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Publication Date

2015

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Santa Barbara

Ecological impacts of mountain yellow-legged frog (*Rana muscosa* and *Rana sierrae*) declines on Sierra Nevada lake communities

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Ecology, Evolution, and Marine Biology

by

Thomas Collier Smith

Committee in charge:
Professor Cheryl J. Briggs, Chair
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Professor Sally J. Holbrook

March 2015

The dissertation of Thomas C. Smith is approved.

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December 2014

The ecological consequences of the decline of the mountain yellow-legged frogs.

by

Thomas C. Smith

ACKNOWLEDGEMENTS

Dr. Cheryl Briggs is an adventurous person and scientist, eager to accept new challenges, and she inspires the same in her students. She has created a respectful, low-stress research group I feel fortunate to have learned from. Dr. Sally Holbrook's goal oriented approach and enthusiasm for my research questions were critical. Dr. Scott Cooper's generosity with his time, attention to detail, and recall of ecological arcana are unrivaled and were indispensible. Dr. Roland Knapp gave me the opportunity to see a lot of lakes; he continues to be a sounding board for new ideas and a role model for perseverance and for great science.

I was helped by awesome undergraduates, and I thank them for their assistance in the laboratory and field, for their enthusiasm, and for entertainment: Michael Hernandez, Kim Riley, Colin Sayre, Teo Pier, Marina Bozinovic, Michael McFarlin, Shelby Geitner, Andrew Chen, Steven DeLisle, Cord Dorcey, Nikki Gantos, Vanessa Hurtado, Madelynn Johnston, Garrison Loope, Sabrina Lum, Michael McFarlin, Nick Meisinger, Yishen Miao, Melinda Mohamed, and Jennifer Taylor, and others.

Thanks also to Karen, Rufus, and Jennifer Smith, hard working role models and patient supporters. To Matt Kelly, Drew and Brent Reilly, Matt Daugherty, Tate Tunstall, Neil Kaufmann, and Julia Rosen, thanks for taking me climbing at exactly the right moments.

And to Dominique,

Thank you.

Vitae of Thomas C. Smith
January 2015

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PUBLICATIONS

1. Smith, Thomas C. 2013. *Pseudostaurosira pseudoconstruens*. in Diatoms of the United States, http://westerndiatoms.colorado.edu/taxa/species/pseudostaurosira_pseudoconstruens
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ABSTRACT

Ecological impacts of mountain yellow-legged frog (*Rana muscosa* and *Rana sierrae*) declines on Sierra Nevada lake communities

by

Thomas Collier Smith

Mountain yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) of California's Sierra Nevada have been extirpated from over 90% of their historic range, initially by introduced trout predators, and more recently by the emergence of the lethal amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). Formerly widespread and abundant, mountain yellow-legged frogs are grazers and highly connected high level predators, living in low diversity, low productivity high elevation lakes. Here, I examine the extent to which frog extinctions impact Sierra Nevada lake communities. First, in a study that combined surveys of benthic macroinvertebrates in 22 lakes over 5 years with a reanalysis of benthic macroinvertebrate community data from over 150 lakes, I found no strong differences in benthic macroinvertebrate community diversity or composition between lakes with frogs vs. lakes without frogs. I also conducted experiments to evaluate the impact of tadpoles as grazers on benthic algae, and found that tadpoles do reduce algal biomass in artificial habitats, but that the effect can be small and may not outweigh the influence that abiotic variability has on algal biomass in lakes. Lastly, while I documented that large and periodic tadpole aggregations create biogeochemical hotspots of dissolved nitrogen, the phenomena may not be widespread enough to drive differences in diatom diversity in lakes with vs. without tadpoles. Within lakes, I found little indication that diatom community diversity and composition were responding to tadpole generated nitrogen hotspots as a fluctuation-dependent mechanisms with potential to enhance diatom coexistence. While none of the ecological effects of mountain yellow-legged frogs and tadpoles that I observed suggests that their extinctions are having large impacts on Sierra Nevada lake communities, they should not be discounted as expendable, because there are many other ways in which these amphibians could be important in their communities.

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INTRODUCTION

Worldwide, biodiversity is declining due to global and local extinctions (Barnosky et al. 2011, Dirzo et al. 2014b). Ecologists understand well the many causes of single-species local extinctions – including physical disturbance, environmental change, stochasticity, species interactions – and can predict their consequences on populations and metapopulations (Harrison 1991, Lande 1993, Chesson and Huntly 1997, Hanski 1998, Murdoch et al. 2003). Ecologists are also well aware that when a species goes locally extinct, the loss of biomass and interactions can precipitate cascading changes in the surrounding community (Pace et al. 1999, Ferretti et al. 2010, Hollings et al. 2014).

Ecological principles can guide predictions about how communities (or ecosystems) might respond to single-species local extinctions. Local extinctions or species removals can release resources from top-down pressure and allow release of resource competitors (Kareiva 1982, Schmitt and Holbrook 1990, Holbrook and Schmitt 1995), or eliminate resources (Lafferty and Kuris 2009). Building on these basic consumer-resource interactions, the concepts of trophic cascades and keystone species suggest that removing one species can directly and indirectly affect the abundances of species in other trophic levels (Carpenter and Kitchell 1993, Jones et al. 1994, Power et al. 1996, Pace et al. 1999). Recent emphasis on the relationship between biodiversity and ecosystem function indicates that extinctions or species removals that reduce species richness can reduce the productivity or stability of communities (Gross and Cardinale 2005, Cardinale et al. 2006). In addition, food web ecology suggests that losses of highly connected, strongly interacting species might have the largest impacts on communities (Paine 1992, Berlow et al. 2009, O’Gorman et al. 2010).

Despite the depth of ecological knowledge of how population dynamics and species interactions shape communities, predicting the ecological impacts of extinction of a particular species remains difficult (Simberloff 2003). While declines and local extinctions of predicted keystone species like wolves, beavers, and otters changed the local ecosystem, declines of other potentially important species like American elm and chestnut may have had little impact (Simberloff 2003). Biodiversity-function studies have generally focused on numbers of species, with less emphasis on identity of species (). Regardless of connectedness, few most interspecific interactions within a community are probably relatively weak (McCann et al. 1998, Berlow 1999), in addition, the impacts of a species may vary in time, across its range, or among communities (Menge 2003). Subsequently, communities may exhibit minimal responses to the loss of any particular species. Synthesis of the effects of extinctions suggests that the ecological impacts of any species' extinction will be discovered best by testing the predictions based on ecological principles with thorough empirical investigation (Kareiva and Levin 2003).

My objective here was to investigate how a non-random, single species local extinction impacted simple, but real, communities. I quantified how disease-driven local extinctions of endemic mountain yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) affected several components of aquatic communities in high elevation lakes in the southern Sierra Nevada of California. These frogs were formerly abundant and widespread in Sierra Nevada lakes, live in low diversity communities, and are grazers as tadpoles and highly connected high-level predators as adults (Grinnell and Storer 1924, Knapp and Matthews 2001, Harper-Smith et al. 2005). As such, they have the potential to impact the abundance of algal resources and of invertebrate competitors and prey. Here, I studied the effects of these frogs on algal biomass, algal diversity, and benthic macroinvertebrate diversity.

While my results highlight one part of the spectrum of expected responses of communities to extinctions, they do so within the contexts of biodiversity loss (Barnosky et al. 2011, Dirzo et al. 2014a), worldwide amphibian declines (Stuart et al. 2004, Wake and Vredenburg 2008), and the declines of particular frog species in the Sierra Nevada (Vredenburg et al. 2010). This study measures the response of large segments of real communities over months to years. I have used experimental and observational, natural experiments to examine top-down and bottom up, negative and positive interactions of frogs, and their influence on community diversity and composition. In addition, I shed light on how wildlife diseases – in particular, the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) – can impact communities. Lastly, this work contributes to the collective knowledge of the ecology and natural history of Sierra Nevada lakes.

My dissertation summarizes three studies and places them in the context of current ecological knowledge. In Chapter 1, I describe a five-year, 22 lake, natural experiment in which I surveyed benthic macroinvertebrate communities in lakes where frogs were either extant, declining due to disease, or locally extirpated by disease. I also describe my reanalysis of a collaborator's similar data from over 150 frog-containing and frogless lakes. In both studies, benthic macroinvertebrate communities differed little with regard to extant, declining, or extinct frog populations. In Chapter 2, I describe two experiments designed to quantify the impact of tadpole grazers on algal resources. In the *in situ* field enclosure experiment, tadpoles had no effect on algal biomass, though they had a moderate but marginal effect in the subsequent mesocosm experiment. Lastly, in Chapter 3 I describe how waste excretion by tadpoles combined with their aggregating behavior has the potential to create hotspots of nutrients, which as a source of environmental heterogeneity, could enhance diversity and productivity of algal communities. Differences among algal communities were subtle, and appear driven more by characteristics of the lake than by the biogeochemical gradients created by tadpoles.

In summary, my findings indicate a very small overall effect of mountain yellow-legged frog extinctions on Sierra Nevada lake communities. While this may seem counterintuitive given the frogs' ecological characteristics and former abundance, it supports the concept that not all species have large impacts, and not all extinctions will have dramatic secondary effects on communities. My results do not preclude the importance of mountain yellow-legged frogs in their lake communities: many of their interactions remain to be examined, and only a thorough investigation of all of their interactions will clarify the ecologically importance of mountain yellow-legged frogs. Lastly, however small the secondary effects of these frogs' extinctions are, they do nothing to minimize the widespread local extinctions of the once ubiquitous and abundant mountain yellow-legged frogs (Grinnell and Storer 1924, Vredenburg et al. 2007, 2010).

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I. DECLINES OF MOUNTAIN YELLOW-LEGGED FROGS HAVE SMALL EFFECTS ON BENTHIC MACROINVERTEBRATE COMMUNITIES

ABSTRACT

Species extinctions have the potential to dramatically reshape ecological communities. In the Sierra Nevada mountains of California, communities are changing following the emergence of a lethal amphibian pathogen (*Batrachochytrium dendrobatidis*), which drives mountain yellow-legged frog (*Rana muscosa* and *Rana sierrae*) populations to local extinction. These frogs and their tadpoles are abundant, highly connected high level predators and grazers. To quantify how lake communities responded to these frog declines and extinctions, I repeatedly surveyed and analyzed aquatic macroinvertebrate communities in 22 alpine lakes in California's Sierra Nevada ("Resurveys"). Each of these lakes was categorized, for the duration of my study, as having extant frog populations, experiencing ongoing disease driven frog declines, or having previously experienced local disease driven frog extinction. In addition, I reanalyzed previously collected data from a large scale synoptic survey ("Snapshot survey") of 157 lakes with and without frogs. My results describe how simple, low diversity aquatic macroinvertebrate communities can respond to a non-random single species extinction. In the Snapshot survey, invertebrate taxonomic richness was 6 - 15% lower in lakes without frogs, but multivariate analyses of community similarity indicated only small differences between lakes with and without frogs. In the Resurveys, taxonomic richness was 17% higher in lakes where frogs were declining or extinct, compared to lakes where frogs were extant. However, multivariate analyses revealed no strong dissimilarities among Resurvey communities, and I found no differences in the abundances of individual taxa with respect to where frogs were extant, declining, or extinct. Overall, disease-driven mountain yellow-legged frog extinctions appear to have small effects on Sierra Nevada lake benthic macroinvertebrate communities, with no large changes in invertebrate abundance, richness or evenness, no clear secondary extinctions or invasions, and only a few taxa showing distinct responses to extinctions of frogs. My study highlights how even for conspicuous, highly connected, omnivorous taxa that are experiencing large, rapid, and widespread declines and extinctions, the ecological effects of extinctions will sometimes be small and subtle.

Keywords: Batrachochytrium dendrobatidis*, consequences of extinctions, amphibian declines,* Rana muscosa/Rana sierrae*, Sierra Nevada.*

INTRODUCTION

Single species extinctions have the potential to dramatically change communities (Pace et al. 1999, Ferretti et al. 2010, Hollings et al. 2014), but that potential depends on the tendency of a species to influence the abundances of others in its community (Menge 2003). In turn, the impact that one species has on the abundance of others can depend on community diversity (McCann 2000), composition (Menge 2003), and connectance (Petchey et al. 2008, Dunne and Williams 2009), on species' interaction strengths (Otto et al. 2008) and abundance (Doak and Marvier 2003), or on the role of top-down and bottom-up processes in a community (Shurin et al. 2002, Borer et al. 2005). All of these properties can vary between communities and species as well as spatio-temporally for both (Harley 2003). Because of this variation and because most species may interact weakly (McCann et al. 1998, Berlow 1999, Emmerson and Yearsley 2004), the various drivers of species extinctions may not always extirpate the most highly connected, most strongly interacting, or most important species (Srinivasan et al. 2014). Thus, it seems plausible that many species will go extinct with little measurable effect on their communities (Simberloff 2003).

Numerous observational studies have explored the effects of recent species declines and extinctions on whole communities, and have demonstrated the importance of particular species in their communities. Local or regional extinctions of Pacific sea otters (Estes et al. 1998), Yellowstone's wolves (Beyer et al. 2007, Beschta and Ripple 2008, Smith and Tyers 2012), Midwestern large-mouth bass (Hall and Ehlinger 1989) and caddisflies (Kohler and Wiley 1997) and Neotropical stream frogs (Ranvestel et al. 2004, Connelly et al. 2008b, Colón-Gaud et al. 2009) all allowed predatory or competitive release of grazers or producers or loss of facilitation, creating measurable changes in community composition and even ecosystem processes like productivity, nutrient flow, and physical processes. These examples in which extinctions strongly affected communities overshadow the quantitative examples in which extinctions have relatively little effect on communities, but which I should expect given the number of currently declining or recently extinct species (McCann et al. 1998, Simberloff 2003, Srinivasan et al. 2014).

Worldwide, biodiversity continues to decline and extinction rates are increasing (Barnosky et al. 2011). Amphibians are the most threatened vertebrate taxa (Wake and Vredenburg 2008), due in large part to the emergence of the lethal amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd hereafter, Stuart et al. 2004, Wake and Vredenburg 2008). One of the most intensively studied disease driven amphibian extinction events is that of the mountain yellow-legged frogs (*Rana muscosa and Rana sierrae,* Wake and Vredenburg 2008, Briggs et al. 2010). Once the most abundant amphibian in the Sierra Nevada (Grinnell and Storer 1924), these frogs first declined as a result of non-native fish introductions. Recently, population declines and extinctions have continued as a direct result of high frog mortality during epizootics of Bd (Rachowicz et al. 2006). In Sierra Nevada lakes, Bd kills only a single species (*R. muscosa* or *R. sierrae*) in any given lake (Vredenburg et al. 2010). Therefore, as populations of these frogs are extirpated by disease, any changes in non-Bd host species abundances and community composition that occur are likely to be in response to local extinctions of mountain yellow-legged frogs.

Several factors suggest that disease-driven mountain yellow-legged frog extinctions may impact Sierra Nevada lake benthic macroinvertebrate communities. In mesocosm experiments, mountain yellow-legged frog tadpole exclusion leads to higher benthic producer abundance (Smith 2015), suggesting that extinctions of these tadpoles might free resources and allow increases in grazing benthic competitors. Whether considered omnivores or ontogenetically separated trophic species (tadpoles are grazers, adults are predators on emerging aquatic insects) their extinction will reduce the number of trophic links throughout the community (Harper-Smith et al. 2005), which can reduce community stability (Borrvall et al. 2000) and increase the chances that secondary extinctions will occur (Dunne et al. 2002). Furthermore, the addition of trophically similar fish to these communities dramatically reduced the abundance and diversity of benthic macroinvertebrate communities (Knapp et al. 2001, 2005, Harper-Smith et al. 2005), so community responses to removal mountain yellow-legged frogs might mimic removal of trout and release macroinvertebrates from top-down control (Knapp et al. 2001, 2005).

On the other hand, there are factors which may reduce the impact of frog extinctions on Sierra Nevada lake communities. Keystone species are not common (Power et al. 1996) and most interspecific interactions within communities may be weak (McCann et al. 1998, Berlow 1999), so statistically it seems unlikely that frogs will be important enough that communities will collapse in their absence. Adult frogs are nearly top predators and no macroinvertebrates depend on the frogs or tadpoles as prey, so secondary extinctions of macroinvertebrates seem unlikely (Dunne and Williams 2009). Frogs can only feed on aquatic invertebrates when they emerge along lake shorelines and become terrestrial, so frogs only have access to a relatively small portion of the insect biomass that leaves the lake and thus may have limited ability to control those prey populations.

The objective of the current study was to describe benthic macroinvertebrate community responses to frog declines and extinctions. I compared communities in Sierra Nevada lakes where mountain yellow-legged frog populations were either uninfected with

Bd, currently declining due to ongoing epizootics, or locally extinct due to previous epizootics. I predicted that a) communities would differ in composition and/or relative abundances of benthic macroinvertebrate taxa, with respect to where frogs are extant versus extinct, b) communities in lakes where frogs were declining would have characteristics intermediate between those in frogs-extant lakes and frogs-extinct lakes and indicate a transitional state that could help me connect cause and effect, and c) when considered as separate trophic species, frogs and tadpoles would have distinct effects on communities which could be responsible for that transitional state (see Table 1 for detailed predictions). I also aimed to characterize differences among communities where frogs were extant vs. extinct or declining across a broad range of habitats in the Sierra Nevada to infer generality of the effects of frog extinctions, and among lakes for which I had definite knowledge of disease as a driver of frog population declines and extinctions over time to link cause and effect. My study complements existing knowledge of how extinctions affect communities, as I followed a single-species extinction of a highly connected omnivore that therefore has the potential to directly influence community dynamics.

METHODS

Study area, design and sampling

My study lakes lie close to and west of the Sierra Nevada crest, clustered in basins that drain westward into the Kings, San Joaquin, Merced, and Tuolumne River watersheds. These small, high elevation (Tables 2, 3) lakes lie in granitic basins near or above tree line, surrounded by small meadows, sparse vegetation, and bare rock. The water in these lakes has low nutrient concentrations and circumneutral pH: nitrate 0 – 10 μmol L^{-1}, total phosphorus

0 – 1 μmol L^{-1} (Sickman et al. 2003); median pH ≅ 7 (Bradford et al. 1998). I selected lakes based on their elevation, depth, area, and on the status of mountain yellow-legged frog populations (extant, declining, extinct). Data on frog populations were obtained from annual visual encounter surveys conducted since the mid-1990s in lakes throughout the southern and central Sierra Nevada. Lake physical characteristics were obtained from a database describing over 8000 Sierra Nevada water bodies (Knapp et al. 2003, Knapp 2005, Davidson and Knapp 2007) that includes information on lake elevation, depth, area, perimeter, latitude and longitude, drainage basin, and substrate composition (see Knapp and Matthews 2000 regarding the characterization of lake physical attributes).

I performed two sets of surveys: a set of 157 snapshot surveys (i.e., one survey per lake) across a broad latitude and elevation range of lakes with known frog presence-absence but unknown disease history (hereafter, the “Snapshot survey”), and a set of surveys repeated over five years in 22 lakes in which I were concurrently documenting frog population abundance and demographics and Bd presence and prevalence (hereafter, the “Resurveys”). By design, the Snapshot survey allowed me to infer generality of the effects of frog extinctions throughout Sierra Nevada lakes, while the Resurveys allowed me to connect the cause of potential differences to observed patterns.

Snapshot survey. – Frog populations in the Sierra Nevada once occurred across broad gradients of elevation, latitude, lake size, and productivity, all of which have been shown to affect community composition and species abundances (Knapp et al. 2001), so this survey allowed me to generalize the potential effect of frog population status in structuring communities across a broad range lake characteristics. I compared macroinvertebrate communities in lakes with frogs to communities in lakes without frogs, using data from benthic macroinvertebrate surveys performed by Knapp et al. (Knapp et al. 2001, 2005). Between 1995 and 2001, Knapp et al. sampled benthic macroinvertebrate communities in lakes throughout the central and southern Sierra Nevada, including in Yosemite National Park (YNP), Kings Canyon National Park (KCNP), and the John Muir Wilderness (JMW). At the time of the survey, these regions contained some of the largest remaining mountain yellow-legged frog populations in the Sierra Nevada (but there are regionally disjunct populations in the Transverse Ranges of southern California, Vredenburg et al. 2007).

Although Knapp et al.'s studies were designed to quantify the impact of introduced trout on lake communities, I analyzed data only from historically and currently fishless lakes to examine differences between communities in lakes with frogs versus lakes without frogs independent of the large effect of trout. From Knapp et al.'s studies, I selected every lake that was fishless and greater than two meters deep, two conditions that are necessary for breeding frog populations (Knapp et al. 2003). Using amphibian survey data, I categorized lakes as either containing frogs ("*frogs-extant*") or lacking frogs ("*frogless*"). For these frogless lakes, I did not know historical frog presence nor did I know the reasons for frogs' absence, thus I did not categorize them as "*frogs-extinct*". As the habitat characteristics of frogless lakes did not differ from those of frogs-extant lakes and are within the ranges that frogs inhabit (Knapp et al. 2003), I assumed that frog absence was very likely due to Bd-caused local extinctions. In total, I examined communities in 157 lakes, including 45 frogs-extant and 51 frogless lakes in YNP and 21 frogs-extant and 40 frogless lakes in KCNP and JMW (Table 2).

Resurvey. – The Resurvey allowed me to look for differences among communities for which I knew that Bd was the proximate driver of frog declines or frog absence. I performed repeat surveys to compare macroinvertebrate communities in lakes that differed in the status and dynamics of their frog populations: lakes with stable, Bd naïve frog populations ("*frogs-extant*")*,* lakes experiencing ongoing frog die-offs due to Bd epizootics ("*frogs-declining*"), and lakes from which frogs were extirpated by Bd prior to 2006 ("*frogs-extinct*"). For the Resurvey, I use the term "frogs-extinct" to highlight that frogs were recently present but I observed their local extinctions, unlike in the Snapshot survey. These 22 Resurvey lakes, all in KCNP and JMW, ranged in elevation from 3300 to 3600 m, were all greater than 2 m deep, and had surface areas greater than 0.5 hectare, providing breeding and overwintering habitats for frogs and tadpoles. I collected 1-3 samples per lake per ice-free season (from mid-June and mid-July through late September) of 2007-2010 and 2012, including in 7 frogs-extant, 6 frogs-declining, and 9 frogs-extinct lakes (Table 3). The Resurveys allowed me to compare communities in frogs-extant, frogs-declining, and frogs-extinct lakes across a period during which Bd epizootics reduced frog abundance in frogs-declining lakes, while frog abundances in frogs-extant lakes were large and did not display trends over the course of the study, and abundances in frogs-extinct lakes remained at zero.

Amphibian density data. ─ For the Snapshot and Resurvey lakes, tadpole and subadult/adult (adults, hereafter) mountain yellow-legged frog population counts were conducted once annually using visual surveys. Observers walked the entire shoreline of each lake and recorded all individuals of each frog life stage observed. In these lakes, clear water, unvegetated shorelines, and basking behavior of frogs and tadpoles allow a single visual survey to provide a repeatable estimate of the presence-absence and relative abundance of tadpoles and frogs in each lake (Bradford 1989, Knapp and Matthews 2000, Knapp et al. 2003). To calculate tadpole and frog densities, I divided the observed abundance of each life stage by the lake perimeter. I calculated lake perimeters using a geographic information system (ArcGIS10, ESRI 2011). I compared densities of adults, subadults, and tadpoles across frog population status levels using ANOVA and Tukey's HSD tests (Quinn and Keough 2002); densities and population demographics were different among frog population status categories in both surveys (Tables 2, 3).

Littoral benthic macroinvertebrate surveys. ─ To assess invertebrate community richness, composition, and taxa abundances, I collected benthic macroinvertebrates in the littoral zone of each lake, and I used identical sampling methods in both the Snapshot surveys and the Resurveys (Knapp et al. 2001, 2005). In the Snapshot surveys, each lake was sampled exactly once in 1995-1997 or 2000-2001, on the same day as amphibian surveys. In the Resurvey study, I collected benthic macroinvertebrates at least twice in each of the 22 lakes between 2007 and 2010 and in 2012; frogs-declining lakes were sampled most frequently, and frogs-extinct lakes were sampled the least (Table 3). This variation in the number of samples from Resurvey lakes was due to weather-imposed time constraints and variability in the timing of snowmelt. In the Resurveys, invertebrate surveys were not conducted on the same days as amphibian surveys. To prevent the dispersal of Bd between lakes, all field gear was disinfected in a 0.01% solution of quaternary ammonia for five minutes after sampling each lake (Johnson et al. 2003).

To obtain a macroinvertebrate sample from a lake, I took 15 standard, 1-m long sweeps with a 30 cm diameter D-net (mesh size 250 µm) at 0.5-1 m depth in the littoral zone around each lake's perimeter. The number of sweeps on a given substratum was taken in proportion to the availability of different substrate types in each lake's littoral zone. Substrates were categorized as silt (< 0.5mm), sand (0.5-2 mm), gravel (>2-75 mm), cobble

(>75-300 mm), boulder (>300 mm), bedrock, and aquatic vegetation, as in Knapp and Matthews (2000). To sample interstices within substrates, I disturbed gravel, cobbles, or small boulders with my feet before taking sweeps.

The contents of all 15 sweeps were pooled, invertebrates in samples were removed in the field, and were preserved in 70% ethanol. These sampling methods are useful for calculating both absolute and relative abundances of taxa in the benthic macroinvertebrate community. In general, I applied similar effort to all samples, examining the entire sample and removing most invertebrate individuals. I consistently subsampled Chironomidae, as they are difficult to see and to capture, and can be extremely abundant, but since my subsampling of Chironomidae was similar across surveys, I underestimated their absolute and relative abundances consistently. If sorting took more than four hours or surveyors were threatened by inclement weather, I stopped sorting and visually estimated the proportion of the sample volume that I had subsampled for both the whole sample and for each taxon. Only 5 out of 106 samples took too long or were cut short by weather, and I may have underestimated the overall abundance of invertebrates in these lakes. Relative abundances should not have been biased by subsampling, except for very low abundance or extremely inconspicuous taxa which might have been less detectable in subsamples. I consistently subsampled Chironomidae, as they are difficult to see and to capture, and can be extremely abundant. My subsampling of Chironomidae was similar between lakes, so while I probably underestimated their absolute and relative abundances, I did so consistently across lakes. These methods were the same as those used by others in previous surveys of Sierra Nevada benthic macroinvertebrates (Bradford et al. 1998, Knapp et al. 2001, 2005).

