HHS Public Access

Author manuscript

Oecologia. Author manuscript; available in PMC 2016 May 05.

Published in final edited form as:

Oecologia. 2015 May ; 178(1): 239–248. doi:10.1007/s00442-014-3165-6.

Effects of nutrient supplementation on host-pathogen dynamics of the amphibian chytrid fungus: a community approach

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SUMMARY

1. Anthropogenic stressors may influence hosts and their pathogens directly or may alter host–pathogen dynamics indirectly through interactions with other species. For example, in aquatic ecosystems, eutrophication may be associated with increased or decreased disease risk. Conversely, pathogens can influence community structure and function and are increasingly recognised as important members of the ecological communities in which they exist.

2. In outdoor mesocosms, we experimentally manipulated nutrients (nitrogen and phosphorus) and the presence of a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), and examined the effects on Bd abundance on larval amphibian hosts (*Pseudacris regilla*: Hylidae), amphibian traits and community dynamics. We predicted that resource supplementation would mitigate negative effects of Bd on tadpole growth and development and that indirect effects of treatments would propagate through the community.

3. Nutrient additions caused changes in algal growth, which benefitted tadpoles through increased mass, development and survival. Bd-exposed tadpoles metamorphosed sooner than unexposed individuals, but their mass at metamorphosis was not affected by Bd exposure. We detected additive rather than interactive effects of nutrient supplementation and Bd in this experiment.

4. Nutrient supplementation was not a significant predictor of infection load of larval amphibians. However, a structural equation model revealed that resource supplementation and exposure of amphibians to Bd altered the structure of the aquatic community. This is the first demonstration that sublethal effects of Bd on amphibians can alter aquatic community dynamics.

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Keywords

Batrachochytrium dendrobatidis; eutrophication; food web; pathogen; trophic cascade

Introduction

The emergence of infectious diseases in wildlife threatens global biodiversity (Daszak, Cunningham & Hyatt, 2000). Environmental stressors such as habitat loss, climate change and contamination may interact with infectious diseases by directly affecting host and pathogen physiology, or through indirect mechanisms such as interactions with other species (Smith, Acevedo-Whitehouse & Pedersen, 2009). The effects of emerging infectious pathogens on hosts are typically investigated in laboratory-based studies, and while these studies are useful for determining susceptibility of hosts, they typically ignore direct and indirect influences of other biotic and abiotic factors. Furthermore, through their effects on hosts, infectious agents can have important effects on the structure and function of ecosystems (reviewed in Hatcher, Dick & Dunn, 2012), but as most laboratory-based studies exclude members of the community other than the host and the pathogen, they cannot detect these effects.

Human-induced modifications of the environment, such as eutrophication due to increased nutrient loading, can have profound effects on community dynamics including interactions between hosts and their pathogens (Johnson *et al.*, 2010). At low to moderate levels, nutrient loading is generally positively associated with disease risk (Johnson & Carpenter, 2008). Nutrient supplementation may promote disease by directly influencing abundance, virulence and survival of disease agents (Smith & Schindler, 2009). Eutrophication may also promote infection through effects on host abundance and susceptibility. By definition, hosts and parasites share common resources, and parasites must compete effectively to obtain the resources needed to sustain themselves, complete their life cycle and avoid host immune responses (Hall *et al.*, 2007, 2010; Smith, 2007). The availability of nutrients can have considerable effects on the host's ability to mount these costly responses (Smith, Jones & Smith, 2005). In addition to direct effects on pathogens and hosts, eutrophication may also affect disease risk through indirect pathways, such as intermediate hosts or vectors, or trophic interactions (Lafferty & Holt, 2003; McKenzie & Townsend, 2007). For example, eutrophication can provide increased resources for growth and reproduction of snails, the first intermediate host of a trematode parasite, leading to increased density of infected snails and increased per capita rate of production of free-swimming stages which infect amphibians (Johnson *et al.*, 2007; Rohr *et al.*, 2008).

