphes1	0	Riffle	2004	15.78	7.67	5.69	0.53
Leptohyphes1	9	Riffle	2004	0	0	0	0
Petrophila2	7	Riffle	2004	416.19	299.67	54.52	39.25
Psephenus1	6	Riffle	2004	10.75	3.77	26.92	17.72
Psephenus2	3	Riffle	2004	56.60	3.57	33.12	11.49
Stenonema1	2	Riffle	2004	6.19	2.10	17.95	NA
Stenonema2	5	Riffle	2004	92.97	88.91	26.00	13.01
Thraulodes1	4	Riffle	2004	5.92	1.43	4.52	NA
Thraulodes2	6	Riffle	2004	33.97	9.71	29.31	7.03
Tricorythodes1	10	Riffle	2004	33.92	21.20	25.86	16.04
Tricorythodes2	2	Riffle	2004	21.56	17.66	22.35	14.71

Taxon		Habitat	Year	*Achnanthes* Mean	*Achnanthes* SE	*Acnanthidium* Mean	*Achnanthidium* SE
Baetis1	7	Riffle	2009	18.47	5.78	9.72	NA
Baetis2	7	Riffle	2009	86.14	53.37	15.52	4.17
Baetodes1	8	Riffle	2009	58.68	23.73	24.09	19.32
Baetodes2	10	Riffle	2009	38.23	27.65	155.41	145.99
Farrodes1	10	Riffle	2009	4.01	NA	0	0
Farrodes2	7	Riffle	2009	39.93	14.04	27.45	10.32
Hagenulopsis1	2	Riffle	2009	23.41	NA	0	0
Leptohyphes1	3	Riffle	2009	8.18	NA	0	0
Petrophila2	9	Riffle	2009	535.89	NA	107.18	NA
Psephenus1	2	Riffle	2009	384.74	202.91	115.03	50.92
Psephenus2	10	Riffle	2009	480.92	323.52	195.22	99.28
Stenonema2	6	Riffle	2009	146.93	110.30	171.49	NA
Thraulodes1	8	Riffle	2009	76.58	5.86	57.44	7.97
Thraulodes2	13	Riffle	2009	180.59	106.58	638.85	603.32
Tricorythodes1	11	Riffle	2009	16.69	5.02	4.17	NA

Taxon	Habitat	Year	*Adlafia* Mean	*Adlafia* SE	*Amphipleura* Mean	*Amphipleura* SE
Hyloscirtus	Riffle	2004	2.71	NA	0	0
Lithobates	Riffle	2004	0	0	0	0
Baetis1	Riffle	2004	0	0	0	0
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	7.71	3.41	0	0
Baetodes2	Riffle	2004	4.12	NA	0	0
Farrodes1	Riffle	2004	3.76	NA	0	0
Farrodes2	Riffle	2004	8.44	NA	0	0
Haplohyphes1	Riffle	2004	4.19	0.11	0.86	NA
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	45.72	30.62	0	0
Psephenus1	Riffle	2004	218.25	NA	0	0
Psephenus2	Riffle	2004	120.34	NA	0	0
Stenonema1	Riffle	2004	36.08	NA	17.95	16.61
Stenonema2	Riffle	2004	545.63	NA	7.00	5.99
Thraulodes1	Riffle	2004	3.87	NA	0	0
Thraulodes2	Riffle	2004	5.02	1.04	1.76	0.29
Tricorythodes1	Riffle	2004	25.66	12.24	0.38	NA
Tricorythodes2	Riffle	2004	4.31	NA	1.34	NA

Taxon	Habitat	Year	*Adlafia* Mean	*Adlafia* SE	*Amphipleura* Mean	*Amphipleura* SE
Baetis1	Riffle	2009	0	0	0	0
Baetis2	Riffle	2009	15.90	7.87	0	0
Baetodes1	Riffle	2009	4.15	NA	1.66	NA
Baetodes2	Riffle	2009	5.51	1.69	0	0
Farrodes1	Riffle	2009	4.01	NA	0	0
Farrodes2	Riffle	2009	5.36	NA	0	0
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	107.18	NA	0	0
Psephenus1	Riffle	2009	110.64	NA	0	0
Psephenus2	Riffle	2009	149.80	NA	1.42	NA
Stenonema2	Riffle	2009	85.74	NA	0	0
Thraulodes1	Riffle	2009	16.49	NA	0	0
Thraulodes2	Riffle	2009	0	0	0	0
Tricorythodes1	Riffle	2009	8.33	NA	0	0

Taxon	Habitat	Year	*Chaempinnularia* Mean	*Chaempinnularia* SE	*Cocconeis* Mean	*Cocconeis* SE
Hyloscirtus	Riffle	2004	10.2	4.19	0.77	NA
Lithobates	Riffle	2004	36.36	10.73	47.75	8.86
Baetis1	Riffle	2004	0	0	22.40	NA
Baetis2	Riffle	2004	0	0	144.58	NA
Baetodes1	Riffle	2004	4.01	0.83	29.48	15.67
Baetodes2	Riffle	2004	0	0	35.51	17.12
Farrodes1	Riffle	2004	0	0	3.87	0.86
Farrodes2	Riffle	2004	0	0	9.82	3.65
Haplohyphes1	Riffle	2004	0	0	28.38	11.15
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	26.38	NA	178.75	73.59
Psephenus1	Riffle	2004	17.69	13.49	179.83	78.74
Psephenus2	Riffle	2004	0	0	657.37	363.09
Stenonema1	Riffle	2004	4.15	NA	19.31	11.30
Stenonema2	Riffle	2004	25.98	NA	20.02	3.36
Thraulodes1	Riffle	2004	0	0	3.80	0.58
Thraulodes2	Riffle	2004	7.61	3.31	60.19	35.09
Tricorythodes1	Riffle	2004	20.79	NA	30.02	20.11
Tricorythodes2	Riffle	2004	0	0	7.93	4.55

Taxon	Habitat	Year	*Chaempinnularia* Mean	*Chaempinnularia* SE	*Cocconeis* Mean	*Cocconeis* SE
Baetis1	Riffle	2009	13.23	NA	2.81	2.28
Baetis2	Riffle	2009	0	0	55.02	27.72
Baetodes1	Riffle	2009	4.15	NA	95.06	77.28
Baetodes2	Riffle	2009	7.21	NA	80.50	43.06
Farrodes1	Riffle	2009	0	0	4.41	NA
Farrodes2	Riffle	2009	9.51	4.15	8.85	3.33
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	5.31	NA
Petrophila2	Riffle	2009	0	0	10.72	NA
Psephenus1	Riffle	2009	32.05	NA	142.67	55.60
Psephenus2	Riffle	2009	22.59	16.83	274.02	84.25
Stenonema2	Riffle	2009	0	0	91.61	88.45
Thraulodes1	Riffle	2009	17.68	NA	22.98	NA
Thraulodes2	Riffle	2009	46.35	NA	23.96	14.53
Tricorythodes1	Riffle	2009	0	0	14.59	NA

Taxon	Habitat	Year	*Diadesmis* Mean	*Diadesmis* SE	*Diploneis* Mean	*Diploneis* SE
Hyloscirtus	Riffle	2004	10.74	0.98	7.76	5.17
Lithobates	Riffle	2004	24.71	4.56	0	0
Baetis1	Riffle	2004	0	0	0	0
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	7.94	NA	0	0
Baetodes2	Riffle	2004	8.23	NA	0	0
Farrodes1	Riffle	2004	1.88	NA	4.13	NA
Farrodes2	Riffle	2004	6.07	1.95	0	0
Haplohyphes1	Riffle	2004	5.93	1.90	0	0
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	28.13	13.96	26.38	NA
Psephenus1	Riffle	2004	80.88	75.01	0	0
Psephenus2	Riffle	2004	0	0	0	0
Stenonema1	Riffle	2004	11.92	3.68	0	0
Stenonema2	Riffle	2004	110.03	97.84	4.06	NA
Thraulodes1	Riffle	2004	11.60	NA	1.55	NA
Thraulodes2	Riffle	2004	6.73	1.42	0	0
Tricorythodes1	Riffle	2004	11.72	4.01	5.31	NA
Tricorythodes2	Riffle	2004	12.93	NA	5.72	NA

Taxon	Habitat	Year	*Diadesmis* Mean	*Diadesmis* SE	*Diploneis* Mean	*Diploneis* SE
Baetis1	Riffle	2009	22.04	NA	0.88	NA
Baetis2	Riffle	2009	20.82	12.48	0.81	NA
Baetodes1	Riffle	2009	118.27	NA	0	0
Baetodes2	Riffle	2009	7.14	1.50	3.00	0.84
Farrodes1	Riffle	2009	12.02	NA	0	0
Farrodes2	Riffle	2009	24.29	6.98	40.69	38.55
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	225.07	NA	0	0
Psephenus1	Riffle	2009	55.32	NA	0	0
Psephenus2	Riffle	2009	61.31	56.95	1156.85	NA
Stenonema2	Riffle	2009	49.19	36.56	6.32	NA
Thraulodes1	Riffle	2009	17.68	NA	14.14	NA
Thraulodes2	Riffle	2009	70.81	24.28	87.20	63.02
Tricorythodes1	Riffle	2009	0	0	0	0