Individual invertebrates in samples were identified in the laboratory under a stereoscope at 60 x magnification, using Merritt and Cummins (1996), Thorp and Covich (2009), and my own reference collection. When species level identifications were not possible or practical, genus or family level identifications were used (as in Knapp et al. 2001), mites and oligochaetes were identified to order (Acari and Oligochaeta, respectively). Once identified, all individuals of each taxon were counted; for samples or taxa (e.g. Chironomidae) that I had subsampled, laboratory observed abundances were adjusted by my in-field estimate of the proportion of each taxon or whole sample that I subsampled.

Analytical Methods

Following the objective of my study, I compared invertebrate communities across frog population status categories or frog densities. I used linear models to describe univariate community characteristics and abundances of individual taxa, and compared multivariate representations of whole communities.

Analysis of univariate community characteristics. ─ I evaluated the effects of frog population status or density, and environmental covariates, on total benthic macroinvertebrate abundance, taxonomic richness, and community evenness using general linear mixed-effects models (Zuur et al. 2009). I did this separately for Snapshot survey lakes in KCNP/JMW versus YNP, because invertebrate abundance, taxonomic richness, and community composition differed significantly for these two regions (see Results).

In the Snapshot survey and in the Resurvey, my independent variable was the effect class variable frog population status, to test my prediction that frog population status can explain variation among communities, and classifies frogs and tadpoles as one omnivorous taxa (Predictions 1 and 2, Table 1). In the Snapshot survey levels were frogs-extant and frogless; in the Resurvey levels were frogs-extant, frogs-declining (to test Prediction 2, Table 1), and frogs-extinct. In the Resurvey, I also performed analyses using both of the continuous variables tadpole density and adult density, which allowed me to test my prediction (Prediction 3, Table 1) that ontogenetic diet shifts in frogs had distinct effects on communities and frogs and tadpoles should be treated as distinct trophic species (similar data were not available for Snapshot surveys). I separated the densities of the two life stages because in declining populations, adult frogs die from Bd infection but tadpoles do not, so a population in a frogs-declining lake has an age structure distinct from those in frogs-extant or –extinct lakes. In addition, tadpoles and adult frogs have different ecological trait. Tadpoles metamorphose in 2-4 years, and are benthic grazers. Adult frogs can live over 10 years (Matthews and Miaud 2009) and are predators, feeding on emerging adult aquatic insects, terrestrial insects, and conspecifics (Finlay and Vredenburg 2007).

My analyses included the categorical covariate drainage basin, and the continuous covariates elevation, latitude, lake perimeter, and siltiness. I rescaled elevation by subtracting the lowest elevation and rescaled latitude (in units of UTMs) by subtracting the minimum and dividing by 10,000. I used lake perimeter as an index of lake size in my analyses because it was correlated with lake depth and surface area (Pearson's correlation, *df* = 155, depth:perimeter: $p < 0.001$, $r = 0.27$; area:perimeter $p < 0.0001$, $r = 0.75$), both of which are related to invertebrate community structure. Previous analyses indicated that frequencies of substrate categories were highly intercorrelated and that the percent of substrate dominated by silt (siltiness, hereafter) was a good predictor of community composition (Knapp et al. 2001), therefore I used siltiness as the covariate to describe substrate characteristics.

Dependent variables: Invertebrate abundance, rarefied richness, and evenness. — I calculated invertebrate abundances were calculated as the total number of each taxa collected in a sample. The total invertebrate abundance in each lake was calculated by summing the estimated abundances of all taxa. In both surveys, invertebrate richness was positively correlated with estimated invertebrate abundance (Snapshot surveys: $r = 0.67$, $p < 0.0001$, $df = 2, 155$, $t = 11.12$; Resurveys: $r = 0.49$, $p < 0.0001$, $df = 2, 104$, $t = 4.9$), so I calculated rarefied richness (Magurran and McGill 2011) for the minimum invertebrate abundance, after excluding the bottom fifth percentile of samples based on abundance. For Snapshot surveys in YNP, I estimated rarefied richness using 56 individuals. For Snapshot surveys in KCNP/JMW, I estimated rarefied richness using 8 individuals; while this seems like a very low number and only allows the maximum rarefied richness to be 8 taxa, the observed richness for these lakes was also low (mean 6.50 ± 0.44 s.e., range: 1-16 taxa). For Resurveys, I estimated rarefied richness using 40 individuals. Community evenness was calculated as the Shannon diversity of a community (H) divided by the natural logarithm of observed richness (S): $H / ln(S)$ (Magurran and McGill 2011). I used the vegan package for R (The R Foundation for Statistical Computing 2012) to calculate rarefied richness and community evenness.

Linear model structures: random and mixed effects, and linear model assumptions. – For the Snapshot survey, I treated individual lakes as independent replicates representing a random sample of the thousands of lakes within KCNP, JMW, and YNP. In this data set, frog population status often differed between lakes within the same drainage basin. For the Resurvey, my replicates also were individual lakes, representing a random subset of alpine lakes in KCNP and JMW (Tables 2, 3). Frog population status varied at the lake basin scale, because Bd epizootics generally drive populations extinct concurrently in all lakes across an entire lake basin (Vredenburg et al. 2010). Therefore, in analyses of Resurvey data, I nested lakes within the random effect of drainage basin. Here my main focus was the overall differences between communities in lakes of different frog population status; rather than either within year or between year dynamics. Exploratory plots suggested that neither sample number within a year, day of year, nor year had an effect on the abundances of each taxon. Therefore, for each lake I used the overall average abundance for each taxon, calculated by averaging relative abundances of each taxon within each year, then across all years sampled.

I assessed normality of dependent variable distributions graphically and using Shapiro-Wilk tests, and where necessary applied transformations to meet the normality assumption of the analyses. In all three datasets, invertebrate abundance met the assumption of normality, after $\log_{10}$ transformation. In all three datasets, I squared invertebrate community evenness to meet the assumption of normality, and rarefied richness met the assumption of normality without transformation. I evaluated equality and homogeneity of variance graphically (Quinn and Keough 2002, Zuur et al. 2009). Using generalized linear models (GLMs), I included different variances of invertebrate abundance, rarefied richness, and evenness between levels of frog population status, and drainage. I compared model fits and parsimony using the Akaike Information Criteria (AIC, Zuur et al. 2009). Univariate analyses were performed using the nlme package in R. I used this approach to establish relationships between my dependent community descriptors invertebrate abundance, rarefied richness, and community evenness, and my independent variables and covariates frog population status or density, lake elevation, lake latitude, lake perimeter, and littoral zone siltiness, as well as drainage basin in the analysis of Resurvey communities. To facilitate interpretation of Snapshot survey invertebrate abundance, I back-transformed mean residual $\log_{10}$ transformed invertebrate abundance.

Composition and similarity of communities. – To compare the similarity of lake communities in different frog population categories and to identify taxa associated with these categories, I performed multivariate analyses for communities in both Snapshot survey and Resurvey lakes. In both surveys, I designated taxa present in over ten percent of lakes as commonly occurring taxa (sensu Gaston 1994, as used in Bradford et al. 1998, Knapp et al. 2001). I used this occupancy based definition of commonness, rather than an abundance based definition (sensu Magurran and McGill 2011), because my impression was that in my samples, many taxa were represented by only one or a few individuals even when widely distributed, and taxa that occurred in very few or single samples often also occurred at very low abundance. Excluding taxa based on abundance severely truncated the richness of my communities.

I based my analyses on community matrices of relative abundance of these commonly occurring taxa. I calculated relative abundance values for each taxon in each lake replicate, then used these matrices to calculate Bray-Curtis multivariate distances between all pairs of lakes in each survey (Magurran and McGill 2011). I compared within and between frog population status group multivariate distances using the Multi-Response Permutation Procedure (MRPP, Quinn and Keough 2002). I visualized similarities between communities, by plotting communities' non-metric multidimensional scaling scores commonly occurring taxa (NMDS, Quinn and Keough 2002). To describe the performance of the NMDS, I calculated stress and correlations between original and ordination distances (Quinn and Keough 2002).

Responses of individual taxa. – To examine the relationship between individual taxa and frog population status, I examined the occupancy of taxa in lakes of each frog population status, calculated correlations of common taxa absolute abundances with NMDS axes, and used my results of those correlations to suggest for which taxa I should test linear models of abundance as a result of frog population status and covariates. In both surveys, I examined the occurrence of individual taxa in lakes of each frog population category, in order to identify potential secondary extinctions or invasions. For YNP Snapshot surveys, I examined only the taxa that were significantly correlated to NMDS axes, due to the very large number (> 50) of common taxa in YNP lakes. For KCNP/JMW Snapshot surveys and for Resurveys, I evaluated the abundances of all common taxa. To describe how the abundance of each common taxon differed between lakes of different frog-population status, I used the absolute abundances of taxa as dependent variables and frog population status variables and environmental covariates as independent variables. For the Resurvey, I summarized abundances of taxa by calculating per-lake averages across all survey years, resulting in one abundance value per taxon per lake (n = 22 lakes). I used GLMs for zero-inflated species count data with negative binomial distributions, using a logit link function (Zuur et al. 2009); my general approach to fitting these models was similar to that described above for models of univariate community dependent variables. After factoring out the effects of environmental covariates, I compared residuals of each taxon's absolute abundance between the levels of frog population status (ANOVA, Tukey's HSD).

RESULTS

Frog Population Status and Invertebrate Abundance, Richness and Evenness. – Invertebrate abundance did not differ between KCNP and JMW, but in YNP abundance was 2.5-4.5 times higher than in KCNP and JMW (ANOVA, $F_{3,154} = 22.45$, $p < 0.001$). Observed richness was three times higher in YNP than in the other regions (ANOVA, $F_{3,154} = 98.01$, $p < 0.001$, Figure 1), and community composition was conspicuously different between YNP and both KCNP and JMW. Therefore, I evaluated lake communities in KCNP and JMW together, and evaluated lake communities in YNP separately.

In the Snapshot surveys, benthic macroinvertebrate richness was higher in frogless lakes. In YNP, frogs-extant lakes had about 1, or 6%, more taxa (rarefied richness) than frogless lakes (Figure 1), but elevation had a negative effect on richness (Table 4). Frog population status was not retained as a fixed effect in the best models of either invertebrate abundance or community evenness in YNP. In KCNP/JMW, frogs-extant lakes had about 1, or 15%, more taxa than frogless lakes (Figure 1), while lake perimeter had a negative effect on richness (Table 4). Abundance was also higher in KCNP/JMW frogs-extant lakes, by 77% after mean residual $\log_{10}$ invertebrate abundance was back-transformed (Figure 2), while elevation had a negative effect on abundance (Table 4). Frog population status was not retained as a fixed effect in models of community evenness in KCNP and JMW.

In the Resurveys, rarefied richness was 17% higher, about 1 taxa, in lakes where frogs were declining or extinct, relative to those where frogs were extant (Figure 3). Notably, this relationship is opposite of the status-richness relationship I observed in Snapshot surveys. No environmental covariates were included in this best-fit model (Table 5). In the Resurveys, frog population status was not included as a fixed effect in best-fit models of either invertebrate abundance or community evenness. Adult frog density was negatively related to invertebrate abundance and positively related to community evenness, but the effects were small and marginal (Table 5). Adult frog density was unrelated to rarefied richness, and tadpole density was unrelated to abundance, richness, and evenness.

Frog population status and invertebrate community structure. – In the Snapshot survey, YNP communities in frogs-extant lakes differed from those in frogless lakes, although the difference was small (Figure 4), but KCNP/JMW communities in the two categories were not different (Figure 5). Yosemite National Park samples included 136 taxa, of which 56 were common and used in the multivariate analysis. Frogs-extant and frogless communities did not differ on NMDS axis one, but did differ on axis two. *Oreodytes sp.*, *Cenocorixa sp.*, and *Callibaetis ferrugineus* were all positively correlated to axis two, suggesting these three taxa were associated with frogs extant communities; no taxa were strongly associated with frogs-extinct lakes in YNP (Figure 4). I observed 36 taxa in KCNP /JMW, of which 22 taxa were common and used in the multivariate analysis. Frogs-extant and frogless lakes differed on NMDS axis one and on axis two, but not on axis three, and overall communities in frog population status groups did not differ (MRPP, $p = 0.1$). *Desmona mono* was associated with frogless communities and *Callibaetis ferrugineus* was associated with frogs-extant communities on axis one; on axis two *Ameletus edmundsi* was associated with frogs-extant communities while Chironomidae was associated with frogless communities in KCNP/JMW Snapshot surveys (Figure 5).

In the Resurveys, benthic macroinvertebrate communities did not differ with respect to frog population status (Figure 6). Of the 40 taxa observed in these surveys, 29 were common and were used in analyses of community structure. Communities did not differ with respect to frog population status on NMDS axes 1 and 2. On NMDS axis three, communities in frogs-declining lakes differed from those in frogs-extant and frogs-extinct lakes. However, this difference among communities with respect to frog population status contributed little to the overall variation among communities, because only 8% of the variation in communities was accounted for by NMDS axis 3. As such, only *Stictotarsus spp.* was associated with frogs-declining communities along NMDS axis 3.

Frog Population Status and the Abundances of Individual Taxa. – In Snapshot surveys there was no evidence of secondary extinctions or invasions in either YNP or KCNP/JMW; all common taxa were present in some lakes in both frog population categories. In YNP, the abundances of *Callibaetis ferrugineus*, *Oreodytes sp.*, *Ameletus edmundsi*, and *Cenocorixa sp.* (the taxa correlated with NMDS axes) did not differ with respect to frog population status. In KCNP/JMW, residual abundances (Figure 7) of Oligochaeta and *Sialis occidens* were higher in frogs-extant lakes than in frogless lakes, and the residual abundances of *Desmona mono* were significantly lower in frogs-extant lakes than in frogless lakes. Frog population status was retained as a fixed effect in best-fit GLMs of the absolute abundance of seven taxa (Table 6), out of 18 taxa tested.

In the Resurveys, two common taxa, *Ecclisomyia sp.* and *Sanfillipodytes sp.* were both absent from all frogs-extinct lakes, representing possible secondary extinctions. Hirudinea was absent from all frogs-declining lakes. Frog population status was retained as a fixed effect in GLMs of the absolute abundance of four taxa (*Ameletus edmundsi*, *Stictotarsus spp.*, *Callibaetis ferrugineus*, and *Sialis occidens*), out of 18 taxa tested. However, residual abundances of those four taxa did not differ with respect to frog population status (Table 5).

DISCUSSION

Overall, declines and local extinctions of mountain yellow-legged frogs had weak effects on benthic macroinvertebrate communities of Sierra Nevada lakes. Lakes where frogs were extant, declining, or extinct sometimes differed in overall invertebrate abundance, richness, and community composition, but the magnitudes and directions of those differences varied within and between my studies. I did observe lower overall macroinvertebrate abundance where frogs were absent, but only in the snapshot survey in Kings Canyon National Park and John Muir Wilderness (KCNP/JMW). While in the Snapshot surveys macroinvertebrate richness was lower in frogless lakes, in the Resurveys richness was higher in lakes where frogs were declining or extinct. In both cases those richness differences were proportionally large, but actually represented just a single taxon. I could not attribute these richness differences to declines, coextinctions, increases or invasions of any particular macroinvertebrate taxa because taxonomic composition of communities did not vary consistently with respect to frog population status. Multivariate analyses of communities revealed no large differences either. Nonetheless, the abundances of some taxa varied with respect to frog population, which may suggest undocumented interactions. I hoped to clarify the ecological effects of both adult and tadpoles lifestages, but the effects of adult density were marginal and there was no detectable effect of tadpole density. My initial predictions were only partially supported by my results (Table 1), and the effects of frog extinctions were weaker than expected.

It is unclear why the trends of macroinvertebrate taxonomic richness trends differed between the two surveys. In Snapshot communities, richness was 6 - 15% lower in frogless lakes than in frogs-extant lakes, while in the Resurvey communities, richness was 17%

percent higher in both frogs-declining and frogs-extinct lakes relative to frogs-extant lakes. I feel confident this discrepancy does not arise from differences in sampling design between the two studies or from variation in sampling intensity among Resurvey lakes. Rather, these opposing patterns of invertebrate richness observed in the two studies might arise due to the different reasons for the absence of frogs from lakes in each study. While I documented epizootics of Bd driving declines and extinctions of frogs in Resurvey lakes (Vredenburg et al. 2010), I had no knowledge of the causes of frog presence-absence in Snapshot survey lakes. Those lakes may actually have been frogless because they do not provide suitable habitat for frog populations. They may also be poor habitat for benthic macroinvertebrates; in these frogless lakes, invertebrate abundance was almost half that observed in frogs-extant Snapshot survey lakes. However, lake characteristics which influence frog occupancy (lake depth, elevation, substrate composition) did not differ with respect to frog presence-absence, so my assumption that Snapshot survey lakes were frogless due to earlier, undocumented disease epizootics still seems valid.

The distinct responses of taxa to the absence of or decline of frog populations suggest undocumented interactions. Frog declines may have released the detritivorous/herbivorous caddisfly *Desmona mono* from competition with tadpoles. By eliminating facilitative interactions, tadpole declines may have harmed two burrowing taxa, predacious alderflies (*Sialis occidens*) and herbivorous annelids (Oligochaeta), which were both less abundant in frogless lakes. In other systems, tadpoles bioturbate sediments (Regester et al. 2006, Wood and Richardson 2010), as they graze over or burrow in soft substrates, which may oxygenate or loosen sediment and enhance habitat for burrowers like *Sialis occidens* (Gallon et al. 2008) and oligochaetes. Though I have not specifically investigated such a mechanism, I speculate that positive interactions between tadpoles and some invertebrate taxa may be responsible for concurrent declines of both.

The small effect of frogs on benthic macroinvertebrate communities may result from the driver of the frog declines I studied. Disease-driven species declines can differ from other disturbances in taxonomic and functional scope of their victims (Srinivasan et al. 2014). For example, while Bd kills only frogs, trout predation kills frogs and concurrently drives most benthic macroinvertebrates to low abundance, regardless of functional roles or taxonomic group (Knapp et al. 2001, 2005). Introduced predators (Pimm 1987) are among a group of stressors, including habitat destruction (Pimm and Raven 2000), contaminants, and climate change (Midgley et al. 2002), that simultaneously cause mortality across taxa and functional groups. Though Bd infects a broad range of amphibian hosts, its effects on its apparently small number of non-amphibian hosts appear to be nonexistent or small (but see McMahon et al. 2013). Thus Bd, like other pathogens, may only indirectly influence a community by reducing the abundance or presence of hosts in the community (Monahan and Koenig 2006).

The most relevant scenarios with which to contrast my results may be the response of Sierra Nevada lake communities to trout introductions – my study community responding to a different disturbance – and the response of Panamanian stream communities to disease driven declines of frogs – a different community responding to the disturbance I studied. Both of these events had stronger impacts on communities than those I observed. When I contrast my study to previous work by Knapp et al. (2001, 2005) I see that communities respond very differently to the presence or absence of frogs versus fish. Despite nearly identical positions and connectance in the Sierra Nevada lake food web (Harper-Smith et al.

2005), and frog biomass density reaching an order of magnitude higher than fish biomass density (Schindler et al. 2001, Sarnelle and Knapp 2005, Knapp unpublished data), my results indicate that these frogs and trout are not equivalent in Sierra Nevada lakes. There must be another constraint that prevents frogs from exerting greater top-down effects on benthic macroinvertebrate abundances. For example, adult frogs feed at the water-shoreline interface on insects emerging from the lakes while trout feed pelagically on virtually any swimming taxa (Finlay and Vredenburg 2007). Adult frogs only have access to aquatic prey during the brief window when these prey emerge to become terrestrial, and this limited feeding opportunity should pose strong constraints on the amount of prey biomass consumed. In contrast, trout have access to their invertebrate prey across all prey life stages and can therefore presumably consume a much higher biomass of these prey. Other mechanisms such as timing or size of insect emergences or feeding rate and assimilation of insect prey by frogs may weaken the predatory function of adult frogs.

As in the Sierra Nevada, Bd epizootics have decimated amphibian abundance and diversity across Central America (Regester et al. 2006); however there frog extinctions are changing montane tropical stream ecosystems (Whiles 2013; Connelly et al. 2014). Frogs and tadpoles in Panamanian streams are also generally predators and grazers, but there the decline of Anurans led to measurable changes in nutrient flux (Ranvestel et al. 2004, Whiles et al. 2012), sediment and detritus characteristics (Rugenski et al. 2012), higher producer abundances (Connelly et al. 2008a), and shifts in relative abundances of several invertebrate taxa (Colón-Gaud et al. 2010, Connelly et al. 2014). These changes in the Neotropical stream invertebrate community seem to be larger than those I have so far observed in Sierra Nevada lake invertebrate communities. First, although mountain yellow-legged frogs and tadpoles occur at densities typical for frogs in high elevation lakes in western North American (Fellers and Drost 1993, Pilliod and Peterson 2001, Pope 2008), but there may be dramatically more frog biomass in Panamanian forest streams, as the reported tadpole densities are up to 2 to 300 times higher than typical densities in the Sierra Nevada (Whiles 2006, Colón-Gaud et al. 2009). Secondly, in Panama, the higher amphibian and invertebrate taxonomic and functional diversity, greater numbers of declining amphibians (Ranvestel et al. 2004, Colón-Gaud et al. 2009), and higher number of trophic connections thus severed or 'constricted' may have been a much larger perturbation to those food webs (Dunne and Williams 2009) than the loss of a single species or two trophic species in the Sierra Nevada. Those studies also highlight how taking a more comprehensive view of community and ecosystem characteristics may increase my perceived importance of a species (Simberloff 2003).

However, the ecological importance of mountain yellow-legged frogs should not be disregarded based on my observations of weak effects on macroinvertebrates. I neglected potential bottom-up effects of frogs on aquatic producers, symbionts, predators, symbionts, or ecosystem processes which may be interrupted by mountain yellow-legged frog extinctions. Tadpoles, create heterogeneity in dissolved nitrogen (Smith 2015) which may enhance producer diversity and productivity (Chesson et al. 2004, Holbrook et al. 2008). Both tadpoles and frogs are patches for micro- and macroparasites communities (Jani and Briggs 2014, personal observation). Mountain yellow legged frogs provide a cross-habitat subsidy that draws garter snakes (Jennings et al. 1992, Matthews et al. 2002), Brewer's blackbirds (Bradford 1991) and Clark's nutcrackers (Bradford 1991), the loss of which could change how these predators use and shape the alpine landscape (Hutchins and Lanner 1982, Epanchin et al. 2009).

While not all species will be ecologically important (McCann et al. 1998, Berlow 1999), that statement can only be made after extensive quantification of a species' interactions (Kareiva and Levin 2003), and even then, the apparent importance of a species may vary with ecological context (Harley 2003). My present study of the ecological effects of amphibian declines highlights how community responses to single species extinctions may vary across species and communities – the mere reduction of diversity does not indicate dramatic changes in communities (Kareiva and Levin 2003). Here, I have shown small consequences of the near complete extinction of a highly-connected omnivore on a part of a low-diversity community. However, as long as some mountain yellow-legged frogs are extant, I may still discover ways in which they are ecologically important. Finally, no matter how weak or strong the secondary community response, Sierra Nevada alpine lakes are profoundly different following the decline and extinction of mountain yellow-legged frogs.

ACKNOWLEDGEMENTS

This work was performed in part at the University of California Natural Reserve System Valentine Eastern Sierra Reserve and supported by a Mildred E. Mathias Graduate Student Research Grant from the UC Natural Reserve System, and a grant from the Valentine Eastern Sierra Reserve. Additional support was provided by grants from the National Science Foundation Ecology of Infectious Disease program (EF-0723563), the Henry Luce Foundation Environmental Science to Solutions Fellowship, the University of California Santa Barbara Graduate Division and the UCSB Department of Ecology, Evolution, and Marine Biology. Research permits were provided by Yosemite and Sequoia/Kings Canyon National Parks, and Sierra and Inyo National Forests, and the UCSB Institutional Animal Care and Use Committee. Staff from Sequoia/Kings Canyon National Park and the Sierra Nevada Aquatic Research Laboratory assisted with critical logistics. A very large number of field assistants contributed to collection of amphibian survey data and collection and processing of macroinvertebrate samples between 1995 and 2012, but I especially thank Michael Hernandez for collecting, identifying, and counting macroinvertebrates from 2008 to 2012. Thanks to members of Cherie Briggs' lab, especially Andrea Jani and Mark Wilber, and thanks also to Scott Cooper and Sally Holbrook who provided comments that shaped the research and the manuscript. I thank two anonymous reviewers who made constructive and unusually detailed comments that helped me improve the manuscript.

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TABLES

Table 1. My predictions of how Sierra Nevada benthic macroinvertebrate communities could respond to mountain yellow-legged frog declines and extinctions, the relevant ecological processes, what I analysed to address the prediction ("Analysis"), summary of results and whether my predictions were supported.

Main predictions	Sub-predictions	Analysis	Result	Support for prediction
1. Frogs extinct communities ≠ frogs extant communities		Invertebrate abundance, richness, evenness; dissimilarity	Richness, abundance sometimes lower, differences small. Communities can be dissimilar.	partial
	a. No tadpoles → Grazers increase, invade	Grazer Abundances	Some grazers more abundant others less so; not all respond.	partial
	b. No tadpoles → predators increase, invade	Predator Abundances	Some predators increase without frogs, others do not.	partial
	c. No adult frogs → emergent insects increase	Insect Abundances	Some increase, others decrease.	partial
	d. Amphibian declines → greater total abundance	Total abundance	Decreases in one survey, no difference in others.	no
	e. No secondary extinctions	Occupancy	Two taxa missing from frogs-extinct lakes.	no
	f. Higher richness	Richness, evenness	Richness was both higher and lower, in different surveys.	Partial yes, partial no
2. Frog decline communities between frogs-extant and -extinct.		Abundance, richness, evenness; dissimilarity	Frogs-declining communities not dissimilar	no
3. Frog life stages have unique effects on communities		Used frog, tadpole abundances as predictors	Adults reduced invertebrate abundance, evenness; no effect of tadpoles	partial

Table 2. Characteristics of lakes sampled for Snapshot surveys of benthic macroinvertebrates throughout Kings Canyon National Park (KCNP), John Muir Wilderness (JMW), and Yosemite National Park (YNP); means ± one standard error (s.e.) for each characteristic. Superscript letters indicate significant differences among frog population status levels for a particular characteristic, and stars (*) indicate differences for between region comparisons (ANOVA and Tukey-HSD post-hoc comparison, $p < 0.05$). All lakes in the Snapshot survey were sampled exactly once.