The emerging infectious disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter Bd), has propelled amphibians to the forefront of the biodiversity crisis (Stuart *et al.*, 2004; Skerratt *et al.*, 2007; Wake & Vredenburg, 2008). The infective stage of the fungus, an aquatic flagellate zoospore, infects keratinised tissues of amphibian hosts, including mouthparts of larval amphibians. This hinders the efficiency with which infected larvae feed on periphyton (including attached algal particles, detritus and other organic matter) (Venesky, Parris & Storfer, 2009; Venesky, Wassersug & Parris,

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2010), which can lead to reduced growth and slower developmental rates (e.g. Parris & Baud, 2004; Parris & Cornelius, 2004). Buck et al. (2012, 2015) suggested that larval amphibians also compete with zooplankton for suspended algal particles (phytoplankton), and this pattern may be augmented by Bd-induced mouthpart damage. Sublethal effects of Bd on larval amphibians suggest the potential for direct and indirect interactions between Bd and other members of the aquatic community.

We investigated the effects of nutrient supplementation on host–pathogen dynamics of Bd within a community context. In outdoor mesocosms containing tadpoles and a complex aquatic community, we manipulated nutrients (nitrogen and phosphorus) and the presence of the pathogen. Nutrient supplementation could exacerbate the effects of Bd on amphibians if (i) direct or indirect benefits to the pathogen or an increase in host survival increased the duration of infectivity and/or transmission rate of the pathogen (Johnson *et al.*, 2010) or (ii) a nutrient-induced phytoplankton bloom shaded periphyton (Rohr *et al.*, 2008; Staley, Rohr & Harwood, 2011; Halstead *et al.*, 2014), a primary resource of scraping tadpoles. Alternatively, nutrient supplementation could mitigate pathological effects on amphibians if (iii) a nutrient-induced phytoplankton bloom tempered the effects of Bd by benefitting cladocerans, which have been shown to consume Bd zoospores (Buck, Truong & Blaustein, 2011; Searle *et al.*, 2013), or 4) nutrient supplementation caused a phytoplankton bloom, minimising competitive effects between tadpoles and zooplankton for this shared resource (Wassersug, 1972; Altig, Whiles & Taylor, 2007; Whiles *et al.*, 2010; Buck *et al.*, 2011, 2015). We also investigated the effects of host–pathogen dynamics on other members of the aquatic community in the mesocosms.

Methods

In a completely randomised 2 × 2 factorial design in artificial ponds (mesocosms), we experimentally manipulated nitrogen and phosphorus concentrations and Bd. We crossed the presence or absence of added nutrients with the presence or absence of the pathogen. Each of the four treatments was replicated eight times for a total of 32 experimental units. The experiment was conducted at the Oregon State University Lewis-Brown Horticulture Research Farm near Corvallis, OR (68 m, Benton County, U.S.A.), and ran from 22 July 2011 to 30 September 2011. Thirty-two plastic tanks (94 cm L × 70 cm W × 33 cm H) were filled with ~120 L of tap water (pH = 8.0) on 1 July and were covered with weighted screen lids. On 5 July, we added 2 g of leaf litter to each tank for habitat heterogeneity. On the same day, we dosed half of the tanks with solutions of nitrogen (NH_4NO_3) and phosphorus (H_3PO_4) to achieve concentrations of 1800 μg L^{-1} and 200 μg L^{-1}, respectively (Johnson *et al.*, 2007). At the end of the experiment, a water sample of 10 mL was collected from each tank and pooled by nutrient treatment. Samples were frozen in pre-cleaned amber glass jars and shipped to Mississippi State Chemical Laboratory (Mississippi State, MS, U.S.A.) for independent analysis of nutrient concentrations. Results of these analyses indicated that nutrient-control pools had average concentrations of 510 μg L^{-1} N and 90 μg L^{-1} P and nutrient-treated pools had average concentrations of 540 μg L^{-1} N and 180 μg L^{-1} P. Thus, although the concentration of N was marginally higher in supplemented pools, our manipulation primarily elevated P concentrations. Environmental sampling has revealed nutrient concentrations as high as 2859 μg L^{-1} N and 348 μg L^{-1} P in eutrophic wetlands

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inhabited by amphibians, so nutrient concentrations in our experiment are ecologically relevant (Johnson *et al.*, 2007). On 12 July, we inoculated each tank with 600 mL of water containing zooplankton, phytoplankton and periphyton from laboratory stocks. Tanks were checked after 7 days to confirm the presence of zooplankton.