Taxon	Habitat	Year	*Encyonema* Mean	*Encyonema* SE	*Eolimna* Mean	*Eolimna* SE
Hyloscirtus	Riffle	2004	4.07	0.26	6.56	1.53
Lithobates	Riffle	2004	266.71	96.66	74.61	29.52
Baetis1	Riffle	2004	0	0	0	0
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	0	0	0	0
Baetodes2	Riffle	2004	0	0	41.17	NA
Farrodes1	Riffle	2004	0	0	0	0
Farrodes2	Riffle	2004	0	0	0	0
Haplohyphes1	Riffle	2004	0	0	3.76	NA
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0	0	0	0
Psephenus1	Riffle	2004	0	0	33.38	15.70
Psephenus2	Riffle	2004	0	0	29.45	NA
Stenonema1	Riffle	2004	0	0	41.19	16.71
Stenonema2	Riffle	2004	0	0	405.74	385.43
Thraulodes1	Riffle	2004	0	0	0	0
Thraulodes2	Riffle	2004	0	0	43.22	28.81
Tricorythodes1	Riffle	2004	0	0	0	0
Tricorythodes2	Riffle	2004	0	0	0	0

Taxon	Habitat	Year	*Encyonema* Mean	*Encyonema* SE	*Eolimna* Mean	*Eolimna* SE
Baetis1	Riffle	2009	0	0	0	0
Baetis2	Riffle	2009	0	0	0	0
Baetodes1	Riffle	2009	4.15	NA	0	0
Baetodes2	Riffle	2009	0	0	0	0
Farrodes1	Riffle	2009	0	0	10.02	2.00
Farrodes2	Riffle	2009	26.18	NA	10.72	NA
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	0	0	0	0
Psephenus1	Riffle	2009	0	0	0	0
Psephenus2	Riffle	2009	39.42	NA	0	0
Stenonema2	Riffle	2009	0	0	0	0
Thraulodes1	Riffle	2009	0	0	0	0
Thraulodes2	Riffle	2009	0	0	0	0
Tricorythodes1	Riffle	2009	0	0	0	0

Taxon	Habitat	Year	*Eunotia* Mean	*Eunotia* SE	*Fallacia* Mean	*Fallacia* SE
Hyloscirtus	Riffle	2004	22.31	8.02	0	0
Lithobates	Riffle	2004	44.39	33.17	0	0
Baetis1	Riffle	2004	88.29	80.34	0	0
Baetis2	Riffle	2004	7.69	NA	0	0
Baetodes1	Riffle	2004	12.00	4.51	0	0
Baetodes2	Riffle	2004	5.86	1.14	2.78	NA
Farrodes1	Riffle	2004	1.33	0.30	0	0
Farrodes2	Riffle	2004	20.33	8.78	4.12	NA
Haplohyphes1	Riffle	2004	18.91	7.76	0	0
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	110.24	85.34	34.72	7.50
Psephenus1	Riffle	2004	21.62	10.16	4.50	NA
Psephenus2	Riffle	2004	55.89	8.20	15.81	8.06
Stenonema1	Riffle	2004	28.39	12.94	0	0
Stenonema2	Riffle	2004	26.68	14.89	0	0
Thraulodes1	Riffle	2004	1.87	0.06	0	0
Thraulodes2	Riffle	2004	69.63	43.47	14.23	NA
Tricorythodes1	Riffle	2004	12.16	2.51	32.85	22.24
Tricorythodes2	Riffle	2004	30.17	20.19	2.85	NA

Taxon	Habitat	Year	*Eunotia* Mean	*Eunotia* SE	*Fallacia* Mean	*Fallacia* SE
Baetis1	Riffle	2009	25.45	7.97	0	0
Baetis2	Riffle	2009	131.05	81.89	0	0
Baetodes1	Riffle	2009	101.08	37.97	0	0
Baetodes2	Riffle	2009	47.63	30.24	1.53	NA
Farrodes1	Riffle	2009	11.42	7.41	0	0
Farrodes2	Riffle	2009	90.96	81.54	1.07	NA
Hagenulopsis2	Riffle	2009	7.89	3.82	0	0
Leptohyphes1	Riffle	2009	2.45	NA	0	0
Petrophila2	Riffle	2009	0	0	53.59	NA
Psephenus1	Riffle	2009	119.53	90.68	0	0
Psephenus2	Riffle	2009	80.95	40.68	0	0
Stenonema2	Riffle	2009	85.74	NA	0	0
Thraulodes1	Riffle	2009	35.17	5.49	0	0
Thraulodes2	Riffle	2009	28.74	10.07	177.40	NA
Tricorythodes1	Riffle	2009	12.06	7.48	0	0

Taxon	Habitat	Year	*Frustulia* Mean	*Frustulia* SE	*Gomphonema* Mean	*Gomphonema* SE
Hyloscirtus	Riffle	2004	3.88	0.84	7.75	2.59
Lithobates	Riffle	2004	140.32	24.67	101.39	34.65
Baetis1	Riffle	2004	4.48	NA	0	0
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	0	0	3.47	0.10
Baetodes2	Riffle	2004	0	0	8.88	2.57
Farrodes1	Riffle	2004	6.39	NA	0	0
Farrodes2	Riffle	2004	6.44	NA	3.51	0.60
Haplohyphes1	Riffle	2004	3.08	0.93	4.90	1.36
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	26.38	NA	0	0
Psephenus1	Riffle	2004	2.52	NA	14.57	2.66
Psephenus2	Riffle	2004	3.88	NA	10.56	2.70
Stenonema1	Riffle	2004	1.23	0.40	12.73	7.33
Stenonema2	Riffle	2004	4.95	4.14	18.19	NA
Thraulodes1	Riffle	2004	1.03	0.13	3.87	NA
Thraulodes2	Riffle	2004	3.79	1.72	7.85	3.28
Tricorythodes1	Riffle	2004	3.60	2.28	2.41	0.71
Tricorythodes2	Riffle	2004	2.56	0.07	11.39	6.36

Taxon	Habitat	Year	*Frustulia* Mean	*Frustulia* SE	*Gomphonema* Mean	*Gomphonema* SE
Baetis1	Riffle	2009	0	0	4.23	NA
Baetis2	Riffle	2009	19.76	11.31	11.81	9.01
Baetodes1	Riffle	2009	33.30	NA	23.93	NA
Baetodes2	Riffle	2009	5.17	1.71	10.03	4.38
Farrodes1	Riffle	2009	4.01	NA	9.62	NA
Farrodes2	Riffle	2009	10.10	4.37	26.18	NA
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	2.45	NA	0	0
Petrophila2	Riffle	2009	0	0	0	0
Psephenus1	Riffle	2009	0	0	110.64	NA
Psephenus2	Riffle	2009	0	0	8.54	NA
Stenonema2	Riffle	2009	0	0	0	0
Thraulodes1	Riffle	2009	2.47	NA	0	0
Thraulodes2	Riffle	2009	20.06	NA	46.35	NA
Tricorythodes1	Riffle	2009	1.25	NA	0	0

Taxon	Habitat	Year	*Gomphosenia* Mean	*Gomphosenia* SE	*Gyrosigma* Mean	*Gyrosigma* SE
Hyloscirtus	Riffle	2004	0	0	0	0
Lithobates	Riffle	2004	16.23	NA	0	0
Baetis1	Riffle	2004	0	0	0	0
Baetis2	Riffle	2004	0	0	1.77	NA
Baetodes1	Riffle	2004	4.84	NA	0.40	NA
Baetodes2	Riffle	2004	0	0	0.41	NA
Farrodes1	Riffle	2004	0	0	0	0
Farrodes2	Riffle	2004	0	0	0.62	0.20
Haplohyphes1	Riffle	2004	0	0	0.87	0.35
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0	0	0	0
Psephenus1	Riffle	2004	4.21	NA	0	0
Psephenus2	Riffle	2004	3.88	NA	3.37	1.05
Stenonema1	Riffle	2004	0	0	15.02	12.56
Stenonema2	Riffle	2004	12.99	NA	13.52	9.86
Thraulodes1	Riffle	2004	0	0	0	0
Thraulodes2	Riffle	2004	0	0	3.99	1.45
Tricorythodes1	Riffle	2004	0	0	2.20	0.29
Tricorythodes2	Riffle	2004	0	0	4.38	0.51