Frog population status	n	Elev. (m) *	Max. Depth (m)	Surface area (ha)	Perimeter (m) *	% silt[1] *	Latitude [2]*	Adult frog density[3]	Subadult frog density	Tadpole density
Kings Canyon National Park / John Muir Wilderness										
Frogs-extant	21	3450 ± 18	13.3 ± 1.7	5 ± 1	1070 ± 90	6 ± 1	4099790 ± 2460 ^{a}	0.090 ± 0.04	0.06 ± 0.02	0.4 ± 0.2
Frogless	40	3429 ± 20	12.2 ± 1.3	6 ± 1	1020 ± 100	8 ± 2	4118640 ± 2640 ^{b}	0	0	0
Yosemite National Park										
Frogs-extant	33	2625 ± 49	11.3 ± 1.1	6 ± 1 ^{a}	1790 ± 260 ^{a}	24 ± 2	4210350 ± 2310 ^{a}	0.009 ± 0.004	0.08 ± 0.05	0.01 ± 0.01
Frogless	63	2662 ± 70	12 ± 1.0	5 ± 0.5 ^{b}	1100 ± 100 ^{b}	24 ± 3	4202100 ± 2390 ^{b}	0	0	0

1. Mean siltiness calculated as mean of percent of lake substrate which is composed of silt
2. Latitude is UTM northings (m)
3. Frog lifestage densities calculated as abundance / m shoreline

Table 3. Characteristics of lakes sampled for Resurveys of benthic macroinvertebrates throughout Kings Canyon National Park and the John Muir Wilderness; means ± one standard error (s.e.) for each physical characteristic. Superscript letters indicate significant differences among frog population status levels for a particular characteristic (ANOVA and Tukey-HSD post-hoc comparison, $p < 0.05$). Characters described in footnote of Table 2.

Frog population status	n	Samples per lake	Elev. (m)	lake depth (m)	lake surface area (ha)	perimeter (m)	% silt	Latitude (UTM)	adult frog density	subadult density	tadpole density
Frogs-extant	7	5 ± 0.5 ^{a}	3419 ± 45	13 ± 3	12 ± 10	1500 ± 850	4 ± 3 ^{a}	4089189 ± 8460	1.04 ± 0.30 ^{a}	0.35 ± 0.08 ^{a}	2.07 ± 0.54 ^{a}
Frogs-declining	6	7 ± 0.9 ^{b}	3507 ± 28	10 ± 2	3 ± 0.5	800 ± 100	5 ± 2 ^{a}	4111250 ± 4080	0.15 ± 0.05 ^{b}	0.11 ± 0.03 ^{b}	3.26 ± 0.55 ^{a}
Frogs-extinct	9	3 ± 0.2 ^{c}	3457 ± 28	6 ± 1	2 ± 0.5	750 ± 130	23 ± 6 ^{b}	4111273 ± 5620	0.0095 ± 0.07 ^{b}	0 ^{b}	0 ^{b}

Table 4. Descriptions of best-fit models of univariate community response variables, in which frog population status or density was retained as a fixed effect for Snapshot surveys. Fixed effects of frog population status and environmental covariates are described by the coefficient, relevant test statistic and degrees of freedom, and p-value for each fixed effect. Random effects (random intercepts and nested effects) are described where they were included in best-fit models, and variance structure describes which covariates had different variances.

Frog variable (region)	Response variable	Fixed effect	Coefficient, test statistic, p-value	Random effects and variance structure
Frog population status (KCNP/JMW)	$\log_{10}$ transformed invertebrate abundance	Frog population status	0.3, $t_{4,57} = 2.2$, $p = 0.03$	none
		Elevation	-0.2, $t_{4,57} = -3.7$, $p < 0.001$	
Frog population status (YNP)	rarefied richness	Frog population status	1.0, $t_{3,93} = 2.0$, $p = 0.05$	Frog population status $\sigma^2_{extant} = 1.00$ $\sigma^2_{frogless} = 1.50$
		Elevation	-0.5, $t_{3,93} = -7.2$, p < 0.0001	
Frog population status (KCNP/JMW)	rarefied richness	Frog population status	0.6, $t_{3,55} = 2.1$, $p = 0.04$	none
		Perimeter	-0.5, $t_{3,55} = -2.1$, $p = 0.04$	

Table 5. Descriptions of best-fit models of univariate community response variables, in which frog population status or density was retained as a fixed effect for Resurveys. Similar to Table 3.

Frog variable	Response variable	Fixed effects	Coefficient, test statistic, p-value	Random effects and variance structure
Frog population status	rarefied richness	Frog population status	0.1, $\chi^2_{3,19} = 4.8$ $p = 0.1$	$\sigma^2_{extant} = 1.1$ $\sigma^2_{declining} = 1.2$ $\sigma^2_{extinct} = 0.9$
Frog density	$\log_{10}$ transformed invertebrate abundance	Adult density	-0.2, $t_{7,15} = -1.9$, $p = 0.1$	intercept differed with respect to frog population status; mean residual: 0.2, $\sigma^2 < 0.001$
		Elevation	-0.2, $t_{7,15} = -2.5$, $p = 0.03$	
		Perimeter	-0.1, $t_{7,15} = -1.6$, $p = 0.1$	
		Siltiness	0.01, $t_{7,15} = 1.5$, $p = 0.2$	
Frog density	squared community evenness	Adult density	0.1, $t_{10,12} = 1.7$, $p = 0.1$	intercept differed with respect to drainage basin; mean residual = 0.1, $\sigma^2 < 0.001$
		Elevation	0.6, $t_{10,12} = 2.4$, $p = 0.03$	
		Latitude	0.03, $t_{10,12} = 2.5$, $p = 0.03$	

Table 6. Taxa for which frog population status was retained as a fixed effect in generalized linear models of absolute abundance, for Snapshot surveys in King's Canyon National Park/John Muir Wilderness. All n = 61. Functional feeding group and typical behavior from Merritt and Cummins (1996) and Thorp and Covich (2009). Significance of frog population status in model of taxon abundance given by log-ratio test of model with and without frog population status; differences in residuals (**bold**) shown when there was a significant difference between frogs-extinct vs. frogless lakes.

Taxon, common name	Functional feeding group / Typical behavior	Frog population status in models of taxon abundance, and with respect to **residuals**	Taxon abundance residuals
Acari mites	predators / swimmers, crawlers	$\chi^2_{7,54} = 215.3$, $p < 0.001$	Frogs-extant > Frogs-extinct
Oligochaeta annelid worms	detritivores, herbivores / burrowers	$\chi^2_{6,55} = 3.9$, $p = 0.05$ **$F_{2,59} = 6.7$, $p = 0.01$**	Frogs-extant > Frogs-extinct
Sialis occidens alderfly	predators / burrowers, climbers, clingers	$\chi^2_{7,54} = 16.2$, $p = 0.0003$, **$F_{2,59} = 4.8$, $p = 0.03$**	Frogs-extant > Frogs-extinct
Limnephilus spp. caddisfly	detritivores, herbivores / climbers, sprawlers, clingers	$\chi^2_{10,51} = 13.2$, $p = 0.004$	Frogs-extant > Frogs-extinct
Psycoglypha sp. caddisfly	detritivores / sprawlers, clingers	$\chi^2_{12,49} < 0.001$, $p = 1$	Frogs-extant > Frogs-extinct
Desmona mono caddisfly	detritivores, herbivores / sprawlers, burrowers	$\chi^2_{14,47} = 12.6$, $p = 0.1$, **$F_{2,59} = 4.3$, $p = 0.04$**	Frogs-extant < Frogs-extinct
Corixidae water-boatmen	herbivores, predators/ swimmers	$\chi^2_{10,51} = 10.2$, $p = 0.001$,	Frogs-extant < Frogs-extinct

Table 7. Taxa for which frog population status was retained as a fixed effect in generalized linear models of absolute abundance, summarized by lake, for Resurveys in King's Canyon National Park/John Muir Wilderness. Function and Habit describe the range of feeding roles and typical behaviors in the lake (Merritt and Cummins 1996, Thorp and Covich 2009). Significance of frog population status in model of taxon abundance given by log-ratio test of model with and without frog population status. None of the taxa differed in abundance with respect to frog-population status (ANOVA of model residuals, Tukey's HSD).

Taxon	Functional feeding group / Typical behavior	Frog status in model of taxon abundance	Taxon abundance residuals
Ameletus spp. mayfly	herbivores / swimmers, clingers	$p = 0.04$ $\chi^2_{8,22} = 6.6$	Extant > Declining < Extinct
Stictotarsus spp. diving beetle	predators / swimmers, climbers	$p = 0.2$ $\chi^2_{6,22} = 3.5$	Extant > Declining > Extinct
Callibaetis ferrugineus mayfly	herbivores / swimmers, clingers	$p = 0.1$ $\chi^2_{8,22} = 3.9$	Extant <Declining < Extinct
Sialis occiden alderfly	predators / burrowers, clingers	$p = 0.03$ $\chi^2_{7,22} = 6.9$	Extinct < Declining < Extant

FIGURES

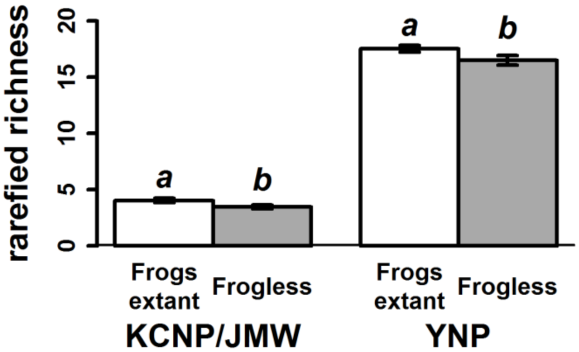

FIG. 1. Mean rarefied richness ± one standard error in communities in Snapshot communities in Kings Canyon National Park and John Muir Wilderness (KCNP/JMW), and in Yosemite National Park (YNP). Letters indicate differences. There was a 15% difference in KCNP/JMW ($t_{3,55} = 2.1$, $p = 0.04$), and a 6% difference in YNP ($t_{3,93} = 2.0$, $p = 0.05$); those differences both equate to about one taxa. Observed richness was 3x higher in YNP than in KCNP/JMW (ANOVA, $F_{3,154} = 98.01$, $p < 0.001$).

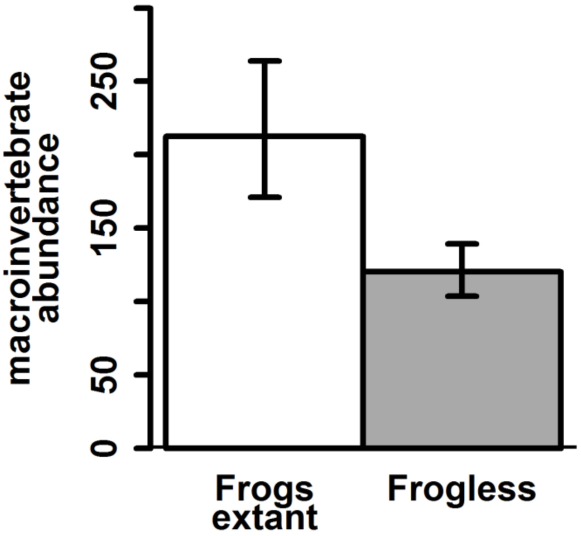

FIG. 2. Benthic macroinvertebrate abundance was 77% higher in Frogs-extant lakes than in frogless lakes in the Snapshot survey in Kings Canyon National Park and John Muir Wilderness, based on mean residual $\log_{10}$ abundance ± one standard error (ANOVA, $F_{1,59}$ = 4.9, p = 0.03); back transformed $\log_{10}$ abundance shown.

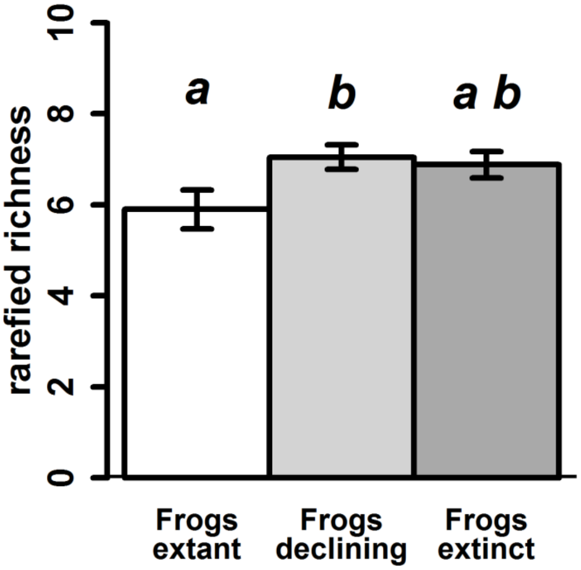

FIG. 3. Mean rarefied richness ± one standard error for Resurvey communities in KCNP/JMW (n = 22). Shared letters indicate groups that do not differ ($p < 0.05$).

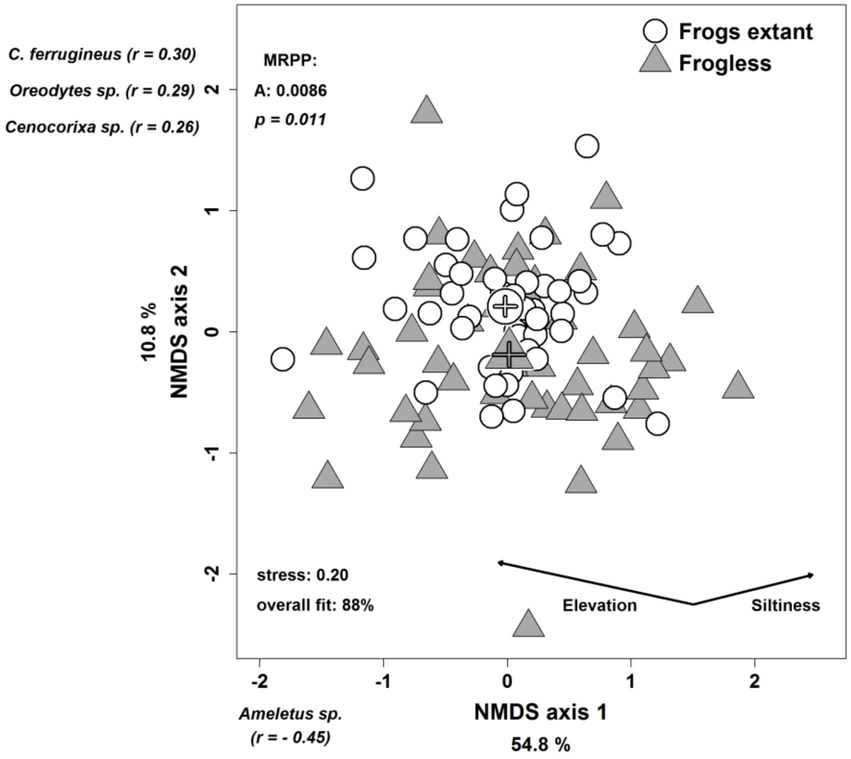

FIG. 4. Ordination plots of Snapshot survey communities (NMDS) in YNP for n = 96 lakes and 56 taxa. Points represent communities in frogs-extant (white circles) and frogless (grey triangles) lakes. Taxa correlated with each NMDS axis are shown (for r > 0.20). Vectors represent direction and relative strength of correlations (r > 0.20) between environmental covariates and community distances. Enlarged symbols indicate centroids and "crosshairs" indicate ± one standard error along each axis for corresponding communities; centroids differ along axis two (ANOVA, $F_{2,94}$ = 9.91, p = 0.004). The variation among communities explained by each axis is shown. Stress and overall fit indicate how well NMDS represents the original distances among communities. Multiple response permutation procedure (MRPP) indicates differences among communities with respect to frog population status.

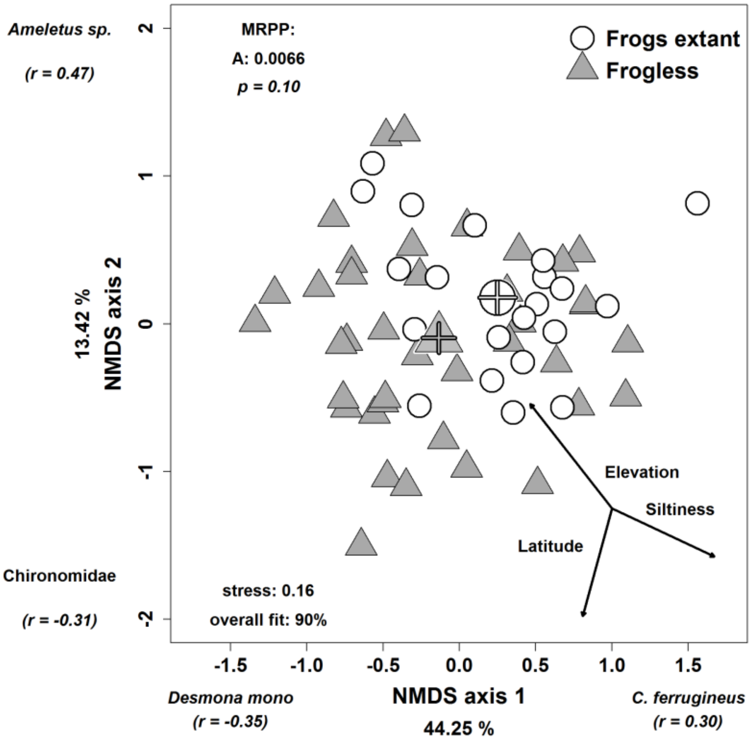

FIG. 5. Ordination plots of Snapshot survey communities (NMDS) in KCNP/JMW NMDS axes 1 and 2 for n = 60 lakes and 22 taxa. Points represent communities in frogs-extant (white circles) and frogless (grey triangles) lakes. Centroids differ with respect to axis 1 (ANOVA, $F_{2,58} = 5.20$, p = 0.03) and axis 2 ($F_{2,58} = 3.59$, p = 0.09). Taxa correlated with each axis are shown (r > 0.20). Communities did not differ along axis 3 ($F_{2,58} = 0.9$, p = 0.4), and it accounted for just 8% of the variation among communities, so it is not shown here. Taxon correlations, environmental vectors, variation among communities explained by each axis, overall fit and stress, and MRPP results are shown, as in Figure 4.

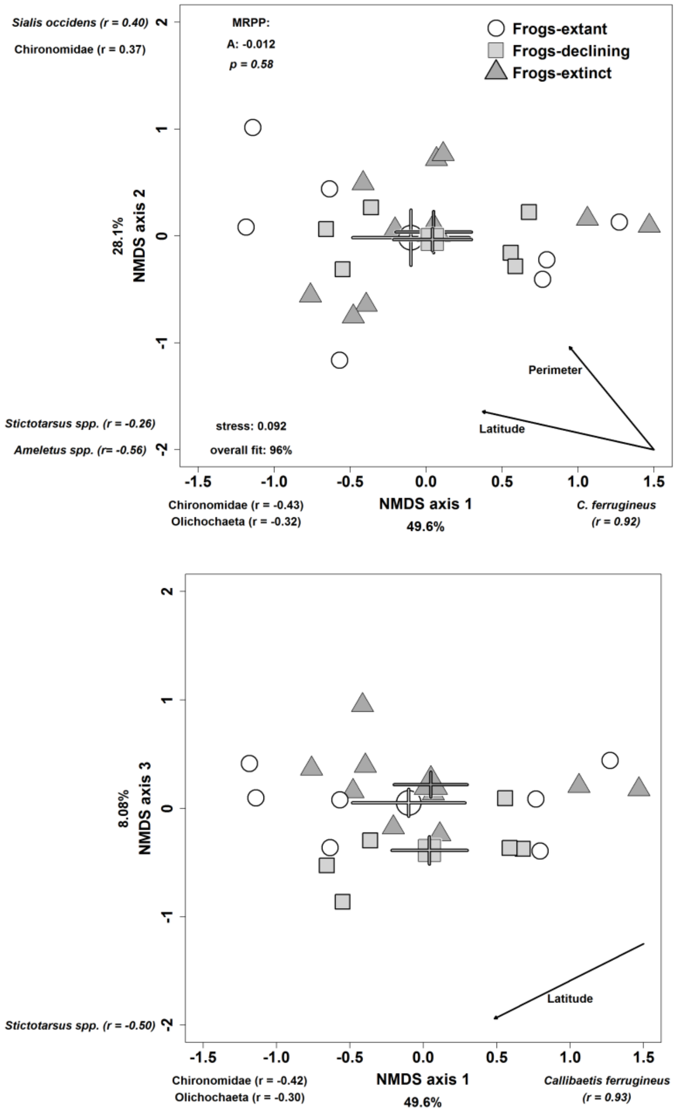

FIG. 6. Ordination plots (NMDS) of Resurvey communities in KCNP/JMW, a) axes 1 and 2, b) axes 1 and 3, for n = 22 lakes and 29 taxa. Points represent communities in frogs-extant (white circles), frogs-declining (light grey squares), and frogs-extinct lakes (dark grey triangles). Along NMDS axis 3, communities differed with respect to frog-population status: $p_{ExtantDeclining} = 0.07$, $p_{Extinct-Declining} = 0.0072$, $p_{Extinct-Extant} = 0.59$ (ANOVA, $F_{3,19} = 6.08$, Tukey's HSD). Centroids and crosshairs, taxon correlations, environmental vectors, variation among communities explained by each axis, overall fit and stress, and MRPP results are shown, as in Figs. 4 and 5.

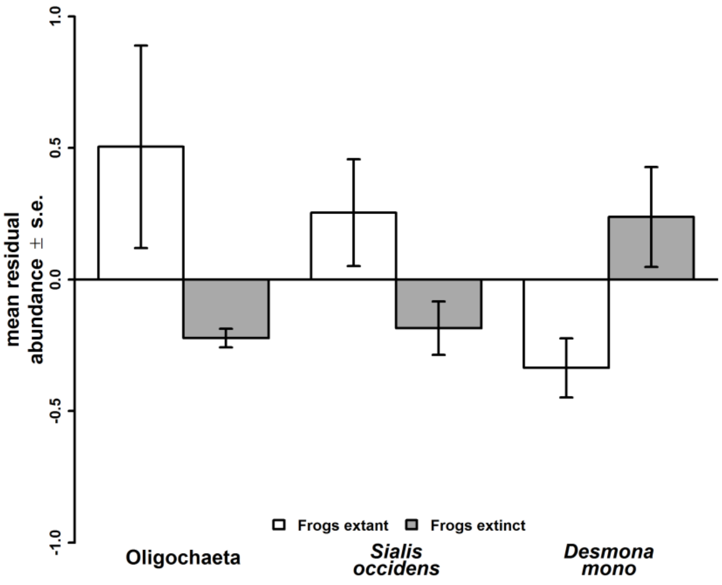

FIG. 7. Mean residual abundances ± one standard error of taxa in KCNP/JMW Snapshot surveys for which frog population status was retained as a fixed effect in best fit GLM models, and in which residual abundances were significantly different between frog population status categories. All within taxa differences were significant (ANOVA, *df* = 59, $p < 0.05$).

II. WEAK INTERACTIONS AMONG ALGAE, MAYFLIES, AND TADPOLES SUGGEST COMMUNITIES RESPOND WEAKLY TO MOUNTAIN YELLOW-LEGGED FROG EXTINCTIONS.

ABSTRACT

Worldwide declines in amphibian populations and diversity have prompted investigations into the ecological roles of amphibian species and the consequences of their extinctions. In the Sierra Nevada of California, mountain yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) are nearly extinct, yet their impacts on, and responses to, other species remain largely unquantified. I performed two experiments to examine mountain yellow-legged frog tadpole grazing impacts on algal communities and outcomes of competitive interactions between tadpoles and mayflies (Baetidae *Callibaetis ferrugineus* and Ameletidae *Ameletus edmundsi*). In three 16-21 day blocks in field enclosures erected in two remote high elevation lakes, algal abundance declined with increasing mayfly, but not tadpole abundance. Because consumer abundances declined throughout blocks, my ability to infer effects of intra- and interspecific competition on consumer body sizes was limited, but in the first time-block tadpole size was unaffected by the abundance of tadpoles or mayflies. Tadpole development increased by 40% in enclosures with higher tadpole abundance, but mayfly loss (emergence and mortality) was unaffected by tadpole abundance. To test the effects of consumers on algal abundance independent of within-lake variability, I also performed a separate mesocosm experiment, in which I manipulated and crossed presence-absence of mesocosms contained no or high densities of tadpoles and mayflies. In this experiment, tadpoles reduced algal abundance by about 50%, but did not significantly reduce algal growth rate. Again, mayfly abundances declined by 50-100%, which was not related to tadpole presence-absence. Overall, my studies indicate that the removal of mountain yellow-legged frog tadpoles might allow benthic producers to reach higher abundance, but grazing effects of tadpole were evident only in the mesocosm experiment and grazing effects of mayflies were evident only in the field experiment. There was no indication of interspecific competition or competitive release of tadpoles or mayflies in the absence of the other.

Keywords: *Ameletus spp.,* amphibian declines, *Callibaetis ferrugineus*, grazing, *Rana muscosa, Rana sierrae,* Sierra Nevada lakes,

INTRODUCTION

Although worldwide amphibian population declines and extinctions (Stuart et al. 2004, Wake and Vredenburg 2008) have been recognized for over 25 years, the ecological consequences of most of these declines remain unquantified (but see Whiles 2006, Connelly et al. 2008, 2014, Colón-Gaud et al. 2009, 2010a, 2010b, Whiles et al. 2009, 2012). Generally, extinctions or species removals can alter communities, in part through the loss of top-down resource control (Hairston et al. 1960, Paine 1966, Carpenter et al. 1985, Chalcraft and Resetarits 2003, Gruner et al. 2008) or through competitive release due to the release of resources (Holbrook and Schmitt 1995). Declines and extinctions of amphibians have the potential to change communities, but the extent to which any species shapes its community via resource consumption is likely to vary idiosyncratically (Menge 2003) depending on several factors, including the impact of a species on its resources (Shurin et al. 2002, Borer et al. 2005, Wollrab et al. 2012)and on other species that share the resources (Murdoch et al. 2003). Therefore, predictions about the ecological effects of declines or extinctions of a species should be based on quantitative measurements of its unique interactions with other community members (Simberloff 2003).