Clutches of *Pseudacris regilla* eggs (23 masses) were collected on 23 June within 48 h of oviposition from a natural pond in the Cascade Mountains (Linn County, elevation = 1140 m). *Pseudacris regilla* is a typical pond-dwelling tadpole, possessing a generalised oral morphology that allows for scraping and suspension feeding on green algae, cyanobacteria, bacteria, diatoms, protozoa, and organic and inorganic debris (Wassersug, 1972, 1976). Eggs were hatched and tadpoles reared in outdoor holding tanks near the experimental site. Tadpoles were fed rabbit chow *ad libitum*. Bd exposure occurred in separate tanks before the experiment. On 6 July, tadpoles were split into two groups. Tadpoles in the Bd exposure group were exposed to Bd three times over a 17-day period (on 7 July, 13 July and 19 July). Bd was grown in pure culture on plastic Petri plates (10 cm diameter) with standard TGhL nutrient agar medium (Longcore, Pessier & Nichols, 1999). We inoculated plates with liquid culture of Bd isolate JEL 274, originally isolated from *Anaxyrus boreas* from Colorado, and incubated them at 22 °C for 8 days prior to use. Each plate was flooded with 15 mL of dechlorinated water and scraped after 5 min. The water from these plates was pooled, and Bd zoospore concentration was quantified with the use of a haemocytometer. The broth was then diluted using dechlorinated water and added to the Bd exposure tank to achieve an average zoospore concentration of 2.0×10^5 zoospores L^{-1}. This dose is within the range of doses normally used to infect tadpoles in mesocosm and laboratory experiments (Searle *et al.*, 2011; Buck *et al.*, 2012, 2015; Hamilton, Richardson & Anholt, 2012). A broth containing water from flooded control plates was diluted and added to the Bd control tank. On 22 July (day 1 of the experiment), 40 tadpoles from either the Bd or the Bd-control exposure group were added to each experimental tank. The initial mass of the tadpoles (mean ± 1 SE) was 82.3 ± 8 mg, and their developmental stage (Gosner, 1960) was 25–27 (hind limb buds beginning to develop).

On day 20 of the experiment, ten tadpoles from each tank were haphazardly chosen, euthanised using an overdose of MS-222 and preserved in 90% ethanol. The mass and Gosner (Gosner, 1960) stage of these individuals was later measured. Following the methods of Boyle et al. (2004), we used real-time quantitative polymerase chain reaction (qPCR) to quantify the infection status of all Bd-exposed individuals (n = 160) and two randomly selected individuals from each control Bd tank (n = 32). Each sample was run in triplicate against a Bd standard titration from 10^{-1} to 10^2 zoospores. qPCR analysis was conducted on an Applied Biosystems StepOne Plus real-time PCR machine (Applied Biosystems, Inc., Waltham, Massachusetts, USA).

Remaining amphibians were removed from tanks upon emergence at Gosner (Gosner, 1960) stages 45–46 (metamorphosis). The first newly metamorphosed amphibian (metamorph) was observed on day 26. Following this initial observation, tanks were checked daily for metamorphs until the end of the experiment on day 71. Metamorphosed individuals were euthanised using an overdose of MS-222 and preserved in 90% ethanol for later

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measurement of mass. At the end of the experiment, all remaining individuals (~9% of all individuals added to mesocosms) were preserved, regardless of Gosner stage.