Taxon	Habitat	Year	*Gomphosenia* Mean	*Gomphosenia* SE	*Gyrosigma* Mean	*Gyrosigma* SE
Baetis1	Riffle	2009	0	0	0	0
Baetis2	Riffle	2009	0	0	0	0
Baetodes1	Riffle	2009	0	0	0	0
Baetodes2	Riffle	2009	0	0	0	0
Farrodes1	Riffle	2009	0	0	0	0
Farrodes2	Riffle	2009	0	0	1.43	NA
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	0	0	107.18	NA
Psephenus1	Riffle	2009	0	0	0	0
Psephenus2	Riffle	2009	0	0	4.74	NA
Stenonema2	Riffle	2009	0	0	0	0
Thraulodes1	Riffle	2009	0	0	0	0
Thraulodes2	Riffle	2009	0	0	0.94	NA
Tricorythodes1	Riffle	2009	0	0	0	0

Taxon	Habitat	Year	*Luticola* Mean	*Luticola* SE	*Navicula* Mean	*Navicula* SE
Hyloscirtus	Riffle	2004	5.73	1.48	19.82	8.96
Lithobates	Riffle	2004	4.16	2.95	874.06	286.74
Baetis1	Riffle	2004	0	0	3.45	2.26
Baetis2	Riffle	2004	0	0	5.91	NA
Baetodes1	Riffle	2004	0	0	10.32	NA
Baetodes2	Riffle	2004	0	0	2.07	1.24
Farrodes1	Riffle	2004	0	0	0.97	0.54
Farrodes2	Riffle	2004	4.34	1.33	17.89	9.65
Haplohyphes1	Riffle	2004	4.17	0.78	8.98	3.36
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	16.81	10.69	66.54	34.78
Psephenus1	Riffle	2004	4.70	NA	14.55	11.44
Psephenus2	Riffle	2004	30.67	29.50	28.35	16.57
Stenonema1	Riffle	2004	18.57	8.34	34.21	18.43
Stenonema2	Riffle	2004	13.69	1.90	71.62	56.99
Thraulodes1	Riffle	2004	3.17	NA	12.76	NA
Thraulodes2	Riffle	2004	4.85	1.23	25.27	11.03
Tricorythodes1	Riffle	2004	8.85	2.76	3.50	0.93
Tricorythodes2	Riffle	2004	22.25	20.11	8.27	3.63

Taxon	Habitat	Year	*Luticola* Mean	*Luticola* SE	*Navicula* Mean	*Navicula* SE
Baetis1	Riffle	2009	0	0	16.06	10.85
Baetis2	Riffle	2009	28.27	22.96	42.30	31.40
Baetodes1	Riffle	2009	200.94	127.54	27.33	7.57
Baetodes2	Riffle	2009	40.28	19.33	56.36	13.79
Farrodes1	Riffle	2009	71.72	38.86	64.51	32.05
Farrodes2	Riffle	2009	50.57	23.94	84.98	32.74
Hagenulopsis2	Riffle	2009	12.37	6.94	29.82	14.08
Leptohyphes1	Riffle	2009	8.18	NA	6.95	NA
Petrophila2	Riffle	2009	107.18	NA	96.46	NA
Psephenus1	Riffle	2009	0	0	24.04	NA
Psephenus2	Riffle	2009	20.08	8.73	17.66	6.86
Stenonema2	Riffle	2009	95.22	76.27	125.57	114.51
Thraulodes1	Riffle	2009	63.00	3.78	40.99	23.31
Thraulodes2	Riffle	2009	46.53	12.96	101.85	33.86
Tricorythodes1	Riffle	2009	36.98	35.73	35.27	29.85

Taxon	Habitat	Year	*Nitzschia* Mean	*Nitzschia* SE	*Nupela* Mean	*Nupela* SE
Hyloscirtus	Riffle	2004	6.85	NA	202.65	101.27
Lithobates	Riffle	2004	21.86	13.74	359.76	29.18
Baetis1	Riffle	2004	0	0	50.04	44.87
Baetis2	Riffle	2004	0	0	634.50	NA
Baetodes1	Riffle	2004	0	0	187.27	113.04
Baetodes2	Riffle	2004	0	0	84.11	75.38
Farrodes1	Riffle	2004	3.76	NA	17.58	12.42
Farrodes2	Riffle	2004	2.58	NA	81.29	35.74
Haplohyphes1	Riffle	2004	16.63	NA	60.70	26.30
Leptohyphes1	Riffle	2004	0	0	38.86	32.59
Petrophila2	Riffle	2004	0	0	3187.09	1461.79
Psephenus1	Riffle	2004	0	0	1192.63	880.67
Psephenus2	Riffle	2004	0	0	2915.53	2329.87
Stenonema1	Riffle	2004	4.74	0.65	254.42	55.24
Stenonema2	Riffle	2004	0	0	583.68	495.90
Thraulodes1	Riffle	2004	0	0	33.91	21.02
Thraulodes2	Riffle	2004	8.54	NA	240.82	78.22
Tricorythodes1	Riffle	2004	2.41	0.21	421.03	253.01
Tricorythodes2	Riffle	2004	0	0	137.67	104.93

Taxon	Habitat	Year	*Nitzschia* Mean	*Nitzschia* SE	*Nupela* Mean	*Nupela* SE
Baetis1	Riffle	2009	0	0	131.74	79.95
Baetis2	Riffle	2009	4.17	NA	490.69	220.81
Baetodes1	Riffle	2009	0	0	425.69	270.08
Baetodes2	Riffle	2009	3.84	NA	803.26	257.29
Farrodes1	Riffle	2009	0	0	115.79	62.10
Farrodes2	Riffle	2009	3.58	NA	153.57	42.73
Hagenulopsis2	Riffle	2009	4.68	NA	70.74	46.32
Leptohyphes1	Riffle	2009	0	0	32.70	NA
Petrophila2	Riffle	2009	0	0	25208.33	NA
Psephenus1	Riffle	2009	0	0	1646.65	717.97
Psephenus2	Riffle	2009	3.91	0.65	2771.96	1428.36
Stenonema2	Riffle	2009	0	0	500.77	433.82
Thraulodes1	Riffle	2009	0	0	252.05	96.23
Thraulodes2	Riffle	2009	10.89	NA	321.41	111.18
Tricorythodes1	Riffle	2009	0	0	159.44	11.83

Taxon	Habitat	Year	*Orthoseira* Mean	*Orthoseira* SE	*Pinnularia* Mean	*Pinnularia* SE
Hyloscirtus	Riffle	2004	0	0	7.38	4.11
Lithobates	Riffle	2004	0	0	66.78	40.34
Baetis1	Riffle	2004	0	0	0.20	NA
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	0	0	0.51	0.08
Baetodes2	Riffle	2004	0	0	0.24	NA
Farrodes1	Riffle	2004	0	0	3.86	3.65
Farrodes2	Riffle	2004	0	0	3.16	1.69
Haplohyphes1	Riffle	2004	1.95	1.06	3.77	1.92
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0	0	34.40	27.60
Psephenus1	Riffle	2004	0	0	0.43	0.07
Psephenus2	Riffle	2004	0	0	8.06	4.22
Stenonema1	Riffle	2004	0	0	13.73	4.10
Stenonema2	Riffle	2004	0	0	27.77	22.89
Thraulodes1	Riffle	2004	0	0	1.74	NA
Thraulodes2	Riffle	2004	1.00	NA	7.13	2.57
Tricorythodes1	Riffle	2004	0	0	3.00	1.56
Tricorythodes2	Riffle	2004	0	0	6.41	5.24

Taxon	Habitat	Year	*Orthoseira* Mean	*Orthoseira* SE	*Pinnularia* Mean	*Pinnularia* SE
Baetis1	Riffle	2009	0	0	5.39	3.62
Baetis2	Riffle	2009	0	0	9.62	5.41
Baetodes1	Riffle	2009	0	0	13.75	2.91
Baetodes2	Riffle	2009	0	0	22.16	8.90
Farrodes1	Riffle	2009	0	0	6.41	2.20
Farrodes2	Riffle	2009	0	0	20.83	7.74
Hagenulopsis2	Riffle	2009	0	0	5.07	3.71
Leptohyphes1	Riffle	2009	0	0	7.36	NA
Petrophila2	Riffle	2009	0	0	466.23	NA
Psephenus1	Riffle	2009	0	0	0	0
Psephenus2	Riffle	2009	0	0	13.79	10.92
Stenonema2	Riffle	2009	0	0	107.18	NA
Thraulodes1	Riffle	2009	0	0	10.72	NA
Thraulodes2	Riffle	2009	0	0	36.13	16.38
Tricorythodes1	Riffle	2009	5.43	NA	4.38	NA