The effects of amphibian declines on freshwater and terrestrial communities will depend in part on the declining species' impact on resources and on other consumers in its community. While over 40% of the 5700 amphibian species are declining in abundance or shrinking in distribution or both (Stuart et al. 2004), declines of anurans (frogs and toads) are the best understood and may be the most extensive. Many have declined in abundance or have been driven extinct by habitat destruction, over-exploitation, disease, or a combination of causes (Stuart et al. 2004). Anurans, can play ecologically important roles, but tadpoles may be especially important because they are aquatic grazers that often occur at very high abundances (Alford 1999). Many tadpoles can reduce the abundance of benthic producers (Kupferberg 1997a, 1997b, Alford 1999, Connelly et al. 2008, 2014), even up to 98% in some cases (Brönmark et al. 1991, Lamberti et al. 1992). This ability to affect resources also allows tadpoles to be strong exploitative competitors, and they can induce declines in abundance, growth, and fecundity of other amphibian, insect, and invertebrate grazers (Brönmark et al. 1991, Kupferberg 1997a, 1997b). As consumers, tadpoles also can interfere with or facilitate the feeding of aquatic insects and other amphibians (Steinwascher 1978a, Kiffney and Richardson 2001, Ranvestel et al. 2004), and can be negatively affected by interspecific competition (Morin et al. 1988).

Like many tadpoles, those of the endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) graze on benthic algae, and are potential competitors with mayfly nymphs, caddisfly larvae, diptera larvae, and other benthic macroinvertebrates (Grinnell and Storer 1924, Zweifel 1955, Finlay and Vredenburg 2007). Because of their historical ubiquity and abundance (Grinnell and Storer 1924), mountain yellow-legged frog tadpoles may have been ecologically important members of Sierra Nevada aquatic ecosystems. Mountain yellow-legged frog and tadpole populations initially declined due to predation by stocked non-native trout (Knapp and Matthews 2000). Even after trout stocking was ended, frog populations continued to decline due to the emergence and spread of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Briggs et al. 2005). Currently, large mountain yellow-legged frog populations are limited to a handful of extremely high elevation lakes in Yosemite and Sequoia/Kings Canyon National Parks and the adjacent John Muir Wilderness in the southern Sierra. In most lakes in the Sierra Nevada, mountain yellow-legged frogs and their tadpoles have gone locally extinct (Briggs et al. 2010, Vredenburg et al. 2010).

To explore how declines and local extinctions of mountain yellow-legged frogs might affect Sierra Nevada lake communities, I examined the impacts of tadpoles on their resources and on potential competitors. Mountain yellow-legged frog tadpoles may reduce algal resources and compete, through exploitative or interference interactions, with co-occurring native insect grazers. I chose to study interactions between mountain yellow-legged frog tadpoles and mayfly nymphs as potential competitors with tadpoles because mayfly nymphs are abundant in Sierra Nevada lakes and can also suppress algal abundance (Hill and Knight 1987, Morin et al. 1988, Dudley 1992, Bradford et al. 1998, Hertonsson et al. 2007, Epanchin et al. 2010). I predicted that in the presence of tadpoles – as in the pristine, fish-free and disease-free state – algal abundance would be lowest; reducing tadpole abundance or presence – mimicking disease driven declines and extinctions – would increase algal abundance. In addition, I predicted that both mayflies and tadpoles would reduce algal abundance, with negative effects on their own and each other’s body sizes. To test these predictions, I performed two experiments which manipulated the presence or abundance of tadpoles to examined their effects on algal abundance and competitor body size. The results of these experiments clarify the role of mountain yellow-legged frog tadpoles in Sierra Nevada lakes, and shed light on how their extinctions might affect lake communities.

METHODS

Experimental methods

I performed two experiments, an *in situ* field experiment and a mesocosm experiment. In the field experiment, I examined interactions between two consumers, mountain yellow-legged frog tadpoles (*Rana muscosa* and *Rana sierrae*) mayfly nymphs (Ephemeroptera: *Callibaetis ferrugineus* and *Ameletus edmundsi*), and their shared food resource, benthic organic matter. This consists largely of diatoms but can also include green algae, cyanobacteria, chrysophytes, detritus, and bacteria. Because of the apparent dominance of diatoms and producers, I hereafter refer to the food resource as algae. In the subsequent mesocosm experiment, I measured the effects of two grazers (*Rana sierrae* tadpoles and *Callibaetis ferrugineus* nymphs) on algal resources in outdoor arenas with standard environmental conditions (nutrients, temperature, and substrates).

Field enclosure experiment. – In the field enclosure experiment, I used a response surface design to characterize the independent and interactive effects of grazers on their algal resources, as well as on themselves and each other (Inouye 2001). I used a full factorial design, in which four densities of tadpoles (0, 2, 10, 20) were crossed with four densities of mayflies (0, 25, 125, 250). The highest abundance treatments were set by the highest density of these consumers that colleagues and I have observed in high elevation Sierra Nevada lakes, with lower densities set at half and 1/10 of these high abundances (Roland A. Knapp, personal communication, and T. Smith diss. 2015). I performed this experiment in two lakes, and in each lake at each time, each treatment was replicated once with the exception of the no-consumer control, which was replicated twice. Treatments were randomly assigned to locations within lakes. Because of the remoteness of my study lakes, it was difficult to set up additional enclosures, so experimental treatments were replicated in three time-blocks (n = 102 data points for the study, based on 17 enclosures/lake × 2 lakes × 3 time-periods, with each period treated as a block in analysis, hereafter called time-blocks).

The two study lakes are remote high elevation lakes in the Kings Canyon National Park backcountry; I refer to these unnamed lakes as LeConte (3221 m elevation, 37°06'58.78" N 118°38'40.16" W) and Spur lakes(48 km to the southeast of LeConte, 3518 m elevation, 36°43'47.49" N, 118°23'38.33" W, Google Earth 2014). These small alpine lakes lie close to and west of the Sierra Nevada crest; while LeConte is surrounded by small meadows, whitebark pine and willow patches, talus, and bare bedrock, Spur is in a basin devoid of vegetation and containing mostly talus and minimal bare bedrock. These lakes have low nutrient concentrations and circumneutral pHs: nitrate 0 – 10 μmol L^{-1}, total phosphorus 0 – 1 μmol L^{-1} (Sickman et al. 2003); median pH ≅ 7 (Bradford et al. 1998). I selected these two lakes because both had large, disease-free cohorts of mountain yellow-legged frog tadpoles and large mayfly nymph populations, and are seldom visited by backpackers.

Seventeen enclosures were placed along each lake's shoreline in the littoral zone where tadpoles feed during the day. Each enclosure was 0.5 m wide x 0.5 m tall at one end and 0.5 m wide x 1.5 m tall at the opposite end, and were 2 m long (bottom area = 1 m^2). Each was oriented perpendicular to the shoreline, so that the tall end sat in deep water, and the short end sat along the shoreline (Figure 1), allowing tadpoles to use deep and shallow water. To accommodate emerging mayflies and tadpoles, enclosures were only partially submerged so a 25 cm tall air space remained in the top of each enclosure, and one rock from outside the lake was placed inside each enclosure to provide above-water substrate for metamorphosed frogs. Enclosures were supported by light weight steel frames (Sturdy Stake #ST6 www.homedepot.com) and guy-lines, and were constructed from synthetic mesh fabric, with pore size approximately 250 µm (Nitex: e.g. SKU 24-C44 www.wildco.com; polyester organza, various sources). This mesh size prevented escape of mayflies and tadpoles and prevented immigration by other benthic macroinvertebrates. We observed the movement into and out of enclosures of sediment and small zooplankton (mostly Copepoda). The movement of small particles like sediment and phytoplankton through the mesh was the source from which algae were introduced into enclosures.

Tadpoles I captured and used in experiments at LeConte lake were *Rana sierrae,* and tadpoles at Spur lake were *Rana muscosa*; I assumed that these allopatric sister species (Vredenburg et al. 2007) are ecologically similar. After weighing and staging tadpoles (Gosner 1960), I placed those between Gosner stages 26 and 38 into enclosures. When individual tadpoles reached stage 39, they were released back into the lake so they did not metamorphose within enclosures during the time-block; each released tadpole was replaced with a younger tadpole. On average, I replaced 1.3 ± 0.3 SE tadpoles per cage per time block (about 12% ± 3 SE of the tadpoles in a cage, Figure 2), so many individual tadpoles remained in the same enclosure throughout all three time-blocks; this was done to minimize the overall number of individuals used in the experiment and to minimize handling of experimental tadpoles. I captured mayflies in the littoral zone using benthic sweeps with a standard D-net (mesh size 250 µm), then separated mayflies without wingpads from other invertebrates in a sorting pan using flexible forceps and a turkey baster. The mayflies in LeConte lake were all *Ameletus edmundsi*; in Spur lake, *Ameletus edmundsi* and *Callibaetis ferrugineus* were present in similar proportions, but since small individuals of these two species can be difficult to distinguish in the field, I did not discriminate as nymphs were added to enclosures.. Emerged adult mayflies were removed from enclosures and replaced with younger individuals, with (0 - 40% (mean 11% ± 0.1 SE) of mayflies emerged per enclosure per time-block (Figure 3).

I measured algal biomass as ash-free dry mass (AFDM) concentration in each enclosure, sampled from organic material accumulated on unglazed porcelain tiles placed on the bottom of each enclosure for the duration of each block (24 tiles, each 2.4 cm x 2.4 cm, 140 cm^2 total area per enclosure). Porcelain tiles, and plastic substrates like my enclosure mesh, do permit algal growth sufficiently for many experimental applications (Aloi 1990). Tiles were not exposed to algal growth prior to the experiment, because lakes could not be accessed until mid-July due to snow-cover. To account for potential variation in algal growth due to unquantified within-lake variation in local algae community composition, nutrient concentrations, temperature, currents, or aspect, I placed 12 tiles in a 15 x 30 cm bag made of the same 250 µm plastic mesh as enclosures, and set that in the littoral zone next to each enclosure (referred to as location-within-lake controls, Figure 1). I recorded natural substrate type below each enclosure. Soft versus hard substrates can strongly influence overlying dissolved nutrient concentrations and producer communities in lake littoral zones (Vincent and Downes 1981, Potapova and Charles 2005, T. Smith dissertation 2015) which could affect nutrient concentration in and algal immigration into the overlying enclosure. Therefore, substrate type was described as percent of the substrate below each enclosure which was composed of silt (defined as particles < 0.5mm, as in Knapp and Matthews 2000). Silt and sediments drifted into and settled in enclosures as a result of benthic disturbance during enlcosure construction. I measured light intensity within and outside each enclosure (photosynthetic photon flux) at the water surface using a quantum meter (Apogee Instruments, Logan, UT www.apogee-inst.com). Mesh reduced light intensity by 24%, from 1977.1 ± 4.2 to 1505.0 ± 25.0 μmol photons m^{-2} s^{-1}(ANOVA, $F_{1,66} = 349.02$, $p < 0.001$).

Experiments began in the early ice-free season (17 July 2009 in LeConte and 21 July 2009 in Spur), and each of three subsequent temporal blocks lasted 16-21 days. At the beginning of each block, I weighed and staged all experimental tadpoles, then placed clean tiles and targeted numbers of tadpoles and mayfly nymphs in enclosures. At the end of each block, I sampled algal, mayfly nymph, and tadpole abundances, and tadpole stages and weights. These data were collected to indicate intra- and interspecific competition; however, they also revealed that treatments were not consistently maintained due to mayfly emergence and mortality and tadpole development (Figs. 2 and 3)

I collected algal samples from tiles in enclosures and from location-within-lake control mesh bags by scrubbing tiles using a soft-bristle toothbrush, suspending organic matter in 60 mL of water, then filtering algal suspensions onto glass fiber filters (1.2 μm pore size). Filters were wrapped in foil and stored in a cool dark place in the field, then transported to and frozen in the laboratory. Filters were dried at 105 °C for 24-48 hours, weighed, combusted at 500 °C for 1 hour, and then weighed again. Ash-free dry mass was calculated as the difference between filter-plus-sample weights before and after combustion (Hauer and Lamberti 2007). My observed algal biomasses are 1-3 orders of magnitude lower than those found in some other studies of high elevation lake periphyton (Vinebrooke and Leavitt 1998) but they are not significantly different than those I observed on artificial substrates outside of enclosures in both lakes and in one lake adjacent to LeConte (ANOVA, $F_{2,209} = 0.09$, $p = 0.9$).

Mesocosm experiment. – I also conducted a mesocosm experiment to further explore the effects of tadpoles and mayflies on algal resources. In this outdoor mesocosm experiment, I used a 2 x 2 factorial design, in which I crossed the presence and absence of tadpoles with the presence and absence of mayfly nymphs, with four replicate mesocosms assigned to each of four treatments (no consumers, 16 tadpoles, 250 mayfly nymphs, and 16 tadpoles + 250 mayfly nymphs.

Mesocosms were located at the Sierra Nevada Aquatic Research Laboratory (SNARL) near Mammoth Lakes, CA (2165 m elevation, 37°36'50.83" N 118°49'57.56" W). and were made from sixteen cube shaped (1 m^3) concrete tanks lined with Thoroseal concrete sealer, with sloping, partially submerged shelves on one side to allow tadpoles and metamorphs to bask (Figure1). Tanks were filled with water from adjacent Convict Creek, in which nitrate and phosphate levels are similar to those observed in many Sierra Nevada lakes, but Convict Creek pH (7.9 – 8.5)is higher than in most Sierra Nevada lakes (Leland et al. 1989, Sickman et al. 2003). Mesocosm pHs (8.7 ± 0.03 SE) were just above the range observed for lakes containing high densities of mayfly nymphs and tadpoles (pH 6.5 - 8.5, Bradford et al. 1998). Mesocosms were filled in April 2010 and thirty-five sets of porcelain tiles (identical to those used in the field enclosures, total area of tiles: 2074 cm^2) were placed in each mesocosm to provide standard substrates for measurement of algal abundance. Thirty tile-sets were placed on the bottom of each mesocosm, and five were placed on each shelf (Figure 1). Colonizing algae came from Convict Creek water.

I collected 160 *Rana sierrae* tadpoles (Gosner stages 34-39) from Marmot Lake (John Muir Wilderness, 3590 m elevation, 37°15'36.33" N 118°41'01.38" W) and transported them to SNARL in seven 4 L containers with portable aerators and cooled by blocks of snow.

About 3000 mayflies (*Callibaetis ferrugineus*) were collected from a small pond in Yosemite National Park (2608 m elevation, 37°53'07.18" N 119°23'39.97" W) using a D-net with 250 µm mesh size, sorted using sieves, pipettes, and turkey basters, and transported to SNARL like tadpoles.

The experiment began on July 26, 2010, when I added consumers to the mesocosms, and it ran for 21 days. Initially, I maintained tadpole abundance by adding younger tadpoles (Gosner stage 34 to 39) to replace metamorphosed individuals, but the experiment ended when tadpole densities could not be maintained because of high levels of tadpole metamorphosis. I was not able to maintain the mayfly treatments; mayflies were undetectable in the mesocosms, either visually or by minimally disruptive benthic sweeps. Thus, I was neither able to measure loss of mayfly individuals during the experiment, nor to compensate for it by adding individuals. However, at the end of the experiment I exhaustively sampled each mesocosm for mayflies, sampling with a D-net until 20 consecutive sweeps of the entire mesocosm bottom produced no additional mayfly nymphs. I then counted the collected mayfly nymphs.

Algal abundance on bottom tiles was determined three times during the mesocosm experiment: once prior to the start of the experiment on July 26 (n = 3 tile sets/mesocosm), and at one (n = 15/mesocosm), and three weeks (10/mesocosm). I did not sample shelf tiles. Algae was removed from each tile using a soft toothbrush and suspended in 60 mL of water, the algal suspensions were filtered through a glass fiber filter. These samples were frozen immediately, and later processed for AFDM as described above.

Analytical methods

Analysis of field enclosure experiment. – For my analysis of field enclosure algal abundance data, the independent variables were the continuous variables mayfly abundance and tadpole abundance, with four levels for each abundance treatment of each consumer. I included categorical covariates for experimental block, with three levels (late July – early August, mid-August, and late August – mid-September), and for lake, with two levels (LeConte and Spur). The lake covariate accounted for differences between lakes such as elevation, temperature, or size. I also included continuous covariates for duration of experimental time-block (days), solar radiation within enclosures, and percent of silt in substrates beneath enclosures.

The response variable was the difference between algal biomass (AFDM m^{-2}) on tile in experimental enclosures and on tiles in location-within-lake controls ($AFDM_{enclosure} - AFDM_{location\text{-}within\text{-}lake\ control}$, hereafter: experiment-control biomass difference), measured at the conclusion of each block. I used linear mixed effects models (Zuur et al. 2009) to test the response of experiment-control biomass difference to variation in consumer abundance. Using a step-down model fitting procedure, I selected the best-fit model based on Akaike Information Criteria (AIC) and visual inspection of model residuals (Zuur et al. 2009). The initial model included the response variable experiment-control biomass difference, the predictor variables tadpole and mayfly abundance, and covariates for per-enclosure substrate siltiness, light intensity, duration of experimental block, lake, and block number. I included an interaction term between consumers, because of the potential for tadpoles to either facilitate or interfere with mayfly grazing. To meet the assumption of normality of residuals, I log transformed the experiment-control biomass difference. I compared models that included random intercepts (for block and for lake), random slopes for consumer effects in different lakes, and allowed variance to differ among experimental blocks, lakes, and levels of mayfly and tadpole abundance (Zuur et al. 2009).

To examine the effect of intraspecific or interspecific competition on tadpoles, I compared average per-enclosure tadpole wet weights at the end of only the first time-block to tadpole and mayfly abundances. I used a similar model selection approach as described above. I was not able to examine the effect of competition on mayfly nymphs. Because treatments were not maintained, but some individuals remained in enclosures while new individuals were introduced, any effects of tadpole or mayfly abundance on individual nymph sizes were confounded by the different durations that individuals were exposed to treatments.

Analysis of mesocosm experiment. – In my analyses of mesocosm algal biomass, the predictor variables were tadpole presence-absence and final mayfly abundance (because the abundance of mayflies declined over the course of the experiment). I included an interaction term between consumers, because of the potential for tadpoles to either facilitate or interfere with mayfly grazing, and covariates for duration of algal growth (days) and for the initial abundance of algae ($\log_{10}$ AFDM) in each mesocosm; I allowed variance to differ with respect to tadpole presence-absence and final mayfly abundance. The response variable was AFDM of benthic organic matter on tiles. I used a similar approach to selecting the best-fit linear model as outlined above. The proportion of mayflies lost in the experiment was compared to zero (no loss) using a one sample t-test, and was compared to tadpole presence-absence using an analysis of variance model, with tadpole presence-absence as the predictor variable and proportion mayflies lost as the response variable. I calculated the growth rate of algae on tiles as the log ratio of algal biomass after 21 days to initial July algal biomass ($\ln(\text{algal AFDM}_{\text{end}}/\text{algal AFDM}_{\text{start}})$/experiment duration). Using an ANOVA, I compared algal biomass growth rate to tadpole presence-absence, final mayfly abundance, and an interaction between consumers. Analyses were performed and visualized using the nlme and ggplot2 packages in R (The R Foundation for Statistical Computing 2012).

RESULTS

Field enclosure experiment. – Many mayfly nymphs in enclosures emerged or died, resulting in 10-100% declines of per-enclosure mayfly abundances (Figure 2), requiring subsequent replacement of lost individuals. The proportion of mayfly nymphs lost during a time-block was not affected by tadpole abundance in enclosures, but decreased marginally with mayfly abundance (Table 1) and differed between the two lakes (Figure 2).Up to 50% of tadpoles per enclosure developed beyond Gosner stage 38, requiring release and replacement of individuals between time-blocks (Figure 3). Individual tadpole sizes at the end of the first block did not differ with respect to the abundance of either tadpoles or mayflies. The proportion of tadpoles in an enclosure that exceeded Gosner stage 38 increased by 0-40% for 5-10 fold increases in tadpole abundance (Figure 3) and declined less than 0.5% with increasing mayfly abundance (Table 2).

Despite the high losses of mayfly nymphs, only mayfly nymph abundance had a negative effect on the experimental-control algal biomass difference (Table 3). The variance in experimental-control biomass difference was an order of magnitude higher in Spur than in LeConte. The best fit linear mixed effects model of controlled algal abundance (Table 4) included fixed effects for mayfly abundance and for duration of block. The model also included a random intercept that allowed the experimental-control biomass difference to vary with respect to experimental block, nested within lake, and allowed variance of experimental-control biomass difference to differ among experimental blocks and between lakes.

Mesocosm experiment. – In the 2010 mesocosm experiment, mayfly nymph abundance declined by 50% – 100% during the experiment (Figure 5), which was a significant decline from the initial abundance of 250 mayfly nymphs per mesocosm (change > 0, one sample t-test, $p = 0.01$, $t_7 = 3.4$). Live mayflies collected from mesocosms at the conclusion of the experiment were not near metamorphosis (they did not have wing pads), nor were exuvia or emerged adults ever observed. This apparent mortality was not related to coexistence with tadpoles (Figure 5, ANOVA, $F_{2,6} = 0.4$, $p = 0.6$).

Tadpole presence reduced algal abundance by about 50% (generalized least squares model, $t_{14} = -2.0$, $p = 0.07$, Figure 6), though the effect was not significant at the $p = 0.5$ level. Mayfly final abundance had no effect on algal abundance. The best-fit generalized least squares model included only a fixed effect for tadpole presence-absence, and allowed variances to differ between tadpole presence-absence treatments ($\sigma_{\text{No tadpoles}} = 0.06^2$ and $\sigma_{\text{Tadpoles}} = 0.11^2$). Algal growth rates did not differ with respect to consumer treatments (Figure 7, ANOVA, $F_{1,14} = 1.3$, $p = 0.3$).

DISCUSSION

Overall, tadpoles and mayfly nymphs both reduced algal biomass by small to moderate amount, though the impact of each grazer differed in the two experiments. Mountain yellow-legged frog tadpoles marginally reduced the biomass of algae in mesocosms but they had no effect on algal biomass in field enclosures. Meanwhile, only mayflies reduced algal biomass in the field experiment. The proportional losses of both consumers – development of tadpoles and mortality and emergence of mayflies – increased at higher abundances of conspecifics, but were unaffected by presence or abundance of the other consumer species. Based on my results in this study, there is little evidence that mountain yellow-legged frog tadpoles exert top-down pressure on algal resources or competitive pressure on some other grazers, suggesting that local extinctions of mountain yellow-legged frog tadpoles may have limited impacts on Sierra Nevada lake communities. The general prediction that removal or local extinction of a grazer can release producers or competitors (Hairston et al. 1960, Paine 1966, Carpenter et al. 1985, Holbrook and Schmitt 1995, Chalcraft and Resetarits 2003, Gruner et al. 2008) was not supported by my results. This may be because either the experiments had limited power to detect strong effects of tadpoles as grazers, or tadpoles do not have strong grazing effects on producers.

Statistical power to detect potentially strong effects of tadpoles and mayflies as grazers may have been reduced by aspects of experimental design and grazer biology. Tadpole grazing impacts appeared to be much stronger in the mesocosm than enclosure experiment, perhaps because pre-treatment algal colonization and growth times were much greater in the mesocosm than enclosure experiment, allowing a greater range of algal biomass for evaluating grazer impacts. I used two mayfly species, and they may have different grazing abilities; mayfly effects were clearest in LeConte lake, which is only occupied by *Ameletus edmundsi*, while *Callibaetis ferrugineus* composed about half of mayfly nymphs in Spur and all in the mesocosms. Mesocosms were much more homogeneous in environmental conditions than the lake enclosures, with high environmental variation both within and between lakes, so consumer effects on their resources could be more clearly discerned against limited environmental variability in the mesocosm experiment. Variation in losses of grazers between enclosures, time-blocks, lakes in the enclosure experiment meant that treatments were neither maintained nor changed consistently, which may have increased variability in algal biomass. However, despite high tadpole losses in the first time-block, virtually no tadpoles were lost in the second and third blocks, but I still observed no effect of tadpole grazing on algal biomass in the enclosure experiment.

Many experiments have concluded that tadpole grazing can reduce algal resources (Brönmark et al. 1991, Kupferberg 1997a, Alford 1999). The generality of control of algae by aquatic grazers may result from the experiments in which those interactions have been studied: in a meta-analysis of grazing experiments, 70% of experiments found that grazers at ambient densities reduced algal biomass (Feminella and Hawkins 1995). Grazer effects were largest in long lab experiments and were smaller for short experiments or field experiments, where variable conditions or environmental heterogeneity are probably more influential (Feminella and Hawkins 1995). This may partially explain my results, that tadpoles had no effect in a field experiment and a moderate effect in mesocosm experiment of the same duration (though mayfly nymphs had the opposite effects).

Manipulative and natural field experiments have detected regulation of algal abundance by tadpoles, and tadpole exclusion or disease-caused extinctions can release algae from top-down regulation. The exclusion of tadpoles from the benthos in Neotropical streams resulted in 111% to 200% increases in algal abundance , and after the amphibian chytrid fungus caused extinction of tadpoles in these same streams, algal abundance rapidly increased 2-6 fold (Ranvestel et al. 2004, Connelly et al. 2008, 2014). To the contrary, in

Pacific northwest streams, algal abundance did not increase where tailed frog tadpoles were excluded, probably because the effects of tadpole exclusion were masked by between stream variability (Lamberti et al. 1992, Mallory and Richardson 2005). This contradiction between the weak effects of mountain yellow-legged frog tadpoles and tailed frog tadpoles versus the strong effects of tropical stream tadpoles reinforces how ecological effects of amphibian declines will vary for each threatened species and with ecological context (Menge 2003). Identifying differences among these frog species and their communities may indicate when and where amphibians exert top-down control or respond to bottom-up processes.

Correlations between consumer and resource abundances may be weakened by competition, long consumer generation times, or low nutritional value of resources weaken (Power 1992). Like many grazers, tadpoles can engage in intraspecific exploitative and interference competition (Steinwascher 1978a, Griffiths et al. 1993, Kupferberg 1997b, Faragher and Jaeger 2011). For tadpoles, the time between grazing and reproduction could be years. Many types of diatoms, and up to 40% of those ingested, pass through tadpole guts unharmed (Peterson et al. 1998, Peterson and Boulton 1999), which must reduce their nutritional value to tadpoles. In the feces of my mesocosm tadpoles, diatoms appeared generally intact, many still containing chloroplasts; the feces of wild caught tadpoles is similar but also contains a high proportion of sand, which could reduce feeding efficiency and disconnect tadpole abundance from producer abundance. Tadpole feces can also supplement tadpole diets (Gromko et al. 1973, Steinwascher 1978a, 1978b), which would further decouple tadpole abundance from algal abundance as tadpoles' diets include some fraction of semi-digested-then-defecated material rather than all new benthic material. These characteristics of mountain yellow-legged frog tadpoles may further clarify why I observed weak top-down effects of tadpoles as consumers.