To determine how treatments affected the pond community, we sampled zooplankton, phytoplankton and periphyton on 28 and 29 July and every 2 weeks thereafter (four times total) following the methods of Buck et al. (2015). Briefly, zooplankton were sampled using a tube sampler and then filtered and preserved for later quantification. Chlorophyll a was extracted from phytoplankton samples, and fluorescence measurements were taken with a Turner Designs fluorometer (model TD-700; Sunnyvale, CA, U.S.A.). To quantify periphyton biomass, the periphyton was scraped from the outer side of a microscope slide mounted vertically in the tank, dried on pre-weighed filters and reweighed.

We deployed iButton temperature probes (Maxim, Sunnyvale, CA, U.S.A.) in 16 tanks at the beginning of the experiment. Each probe logged temperature every 4 h over the course of the experiment. Temperature measurements were averaged by week. Dissolved oxygen and pH measurements were taken using digital meters (Oakton Instruments, Vernon Hills, IL, U.S.A.) on day 14 of the experiment. ANOVAs were conducted to test for the effects of treatments on temperature, dissolved oxygen and pH.

Statistical analyses

Response variables for tadpoles included mass and developmental stage (Gosner, 1960). Response variables for metamorphs included mass, development (larval period) and survival to metamorphosis. We used linear mixed-effects models to determine the effects of nutrients, Bd exposure and their interaction on mass and development of amphibians. We used a Cox mixed-effects model to determine the effects of nutrients, Bd exposure and their interaction on survival of amphibians to metamorphosis. Individuals were nested by tank to avoid pseudoreplication. Mixed-effects models were constructed using the nlme and coxme packages in R version 3.0.2. (2013). We also conducted qPCR analysis on all 10 Bd-exposed animals from each tank that were preserved on day 20 of the experiment ($n = 160$), and two randomly selected unexposed animals from each tank ($n = 32$). We used an ANOVA to test for the effects of the nutrient treatment on infection load.

Abundance of cladocerans, abundance of phytoplankton and periphyton biomass were log-transformed to meet parametric assumptions. We performed a series of repeated-measures ANOVAs to determine the effects of nutrient supplementation, Bd and their interaction on community response variables. We used Mauchly's test for sphericity to test the assumption that the variances of the differences between the repeated measurements were approximately the same, and we report Greenhouse-Geisser-corrected *P*-values. Repeated-measures ANOVAs were conducted using the ez package in R version 3.0.2. (2013).

We employed structural equation modelling (SEM) to test the effects of treatments on the aquatic community. SEM, which is a statistical technique based on the analysis of variance–covariance matrices (Grace, 2006), is well suited to testing web-like hypotheses because it allows variables to serve simultaneously as independent and dependent variables. For the aquatic community, measured variables included in our model were abundance of cladocerans, abundance of phytoplankton and biomass of periphyton, all measured on the

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last sampling occasion. All community variables were log-transformed to meet parametric assumptions. We constructed a full model, which included fourteen paths to be tested (a-n). A latent variable, tadpole performance, was associated with the indicator variables log mass, development (log larval period) and survival of amphibians to metamorphosis (arcsine square-root transformed). Using the lavaan package in R version 3.0.2 (2013), we tested among nineteen hypothesised path models that removed various sets of paths from the full model. These models were ranked using the corrected Akaike information criterion (AICc) using the AICcmodavg package in R version 3.0.2. (2013).

Results

We measured mass and quantified Gosner stage for the tadpoles preserved on day 20 of the experiment. Linear mixed-effects models revealed that mass of tadpoles was increased by nutrient supplementation ($P < 0.001$), but was not significantly affected by Bd exposure ($P = 0.70$) or the interaction between nutrients and Bd exposure ($P = 0.71$) (Table 1, Fig. 1a). Nutrient additions caused tadpoles to develop faster ($P = 0.0088$), but Gosner stage at day 20 was not significantly affected by Bd exposure ($P = 0.31$) or the interaction between nutrients and Bd exposure ($P = 0.56$) (Table 1, Fig. 1b).