Taxon	Habitat	Year	*Placoneis* Mean	*Placoneis* SE	*Planothidium* Mean	*Planothidium* SE
Hyloscirtus	Riffle	2004	0	0	17.86	NA
Lithobates	Riffle	2004	0	0	37.55	16.04
Baetis1	Riffle	2004	0	0	2.04	NA
Baetis2	Riffle	2004	0	0	0	0
Baetodes1	Riffle	2004	1.72	NA	1.19	NA
Baetodes2	Riffle	2004	0	0	0	0
Farrodes1	Riffle	2004	0	0	4.23	NA
Farrodes2	Riffle	2004	0	0	9.02	4.90
Haplohyphes1	Riffle	2004	0.96	NA	4.46	1.40
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0	0	204.32	174.20
Psephenus1	Riffle	2004	0	0	5.00	NA
Psephenus2	Riffle	2004	0	0	63.90	24.63
Stenonema1	Riffle	2004	3.73	NA	6.65	NA
Stenonema2	Riffle	2004	0	0	4.30	3.49
Thraulodes1	Riffle	2004	2.71	NA	2.32	NA
Thraulodes2	Riffle	2004	0	0	12.92	8.43
Tricorythodes1	Riffle	2004	0.76	NA	29.73	24.78
Tricorythodes2	Riffle	2004	0	0	2.31	1.50

Taxon	Habitat	Year	*Placoneis* Mean	*Placoneis* SE	*Planothidium* Mean	*Planothidium* SE
Baetis1	Riffle	2009	0	0	0	0
Baetis2	Riffle	2009	0	0	4.63	NA
Baetodes1	Riffle	2009	0	0	0	0
Baetodes2	Riffle	2009	3.82	NA	4.22	1.55
Farrodes1	Riffle	2009	0	0	4.41	NA
Farrodes2	Riffle	2009	0	0	14.70	11.48
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	0	0	107.18	NA
Psephenus1	Riffle	2009	0	0	0	0
Psephenus2	Riffle	2009	0	0	29.40	25.04
Stenonema2	Riffle	2009	0	0	150.05	NA
Thraulodes1	Riffle	2009	0	0	0	0
Thraulodes2	Riffle	2009	0	0	0	0
Tricorythodes1	Riffle	2009	0	0	1.25	NA

Taxon	Habitat	Year	*Rhoicosphenia* Mean	*Rhoicosphenia* SE	*Rhopalodia* Mean	*Rhopalodia* SE
Hyloscirtus	Riffle	2004	2.58	NA	0	0
Lithobates	Riffle	2004	35.51	9.99	0	0
Baetis1	Riffle	2004	0	0	1.22	NA
Baetis2	Riffle	2004	0	0	0.59	NA
Baetodes1	Riffle	2004	0	0	0	0
Baetodes2	Riffle	2004	0	0	0.82	NA
Farrodes1	Riffle	2004	0	0	0.59	0.16
Farrodes2	Riffle	2004	0	0	0	0
Haplohyphes1	Riffle	2004	0	0	1.32	0.67
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0	0	0	0
Psephenus1	Riffle	2004	0	0	0	0
Psephenus2	Riffle	2004	0	0	0	0
Stenonema1	Riffle	2004	8.29	NA	2.99	2.33
Stenonema2	Riffle	2004	2.03	NA	2.60	NA
Thraulodes1	Riffle	2004	0	0	0	0
Thraulodes2	Riffle	2004	0	0	1.00	0.23
Tricorythodes1	Riffle	2004	0	0	4.44	NA
Tricorythodes2	Riffle	2004	0	0	1.49	1.09

Taxon	Habitat	Year	*Rhoicosphenia* Mean	*Rhoicosphenia* SE	*Rhopalodia* Mean	*Rhopalodia* SE
Baetis1	Riffle	2009	0	0	0	0
Baetis2	Riffle	2009	0	0	1.68	NA
Baetodes1	Riffle	2009	0	0	0	0
Baetodes2	Riffle	2009	0	0	0	0
Farrodes1	Riffle	2009	0	0	4.41	NA
Farrodes2	Riffle	2009	47.69	NA	2.67	0.88
Hagenulopsis2	Riffle	2009	0	0	0	0
Leptohyphes1	Riffle	2009	0	0	0	0
Petrophila2	Riffle	2009	0	0	0	0
Psephenus1	Riffle	2009	0	0	0	0
Psephenus2	Riffle	2009	0	0	0.76	0.19
Stenonema2	Riffle	2009	0	0	0	0
Thraulodes1	Riffle	2009	0	0	0	0
Thraulodes2	Riffle	2009	0	0	36.75	NA
Tricorythodes1	Riffle	2009	0	0	0	0

Taxon	Habitat	Year	*Synedra* Mean	*Synedra* SE	*Terpsinoe* Mean	*Terpsinoe* SE
Hyloscirtus	Riffle	2004	1.11	0.79	3.18	0.37
Lithobates	Riffle	2004	54.53	12.27	3.23	0.39
Baetis1	Riffle	2004	0.41	NA	0	0
Baetis2	Riffle	2004	0.59	NA	0	0
Baetodes1	Riffle	2004	1.00	0.79	0	0
Baetodes2	Riffle	2004	0.32	0.09	0	0
Farrodes1	Riffle	2004	2.82	NA	0	0
Farrodes2	Riffle	2004	0.84	0.32	0	0
Haplohyphes1	Riffle	2004	2.12	0.49	0.52	0.20
Leptohyphes1	Riffle	2004	0	0	0	0
Petrophila2	Riffle	2004	0.88	NA	0	0
Psephenus1	Riffle	2004	0.71	0.13	0	0
Psephenus2	Riffle	2004	0.19	NA	0	0
Stenonema1	Riffle	2004	6.75	5.93	5.04	1.76
Stenonema2	Riffle	2004	1.89	0.06	6.24	4.94
Thraulodes1	Riffle	2004	0.19	NA	0	0
Thraulodes2	Riffle	2004	2.27	1.01	0.62	NA
Tricorythodes1	Riffle	2004	1.35	0.58	8.19	NA
Tricorythodes2	Riffle	2004	0.20	NA	0.59	0.21

Taxon	Habitat	Year	*Synedra* Mean	*Synedra* SE	*Terpsinoe* Mean	Terpsinoe SE
Baetis1	Riffle	2009	9.64	3.29	0	0
Baetis2	Riffle	2009	13.13	4.79	0.42	0.00
Baetodes1	Riffle	2009	88.00	41.67	0	0
Baetodes2	Riffle	2009	46.75	28.62	3.84	NA
Farrodes1	Riffle	2009	33.05	20.23	0.80	NA
Farrodes2	Riffle	2009	36.24	13.67	0.27	NA
Hagenulopsis2	Riffle	2009	8.73	0.05	0	0
Leptohyphes1	Riffle	2009	1.84	NA	0	0
Petrophila2	Riffle	2009	246.51	NA	0	0
Psephenus1	Riffle	2009	22.13	NA	0	0
Psephenus2	Riffle	2009	5.13	3.59	0.24	NA
Stenonema2	Riffle	2009	111.47	NA	0	0
Thraulodes1	Riffle	2009	22.26	NA	0	0
Thraulodes2	Riffle	2009	30.52	12.15	0	0
Tricorythodes1	Riffle	2009	60.30	58.01	0	0

Taxon	Habitat	Year	*Tryblionella* Mean	*Tryblionella* SE
Hyloscirtus	Riffle	2004	3.81	0
Lithobates	Riffle	2004	0	0
Baetis1	Riffle	2004	0	0
Baetis2	Riffle	2004	0	0
Baetodes1	Riffle	2004	0	0
Baetodes2	Riffle	2004	0	0
Farrodes1	Riffle	2004	1.13	NA
Farrodes2	Riffle	2004	4.95	NA
Haplohyphes1	Riffle	2004	3.84	NA
Leptohyphes1	Riffle	2004	0	0
Petrophila2	Riffle	2004	0	0
Psephenus1	Riffle	2004	0	0
Psephenus2	Riffle	2004	0	0
Stenonema1	Riffle	2004	3.29	0.80
Stenonema2	Riffle	2004	0	0
Thraulodes1	Riffle	2004	2.32	NA
Thraulodes2	Riffle	2004	1.16	NA
Tricorythodes1	Riffle	2004	1.31	NA
Tricorythodes2	Riffle	2004	12.63	NA

Taxon	Habitat	Year	*Tryblionella* Mean	*Tryblionella* SE
Baetis1	Riffle	2009	0	0
Baetis2	Riffle	2009	2.09	NA
Baetodes1	Riffle	2009	0	0
Baetodes2	Riffle	2009	3.06	0.01
Farrodes1	Riffle	2009	0	0
Farrodes2	Riffle	2009	0	0
Hagenulopsis2	Riffle	2009	11.71	NA
Leptohyphes1	Riffle	2009	0	0
Petrophila2	Riffle	2009	107.18	NA
Psephenus1	Riffle	2009	0	0
Psephenus2	Riffle	2009	2.10	NA
Stenonema2	Riffle	2009	0	0
Thraulodes1	Riffle	2009	0	0
Thraulodes2	Riffle	2009	0	0
Tricorythodes1	Riffle	2009	0	0

APPENDICES CHAPTER 4

Appendix A

Table 1. Mean annual biomass [mg ash-free dry mass (AFDM) m^{-2} ± 95% CI] of macroinvertebrates in the riffle habitat pre- (2004) and post- (2009) amphibian declines. N = 15 (5 samples each for 3 mo.) for each year.