The weak top-down effects of mountain yellow-legged frog tadpoles may have little impact on their communities, but the loss of unstudied facilitative and bottom-up roles of tadpoles may prove to have greater impacts on communities (Bruno et al. 2003, Ellison et al. 2005, Lafferty and Kuris 2009). Tadpoles can benefit other species' grazing by bioturbating silt that covers substrates, increasing access of invertebrate grazers to algal resources (Ranvestel et al. 2004). In addition, tadpole excretion creates nutrient hotspots which could increase quality, quantity and diversity of algal resources for themselves and other grazers (Seale 1980, Vanni 2002, Smith diss. 2015). Tadpoles can be important prey for adult frogs, garter snakes, and Clark's nutcrackers (Jennings et al. 1992, Matthews et al. 2002, Pilliod 2002, Finlay and Vredenburg 2007). In addition, tadpoles and adults host parasite and microbial communities that could be lost when frogs and tadpoles go extinct (Lafferty and Kuris 2009, Jani and Briggs 2014). Although my results indicate that frog extinctions would have limited effects on lake benthic algal biomass and invertebrate communities, other aspects of their roles in food web networks demand additional exploration.

The importance of the endangered mountain yellow legged frogs and tadpoles is not precluded by the equivocal effects of tadpoles on benthic producers observed in this study or the weak effects of frogs and tadpoles on macroinvertebrate communities that I have also observed (Smith diss. 2015). While my current work provides equivocal support for my prediction that extinctions of frogs and tadpoles would release communities from top-down control and exploitative competition, it does not mean that mountain yellow-legged frogs are unimportant in lakes. There are unquantified processes in which frogs and tadpoles may be important; and until these are documented, frogs and tadpoles cannot be called expendable (Simberloff 2003).

ACKNOWLEDGEMENTS

The use of tadpoles in these experiments was approved by the University of California, Santa Barbara Institutional Animal Care and Use Committee (protocol #6-08-762). Sequoia-Kings Canyon and Yosemite National Parks permitted the research, and I thank Danny Boiano for providing logistical support in the field. I thank Dan Dawson, Roland Knapp, and the staff at the Sierra Nevada Aquatic Research Laboratory for providing research and housing facilities and for logistical support. This research was supported by National Science Foundation Ecology and Evolution of Infectious Disease program (EF-0723563), by the University of California Natural Reserve System Mildred E. Mathias Grant for Graduate Student Research, the Henry Luce Foundation Environmental Science to Solutions Fellowship, and UCSB Department of Ecology, Evolution, and Marine Biology. Marina Bozinovic (UCSB EEMB Aquatic Biology ‘15) and Yishen Miao (UCSB CCS Biochemistry ‘15) contributed to the research, analysis, and text, and edited the manuscript. Stephen DeLisle, Michael McFarlin, Garrison Loope, Michael Hernandez and Madelynn Johnston, Cord Dorcey, Andrea Jani, Mary Toothman, and Tate Tunstall assisted with field and/or laboratory work. Nikki Gantos, Patricia Monie, and Dominique Monie sewed the enclosures. Sally Holbrook and Scott Cooper provided valuable comments that enhanced the research and the manuscript.

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TABLES

Table 1. Summary of best fit model of proportion of mayfly loss (final abundance/initial abundance) in all time-blocks in field enclosures.

	Linear model coefficient	t_{66}	p-value	Random intercept	Random slope
Mayfly abundance	-0.0004	-2.1	0.04		
Time-Block					$\sim N(0, 0.29^2)$
Lake				$\sim N(0, 0.9^2)$	

Table 2. Summary of best fit model of per-enclosure log proportion of tadpoles greater than Gosner stage 38 at end of first time-blocks in field enclosure experiment.

	Linear model coefficient	t_{20}	p-value	Random intercept	Variance
Tadpole abundance	0.09	7.6	< 0.001		$\sigma_0 \sim N(0, 1.9^2)$ $\sigma_{10} \sim N(0, 2.4^2)$ $\sigma_{20} \sim N(0, 0.58^2)$
Mayfly abundance	-0.004	-7.8	< 0.001		$\sigma_0 \sim N(0, 0.19^2)$ $\sigma_{25} \sim N(0, 0.27^2)$ $\sigma_{125} \sim N(0, 0.58^2)$ $\sigma_{250} \sim N(0, 0.11^2)$
Lake				$\sim N(0, 0.000006^2)$	

Table 3. Description of best-fit model of log transformed experiment-control algal biomass difference in 2009 field enclosure experiment. Following a backwards step-down model selection approach, the initial model included predictor variables tadpole and mayfly abundance (and their interaction), and covariates for per-enclosure substrate siltiness, light intensity, duration of time- block, lake, and time-block.

	Linear model coefficient	t_{96}	p-value	Random intercept	Combined variance structure
Mayfly Abundance	-0.0011	-3.7	0.0004		
Duration of Block	-0.07	-1.2	0.22		
Lake					$\sigma_{LeConte} = 0.33^2$ $\sigma_{Spur} = 3.36^2$
Block				$\sim N(0, 0.48^2)$	$\sigma_{block\ 1} \sim N(0, 0.48^2)$ $\sigma_{block\ 2} \sim N(0, 0.27^2)$ $\sigma_{block\ 3} \sim N(0, 0.22^2)$

Table 4. Models of log transformed experiment-control algal biomass difference for 2009 field enclosure experiment. The initial model included predictor variables tadpole and mayfly abundance (and their interaction), and covariates for per-enclosure substrate siltiness, light intensity, duration of time-block, lake, and time-block. Δ AIC compares the model to the best-fit model.

Fixed effects	Random effects	Heterogeneity of variances	Δ AIC
Tadpole Abundance x Mayfly Abundance + Lake + Siltiness + Radiation+No. of Days			136.90
Tadpole Abundance x Mayfly Abundance + Lake + Siltiness + Radiation + No. of Days	Block		140.6
Tadpole Abundance x Mayfly Abundance + Lake + Siltiness + Radiation	Random slope for No. of Days nested within random intercept for Block		145.2
Tadpole Abundance x Mayfly Abundance + Lake + Siltiness + Radiation + No. of Days	Block	Lake, Block	5.9
Mayfly Abundance + No. of Days	Block	Lake, Block	0

FIGURES

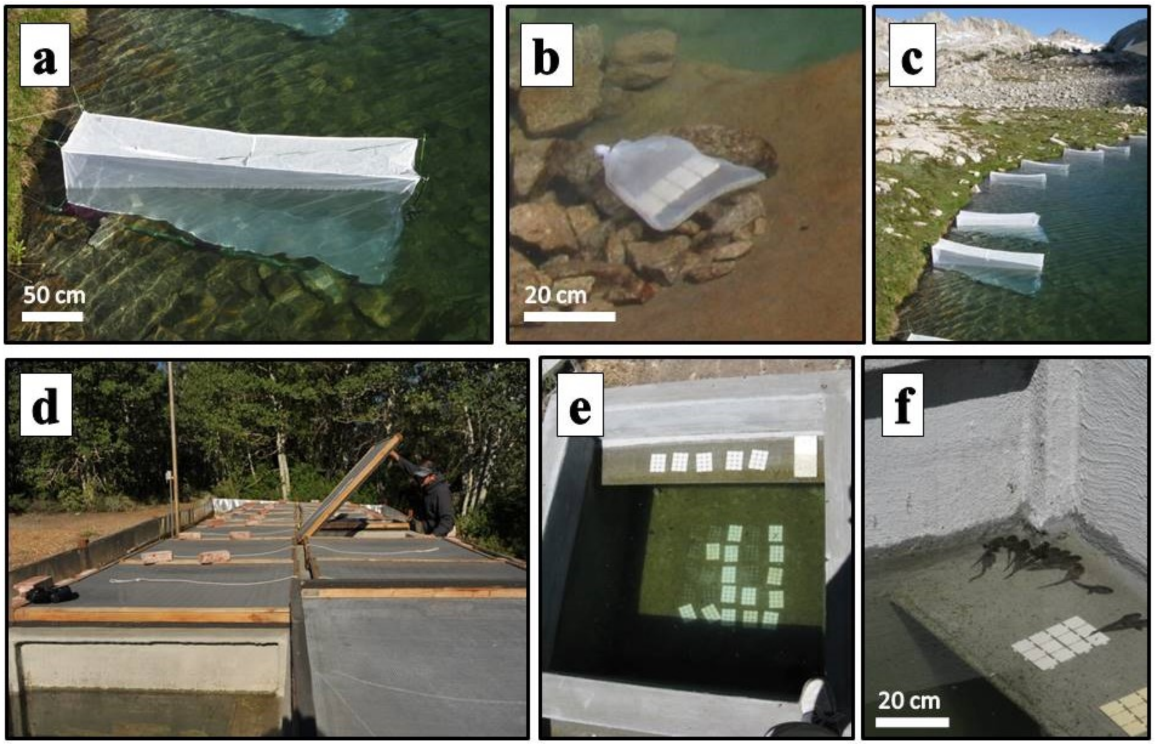

FIG. 1. a) *in situ* experimental mesh enclosure in LeConte lake, b) no consumer location-within-lake control tiles in bag of same mesh as enclosure and placed next to enclosure, c) Field enclosures in LeConte lake in Kings Canyon National Park, d) mesocosms located at Sierra Nevada Aquatic Research Laboratory in Mammoth Lakes, CA, e) view of experimental tiles and algal growth in one mesocosm, and f) tadpoles basking on shelf in a mesocosm. A map of lakes may be viewed in Google Earth, using the following URL and clicking “View raw”: https://github.com/TomCSmith/manuscript-support-files/blob/master/ThomasCSmith_LeConteSpur_map.kmz

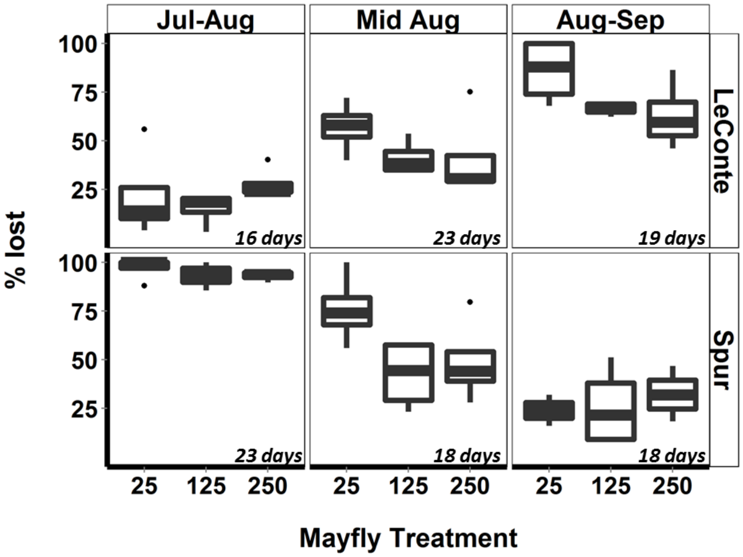

FIG. 2: Proportion of in mayfly nymphs lost (by mortality or emergence) in field experiment enclosures, with respect to mayfly density treatments (nymphs per enclosure) and experimental time-blocks, lakes; these mayflies were subsequently replaced with new individuals. n = 12 mayfly containing enclosures per lake-time-block. Losses differed between all time-blocks within each lake, and differed between lakes in time-blocks 1 and 3 (ANOVA, $F_{5,65} = 30.4$, $p < 0.001$, Tukey's HSD, all $p < 0.02$); losses did not differ for mayfly treatments within any lake-time-block. Heavy bars indicate medians, boxes include points within the first and third quartiles, whiskers include data points that lie within 1.5 x the inter-quartile range, points lie outside that range.

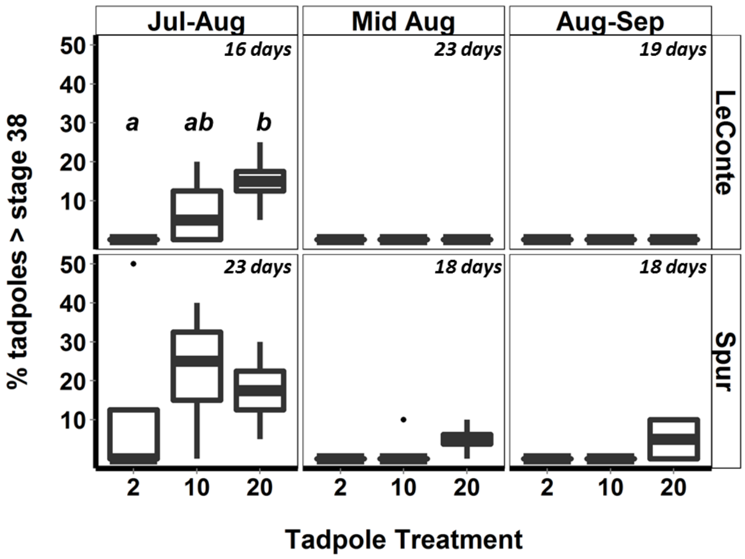

FIG. 3. Percent of tadpoles exceeding Gosner stage 38, with respect to tadpole density treatments (tadpoles per enclosure), and lake-time-blocks; these tadpoles were subsequently removed from enclosures and replaced by younger individuals. n = 12 tadpole-containing enclosures per lake-time-block (n = 4 per tadpole treatment). Tadpole development was higher in Spur (ANOVA, $F_{5,65} = 16$, Tukey's HSD, $p = 0.02$) and highest in the late July-early August block (ANOVA, $F_{5,65} = 16$, Tukey's HSD, $p < 0.001$). Letters indicate within lake-time-block differences among tadpole treatments (ANOVA, $F_{5,65} = 16$, Tukey's HSD, $p < 0.05$). Heavy bars indicate medians, boxes include points within the first and third quartiles, whiskers include data points that lie within 1.5 x the inter-quartile range, points lie outside that range.

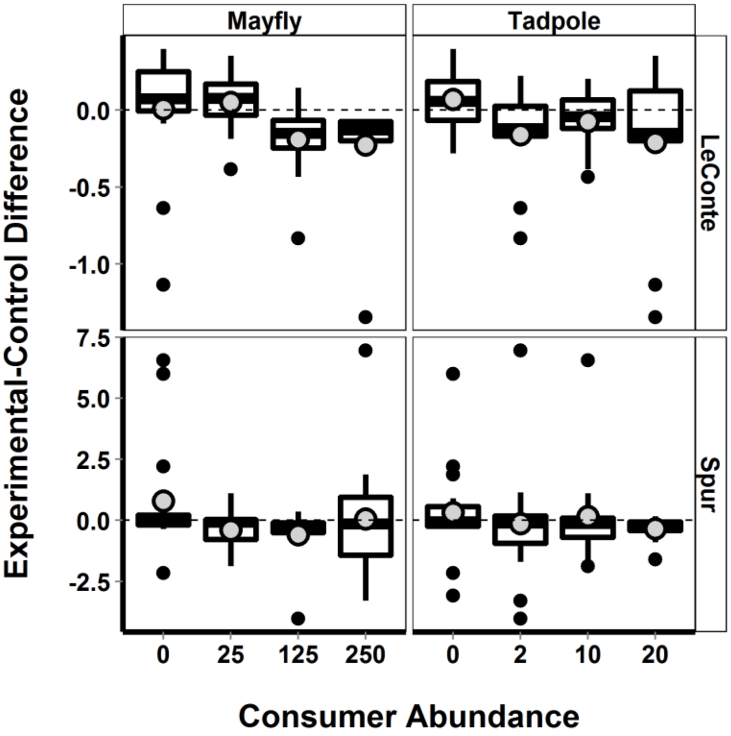

FIG. 4. For 2009 field enclosure experiment, difference in algal biomass (mg AFDM) on tiles in experimental enclosures relative to algal biomass on location-within-lake control tiles, with respect to lake and to each consumer. n = 51 AFDM samples per lake, over all three time-blocks. Grey circles indicate means, heavy bars indicate medians, boxes include points within the first and third quartiles, whiskers include data points that lie within 1.5 x the inter-quartile range, points lie outside that range.

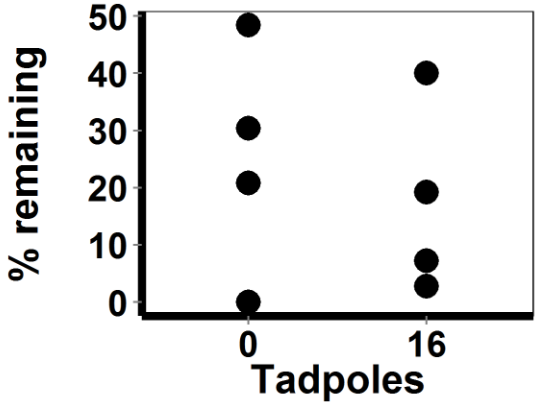

FIG. 5. Mayfly nymph abundances at the end of the mesocosm experiment were significantly lower than the initial abundance of 250 nymphs (one-sample t-test, $t_7 = 3.4$, $p = 0.01$,). Tadpole presence-absence had no effect on the loss of mayfly nymphs (ANOVA, $F_{2,6} = 0.4$, $p = 0.6$).

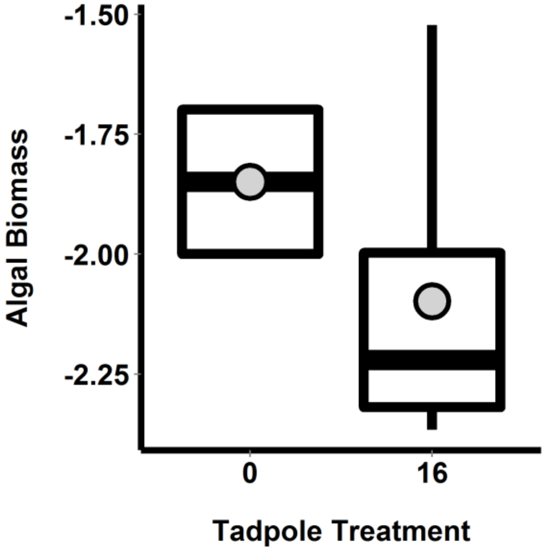

FIG. 6. Algal biomass ($\log_{10}$ AFDM) in 2010 mesocosms, n = 16. Algal biomass was not significantly lower in the presence of tadpoles (generalized least squares model, $t_{14} = -2.0$, p = 0.07). The model allowed variance in algal biomass to differbetween tadpole treatments, with $\sigma_{\text{No tadpoles}} = 0.06^2$ and $\sigma_{\text{Tadpoles}} = 0.11^2$. Grey circles indicate means, heavy bars indicate medians, boxes include points within the first and third quartiles, whiskers include data points that lie within 1.5 x the inter-quartile range, points lie outside that range.

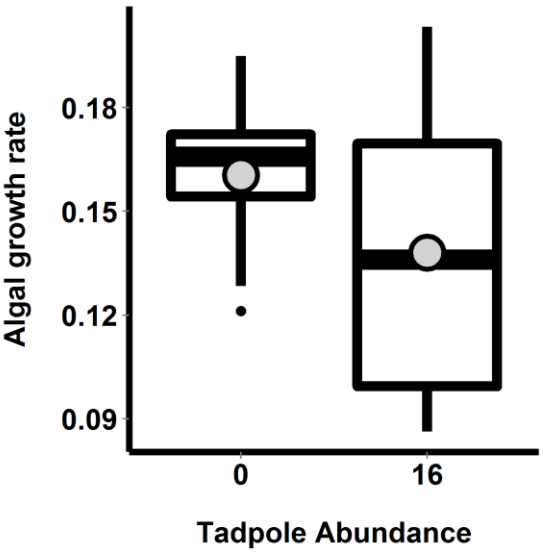

FIG. 7. Growth rates ($\ln(AFDM_{end}/AFDM_{start})/21$ days) for algae on experimental tiles in mesocosms (n=16). Growth rates in the presence of tadpoles were not significantly lower (ANOVA, $F_{1,14} = 1.3$, $p = 0.3$). Grey circles indicate means, heavy bars indicate medians, boxes include points within the first and third quartiles, whiskers include data points that lie within 1.5 x the inter-quartile range, points lie outside that range.

III. PERIODIC AGGREGATIONS OF TADPOLES AS A FLUCTUATION-DEPENDENT DRIVER OF BENTHIC COMMUNITY DIVERSITY

ABSTRACT

As biodiversity declines worldwide, ecologists investigate with more urgency both the processes that maintain that diversity, and the consequences of its loss. In high elevation lakes in California's Sierra Nevada, tadpoles of the endangered mountain yellow-legged frogs periodically form large aggregations, which, as thousands of tadpoles excrete in the same spot throughout a day, can create ammonia hotspots. This potential for localized and daily buildup of ammonia suggests that fluctuation-dependent mechanisms could act to enhance the diversity of nitrogen-consuming benthic producers. In four lakes, I investigated the extent to which tadpoles aggregate, how those aggregations affected spatial and temporal variability of ammonia, and in two of those lakes I examined in detail how ammonia variability affected diversity of benthic producer communities, as well as the effects of tadpole grazing. Most notably, tadpoles did not aggregate as consistently as anticipated, and heterogeneity in temporal variance of NH_3 and NO_3+NO_2 occurred regardless of the presence or abundance of tadpoles. Diatom diversity declined across these nutrient gradients regardless of the abundance of tadpoles in a site. While diatom community diversity and composition did not differ with respect to tadpole grazing, diatom abundance was higher in areas where tadpoles were experimentally excluded. Overall, diatom communities did not differ between sites in lakes where tadpoles aggregated versus those where tadpoles did not aggregate. Diatom diversity declined far from the center of sites, and communities at the centers differed from those at the edges, but due to the lack of correlation between tadpole abundance and nutrient concentrations, those difference cannot be linked back to the proposed fluctuation dependent mechanism of tadpole-generated nitrogen heterogeneity.

However, the observed differences in diversity and community composition may result from other tadpole generated processes, like benthic disturbance or producer dispersal.

Keywords: alpine lake, ammonia, consequences of extinctions, diatoms, epiphyton, heterogeneity, resource subsidy, *Rana muscosa*, *Rana sierrae,* Sierra Nevada

INTRODUCTION

Ecologists continue to refine and seek support for our understanding of the processes that influence community diversity (Levine and HilleRisLambers 2009, Siepielski and McPeek 2010, Allesina and Levine 2011, Gravel et al. 2011). Classical ecological theory suggests that the number of species coexisting cannot exceed the number of limiting resources (Hardin 1960, MacArthur and Levins 1967), and in a constant environment (under equilibrium conditions) coexistence of multiple competitors requires the presence of some mechanism, such as resource partitioning (MacArthur 1970, Chesson 1994) or frequency-dependent predation (Gendron 1987, Huntly 1991), that effectively increases the number of resources (Tilman 1982, Grover 1997). Under varying conditions, however, a number of "fluctuation-dependent" mechanisms can promote coexistence of multiple species (Stewart and Levin 1973, Chesson 1994, 2000, Gravel et al. 2011).

Fluctuation-dependent mechanisms include relative nonlinearity of competition and the storage effect (Chesson 2000). In relative nonlinearity, coexistence is possible due to species differing in their nonlinear responses to a shared limiting resource that fluctuates either due to the biological interaction itself having an unstable equilibrium (e.g. consumer-resource cycles; Armstrong and McGehee 1976, 1980), or due to external environmental forcing (Sommer 2002, Descamps-Julien and Gonzalez 2005). In the storage effect, species can coexist in a variable environment through a form of temporal niche partitioning, in which the interaction between competition and the environment causes the effects of intraspecific competition to exceed interspecific competition. Temporal variability in resource availability can also have the opposite effect, increasing the risk of extinction if it results in fluctuations in the dynamics of some species to low densities (Holt 2008).

In lakes in the Sierra Nevada, mountain yellow-legged frog tadpoles can create large amplitude spatial and temporal variation in dissolved nutrients (T.C. Smith, unpublished data), but that mechanism may become less prevalent as their populations decline. Mountain yellow-legged frogs (*R. muscosa* and *R. sierrae*) are endemic to California's Sierra Nevada (Vredenburg et al. 2007). Once the most abundant vertebrate in this ecosystem (Grinnell and Storer 1924), populations of these frogs have been fragmented and extirpated by the introduction of non-native trout (Knapp and Matthews 2000) and by the emergence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd hereafter, Vredenburg et al. 2010). Trout have become widespread, and exclude frogs from lakes by predation. Less than ten percent of historic frog habitat remains due to the presence of trout (Vredenburg et al. 2007). In remaining habitats, populations are threatened by, or have been recently extirpated by, the emergence of Bd. For mountain yellow-legged frogs, infection with Bd is usually lethal and mortality in a population often approaches 100%. Populations usually decline to just a few or no individuals within a few years of the appearance of Bd (Briggs et al. 2010, Vredenburg et al. 2010).

Mountain yellow-legged frog tadpoles are generally benthic feeding grazers, and diatoms form a substantial portion of their gut contents (T.C. Smith, unpublished data). Following digestion and metabolism, tadpoles excrete nitrogen as ammonia through their gills (McDiarmid and Altig 1999). Where tadpoles aggregate, ammonia concentration fluctuates dramatically on a daily basis. Where tadpoles have been extirpated, we anticipate there will be no 'hotspots' of ammonia and that ammonia levels throughout the lake will be homogenous, minimal, and similar to levels of nitrate.

Ammonia can be important to primary producers in the lake benthos. In Sierra Nevada lakes, epiphyton is composed almost entirely of diatoms (T.C. Smith, this study). In this region, silicon is unlikely to be the limiting resource to diatom growth, because it is abundant due to geologic processes (Werner 1977, Barmuta et al. 1990, Williams and Melack 1991). While algal growth in lakes is commonly thought of as phosphorus limited (Sterner 2008), epiphyton growth in shallow high elevation lakes can be limited (or co-limited) by carbon, phosphorus, or nitrogen (Maberly et al. 2002, Nydick et al. 2004, Saros et al. 2005), and in the Sierra Nevada, phytoplankton in lakes may be nitrate limited (Sickman et al. 2003). Therefore, additions of nitrogen as ammonia could benefit producer communities, especially if diatoms prefer dissolved ammonia to nitrate as a nitrogen source and are stimulated to grow by ammonia enrichment (e.g. McCarthy et al. 1977, Werner 1977, Axler et al. 1982).