We measured mass, length of the larval period and percentage survival to metamorphosis for the individuals preserved at metamorphosis. Linear mixed-effects models revealed that mass of metamorphs was increased by nutrient supplementation ($P < 0.001$), but was not significantly affected by Bd exposure ($P = 0.91$) or the interaction between nutrients and Bd exposure ($P = 0.24$) (Table 2, Fig. 2a). The larval period of amphibians was reduced by nutrient supplementation ($P < 0.001$) and Bd exposure ($P = 0.015$), but was not significantly affected by their interaction ($P = 0.88$) (Table 2, Fig. 2b). Survival to metamorphosis was 76% overall and was increased by nutrient additions ($P = 0.0095$), but was not significantly affected by Bd exposure ($P = 0.91$) or the interaction between nutrients and Bd exposure ($P = 0.54$) (Table 2, Fig. 2c).

qPCR analysis was conducted on all 10 Bd-exposed animals from each tank that were preserved on day 20 of the experiment ($n = 160$), and two randomly selected unexposed animals from each tank ($n = 32$). Seventy-eight of 160 (49%) Bd-exposed larvae were infected, and average infection intensity was 0.1–2.2 Bd genome equivalents. This infection intensity is low compared to infection loads detected in many laboratory studies (e.g. Searle *et al.*, 2011), but is comparable to infection intensities observed in mesocosm experiments (Buck *et al.*, 2012, 2015; Hamilton *et al.*, 2012). All unexposed individuals that were tested were uninfected. Nutrient supplementation was not a significant predictor of infection status or load of amphibians. Bd-exposed tadpoles in nutrient-supplemented tanks ($n = 80$) had an average infection intensity of 0.68 ± 0.22 genome equivalents, and Bd-exposed tadpoles in non-supplemented tanks ($n = 80$) had an average infection intensity of 0.65 ± 0.17 genome equivalents.

We measured the abundance of cladocerans, the abundance of phytoplankton and the biomass of periphyton every 2 weeks over the course of the experiment. Repeated-measures ANOVAs revealed that the abundance of cladocerans fluctuated over time ($P < 0.001$), but

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was not significantly affected by nutrient additions ($P = 0.58$) or Bd exposure status of amphibians ($P = 0.36$) (Table 3, Fig. 3a). Neither phytoplankton nor periphyton was significantly affected by the Bd treatment, but both were affected by the interaction of time and nutrient additions (Table 3). Relative to control tanks, phytoplankton bloomed in nutrient-supplemented tanks in week 2 and then subsided for the rest of the experiment (Fig. 3b), whereas periphyton biomass in nutrient-supplemented tanks exhibited the opposite temporal pattern, with low levels early and a peak at the end of the experiment (Fig. 3c). In nutrient-supplemented tanks, periphyton biomass was negatively associated with larval period of the tadpoles ($R^2 = 0.25$, Fig. 4).

Our structural equation model (Fig. 5a) confirmed the effects of nutrients ($P < 0.001$) and Bd ($P = 0.008$) on tadpole performance. Based on AICc, the SEM with the greatest support (Table 4, Fig. 5b) included a positive association between nutrients and periphyton ($P < 0.001$) and a negative association between nutrients and phytoplankton ($P = 0.013$). Tanks with Bd-exposed tadpoles had less phytoplankton at the end of the experiment than tanks with unexposed tadpoles ($P = 0.021$). We also detected negative associations between cladocerans and periphyton ($P = 0.011$) and between tadpoles and cladocerans ($P = 0.028$). This model accounted for 75% of the model weights.

Average weekly temperatures in the experimental tanks ranged from 13.7 to 20.5 °C, which is suitable for the growth of Bd (Piotrowski, Annis & Longcore, 2004). Dissolved oxygen and pH ranged from 7.0 to 8.3 ppm and from 7.9 to 8.0, respectively. Separate ANOVAs indicated that temperature, dissolved oxygen and pH were not affected by treatments.