Table 1.

Taxa	2004 Mean (± CI)	2009 Mean (± CI)
Baetis	1.34 (0.43)	2.41 (0.61)
Baetodes	*3.31 (1.22)*	*8.85 (2.09)*
Stenonema	6.31 (3.23)	0.89 (0.58)
Thraulodes	10.42 (2.09)	16.09 (3.32)
Hagenulopsis	1.06 (0.48)	2.06 (0.85)
Farrodes	14.01 (2.77)	11.27 (2.26)
Haplohyphes	2.31 (1.05)	1.59 (0.57)
Leptohyphes	2.43 (1.21)	1.05 (0.37)
Tricorythodes	8.44 (3.01)	5.31 (1.35)
Anacroneuria	2.12 (1.11)	3.33 (2.35)
Neurocordulia	13.44 (12.04)	0.27 (0.18)
Argia	2.56 (2.56)	0.00 (0.00)
Philogenia	16.07 (12.84)	22.45 (6.39)
Heteragrion	*3.58 (1.03)*	*13.79 (3.78)*
Cora	1.23 (0.82)	2.25 (1.05)
Hetaerina	3.44 (3.31)	0.00 (0.00)
Heterelmis	2.48 (1.09)	2.30 (0.80)
Neoelmis	3.05 (1.22)	0.00 (0.00)
Hexacylloepus	3.79 (1.69)	0.69 (0.30)
Phanocerus	2.70 (0.69)	2.01 (0.77)
Anchytarsus	210.50 (41.15)	54.98 (7.48)
Psephenus	14.79 (2.88)	6.02 (1.46)
Leptonema	13.69 (6.53)	4.12 (2.59)
Macronema	2.26 (1.49)	0.02 (0.02)
Smicridea	0.00 (0.00)	2.77 (1.73)
Wormaldia	3.07 (1.31)	1.06 (0.61)
Chimarra	3.70 (2.69)	0.83 (0.51)
Tanypodinae	6.25 (1.39)	4.79 (0.89)
Non-tanypodinae	7.86 (2.18)	10.98 (3.31)
Hexatoma	0.12 (0.12)	14.56 (3.50)
Tipula	3.48 (1.17)	0.00 (0.00)
Simulium	4.09 (1.49)	22.35 (11.94)
Petrophila	4.19 (1.60)	0.82 (0.55)

Table 2. Mean annual biomass [mg ash-free dry mass (AFDM) m^{-2} ± 95% CI] of macroinvertebrates in the pool habitat pre- (2004) and post- (2009) amphibian declines.

Table 2.

	2004 Mean (± CI)	2009 Mean (± CI)
Thraulodes	4.14 (2.27)	0.33 (0.28)
Farrodes	6.79 (2.93)	6.79 (2.36)
Tricorythodes	4.93 (1.86)	1.00 (0.47)
Philogenia	5.60 (4.81)	6.43 (6.34)
Heteragrion	2.52 (1.24)	10.11 (6.34)
Hetaerina	11.11 (11.06)	0.16 (0.16)
Heterelmis (L)	4.58 (1.58)	9.97 (5.90)
Neoelmis (L)	3.53 (2.04)	0.00 (0.00)
Hexacylloepus (L)	10.57 (2.92)	4.97 (1.61)
Macrelmis (L)	0.11 (0.11)	8.10 (8.10)
Anchytarsus	351.30 (85.36)	84.14 (26.28)
Psephenus	8.50 (3.01)	0.67 (0.44)
Leptonema	20.50 (19.97)	0.00 (0.00)
Macronema	11.42 (5.21)	0.19 (0.15)
Tanypodinae	7.52 (2.89)	9.92 (2.71)
Non-tanypodinae	4.36 (0.96)	7.03 (2.39)
Hexatoma	6.85 (3.64)	32.69 (10.51)
Tipula	4.68 (3.11)	0.00 (0.00)

Appendix B

Table 1 and 2. Mean number of diatom valves m^{-2} in riffles (Table 1) and pools (Table 2) pre- (2004) and post- (2009) amphibian declines.

Table 1. Riffles

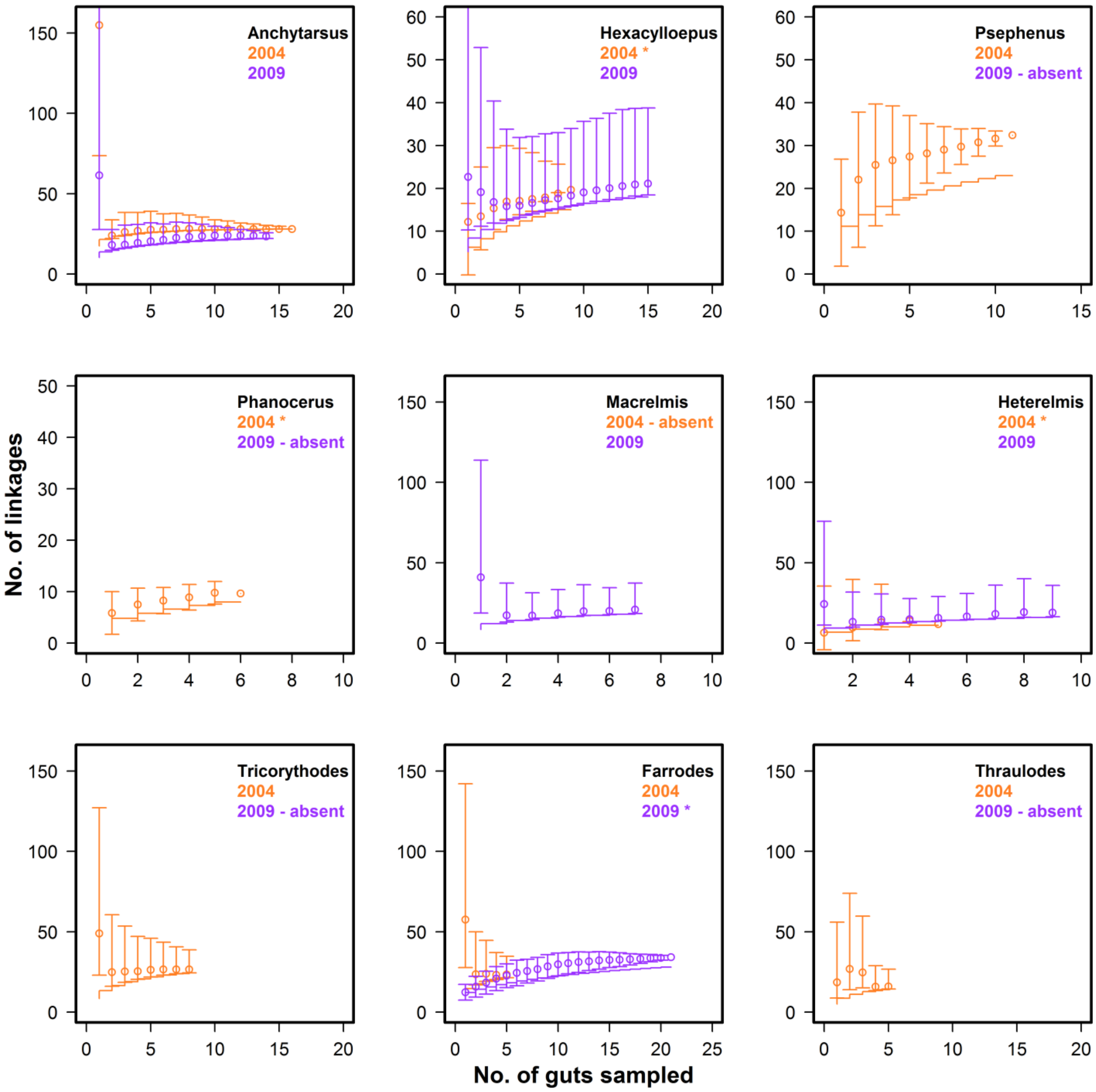
Taxa	2004 Mean (± SE)	2009 Mean (± SE)
Nupela	1922.80 (685.34)	6661.67 (1010.72)
Synedra	32.32 (17.67)	141.74 (40.53)
Nitzschia	89.96 (34.76)	258.78 (65.25)
Eolimna	120.94 (54.59)	341.35 (96.59)
Orthosiera	0.50 (0.50)	4.31 (2.04)
Achnanthes	211.30 (88.29)	401.37 (110.97)
Melosira	8.51 (7.43)	37.80 (20.92)
Luticola	177.18 (52.49)	330.93 (98.61)
Amphipleura	5.01 (5.01)	17.35 (7.89)
Adlafia	24.25 (12.74)	119.50 (76.97)
Rhoicosphenia	3.00 (2.50)	8.89 (4.35)
Planothidium	20.40 (10.19)	35.05 (10.84)
Frustulia	55.46 (22.78)	92.71 (31.28)
Tryblionella	42.01 (26.62)	67.23 (22.62)
Gomphonema	33.57 (13.68)	53.83 (24.28)
Placoneis	6.74 (6.20)	12.35 (5.69)
Gomphosenia	6.50 (3.25)	11.82 (6.74)
Diploneis	2.17 (1.69)	3.67 (1.91)
Rhopalodia	0.50 (0.50)	1.67 (1.67)
Navicula	530.03 (235.68)	668.41 (175.42)
Surirella	0.00 (0.00)	1.67 (1.67)
Stenoterobia	0.00 (0.00)	0.42 (0.42)
Terpsinoe	40.50 (11.87)	44.66 (13.68)
Chaempinnularia	2.50 (2.50)	3.34 (2.27)
Neidium	3.00 (2.50)	2.67 (1.89)
Achnanthidium	55.53 (28.92)	48.53 (13.84)
Pinnularia	117.54 (51.40)	99.91 (25.39)
Planothidium	18.14 (10.33)	12.77 (9.10)
Diadesmis	53.46 (31.70)	41.96 (13.09)
Fallacia	13.59 (8.18)	8.58 (3.66)
Cyclotella	6.01 (2.56)	3.83 (2.11)
Gyrosigma	45.53 (13.70)	28.87 (10.72)
Eunotia	113.74 (52.82)	55.44 (20.66)
Cocconeis	88.95 (21.98)	57.97 (13.43)
Amphora	21.29 (10.28)	2.15 (1.70)