I propose that fluctuation-dependent mechanisms including either relative nonlinearity of competition, the storage effect, or both could act to enhance diversity in areas where the tadpoles aggregate. For the storage effect to promote coexistence of competitors on a fluctuating resource, three features must be present (Chesson 2000): (a) the species respond differently to temporal variability in the environment, (b) covariance between the environment and competition (this causes intraspecific competition to be strongest when the environmental conditions are favorable for a given species), and (c) buffered population growth, such as that resulting from a life-history stage (e.g. a resting stage in diatoms; McQuoid and Hobson 1996) that is invulnerable to the effects of competition and allows each species to persist during periods of unsuitable environmental conditions. There is the potential for the storage effect to act in epiphyton communities because responses to fluctuations in nutrients vary among diatom species (Werner 1977), and many diatom species have resting stages (McQuoid and Hobson 1996). The present study complements previous laboratory tests of coexistence through fluctuation-dependent mechanisms in algal communities (Robinson and Sandgren 1983, Sommer 1985, Grover 1988, Spijkerman and Coesel 1996, Flöder et al. 2002, Descamps-Julien and Gonzalez 2005) by examining natural algal communities in lakes.

In the present study, my objectives (Table 1) were to determine the extent to which tadpoles influence the abundance and distribution of dissolved ammonia, the extent to which algal diversity is influenced by the temporal and spatial variation in nutrients (ammonia), and to determine the extent to which fluctuation-dependent coexistence mechanisms influence the patterns of algae diversity within tadpole-containing lakes. While tadpole grazing could concurrently and synergistically influence algal diversity (Olff and Ritchie 1998, Proulx and Mazumder 1998, Liess et al. 2009) this study focuses on the fluctuation dependent mechanisms. I predicted that the heterogeneity in ammonia that tadpoles can create can locally enhance the diversity of benthic producers in low-diversity Sierra Nevada alpine lakes. This study adds to the growing body of literature that documents how organisms create resource subsidies and resource heterogeneity, and clarifies some of the ways in which communities can respond to species extinctions.

METHODS

Study system. – In this system, tadpoles are both *Rana sierrae* and *Rana muscosa,* sister taxa in the mountain yellow-legged frog species complex (Vredenburg et al. 2007) which I assume are ecologically similar. Benthic producers are diatoms, green algae, blue-green algae (cyanobacteria), and chrysophytes (yellow-green algae); these communities also contain non-photosynthetic bacteria and unidentifiable detritus. Lying in high elevation granitic basins, Sierra Nevada lakes are oligotrophic and characterized by low dissolved nutrient concentrations. Silicon is abundant (Barmuta et al. 1990, Williams and Melack 1991) but phosphorus (total phosphorus 0 – 1 μmol L^{-1}) and nitrogen (nitrate 0 – 10 μmol L^{-1}) are scant and can limit productivity (Sickman et al. 2003). Though lakes were historically phosphorus limited, some evidence suggests a trend towards nitrogen limitation (Sickman et al. 2003). pH is typically circumneutral (median pH $\cong$ 7, Bradford et al. 1998).

Excretion rates for mountain yellow-legged frog tadpoles average ca. 0.2 mg ammonia per liter per hour (T.C. Smith, unpublished data). Each day, tadpoles form dense aggregations in a few warm, shallow areas of many lakes. These aggregations can form in the same locations or patches most days of the ice-free season, and the densities can reach ca. 50 tadpoles per liter of lake water, with aggregations containing hundreds to thousands of individuals. Tadpoles disperse in the evening when water cools, and forage throughout the lake. We estimate that by the end of one day, a large aggregation of tadpoles might excrete over 30 kilograms of ammonia. These observations suggest that where tadpoles aggregate, ammonia can be orders of magnitude more abundant than in the rest of the lake, and can be more abundant than nitrate.

Pilot study of nutrient enrichment by tadpoles. – In 2010, I sampled tadpole aggregations in two lakes, Columbine and Marmot Lakes (Table 2). Based on observations made during several previous visits, I identified locations in the littoral zones of lakes where tadpoles consistently aggregated. To establish whether tadpoles affected nutrient concentrations, I sampled water in the centers of the locations, in the morning and in the afternoon. I also collected water samples at one 1m intervals along two transects radiating from the aggregation, one parallel to the shoreline and one perpendicular to the shoreline. To remove particulates, I filtered water through glass fiber filters with 1.2 µm pore size using a hand powered vacuum pump, and collected filtered water. This filtered water was analyzed for nitrite, nitrite + nitrate, and ammonia, by the University of California, Santa Barbara Marine Science Institute Analytical Laboratory, using flow injection analysis (MSI Analytical Laboratory http://msi.ucsb.edu/services/analytical-lab/seawater-nutrients-fia). The results of this sampling indicated that there was spatial and temporal heterogeneity in ammonia concentration, which was correlated with tadpole aggregation behavior (Figure 3).

Field experiment. –, I designed an experiment that could establish the relationships between tadpole aggregation density, nutrient concentration and heterogeneity, and algal community diversity patterns. I identified four lakes where tadpoles were abundant and likely to aggregate; all are unnamed lakes, hereafter referred to as Barrett, Center, LeConte and Roland's Aquarium These are all small high elevation, remote, backcountry lakes in King's Canyon National Park (Table 2).

To compare algal diversity and nutrient concentration patterns where tadpoles aggregate to those in the rest of the littoral zone, I located 1-2 sites within the littoral zone of each lake where I observed aggregations during my first visit to each lake (very early in the ice-free season of 2011, while lakes still had snow obscuring 10% to > 50% of shoreline). I also identified in each lake one benthically similar site where I did not, and had never previously, observed tadpole aggregations, to serve as a no aggregation site (two site types were Tadpole Aggregation and No Tadpole). I addressed my second objective, to compare algal diversity patterns across the gradient of tadpole abundance and nutrient concentrations, by establishing transects within each site. From a central point, 1 to 3 transects radiated parallel or perpendicular to the shoreline. Sampling points were established at the origin (0 m), and at 0.5, 1, 2, 3, and 5 m intervals, for later sampling of nutrients and algal communities (Figure 1). My third objective was to differentiate between the effects on algal communities of top-down grazing versus bottom-up nutrient effects of tadpoles, so I placed one tadpole exclosure and one exclusion control at each sampling point along each transect, and identified an adjacent patch of similar size with similar depth and substrate for later sampling of benthos exposed to grazing ("no control"). Thus, the experimental design consisted of nested sampling levels: grazing treatments (3 per point, of types Tadpole Exclusion, Exclusion Control, and No Control) within points (4-6 per transect), within transects (1-3 per site), within sites (2-3 per lake, of types Tadpole Aggregation and No Tadpole), within lakes (n = 4); total treatment-point-transect-site-lake combinations = 123(Figure 1).

Tadpole exclosures were small, shallow mesh cones, which each covered 250 cm^2 of the benthos (10 cm diameter x 5 cm tall, Figure 2). The exclosures were constructed of black plastic mesh (pest control net OV7822-168, Industrial Netting www.industrialnetting.com), irrigation tubing (1/4" vinyl micro tubing, DIG Corp., ww.digcorp.com), and zip ties. Each was secured to the benthos by plastic stakes (P33B, DIG Corp.) or weighted with rocks. Exclusion-controls were of identical design but with tadpole sized (2 cm) holes cut in the mesh on each side. Tadpoles were observed freely entering and exiting exclusion controls.

Measurements were made of dissolved nutrients and tadpole abundance at all points along transects and in all sites, in both the mornings and afternoons. This allowed comparison of algal community patterns across continuous variables of tadpole abundance, nutrient concentration, and distance from tadpole aggregation, and comparisons across categorical variables site-type and grazing treatment. I also measured water depth at each point and described substrate below each treatment by visually estimating, in the field, particle size (silt: particles < 0.5mm, sand: 0.5-2 mm, gravel: >2-75 mm, and rock: >75-300 mm).

I began the experiment in late July 2011, just as lake shorelines were becoming ice-free and tadpole aggregations began forming, and ended the experiment in mid-September as littoral zones cooled to near freezing at night and tadpoles remained in the warmer depths of the lakes. In the middle of the season, when lakes were warm and snow melt had ended, I counted tadpoles and collected nutrient samples on three successive days (6 lake-sample-times), at Center, LeConte, and Roland's Aquarium lakes but only two successive days (4 lake-sample-times) at Barrett lake due to weather. After arrival at a lake each morning, I counted tadpoles at the center of each site. I then collected water samples from each point on each transect in each site. As in the pilot study, I immediately filtered water through glass fiber filters with 1.2 µm pore size, and collected filtered water. I repeated tadpole surveys and nutrient sampling in late afternoon when aggregations are typically largest. Water samples could not be frozen in the backcountry, so I buried them in snow fields to keep them dark and at a cool constant temperature until I could freeze them in the laboratory. Thus, for samples collected on the first day of sampling, they were unfrozen up to four days. As before, filtered water samples were analyzed for NH_3, NO_3+NO_2, and PO_4 by the University of California, Santa Barbara Marine Science Institute Analytical Laboratory, using flow injection analysis.

At the conclusion of the experiments in September, I sampled benthic producers at each grazing treatment at each sampling point. I used a modified Loeb sampler (e.g. Flower 2007; Loeb 1981, Peters et al. 2005) to collect 60 cm^2 of benthic material suspended in 20 mL of lake water, which I added 2 mL of 10% formalin as a preservative. To prevent dispersal of *Batrachochytrium dendrobatidis*, I cleaned hard field gear with 70% ethanol and allowed it to dry completely, and I disinfected soft field gear and footwear in a 0.01% solution of quaternary ammonia for five minutes after sampling each lake each day (Johnson et al. 2003).

Algal community description. – To establish the abundances of all benthic producers with respect to tadpole aggregations algal cells were counted from samples from all 0 m, 0.5 m, and 5 m sample points in all lakes. Viewing 9 µL of well mixed sample on a haemocytometer at 400x magnification, cells were counted and classified as diatoms, green algae, blue-green algae (cyanobacteria), yellow-green algae (chrysophytes), or other material. Diatoms were the most abundant algal type (Figure 5).

To quantify diatom community composition and diversity, I made and examined slides of cleaned and mounted diatom frustules. I cleaned 5 mL of sample in 50% hydrogen peroxide followed by 10% hydrochloric acid (Danuta M. Bennett, personal communication). Cleaned samples were settled onto cover slips and dried, then mounted to slides with Naphrax (http://www.brunelmicroscopes.co.uk/naphrax.html). I viewed slides under oil immersion at 1000x magnification (Leica DM6000). I counted over 375 valves (diatom cells) per sample; other researchers of Sierra Nevada diatom communities have counted over 500 valves (Holmes et al. 1989, Sickman et al. 2013). My preliminary analysis suggested that counting 100-200 additional cells would take 33% longer and detect only 5-10% more species (3-6 species). To further streamline the workload, I described communities from only sample points at 0, 0.5, and 5 m. If no differences existed between these communities, it seemed unlikely that intermediate communities would differ. In addition, I only examined all of the samples from two lakes (Barrett and Center) because some authors recommend against comparing diatom communities collected from dissimilar substrate types (Potapova and Charles 2005); my preliminary analyses confirmed that substrate drove much of the variation among communities, and sample points in these two lakes had the least variation in substrate.

I identified diatoms to species, except for two groups of taxa, in each of which I could not differentiate individual diatoms into discrete species. I designated a group of similar eunotioid diatoms as *Eunotia arcus* morphotype and group of similar nitzschioid diatoms as *Nitzschia acicularoides* morphotype, and assigned diatoms to each based on morphological similarities. For the *Achnanthidium minutissimum* group of species, I adhered to morphotypes (similar to those suggested by Potapovae and Hamilton, 2007). I used many references for diatom taxonomy (Table 22), but when possible, I prioritized references based on North American specimens (e.g. Patrick and Reimer 1966, Spaulding et al. 2010). I used open nomenclature naming procedures, indicating uncertainty in identifications with *cf.* and indicating possible new species resembling existing species with *aff.* I assigned unofficial names to taxa for which I could find no similar taxa. All identifications were made by the same observer (myself, T.C. Smith).

Analytical methods. – In both linear models of univariate variables and multivariate analyses, I used continuous predictor variables for size of tadpole aggregation, nutrient concentrations, and distance from the center of sites, and categorical predictor variables for presence of tadpole aggregations at a site, and grazing exposure. Tadpole Abundance was a continuous variable describing the abundance of tadpoles in the aggregation at the 0 m sampling point. Distance was a continuous variable describing the distance from the 0 m sampling point, with predefined levels 0.5, 1, 2, 3, 5 m. I described variation in nutrients with the three continuous variables temporal variance of ammonia (σ^2 NH_3), maximum of ammonia, and maximum of NO_3+NO_2. I selected these three variables as a result of a principal components analysis (Table 4; Johnson and Wichern 2007, calculated in the `vegan` package in R) of 12 potential nutrient variables, which summarized nutrient concentrations in all 4-6 measurements at each sampling point. I also included several categorical variables; site-type was a categorical variable describing the presence of a tadpole aggregation, with levels "tadpole aggregation" and "no aggregation"; treatment described grazing exposure with three levels: tadpole exclusion, exclusion control, and no exclusion. I also included in my analyses covariates for depth (continuous) and for substrate (categorical). Categorical variables for transect, site, and lake were included as potential nested random effects.

I fit linear models to the abundance of tadpoles in aggregations, to the σ^2 NH_3 and the NO_3+$NO_{2\,max}$ concentrations, and for the community response variables overall diatom abundance, rarefied richness (for 375 individuals), community evenness (Shannon evenness), and Shannon diversity (Magurran et al. 2011). I calculated richness, evenness, and diversity for all species in the communities using the `vegan` package for R (Oksanen et al. 2013).

When selecting best-fit linear models, I assessed normality of residuals distributions graphically and where necessary applied transformations to the response variable to meet the normality assumption of the analyses. Model residuals for variance in σ^2 NH_3 and the NO_3+$NO_{2\,max}$ and diatom abundance met the assumption of normality after $\log_{10}$

transformation; tadpole abundance, diatom community richness, evenness and diversity did not require transformation. I evaluated equality and homogeneity of variance graphically (Quinn and Keough 2002, Zuur et al. 2009). Using generalized linear models (GLMs), I allowed variances of most response variables to differ among levels categorical covariates substrate, grazing treatment, transect, site, and lake. I compared model fits and parsimony using the Akaike Information Criteria (AIC, Zuur et al. 2009). Univariate analyses were performed using the `nlme` package in R. I used this approach to establish relationships between the response variables tadpole abundance, nutrient concentrations, diatom abundance, richness, evenness, and diversity, and independent variables and covariates tadpole abundance, nutrient concentrations, distance from aggregation, site-type, grazing treatment, and covariates depth, substrate, transect, site, and lake.

To facilitate multivariate comparisons of variability among diatom communities, I removed rare species from the data, defining rare as a combination of low occurrence and low abundance. I defined rarity as a combination of low occurrence (sensu Gaston 1994) - species occurring in less than 5% of sites – and low abundance (Magurran and McGill 2011) – species composing less than 1.5% of the cells counted per sample (about five cells). A species had to meet both criteria to be considered rare and be excluded. Using the resulting common-species community matrix, I calculated pairwise distances among each community using Morisita-Horn distances (or overlap). This distance index emphasizes abundant species and gives less weight to low-abundance species, whose abundances will be most affected by under sampling; in addition, it is not affected by variable community sizes (as is the commonly used Bray-Curtis distance index, Magurran et al. 2011). However, were the response of diatoms to resource heterogeneity reflected most in rare species, the Morisita-

Horn index might mask those responses. Visual inspection of NMDS plots of communities based on both distance indices indicated that communities were not conspicuously different.

I used non-metric multidimensional scaling (NMDS, Quinn and Keough 2002) to visualize differences between communities. In all cases, I visualized community differences using two NMDS axes, even if major reductions in stress were achieved by using more axes. I plotted correlations between samples and either continuous environmental variables ($r > 0.1$ and $p < 0.05$ for diatom communities, $r > 0.05$ and $p < 0.05$ for algae type communities), algae types ($r > 0.05$, $p < 0.1$) and species abundances ($r > 0.3$ and $p < 0.001$), calculated using the `envfit` function in the `vegan` package in R. I plotted centroids for categorical environmental variables (lake, site-type, treatment, substrate), and tested differences among centroids using a multiple response permutation procedure (MRPP, Quinn and Keough 2002).

RESULTS

Predictor variables: tadpole abundance and nutrient concentrations. – Tadpoles occurred in No-Tadpole sites, indicating that the *a priori* designation of site-types was not maintained; furthermore, daily mean tadpole abundance was only 12.9 tadpoles higher at tadpole-aggregation sites than at no aggregation sites (Figure 4), and varied slightly between lakes and within lakes (Table 4);. The nutrient variables that best described variation among sites were variance of ammonia over time ($\sigma^2 NH_3$) and maximum nitrate + nitrite (NO_3+$NO_{2\ max}$), based on principal components analysis of twelve PO_4, NO_3+NO_2, and NH_3 variables (Table 3). According to the best fit linear mixed effects model (Table 5), $\sigma^2 NH_3$ declined farther from the center of sites and was lower on transects parallel to the shoreline; it also varied relative to substrate type, highest on rock and lowest on gravel (Table 6). Maximum NO_3+NO_2 increased with depth, declined farther from the centers of sites, and was negatively related to $\sigma^2 NH_3$, but the effect was small (Tables 7, 8). Neither $\sigma^2 NH_3$ or $NO_3+NO_{2\,max}$ differed with respect to tadpole abundance or site type.

Algal community composition. – Diatoms dominated whole algae communities in abundance (Figure 5) and in their contribution to variability among communities (Table 9, Figure 6). Green algae and other benthic material were the next most abundant algal types, and were also correlated with variation among communities. Blue-green algae and yellow-green algae were rare and uncorrelated with community variation. Environmental variables correlated with variation among communities included $\sigma^2 NH_3$, $NO_3+NO_{2\,max}$ concentration, distance from center of site, and water depth. These were all weak correlations (Table 9), but while green algae abundance was negatively correlated with these variables, diatom abundance was largely independent of them (Figure 6).

Algal communities were distinctly separated by substrate type and to a lesser extent by lake, though there appears to be high correlation between substrate type and lake (Figure 6). LeConte and Barrett lakes, which had mostly gravel, rock, and sand substrates had low overall abundances of algae, but diatoms and green algae were abundant on the silty substrates more characteristic of Center and Roland's Aquarium lakes (Figure 6). Tadpole variables appear to have little effect on the abundances of algal types; tadpole abundance was uncorrelated to variation in communities described by algal types, and communities did not separate by the categorical variables grazing treatment and site-type.

Diatom Abundance. – The response of diatom abundance to tadpole abundance depended on site-type (tadpole aggregation or no tadpoles) and lake (Figure 7). There was a significant interaction between tadpole abundance and site-type, and tadpole abundance had a negative effect on algal abundance only in no-aggregation sites (Table 11). The best fit mixed effects linear model of diatom abundance (Table 10) also included random intercepts for lake and site and allowed the variance of diatom abundance to differ by substrate, with the highest variance on rock substrate. Though grazing treatment was retained as a non-significant fixed effect, diatom abundance was highest in tadpole exclusions (Table 11).

Diatom Richness, Evenness, and Diversity. – Diatom community variables richness, evenness, diversity, and composition were calculated only for Barrett and Center lakes. I identified 36,438 individual diatom valves in 338 morphospecies from 81 community samples. Observed richness ranged from 41 – 71 morphospecies (mean ± SE 52.1 ± 0.7); richness rarefied at 375 individuals was 37-67 morphospecies (mean: 49.48 ± 0.67). Shannon evenness ranged from 0.68 – 0.88 (mean: 0.81 ± 0.004), and Shannon diversity ranged from 2.6 – 3.7 (mean: 3.2 ± 0.02). Site-wise species pools included 115, 122, and 173 taxa in Barrett and 159, 167, and 143 species in Center, and lake-wise species pools included 214 and 256 species in Barrett and Center, respectively (sampling effort was not equal among sites or lakes).

The best-fit linear mixed effects model of diatom community richness (Table 12) included distance, grazing treatment, and variance of ammonia concentration, all of which we consider potentially related to effects of tadpole aggregations. Distance and variance of ammonia concentration had negative effects on diatom richness; but richness was highest where tadpoles were excluded. NO_3+NO_2 max concentration had a large negative effect on richness and rock substrates had higher diatom richness than sand and gravel substrates. The model also included nested random intercepts for transect, site, and lake, and allowed variance to differ among levels of grazing treatment and substrate (Table 13).

Diatom community evenness declined slightly with σ^2 NH_3 and distance (Tables 14, 15). The model also allowed for different intercepts for sites, nested within lakes, and different variances for lakes and substrate types. Shannon diversity, like evenness, declined with distance and variance in ammonia concentration. The best fit random effects linear model (Table 16) included nested random effects for site, transect, and lake (Table 17). At the whole patch level, there were clearly no differences in richness, evenness or diversity in tadpole aggregation vs. no aggregation sites.

Diatom Communities. – Diatom communities differed much more between lakes than within lakes (MRPP, Figure 8), so I analyzed communities in each lake separately. Similarity of diatom communities in Barrett Lake was weakly correlated to distance from tadpole aggregation and to depth (Table 18), and communities differed weakly but significantly with respect to site-type (tadpole aggregation vs. no tadpoles), and strongly with respect to substrate; communities did not differ with respect to grazing treatment (Figure 9). Notably, there is a distinct group of diatom species negatively correlated with depth and distance and associated more with no-tadpole-aggregation sites and sandy substrates; this group is dominated by species in the genus *Pinnularia* (Figure 9, Table 19).

Similarities of diatom communities in Center Lake were strongly correlated with NO_3+$NO_{2\ max}$ concentration, distance from aggregation, abundance of tadpoles in aggregation, and weakly with depth (Table 20). There was some dissimilarity of communities in tadpole aggregation sites vs. no tadpole sites (MRPP, Figure 10); those in tadpole aggregation sites appear to be a subset of no tadpole aggregation sites. The association of communities with tadpole aggregation sites appears largely independent of the correlation of communities with the abundance of tadpoles in aggregations. There were no differences with respect to grazing treatment or substrate (Figure 10). In Center, there was a group of species negatively correlated with tadpole abundance and NO_3+NO_2 concentration (Figure 10, Table 21) but those taxa widely differ in genera and basic morphotype (includes araphid, monoraphid, symmetrical and asymmetrical biraphid diatoms).

DISCUSSION

Overall, producer communities did not respond as expected to the spatial and temporal nutrient variability that can be caused by the feeding and aggregating behavior of mountain yellow-legged frog tadpoles. Diatom diversity declined at points far from the centers of sites while it also declined as ammonia variance increased, regardless of the presence or abundance of tadpoles. As a result, at a larger scale, there was no difference in richness, evenness, or diversity between patches with versus those without tadpoles. Under the hypothesis that tadpole-generated nutrient heterogeneity should increase local producer diversity, these two results contradict one another. In addition, the occurrence of those patterns both where tadpoles did and did not aggregate does not support tadpole-generated heterogeneity as a driver of diatom community diversity. However, composition of diatom communities did differ between centers and edges of sites, and tadpole abundance and NO_3+NO_2 max concentration were also correlated with community differences in one lake.

Grazing exposure and tadpole abundance reduced algal and diatom abundance, but had no effect on community composition. My predictions that tadpole aggregations could create nutrient heterogeneity and producer community diversity would be enhanced by that heterogeneity were not strongly supported.

The lack of observed relationships between tadpole aggregations and diatom diversity could result from a lack of two steps in the proposed mechanism, either tadpoles do not aggregate and so do not create heterogeneity, or tadpoles do aggregate but whatever heterogeneity they create does not outweigh the influence of other processes in determining diatom community diversity and composition. The lack of the first step has some support, as the tendency to aggregate was not as widespread or consistent as previously thought. But even if tadpoles form aggregations infrequently, aggregations could still facilitate fluctuation-dependent mechanisms of coexistence of ammonia and nitrogen consumers, via storage effects of species capable of persisting or resting between ammonia pulses (Chesson 2000). Although I have observed the aggregations in Barrett lake in the same location on at least 30 days over the past decade, my current study and a follow-up synoptic survey I conducted in almost every large tadpole population in Kings Canyon National Park both indicate that aggregations do not regularly occur in every lake where tadpoles are abundant (T. Smith, unpublished data). So, in many lakes, the tadpole-generated driver of benthic producer diversity probably does not occur.

For those lakes and locations where tadpoles do aggregate, I still did not observe heterogeneity in ammonia and nitrogen that differed from locations without tadpole aggregations. But a large number of tadpoles in one area must produce a large amount of ammonia – which suggests the ammonia tadpoles excrete is quickly moved, used, or converted. Convective currents generated by wind or temperature (MacIntyre and Melack 1995) could carry ammonia out of littoral zones. Much of the ammonia could be consumed rapidly by nitrogen limited bacteria and producers, and ammonia is also rapidly converted to nitrites by bacteria (Bernhard 2010). Lastly, even if the ammonia subsidy is created by tadpole aggregations, it may be small relative to the ammonia in the lake. Sediments are a source for ammonia in lakes (Vincent and Downes 1981) and in the shallow littoral zones where tadpoles aggregate, the transition of sediment ammonia and nitrogen into the overlying water could overshadow the subsidy of ammonia excreted by tadpoles.

In many situations, nutrients excreted by animals do constitute a resource subsidy for surrounding communities, and while this nutrient cycling generally enhances primary productivity, its effects on producer diversity appear mixed or scale dependent. In experiments with phytoplankton, *Daphnia* and roach, nutrient cycling by grazers enhanced phytoplankton abundance, but it reduced phytoplankton richness and evenness (Attayde and Hansson 1999). Nitrogen and phosphorus subsidies by tropical tadpoles and freshwater fish alter community composition and enhance productivity (Vanni and Layne 1997, Knoll et al. 2009), though the top-down effects of grazing on algal abundance do seem to outweigh the bottom-up benefits of the grazer's excreta (Knoll et al. 2009). Due to differences in species composition and stream morphology, these subsidies are heterogeneous (McIntyre et al. 2008), and so could enhance diversity and differentiate species composition of producers at stream-reach or larger scales. Guano of sea-birds is also a large subsidy in coastal communities. On tropical islands, native and non-native trees respond distinctly to the nitrogen pulse from guano, such that the non-native trees may only be able to dominate where seabirds do not aggregate (Young et al. 2011). However, in small Arctic ponds, nitrogen from seabirds reduced diversity slightly, probably because concentrations were extreme enough to select against less nitrogen tolerant species and increase dominance; nonetheless, composition differed between ponds with and without the guano subsidy (Keatley et al. 2008). In these cases, the presence of nutrient rich excreta may enhance producer diversity over larger spatial scales, by driving differences in species composition between patches.