Discussion

The addition of nitrogen and phosphorus to experimental mesocosms caused changes in algal growth. A bloom of phytoplankton occurred within 2 weeks of nutrient additions. No such effect was observed for periphyton, possibly because of shading by phytoplankton (Rohr *et al.*, 2008; Staley *et al.*, 2011; Halstead *et al.*, 2014), or because of suppression by tadpoles. By day 20 of the experiment, positive effects of nutrient supplementation on tadpole mass and development were evident. These effects were maintained through metamorphosis; amphibians from nutrient-supplemented tanks attained higher mass, metamorphosed faster and had higher survival rates than amphibians from control tanks. These results are in accordance with previous studies. Nutrient supplementation typically benefits larval amphibians by increasing the growth of periphyton (e.g. Leibold & Wilbur, 1992). Tadpoles may have also benefitted from the phytoplankton bloom that occurred early in the experiment. Like many species of rasping tadpoles, *P. regilla* incorporates significant amounts of suspended algal particles into its diet, in addition to attached algal particles scraped from surfaces (Wassersug, 1972, 1976; Altig *et al.*, 2007; Whiles *et al.*, 2010).

In contrast to nutrient supplementation, exposure to Bd did not detectably affect tadpole mass or development or other members of the aquatic community early in the experiment. qPCR analysis revealed that about half of Bd-exposed tadpoles were infected by day 20 of the experiment, but the infection had not yet progressed far enough to cause detectable changes in mass and development. However, by metamorphosis, the effects of Bd on

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development were evident, with Bd-exposed tadpoles metamorphosing sooner than unexposed individuals, even though mass was not affected by Bd exposure. Although qPCR analysis detected infections in only approximately half of the tadpoles exposed to Bd, previous studies have shown that exposure can incur costs, even in the absence of detectable infections (Rohr *et al.*, 2013), and that Bd exposure may be a better predictor of effects than Bd load (Hanlon *et al.*, 2015). In response to Bd infection, larval amphibians generally delay metamorphosis (Parris & Baud, 2004; Parris & Cornelius, 2004). The mechanism proposed for such a delay is that Bd causes mouthpart deformities that reduce larval feeding efficiency (Venesky *et al.*, 2009, 2010), thus reducing growth and delaying metamorphosis. While it is possible that Bd infection damaged mouthparts, making suspension feeding more obligatory than facultative, it is unlikely that this damage could cause the observed accelerated metamorphosis without a reduction in body size.

An alternative explanation for the earlier metamorphosis of Bd-exposed amphibians is that Bd exposure stressed the tadpoles. Unfavourable conditions or high mortality risk in the larval environment often induces developmental plasticity in amphibians, including earlier metamorphosis (Newman, 1992). Plasticity of amphibian development in response to pathogen exposure has been documented for ranavirus (Warne, Crespi & Brunner, 2011) and the water mould *Saprolegnia* (Touchon, Gomez-Mestre & Warkentin, 2006; Uller, Sagvik & Olsson, 2009). Bd infection is associated with (Gabor, Fisher & Bosch, 2013; Peterson *et al.*, 2013) and may cause (Searle *et al.*, 2014) elevated corticosterone levels in tadpoles, and this stress hormone can accelerate metamorphosis (Denver, 2009). Although Bd was associated with accelerated metamorphosis, it was not accompanied by smaller body size, suggesting that this hypothesis is also incapable of fully explaining the observed patterns.

It is possible that with increased statistical power, we would have detected Bd-induced changes in mass in addition to changes in development. However, the most parsimonious explanation for accelerated metamorphosis of Bd-exposed amphibians without accompanying changes in body size might be consumption of Bd zoos-pores by tadpoles. Facultative suspension-feeding tadpoles, including Hylids, have been shown to filter Bd zoospores from the water column (Venesky *et al.*, 2014). Zoospores, which are rich in nutrients (Gleason *et al.*, 2008), may represent a food resource that might have accelerated amphibian metamorphosis and caused a shift to increased suspension feeding. Fungi are a common food item of tadpoles (e.g. Altig *et al.*, 2007). Thus, tadpoles might incorporate Bd into their diet because of phylogenetic inertia, in spite of the fact that this would increase contact rates between zoospores and keratinised mouthparts, increasing infection risk. This evolutionary trap hypothesis might explain why so many amphibians are infected with Bd and certainly warrants further exploration. However, this explanation assumes that sufficient quantities of zoospores were shed by infected tadpoles to serve as a resource.