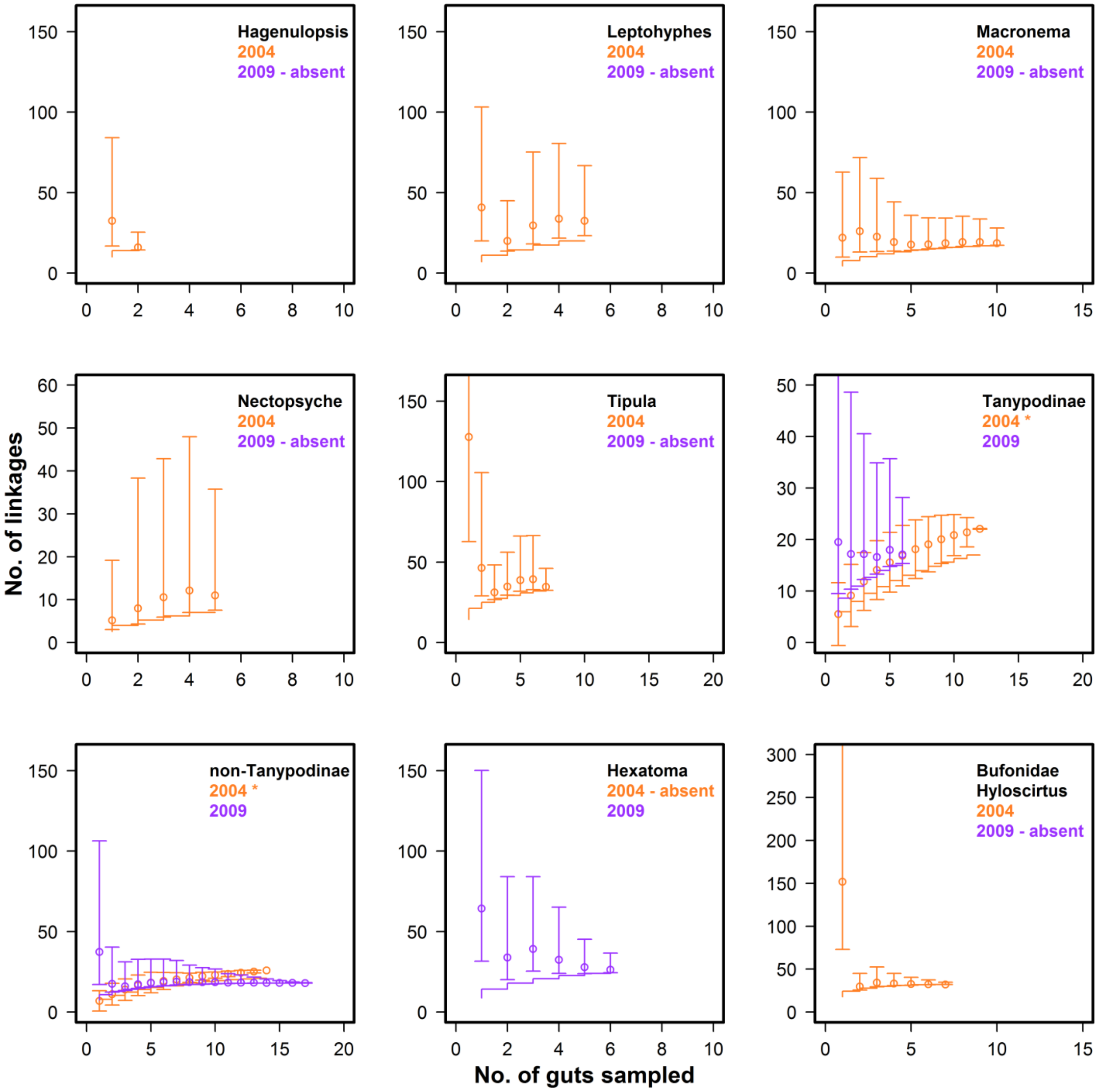
Table 2. Pools

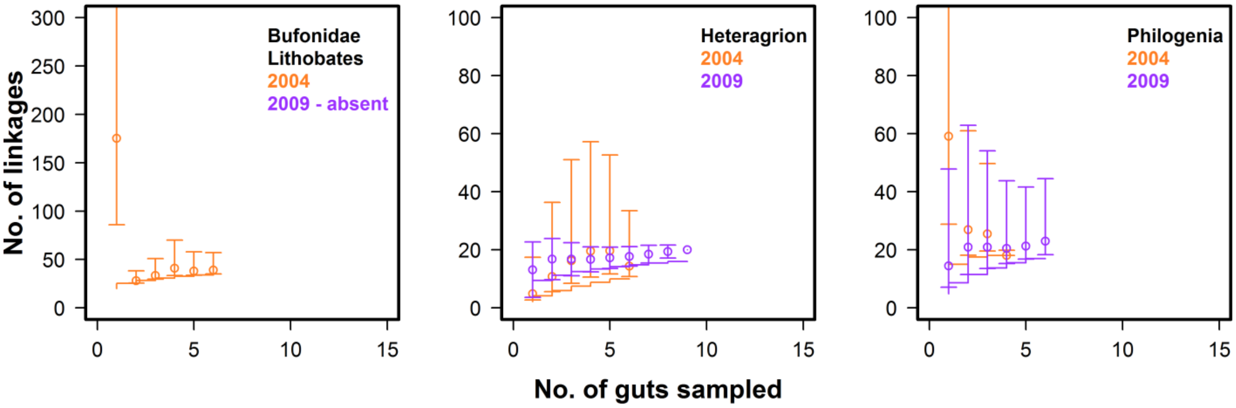
Taxa	2004 Mean (± SE)	2009 Mean (± SE)
Terpsinoe	367.04 (112.80)	1906.07 (574.21)
Synedra	679.03 (209.78)	4858.91 (2197.04)
Nupela	52720.89 (7697.34)	110429.40 (27803.97)
Eolimna	4395.32 (941.30)	9076.62 (2257.56)
Cyclotella	0.00 (0.00)	60.93 (30.21)
Melosira	0.00 (0.00)	1731.65 (1060.39)
Frustulia	7157.30 (2682.06)	17403.08 (5994.27)
Rhoicosphenia	0.00 (0.00)	53.32 (37.28)
Surirella	27.53 (27.53)	196.43 (121.11)
Pinnularia	3106.09 (1166.24)	9682.25 (5483.23)
Navicula	19700.94 (3894.36)	31726.43 (9919.58)
Luticola	7345.41 (1500.19)	11236.54 (3515.37)
Tryblionella	972.66 (419.43)	1757.17 (735.63)
Planothidium	467.98 (96.19)	677.32 (211.09)
Diadesmis	1426.87 (384.74)	1929.27 (657.95)
Planothidium	0.00 (0.00)	8.93 (8.93)
Diploneis	169.76 (70.43)	204.36 (70.77)
Gomphosenia	385.39 (143.33)	477.34 (245.90)
Achnanthes	8607.12 (1796.71)	9603.47 (3702.97)
Amphipleura	527.62 (220.91)	620.49 (471.28)
Caloneis	128.46 (72.64)	155.99 (113.08)
Nitzschia	10639.61 (4185.86)	11405.43 (4345.11)
Neidium	128.46 (109.60)	137.02 (47.08)
Stenoterobia	2349.06 (2134.21)	2388.15 (1401.98)
Adlafia	1312.17 (492.83)	1318.05 (373.96)
Orthosiera	27.53 (27.53)	27.28 (19.83)
Chaempinnularia	233.99 (113.31)	220.89 (65.37)
Rhopalodia	59.64 (32.12)	36.46 (21.01)
Amphora	165.17 (117.51)	49.85 (31.47)
Gyrosigma	935.96 (415.08)	420.62 (129.85)
Encyonema	275.28 (220.99)	0.00 (0.00)
Fallacia	467.98 (224.95)	128.46 (57.72)
Achnanthidium	3275.84 (825.28)	1973.45 (280.38)
Eunotia	3005.15 (711.67)	1310.90 (351.15)
Placoneis	1495.69 (570.60)	239.36 (104.38)
Gomphonema	1876.50 (488.45)	517.23 (176.14)
Cocconeis	3252.90 (1030.29)	512.76 (181.26)