In many cases, the removal or extinction of any of these consumers would change the nutrient supply to parts of their habitats, and we might anticipate that those differences between-patch differences would also disappear. Without the spatial or temporal resource variability created by animal behavior and metabolism, fluctuation-dependent mechanisms of coexistence could break down, leading to homogenization of communities. My objective in this study was to investigate that potential with respect to local extinctions of a nutrient cycling tadpole. While variability created by mountain yellow-legged frog tadpoles occurs, it is not nearly as consistent or general as we initially predicted. That is probably the reason for my inability to detect dramatic differences among algal and diatom communities with respect to spatial and temporal variability in tadpole abundance, nitrogen concentrations, and proximity to aggregations. Without the influence of this biological interaction, producer communities seem to be shaped by abiotic interactions. The roles that species play in communities are not always strong relative to the role the environment plays, even when species can create “bottom-up” variation in the form of nutrient heterogeneity. While indirect positive interactions like this can be important in shaping communities (Stachowicz 2001) their importance probably depends on the community composition and relative influence of environmental variables (Harley 2003).

ACKNOWLEDGEMENTS

Sequoia-Kings Canyon National Park permitted the research, and I thank Danny Boiano for providing logistical support in the field. I thank Dan Dawson and the staff at the Sierra Nevada Aquatic Research Laboratory for providing research and housing facilities and for logistical support, and the Valentine Fund for financial support. This research was also supported by National Science Foundation Ecology and Evolution of Infectious Disease program (EF-0723563), by the Henry Luce Foundation Environmental Science to Solutions Fellowship, and UCSB Department of Ecology, Evolution, and Marine Biology. I thank Teo Pier for assistance in the field, Colin Sayre for assistance in the laboratory, and Kim Riley for processing and compiling diatom images. Sally Holbrook and Scott Cooper provided valuable comments that enhanced the research and the manuscript.

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Table 1. Descriptions of predictions, tests, and results.

Prediction:	Results:	Support for predictions?
Tadpoles create nutrient heterogeneity.	Tadpole abundances higher in aggregation patches. σ^2 NH3 and NO_3+NO_2 declined on the edges of sites, not correlated to tadpole presence or abundance.	Partial: Tadpole aggregations did not drive ammonia variability as clearly as predicted. ***Tables 4, 6, 7, 8.***
Producer diversity responds to heterogeneity a) richness increases b) evenness increases	Algae communities dominated by diatoms, differences correlated to σ^2NH3 and NO_3+NO_2 but not tadpole abundance. Diatom richness negatively related to σ^2NH3 and NO_3+NO_2, distance from center of site,. Evenness negatively related to distance and σ^2NH3. Community composition correlated to Distance, Tadpole Abundance, and NO_3+NO_2.	Partial: Richness responded to space as predicted, but not to actual nutrient concentrations. Correlations of distance, abundance, and nutrient concentrations with community composition existed, but contradicted predictions. ***Tables 9-21, Figs 2-7.***
Grazing reduces diatom abundance but increases richness and diversity.	Abundance negatively but weakly correlated with tadpole abundance, grazing exposure. Diversity and composition not different with respect to grazing exposure.	No.
Algal diversity increases where tadpoles aggregate.	Neither total richness, overall evenness nor diversity differed between site types.	No.
Between-site differences in algal communities enhance algal diversity in lakes where tadpoles are extant.	*Additional sampling of whole lakes is required. Samples have been collected.*	

Table 2. Characteristics of study lakes. A map can be viewed in Google Earth using this link, and selecting “View Raw”: https://github.com/TomCSmith/manuscript-support-files/blob/master/ThomasCSmith_NutrientHeterogeneityLakes_map.kmz

Lake Name	Elevation (m)	Max Depth (m)	Area (ha)	Perimeter (m)	East UTM (m)	North UTM (m)	Lake Substrate Composition (%)				Tadpoles‡
							Rock	Gravel	Sand	Silt	
LeConte	3298	14.9	2.6	657	354133	4109665	86	8	2	4	2038
Roland’s Aquarium	3388	12.8	2.9	762	362853	4103661	60	19	17	5	1160†
Columbine	3554	5.2	1.1	441	363520	4105613	84	3	3	11	1656†
Barrett	3495	14.8	4.0	935	363745	4105233	74	16	9	0	595†
Center	3430	1.8	0.9	358	379849	4064521	53	0	0	47	521
Marmot Lake*	3583	7.8	3.1	833	350765	4124856	85	9	4	1	2550†

* This is an official lake name; all others are colloquial or created for the manuscript.

† Maximum tadpole abundances pre-Bd decline: Barrett: 5300, Roland’s Aquarium: 2200, Columbine: 3400, Marmot: 4400.

‡ Tadpoles observed in most recent survey prior to this study.

Table 3. Principal components describing variability in nutrients among sampling points in Barrett and Center. Based on these PCA results we used variance of ammonia over time (σ^2 NH_3) and maximum nitrate + nitrite concentration (µmol L^{-1}, NO_2+NO_3) in subsequent linear models of diatom community diversity.

	PC 1	PC 2	PC 3
	98%	1.5%	0.7%
Minimum PO_4			
Maximum PO_4			
Mean PO_4			
σ^2 PO_4			
Minimum NO_2+NO_3		0.48	-0.28
Maximum NO_2+NO_3		**0.59**	-0.19
Mean NO_2+NO_3		0.53	-0.21
σ^2 NO_2+NO_3			
Minimum NH_3			
Maximum NH_3	0.18	0.36	0.90
Mean NH_3			
σ^2 NH_3	**0.98**	-0.11	-0.16

Table 4. Description of model of daily mean tadpole abundance at sites within lakes. Two Site Types are Tadpole Aggregation, where tadpole aggregations were observed at the beginning of 2011 ice-free season, and No Aggregation, where tadpole aggregations were not seen prior to the start of the experiment.

	Effect	t_{113}	p	Random intercept
Site Type	12.9	2.6	0.048	
Lake				~ N(0, 0^2)
Site				~ N(0, 27^2)

Table 5. Model selection for temporal variance in ammonia concentration (σ^2 NH_3)as a response variable and number of tadpoles at the center of an aggregation as a predictor variable, with potential covariates distance from center of aggregation (Distance), Lake, Site, Transect, Site Type (Tadpole aggregation vs. No aggregation), and Substrate.

Fixed Effects	Response variable	Random effects	Variance structure	Δ AIC
Tadpole Abundance + Lake + Site + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3			941
Tadpole Abundance + Lake + Site + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3 ⇑			49
Tadpole Abundance + Site + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Lake ⇑		68
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake ⇑		74
Tadpole Abundance + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Transect / Site / Lake ⇓		36
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Lake	72
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Site	54
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Transect	37
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Point	60
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Substrate	57
Tadpole Abundance + Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Lake and Site	2.7
Transect + Distance + Site Type + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Lake and Site	0.99
Transect + Distance + Substrate	$\log_{10} \sigma^2$NH3	Site / Lake	Lake and Site	0

⇑⇓ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

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Table 6. Description of best fit model of variance of ammonia concentration ($\log_{10} \sigma^2 NH3$). For transects, parallel indicates those that were approximately parallel to the shoreline, while perpendicular indicates those that were approximately perpendicular and extended into the lake.

	Effect	Test statistic*	p	Random intercept	Residual variance
Distance	-1.2	$t_{105} = -11.9$	$P < 0.001$		
Substrate	Rock > Sand = Silt > Gravel	$\chi^2_{14} = 14.4$	$P = 0.002$		
Transect	Perpendicular > Parallel	$\chi^2_{14} = 45.8$	$P < 0.001$		
Lake				$\sim N(0, 0.71^2)$	$\sigma^2_{LeConte} = 1.3^2$ $\sigma^2_{Roland's\ Aquarium} = 0.17^2$ $\sigma^2_{Barrett} = 0.24^2$ $\sigma^2_{Center} = 1.1^2$
Site				$\sim N(0, 0.69^2)$	$\sigma^2 = 0.45^2 – 4.6^2$

*For categorical variables Substrate and Transect, significance level in model given by likelihood ratio test using a Chi-square test statistic.

Table 7. Model selection for NO3+NO2 max concentration as a response variable and number of tadpoles at the center of an aggregation as a predictor variable, with potential covariates distance from center of aggregation (Distance), Lake, Site, Transect, Site Type (Tadpole aggregation vs. No aggregation), substrate, and variance of ammonia concentration.

Fixed Effects	Response variable	Random effects	Variance structure	Δ AIC
Tadpole Abundance + σ^2 NH_3+ Lake + Site + Transect + Distance + Site Type + Substrate + Depth	Max. NO_3+NO_2			619.3
Tadpole Abundance + σ^2 NH_3+ Lake + Site + Transect + Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2) ⇑			153.8
Tadpole Abundance + σ^2 NH_3+ Site + Transect + Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake ⇑		181.0
Tadpole Abundance + σ^2 NH_3+ Transect + Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site ⇑		170.7
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect ⇑		75.3
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Lake	61.1
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Site	30.4
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Transect	82.1
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Substrate	50.5
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Lake and Site	12.4
Tadpole Abundance + σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Site and Substrate	4.0
σ^2 NH_3+ Distance + Site Type + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Site and Substrate	2.0
σ^2 NH_3+ Distance + Substrate + Depth	$\log_{10}$(Max. NO_3+NO_2)	Lake / Site / Transect	Site and Substrate	0

⇑⇓ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

Table 8. Description of best fit model of NO3+NO2 max concentration.

	Linear model coefficient	Test statistic*	p	Random intercept	Residual variance
Distance	-0.005	$t_{91} = 2.7$	< 0.001		
Depth	0.003	$t_{91} = 7.5$	< 0.001		
σ^2 NH_3	-0.0003	$t_{91} = -2.8$	0.007		
Substrate	Gravel = Rock > Sand = Silt	$\chi^2_{14} = 7.8$	0.05		$\sigma^2_{Gravel} = 0.04^2$ $\sigma^2_{Rock} = 0.04^2$ $\sigma^2_{Sand} = 0.007^2$ $\sigma^2_{Silt} = 0.007^2$
Lake				$\sim N(0, 0.34^2)$	
Site				$\sim N(0, 0.08^2)$	$\sigma^2 = 0.002^2 - 0.04^2$
Transect				$\sim N(0, 0.07^2)$	

*For categorical variable Substrate, significance level in model given by likelihood ratio test using a Chi-square test statistic, with 14 degrees of freedom.

Table 9. Algae type relative abundance correlations with NMDS axes. Stress = 0.06.

	NMDS 1	NMDS 2	r^2	p
Algae Types				
Diatoms	-0.61	0.41	0.54	0.001
Green algae	-0.15	-0.58	0.36	0.001
Other	-0.25	0.41	0.23	0.001
Distance	0.09	0.25	0.07	0.03
Yellow algae	-0.16	-0.20	0.06	0.06
Blue-green algae	-0.05	-0.05	0.00	0.80
Environmental Variables				
$NO_3 + NO_2$	-0.02	0.34	0.12	0.01
Maximum NH_3	0.02	0.27	0.08	0.02
Depth	-0.03	0.27	0.07	0.03
σ^2 NH_3	0.03	0.22	0.05	0.09
Tadpole Abundance	0.02	-0.18	0.03	0.21

Table 10. Model selection for diatom abundance as a response variable and tadpole abundance (average at the center of the site) as a predictor variable, with potential covariates ammonia variance (σ2NH3), maximum Nitrate + Nitrite (NO_3+NO_2), Lake, Site, Transect, Distance from center, Site Type (Tadpole aggregation vs. No aggregation), Grazing Treatment, and Substrate.

Fixed Effects	Response variable	Random effects	Variance structure	ΔAIC
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	Diatom Abundance			1273
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance ⇑			7.5
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance	Lake ⇑		28.8
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance	Lake / Site ⇑		22.2
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance	Lake / Site	Lake ⇓	27.2
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance	Lake / Site	Site	22.5
Lake+Site+Transect+Point+Treatment × SiteType × Tadpole Abundance + Depth + Substrate + σ^2 NH_3+ NO_3+NO_2	$\log_{10}$ Diatom Abundance	Lake / Site	Substrate	7.7
Treatment + SiteType × Tadpole Abundance + Substrate	$\log_{10}$ Diatom Abundance	Lake / Site	Substrate	0

⇑⇓ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

Table 11. Description of best fit model of $\log_{10}$ diatom abundance.

	Effect	Test statistic*	p	Random intercept	Variance
Site Type × Tadpole Abundance	0.02	$\chi^2_{12} = 16.1$	0.01		
Tadpole: Tadpole Abundance	-0.002	$\chi^2_{12} = 0.39$	0.53		
No Tadpole: Tadpole Abundance	-0.03	$\chi^2_{12} = 9.6$	0.002		
Treatment	Exclusion > Exclusion Control > No Control	$\chi^2_{14} = 2.2$	0.33		
Substrate	Silt = Sand > Gravel > Rock	$\chi^2_{14} = 21.9$	< 0.001		$\sigma^2_{Rock} = 0.68^2$ $\sigma^2_{Sand} = 0.21^2$ $\sigma^2_{Silt} = 0.41^2$ $\sigma^2_{Gravel} = 0.29^2$
Lake				$\sim N(0, 0.25^2)$	
Site				$\sim N(0, 0.00005^2)$	

Table 12. Model selection for diatom community richness, using richness rarefied for 375 individuals as a response variable and distance from center of aggregation (Distance) as a predictor variable, with potential covariates ammonia variance (σ2NH3), maximum Nitrate + Nitrite (NO_3+NO_2), Lake, Site, Transect, Site Type (Tadpole aggregation vs. No aggregation), Grazing Treatment, and Substrate.

Fixed Effects	Random effects	Variance structure	Δ AIC
Lake + Site + Transect + Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2			7.0
Lake + Site + Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Transect		7.0
Lake + Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect		10.9
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake ⤊		22.5
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Lake	20.7
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Substrate	5.3
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Lake and Substrate	7.1
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Treatment	9.1
Distance + Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Substrate and Treatment	1.3
Distance + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site / Transect / Lake	Substrate and Treatment	0

⤊⤋ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

Table 13. Description of best fit model of diatom community richness, rarefied for 375 individuals.

	Linear model coefficient	Test statistic*	p	Random intercept	Residual variance
Distance	-0.76	$t_{58} = -4.4$	< 0.001		
σ^2 NH_3	-0.04	$t_{58} = -3.1$	0.003		
NO_3+NO_2	-3.3	$t_{58} = -5.7$	< 0.001		
Treatment	Exclusion Control > Exclusion > No Exclusion	$\chi^2_{14} = 9.8$	0.01		$\sigma^2_{\text{Exclusion Control}} = 0.55^2$ $\sigma^2_{\text{Exclusion}} = 1.4^2$ $\sigma^2_{\text{No exclusion}} = 1.3^2$
Substrate	Rock > Sand > Gravel	$\chi^2_{13} = 12.0$	0.003		$\sigma^2_{\text{Rock}} = 3.6^2$ $\sigma^2_{\text{Sand}} = 1.4^2$ $\sigma^2_{\text{Gravel}} = 1.6^2$
Lake				~ $N(0, 13^2)$	
Site				~ $N(0, 0.0003^2)$	
Transect				~ $N(0, 4.0^2)$	

*For categorical variables Treatment and Substrate, significance level in model given by likelihood ratio test using a Chi-square test statistic.

Table 14. Model selection for diatom community evenness, using Shannon Evenness (J) as a response variable and distance from center of aggregation (Distance) as a predictor variable, with potential covariates ammonia variance (σ2NH3), maximum Nitrate + Nitrite (NO_3+NO_2), Lake, Site, Transect, Site Type (Tadpole aggregation vs. No aggregation), Grazing Treatment, and Substrate.

Fixed effects	Random effects	Variance Structure	Δ AIC
Lake + Site + Transect + Distance +Depth+Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2			16.2
Site + Transect + Distance +Depth+Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Lake		17.1
Site + Transect + Distance +Depth+Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Lake/Site		22.8
Transect + Distance +Depth+Site Type + Treatment + Substrate + σ^2 NH_3+ NO_3+NO_2	Site/Lake	Lake and Substrate	11.7
Distance + σ^2 NH_3+ NO_3+NO_2 + Substrate +Site Type + Depth + Transect	Site/Lake	Lake and Substrate	8.5
Distance + σ^2 NH_3+ NO_3+NO_2 + Substrate +Site Type + Depth	Site/Lake	Lake and Substrate	4.9
Distance + σ^2 NH_3+ NO_3+NO_2 + Substrate +Site Type	Site/Lake	Lake and Substrate	4.0
Distance + σ^2 NH_3+ NO_3+NO_2 + Substrate	Site/Lake	Lake and Substrate	2.3
Distance + σ^2 NH_3+ NO_3+NO_2	Site/Lake	Lake and Substrate	1.5
Distance + σ^2 NH_3	Site/Lake	Lake and Substrate	0

⇑⇓ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

Table 15. Description of best fit model of diatom community Shannon Evenness.

	Linear model coefficient	t_{73}	p	Random intercept	Residual variance
Distance	-0.007	-5.1	< 0.001		
σ^2 NH_3	-0.0003	-2.6	0.01		
Site				$\sim N(0, 0.008^2)$	
Lake				$\sim N(0, <0.001^2)$	$\sigma^2_{Barrett} = 0.02^2$ $\sigma^2_{Center} = 0.03^2$
Substrate					$\sigma^2_{Rock} = 0.03^2$ $\sigma^2_{Sand} = 0.02^2$ $\sigma^2_{Gravel} = 0.02^2$

Table 16. Model selection for diatom community diversity, using Shannon diversity as a response variable and distance from center of aggregation (Distance) as a predictor variable, with potential covariates ammonia variance (σ2NH3), maximum Nitrate + Nitrite (NO_3+NO_2), Lake, Site, Transect, Site Type (Tadpole aggregation vs. No aggregation), Grazing Treatment, and Substrate.

Fixed Effects	Random intercept	Δ AIC
Lake + Site + Transect + Distance + Site Type + Treatment + Substrate + σ^2 NH_3 + NO_3+NO_2		-9.2
Lake + Site + Distance + Site Type + Treatment + Substrate + σ^2 NH_3 + NO_3+NO_2	Transect	-8.5
Lake + Distance + Site Type + Treatment + Substrate + σ^2 NH_3 + NO_3+NO_2	Transect / Site	-2.3
Distance + Site Type + Treatment + Substrate + σ^2 NH_3 + NO_3+NO_2	Transect / Site / Lake ⇑	7.0
Distance + Site Type + Treatment + Substrate + σ^2 NH_3	Transect / Site / Lake	5.0
Distance + Treatment + Substrate + σ^2 NH_3	Transect / Site / Lake	3.0
Distance + Substrate + σ^2 NH_3	Transect / Site / Lake	1.1
Distance + σ^2 NH_3	Transect / Site / Lake	0

⇑⇓ arrows indicate a conspicuous increase or decrease in the homogeneity of residuals, assessed graphically.

Table 17. Description of best fit model of diatom community Shannon diversity.

	Linear model coefficient	t_{63}	p	Random intercept
Distance	-0.04	-4.6	<0.001	
σ^2 NH_3	-0.001	-2.0	0.05	
Lake				~ N(0, 0.000022)
Site				~ N(0, 0.092)
Transect				~ N(0, 0.042)

Table 18. Correlations of environmental variables with variation among diatom communitt samples in Barrett lake, on two NMDS axes. Stress = 0.14

Environmental Variables	NMDS 1	NMDS 2	r^2	p
Depth	0.92	0.40	0.15	0.04
Distance	0.95	-0.32	0.14	0.04
$NO_3 + NO_2$	0.99	0.10	0.03	0.59
Tadpole Abundance	0.74	0.67	0.02	0.61
Maximum NH_3	-0.99	-0.16	0.02	0.66
σ^2 NH_3	-0.96	-0.27	0.01	0.90

Table 19. Correlations ($p < 0.05$) of diatom taxa with variation among community samples in Barrett lake

Species	NMDS 1	NMDS 2	r^2	P
Psammothidium helveticum (Hustedt) Bukhtiyarova et Round 1996	0.65	-0.76	0.79	0.00001
Encyonema perpusillum (A. Cleve) Mann in Round, Crawford & Mann 1990	-0.90	-0.44	0.74	0.00001
Pinnularia pseudogibba Krammer 1992	-0.86	-0.51	0.70	0.00001
Aulacoseira alpigena (Grunow) Krammer	0.12	0.99	0.69	0.00001
Eunotia arcus morphotype group	-0.88	-0.47	0.66	0.00001
Pinnularia divergens var. sublinearis	-0.95	-0.30	0.63	0.00001
Pinnularia cf. rupestris Hantzsch in Rabenhorst 1861 TCS4428	-0.97	-0.23	0.63	0.00001
Pinnularia acuminata W. Smith 1853	-0.86	-0.51	0.50	0.00002
Psammothidium curtissimum (J.R. Carter) Aboal in Aboal, Alvarez-Cobelas, Cambra & Ector 2003	0.97	0.26	0.48	0.00002
Encyonema sp. SND61	-0.04	1.00	0.41	0.00003
Craticula submolesta (Hustedt) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996	0.92	-0.39	0.35	0.0002
Psammothidium acidoclinatum (Lange-Bertalot in Lange-Bertalot & Metzeltin) H. Lange-Bertalot 1999	0.97	0.26	0.33	0.0002
Pinnularia divergentissima (Grunow in Van Heurck) Cleve 1895	-0.88	-0.47	0.34	0.0004
Pinnularia rhombarea Krammer in Metzeltin & Lange-Bertalot 1998	-0.98	-0.22	0.38	0.0005
Pinnularia divergens var. *linearis*	-0.99	0.16	0.32	0.0009
Pinnularia microstauron var. *rostrata* K. Krammer 2000	-0.99	-0.11	0.27	0.002
Surirella linearis W. Smith 1853	0.25	0.97	0.26	0.002
Pinnularia cf. lata (Brébisson) W. Smith 1853	-1.00	-0.10	0.28	0.0024
Gomphonema productum (Grunow) Lange-Bertalot & Reichardt	-0.94	-0.35	0.26	0.002
Pinnularia borealis var. *subislandica* K. Krammer 2000	-0.87	-0.50	0.27	0.004
Neidium ampliatum (Ehrenberg) Krammer in Krammer & Lange-Bertalot 1985	-0.61	-0.80	0.24	0.004
Pinnularia microstauron var. *angusta* K. Krammer 2000	0.43	0.90	0.25	0.006
Achnanthidium minutissimum NL	-0.91	0.41	0.25	0.007
Pinnularia viridiformis Krammer 1992	-0.96	0.27	0.25	0.007
Pinnularia borealis var. *islandica* K. Krammer 2000	-0.82	0.57	0.24	0.008
Stenopterobia delicatissima (Lewis) Van Heurck 1896	0.97	0.24	0.20	0.01
Fragilaria cf. crotonensis Kitton 1869	-0.81	0.58	0.25	0.01
Eunotia praerupta Ehrenberg 1843	-0.82	-0.57	0.20	0.01
Caloneis cf. lauta Carter 1981	-0.90	-0.44	0.19	0.01
Chamaepinnularia mediocris (Krasske) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996	0.20	0.98	0.19	0.01
Fragilaria exigua Grunow in Cleve & Möller 1878	-0.71	0.70	0.23	0.01
Brachysira brebisonii Ross in Hartley 1986	-0.74	0.68	0.18	0.02
Pinnularia aff. *soehrensis* var. *linearis* (Krasske) Petersen	0.38	-0.92	0.22	0.02
Encyonema cf. kalbei Krammer 1997	0.10	0.99	0.18	0.02
Psammothidium altaicum Bukhtiyarova in Bukhtiyarova & Round 1996	0.33	-0.94	0.20	0.02
Encyonopsis lanceola (Grunow) Krammer 1997	-0.99	0.16	0.18	0.02
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round 1996	0.96	0.28	0.17	0.02
Gomphonema micropus Kützing 1844	0.41	0.91	0.17	0.02
Nitzschia acicularoides morphotype group	0.38	0.93	0.15	0.04
Navicula sp. SND76 hour-glass shaped central area	0.27	0.96	0.17	0.04
Frustulia saxonica Rabenhorst 1848-1860	0.08	1.00	0.15	0.04
Pinnularia obscura Krasske 1932	-0.82	0.58	0.18	0.04

Table 20. Correlations of continuous environmental variables to variation among community samples in Center lake. Stress = 0.15.