Tadpoles in our experiment were exposed to Bd in separate tanks before the start of the experiment, and Bd was not added directly to experimental tanks. Therefore, any associations between Bd and other members of the aquatic community must be indirect, through its effects on amphibians. The effects of Bd on tadpoles probably explain the negative association between Bd and phytoplankton in our structural equation model. Bd-exposed tadpoles may consume more phytoplankton than unexposed tadpoles (Buck *et al.*,

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2012, 2015) because nutritious zoospores cause a shift to increased suspension feeding, or because the infection reduces feeding efficiency on attached algal particles (Venesky *et al.*, 2009, 2010), forcing a shift to suspended algal particles. Indirect effects through amphibians may also explain the negative association between nutrients and phytoplankton detected in our structural equation model. Tadpoles in the nutrient supplementation treatment exhibited increased mass, development and survival, increasing their ability to consume algal resources, and this may have led to the negative association.

We did not detect any response by cladoceran populations to the phytoplankton bloom that occurred in nutrient-supplemented tanks near the beginning of the experiment. This result counters findings from the previous studies (Leibold & Wilbur, 1992; McMahon *et al.*, 2012; Halstead *et al.*, 2014). However, it is possible that larval amphibians suppressed cladoceran populations indirectly through competition for shared phytoplankton resources, as previously suggested by Buck et al. (2012, 2015). Indeed, we detected a negative association between tadpole performance and cladocerans at the end of the experiment. Competition between cladocerans and tadpoles for phytoplankton could also explain the negative association between cladocerans and periphyton at the end of the experiment: below a certain concentration of suspended algal particles, Daphnia may switch to periphyton as an alternative food source (Siehoff *et al.*, 2009).

We also detected a periphyton bloom in nutrient-supplemented tanks at the end of the experiment. The negative correlation between larval period and periphyton biomass in nutrient-supplemented tanks suggests that this was caused by earlier metamorphosis of amphibians in nutrient-supplemented tanks and continued consumption pressure from amphibians in non-supplemented tanks.

Our results show that nutrient supplementation increased algal growth and induced higher rates of growth and development in larval amphibians, consistent with the previous studies (e.g. Halstead *et al.*, 2014). Exposure to Bd increased development, but not mass, of amphibians, which may have been caused by a shift to a diet rich in suspended particles including algae and Bd zoospores. We hypothesised that resource supplementation would minimise negative effects of Bd on amphibians, but we did not find interactive effects of the treatments in this experiment. Instead, resource supplementation and exposure of amphibians to Bd altered the structure of the aquatic community. Pathogens and parasites are integral members of ecosystems and have been shown to play an important role in shaping community structure and function (reviewed in Hatcher *et al.*, 2012). For example, Bd-induced population declines of amphibians have altered communities of primary producers (Connelly et al., 2008) and have even caused changes in nitrogen cycling (Whiles *et al.*, 2013) in Neotropical streams. However, these effects are due to extinction of tadpoles (dominant grazers) in these streams, rather than sublethal effects of the pathogen on amphibians. Although previous studies have shown that Bd exposure alters the consumption of algal resources by tadpoles (Venesky *et al.*, 2009, 2010), suggesting the potential for effects on the community, ours is the first study to demonstrate that sublethal effects of the pathogen on amphibians can alter aquatic community dynamics.

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Acknowledgments

We thank E. Hunt and P. Buck for field assistance. Members of the Blaustein laboratory provided advice regarding experimental design and execution, data analysis, and comments on the manuscript. We also thank the R. Tanguay, PISCO and S. Hacker laboratories, and E. Scheessele for the use of equipment and protocol, and S. Robbins and D. Hinds-Cook for assistance at the Horticulture Farm. This material is based upon work supported by the National Science Foundation under Grant No. 1210520. Supplementary funding was provided by the OSU Zoology Research Fund and the Society of Wetland Scientists.