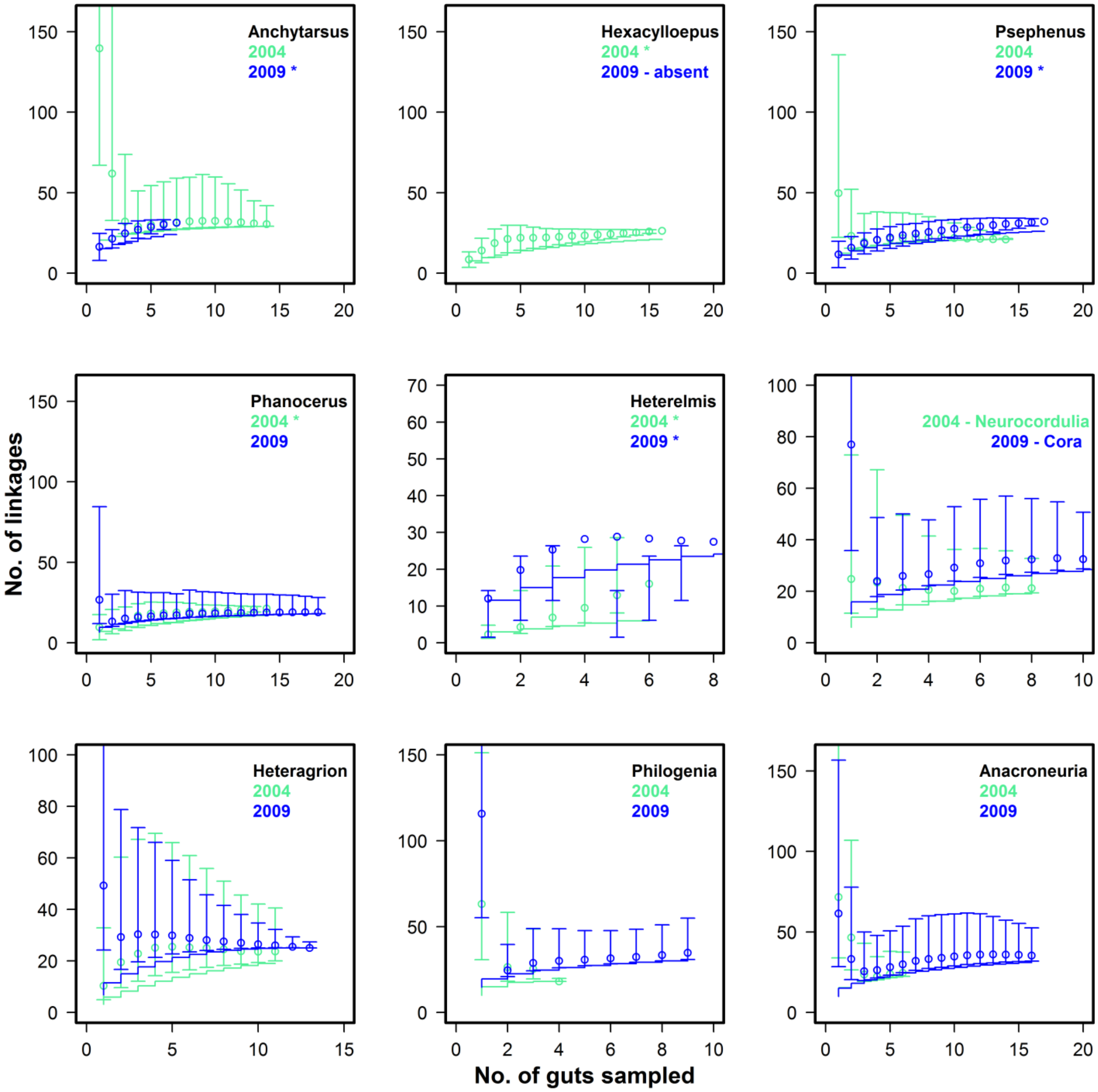
Appendix C

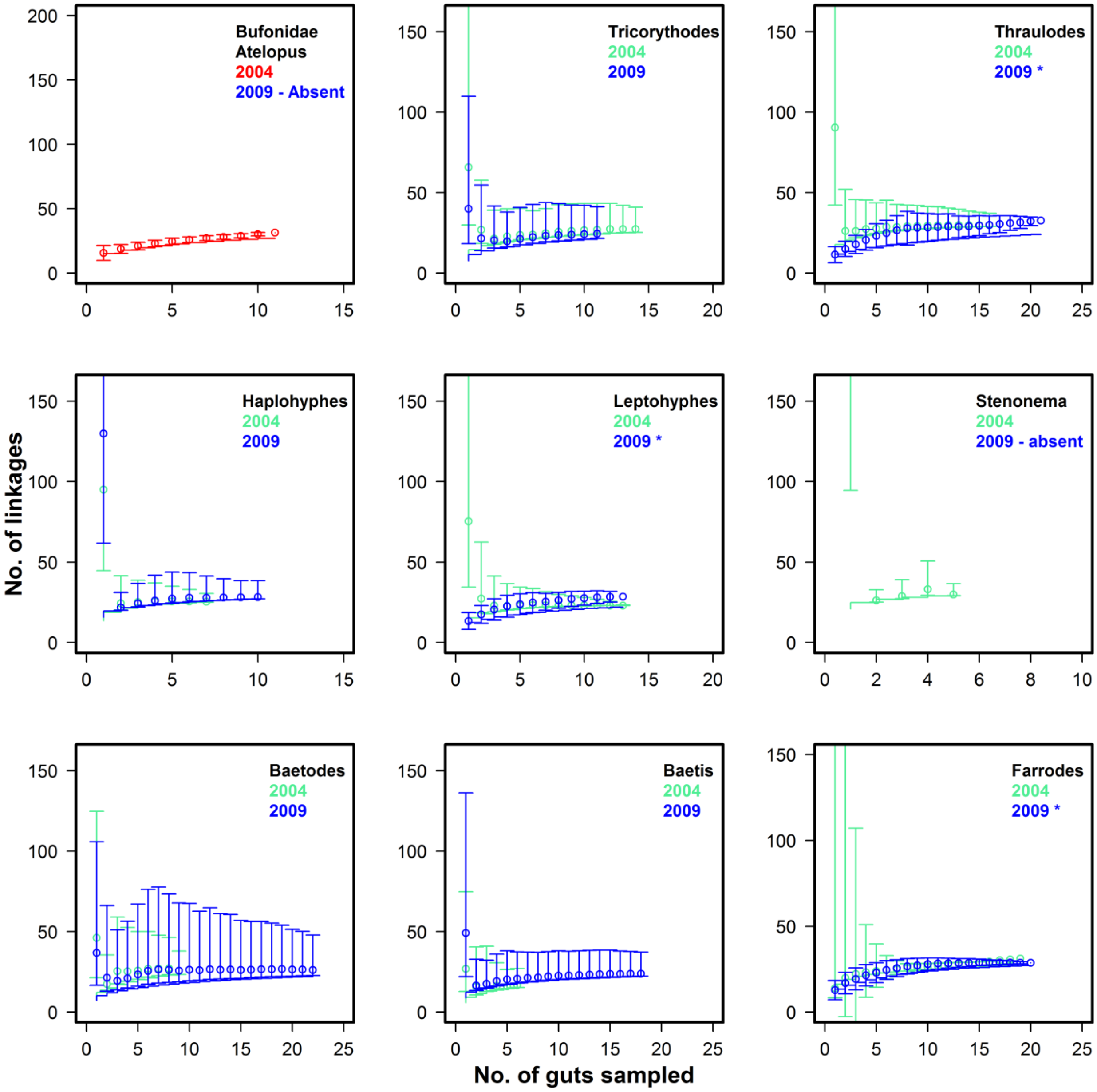
Rarefaction curves (solid lines) of the taxa sampled for gut content analysis from two habitats (riffles and pools) and two points in time (2004 and 2009). A species estimator curve (hollow circles with error bars) using either the Chao1 or Abundance Based Coverage Estimator (ACE) was used to estimate how many additional linkages would be identified with additional sampling. An asterisk next to the taxon name indicates that the coefficient of variation (CV) is greater than 0.5 and that the Classic estimator was used instead of the Bias-Corrected estimator to estimate abundance-based richness using the greater value of the Chao 1 or ACE estimator (Shen et al. 2003). Error bars are SE for 500 runs in EstimateS 8.2 (Colwell 2009). Taxa from pools are in purple and orange, while taxa from riffles are in green and blue. Taxa where the species estimator curve meets the rarefaction curve indicates that further sampling of that taxon would be unlikely to yield any new linkages. Taxa where the species estimator curve does not meet the rarefaction curve suggests that taxon is a specialist, with many singletons and doubletons observed. Additional linkages that were not observed were not factored into the food web analysis because it was unknown where the linkage would connect (e.g. would the prey be an insect or diatom?)