Environmental Variables	NMDS 1	NMDS 2	r^2	p
$NO_3 + NO_2$	-0.57	-0.82	0.77	0.001
Distance	-0.90	0.43	0.53	0.001
Tadpole Abundance	-0.71	-0.70	0.46	0.001
Depth	-0.09	1.00	0.17	0.04
Maximum NH3	0.88	-0.47	0.05	0.44
σ^2 NH3	0.49	-0.87	0.04	0.55

Table 21. Correlations ($p < 0.05$) of diatom taxa with variation among community samples in Center lake

Species	NMDS 1	NMDS 2	r^2	Pr(>r)
Staurosirella pinnata (Ehrenberg) Williams & Round 1987	0.07	-1.00	0.60	0.00001
Diploneis elliptica (Kützing) Cleve 1894	-1.00	-0.08	0.55	0.00001
Brachysira brebisonii Ross in Hartley 1986	0.83	0.56	0.54	0.00001
Fragilaria exigua Grunow in Cleve & Möller 1878	0.94	0.35	0.54	0.00001
Cymbopleura naviculiformis (Auerswald) Krammer 2003 var. naviculiformis	0.98	0.18	0.50	0.00001
Navicula pseudobryophila Hustedt 1942	0.39	0.92	0.45	0.00003
Aulacoseira italica (Ehr.)	0.99	-0.11	0.35	0.00003
Encyonema lunatum var. borealis Krammer 1997	0.96	0.27	0.46	0.00005
Frustulia saxonica Rabenhorst 1848-1860	0.91	0.41	0.41	0.00007
Denticula kuetzingii Grunow	-0.82	-0.57	0.37	0.00007
Sellaphora pupula (Kützing) Mereschkowsky 1902	0.82	-0.58	0.42	0.0001
Navicula densilineolata (Lange-Bertalot) Lange-Bertalot 1993	-0.91	-0.42	0.42	0.0001
Psammothidium altaicum Bukhtiyarova in Bukhtiyarova & Round 1996	0.77	0.64	0.38	0.0002
Pinnularia tirolensis (Metzeltin & Krammer) K. Krammer 2000	1.00	-0.06	0.38	0.0003
Chamaepinnularia begeri (Krasske) Lange-Bertalot in Lange-Bertalot & Metzeltin 1996	0.93	0.36	0.34	0.0005
Caloneis thermalis (Grunow) Krammer 1985	-0.51	0.86	0.34	0.0009
Psammothidium acidoclinatum (Lange-Bertalot in Lange-Bertalot & Metzeltin) H. Lange-Bertalot 1999	0.93	0.37	0.33	0.0009
Adlafia bryophila (Petersen) Moser, Lange-Bertalot and Metzeltin 1998	-0.71	-0.70	0.31	0.001
Aulacoseira alpigena (Grunow) Krammer	0.71	0.70	0.30	0.001
Gomphonema micropus Kützing 1844	0.98	-0.18	0.27	0.001
Encyonopsis cesatii (Rabenhorst) Krammer 1997	-0.10	0.99	0.30	0.002
Nupela fennica (Hustedt) Lange-Bertalot 2004	-0.85	0.52	0.30	0.002
Stauroneis acidoclinata H. Lange-Bertalot & M. Werum in Werum & Lange-Bertalot 2004	-0.91	0.42	0.30	0.002
Psammothidium daonense (Lange-Bertalot in Lange-Bertalot & Krammer) H. Lange-Bertalot 1999	0.99	-0.16	0.25	0.004
Brachysira cf. *microcephala* (Grunow) Compere 1986 rounded-subcapitate morphotype	-0.05	1.00	0.27	0.005
Pinnularia microstauron var. *rostrata* K. Krammer 2000	-1.00	0.01	0.25	0.007
Araphid two dots	0.13	0.99	0.25	0.008
Pseudostaurosira robusta (Fusey) Williams & Round 1987	0.93	-0.37	0.22	0.008
Achnanthidium minutissimum NL-COARSE	-0.90	0.43	0.22	0.009
Staurosirella martyi (Héribaud) E.A. Morales & K.M. Manoylov 2006	-0.77	-0.64	0.24	0.01
Encyonema minutum (Hilse in Rabenhorst) Mann in Round, Crawford & Mann 1990	-0.73	-0.68	0.23	0.01
Chamaepinnularia hassiaca (Krasske) Cantonati & Lange-Bertalot 2009	-0.27	0.96	0.21	0.02
Staurosirella oldenburgiana (Hustedt) Morales 2005	0.74	-0.68	0.21	0.02
Encyonema subminutum Krammer 1997	-0.53	0.85	0.21	0.02
Pinnularia divergens var. *sublinearis*	0.35	0.93	0.20	0.02
Aulacoseira distans (Ehr.)	0.87	0.50	0.19	0.03
Encyonopsis cf. *cesatii* (Rabenhorst) Krammer 1997 cf. subspicula Krammer 1997	-0.61	-0.79	0.19	0.03
Achnanthidium minutissimum species complex (Potapovae and Hamilton 2007)	-0.30	-0.95	0.18	0.03
Amphora cf. *veneta* Kutzing	-0.78	-0.62	0.18	0.03
Cymbopleura cf. *incerta* (Krammer) Krammer 1997	0.25	-0.97	0.18	0.03
Diadesmis cf. *ingraeformis*	-0.99	0.14	0.18	0.03
Staurosirella cf. *oldenburgiana* (Hustedt) Morales 2005	-0.87	-0.50	0.18	0.03
Eunotia meisteri Hustedt 1930	0.99	0.13	0.17	0.03

Table 22. A partial list of references used for diatom taxonomy.

1. Achnanthes, eine Monographie der Gattung : mit Definition der Gattung Cocconeis und Nachträgen zu den Naviculaceae. (Lange-Bertalot 1989)
2. Brachysira : monographie der Gattung wichtige Indikator - Species für das Gewässer-Monitoring und Naviculadicta nov. gen. ein Lösungsvarschlag zu dem Problem Navicula sensu lato ohne. (Lange-Bertalot 1994)
3. Diatomeen im Süsswasser-Benthos von Mitteleuropa : Bestimmungsflora Kieselalgen für die ökologische Praxis : über 700 der häufigsten Arten und ihre Ökologie. (Hofmann 2011)
4. Diatoms from British Columbia (Canada) lakes and their relationship to salinity, nutrients and other limnological variables. (Lange-Bertalot 1995)
5. Diatoms of Europe (Lange-Bertalot and Krammer 2000a)
6. Diatoms of Europe : diatoms of the European inland waters and comparable habitats, vol. 1-6. (Lange-Bertalot 2011)
7. Diatoms of the Andes: from Venezuela to Patagonia Tierra del Fuego and two additional contributions. (Rumrich 2000)
8. Diatoms of the United States. (Spaulding et al. 2010)
9. Die cymbelloiden Diatomeen: eine Monographie der weltweit bekannten Taxa. (Krammer 1997)
10. Eunotia Ehrenberg (Bacillariophyta) of the Great Smoky Mountains National Park, USA (Furey 2011)
11. Naviculaceae : neue und wenig bekannte Taxa, neue Kombinationen und Synonyme sowie Bemerkungen zu einigen Gattungen. (Krammer 1985)
12. Süsswasserflora von Mitteleuropa, Bd. 1-4; (Lange-Bertalot and Krammer 2000b)
13. The Diatoms of the United States (exclusive of Alaska and Hawaii), Vol. 1: Fragilariaceae, Eunotiaceae, Achnanthaceae, Naviculaceae (Patrick and Reimer 1966)
14. The diatoms of the United States (exclusive of Alaska and Hawaii). Vol. 2: Entomoneidaceae, Cymbellaceae, Gomphonemaceae, and Epithemiales (Patrick and Reimer 1975)
15. The Diatoms: Biology and Morphology of the Genera (Round et al. 1990)
16. The genus Stauroneis in the Arctic and (sub)-Antarctic regions. (Vijver 2004)

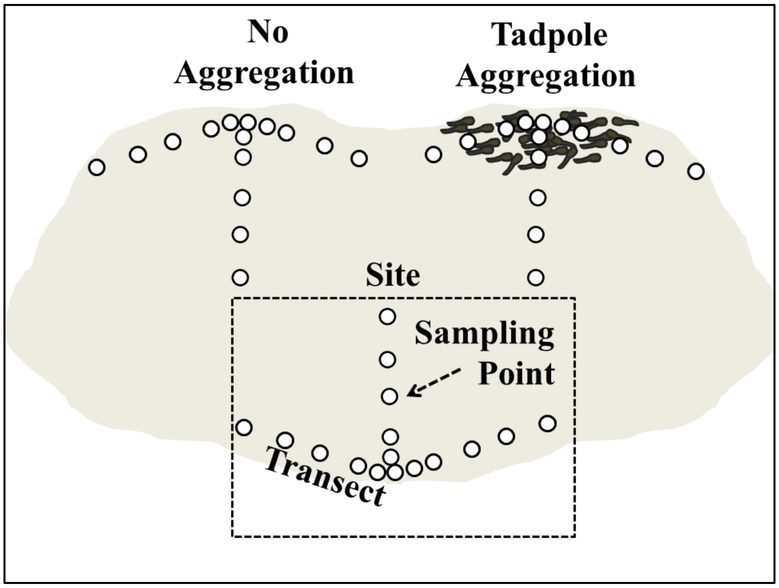

Figure 1. Sampling design in a high elevation lake.

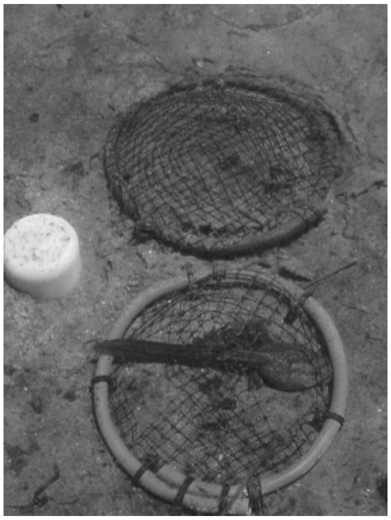

Figure. 2. Tadpole exclusions on the benthos in a lake. A *Rana sierrae* tadpole is shown having swum into the exclusion control.

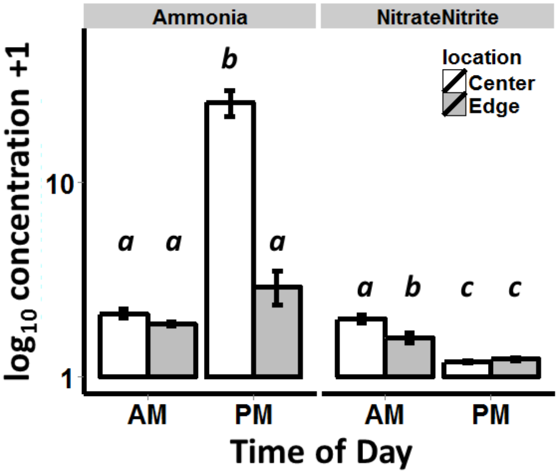

Figure 3. Preliminary measurements of nitrogen (µM Ammonia and Nitrate+Nitrite) in and around tadpole aggregations, in the morning and in the afternoon. Letters indicate differences ($p < 0.05$, ANOVA, Tukey HSD).

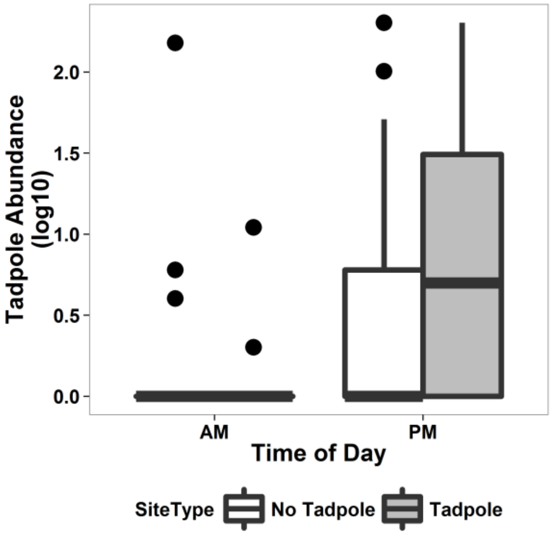

Figure 4. Abundance of tadpoles in No Tadpole (4 sites) and Tadpole aggregation sites (7 sites) in 4 lakes. Every site had at least 20 tadpoles on at least one day. Boxplots indicate distribution of data in each category, in the mornings (AM) and afternoons (PM); thick bars are medians, boxes include 50% of the data, and whiskers include the most extreme data points within 1.5 x the length of the box, points lie outside that distance.

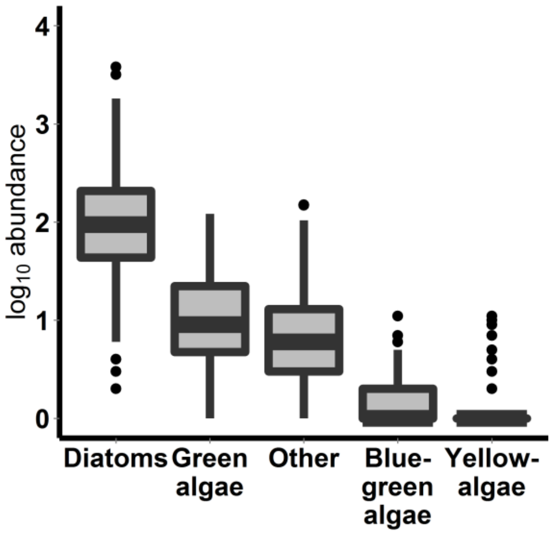

Figure 5. Abundance of algae types in benthic producer samples; thick bars are medians, boxes include 50% of the data, and whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box. Subsequently, we restricted our analyses to diatoms.

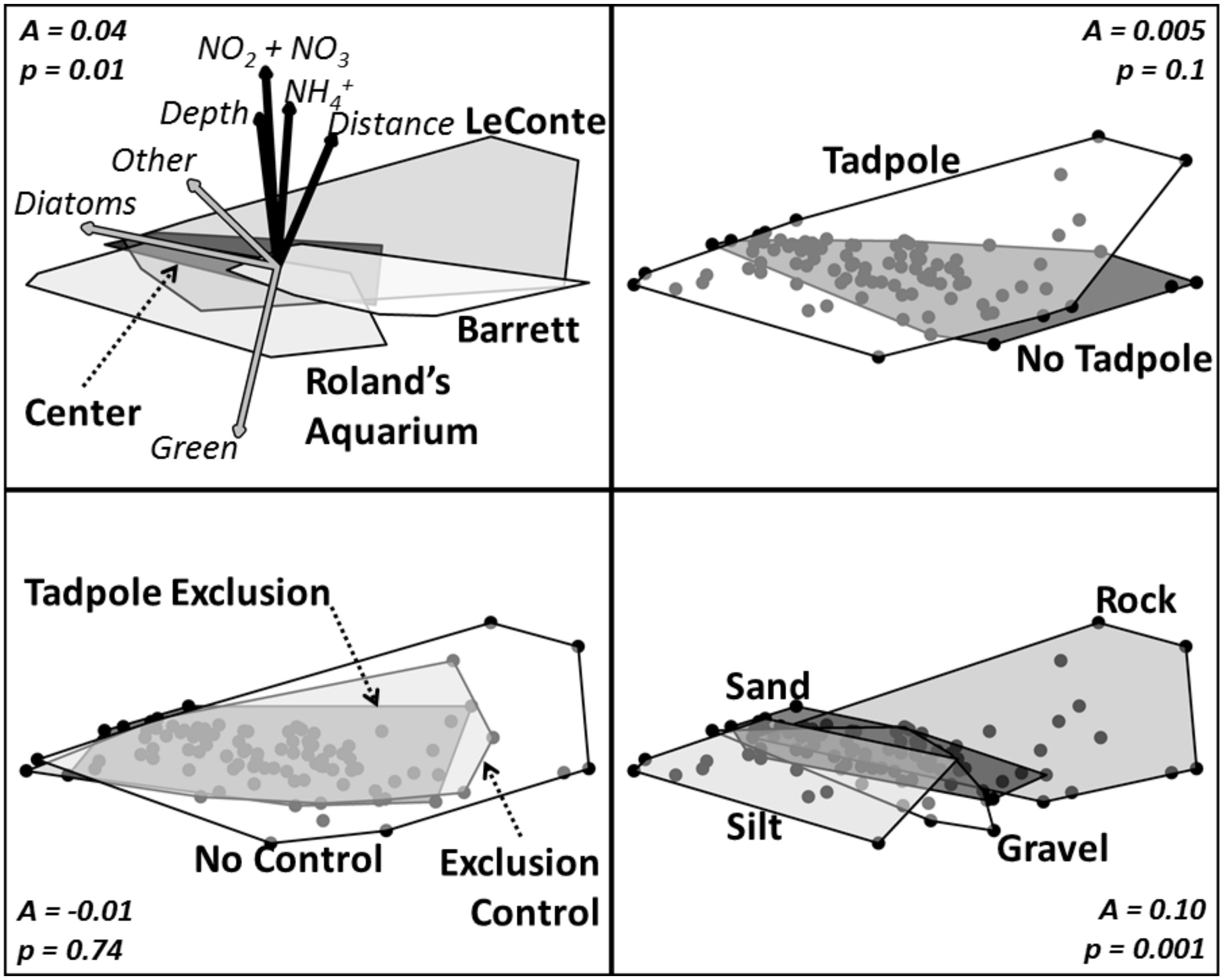

Figure 6. Ordination of relative abundances of algae types in communities. Points are in the same locations in each panel (omitted from upper left for clarity). Shaded polygons and bold text indicate the scope of each factor level; factors were, clockwise from top left Lake, Site Type (Tadpole aggregation or No tadpole aggregation), substrate, or grazing treatment. In upper left panel, vectors and italicized text indicate correlations of continuous environmental variables (black vectors) and of abundances of algae types (all correlations $p < 0.05$, Table 9). Results for comparison of group differences (MRPP) are shown in outer corners of each panel. Stress for ordination in two axes = 0.06.

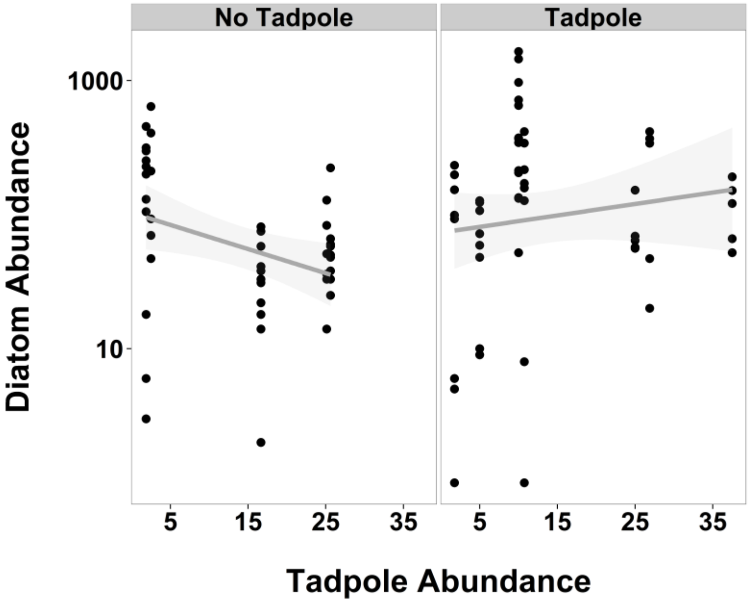

Figure 7. Diatom abundance with respect to interaction between site type (Tadpole aggregation vs. no aggregation) and mean tadpole abundance. Lines and shaded regions are linear fits with 95% confidence regions.

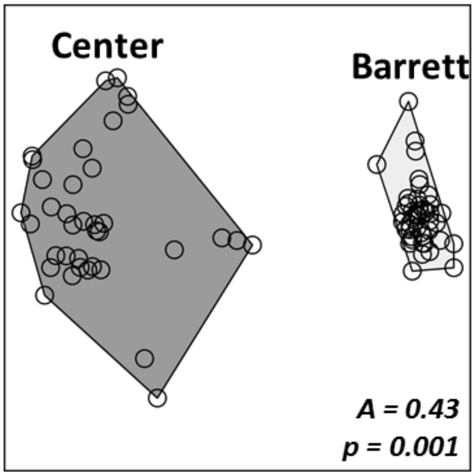

Figure 8. Ordination of diatom communities in in the two lakes for which we studied diatom community composition (NMDS), stress = 0.06, correlation of original to ordination distances = 98%. Differences between diatom communities calculated by MRPP using absolute abundance to describe communities. Between-group distance = 0.93, within group distances = 0.25 and 0.50 for Barrett and Center respectively. Subseqeuntly, we made only within-lake comparisons of diatom communities.

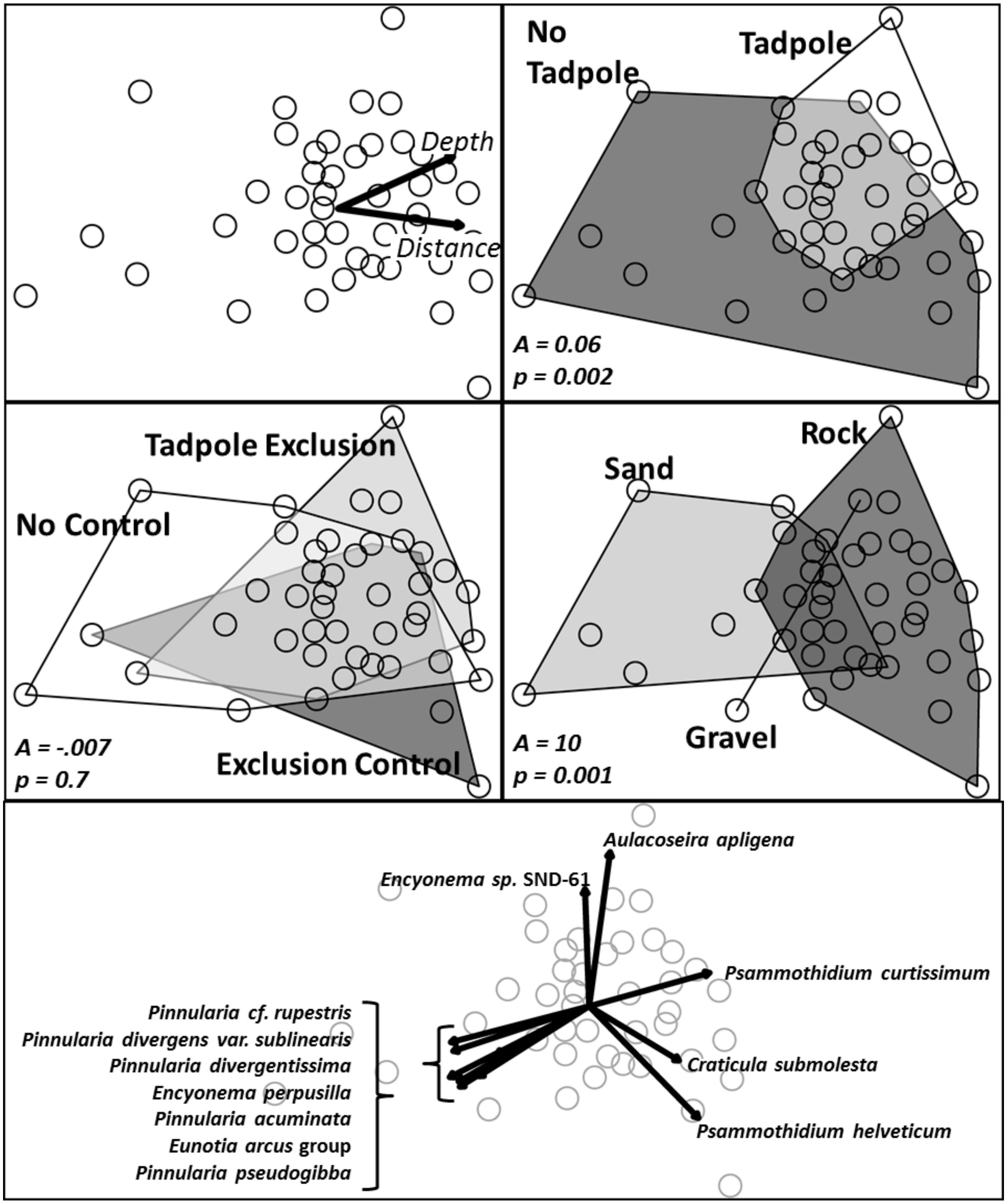

Fig 9. Ordinations (NMDS) of diatom community differences in Barrett, with respect to continuous environmental covariates ($p<=0.05$), categorical frog variables and environmental covariates (MRPP), and with respect to diatom taxa abundances ($p < 0.001$). Stress = 0.14.

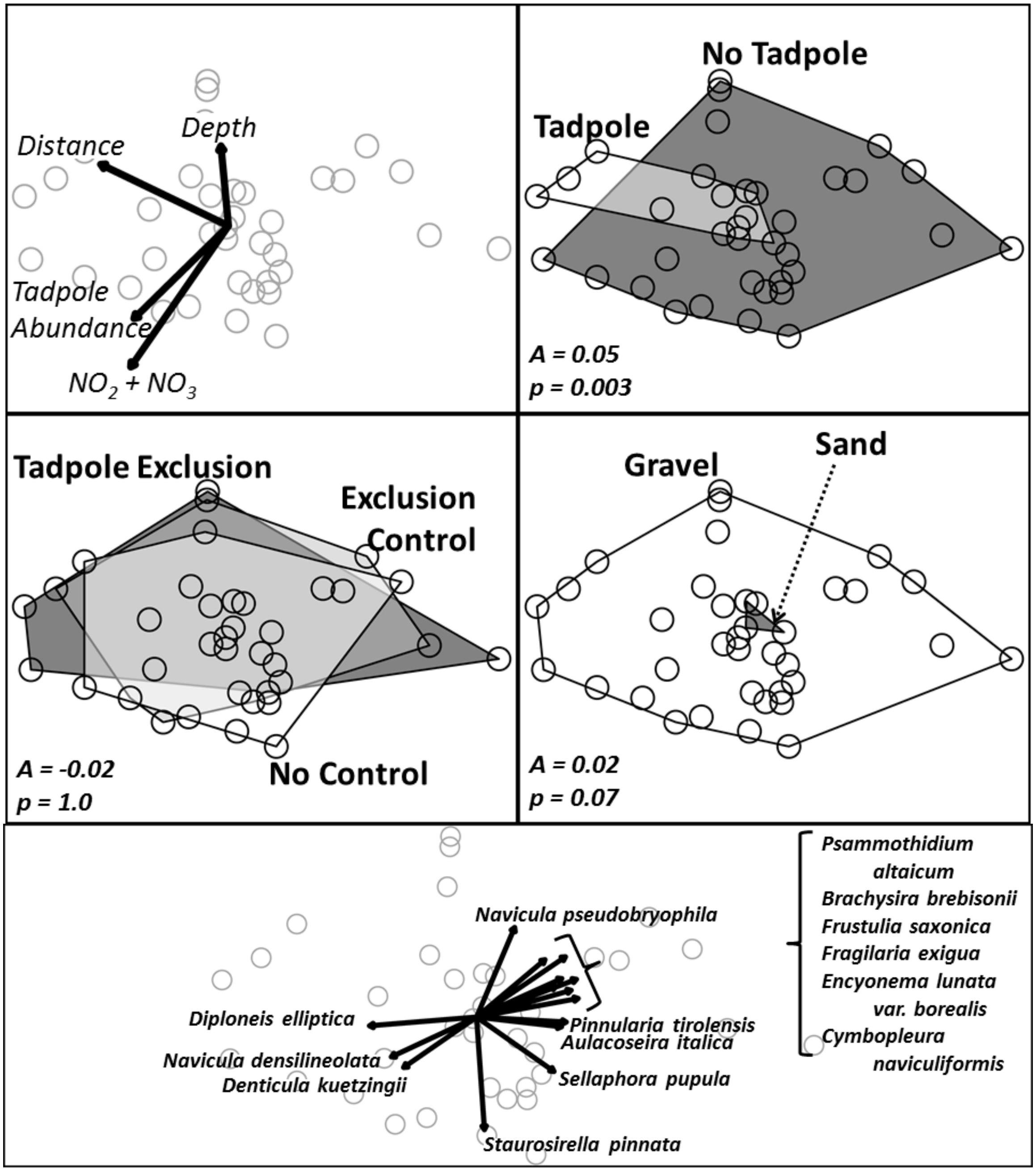

Fig 10. Ordinations (NMDS) of diatom community differences in Center, with respect to continuous environmental covariates ($p<=0.05$), categorical frog variables and environmental covariates (MRPP), and with respect to diatom taxa abundances ($p < 0.001$). Stress = 0.15.