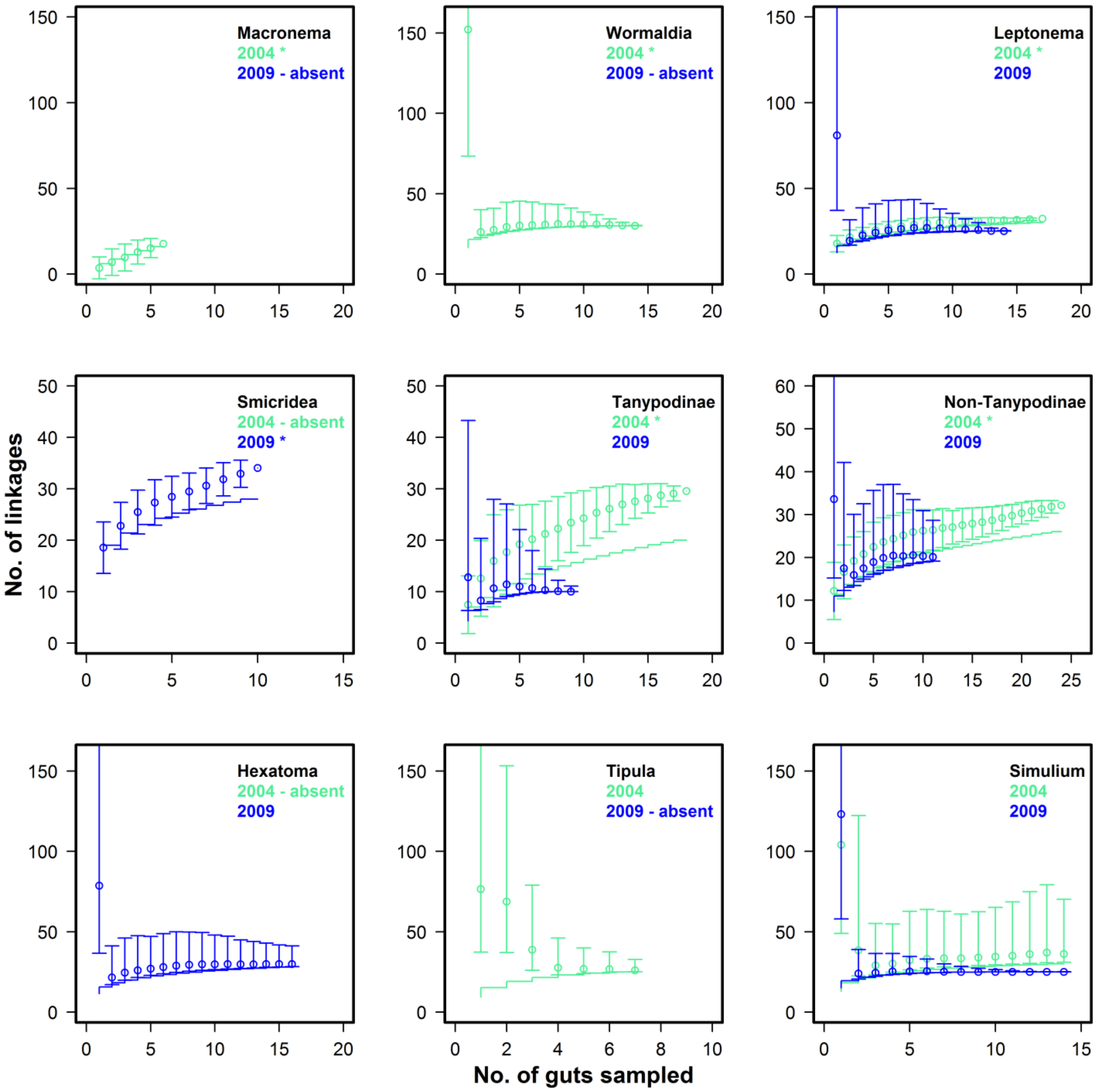

Anchytarsus
2004
2009
Hexacylloepus
2004 *
2009
Psephenus
2004
2009 - absent
Phanocerus
2004 *
2009 - absent
Macrelmis
2004 - absent
2009
Heterelmis
2004 *
2009
Tricorythodes
2004
2009 - absent
Farrodes
2004
2009 *
Thraulodes
2004
2009 - absent
No. of linkages
No. of guts sampled
0
50
100
150
0
10
20
30
40
50
60
0
5
10
15
20
25
0
2
4
6
8
10


Hagenulopsis
2004
2009 - absent
Leptohyphes
2004
2009 - absent
Macronema
2004
2009 - absent
Nectopsyche
2004
2009 - absent
Tipula
2004
2009 - absent
Tanypodinae
2004 *
2009
non-Tanypodinae
2004 *
2009
Hexatoma
2004 - absent
2009
Bufonidae
Hyloscirtus
2004
2009 - absent
No. of linkages
No. of guts sampled
0
50
100
150
0
10
20
30
40
50
60
200
250
300
0
2
4
6
8
10
5
15
20


No. of linkages
No. of guts sampled
Bufonidae
Lithobates
2004
2009 - absent
Heteragrion
2004
2009
Philogenia
2004
2009
300
250
200
150
100
50
0
100
80
60
40
20
0
0
5
10
15


Anchytarsus
2004
2009 *
Hexacylloepus
2004 *
2009 - absent
Psephenus
2004
2009 *
Phanocerus
2004 *
2009
Heterelmis
2004 *
2009 *
2004 - Neurocordulia
2009 - Cora
Heteragrion
2004
2009
Philogenia
2004
2009
Anacroneuria
2004
2009
No. of linkages
No. of guts sampled


Bufonidae
Atelopus
2004
2009 - Absent
Tricorythodes
2004
2009
Thraulodes
2004
2009 *
Haplohyphes
2004
2009
Leptohyphes
2004
2009 *
Stenonema
2004
2009 - absent
Baetodes
2004
2009
Baetis
2004
2009
Farrodes
2004
2009 *
No. of linkages
No. of guts sampled
0
50
100
150
200
0
5
10
15
20
25
2
4
6
8



Macronema
2004 *
2009 - absent
Wormaldia
2004 *
2009 - absent
Leptonema
2004 *
2009
Smicridea
2004 - absent
2009 *
Tanypodinae
2004 *
2009
Non-Tanypodinae
2004 *
2009
Hexatoma
2004 - absent
2009
Tipula
2004
2009 - absent
Simulium
2004
2009
No. of linkages
No. of guts sampled
0
50
100
150
0
10
20
30
40
50
60
0
5
10
15
20
25
0
2
4
6
8
10

Appendix D.

Figure 1. A comparison of the number of diatom valves and filamentous algae cells from insect guts in pools (a) and riffles (b) between pre- and post-decline. Predator taxa are *Anacroneuria*, *Heteragrion*, *Philogenia*, Tanypodinae, and *Hexatoma*. *Leptonema* and *Simulium* are filter feeders.