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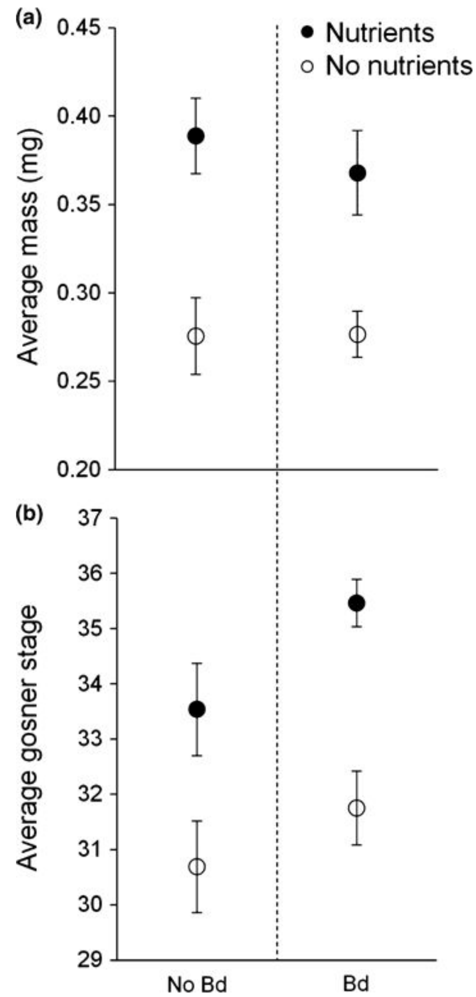

Fig. 1.

Response variables for tadpoles: (a) mass and (b) developmental stage of *Pseudacris regilla* tadpoles in the absence (open symbols) and presence (filled symbols) of added nutrients and not exposed (left) and exposed (right) to Bd. Values plotted are means ± SE (n = 32 tanks).

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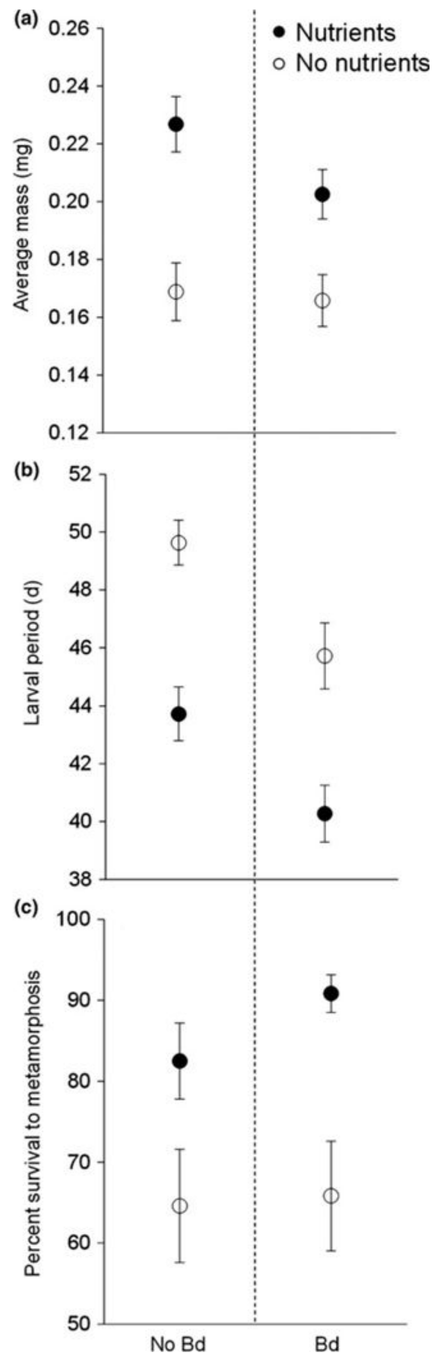

Fig. 2.

Response variables for metamorphs: (a) mass, (b) development and (c) survival of *Pseudacris regilla* tadpoles in the absence (open symbols) and presence (filled symbols) of added nutrients and not exposed (left) and exposed (right) to Bd. Values plotted are means ± 1 SE (n = 32 tanks).

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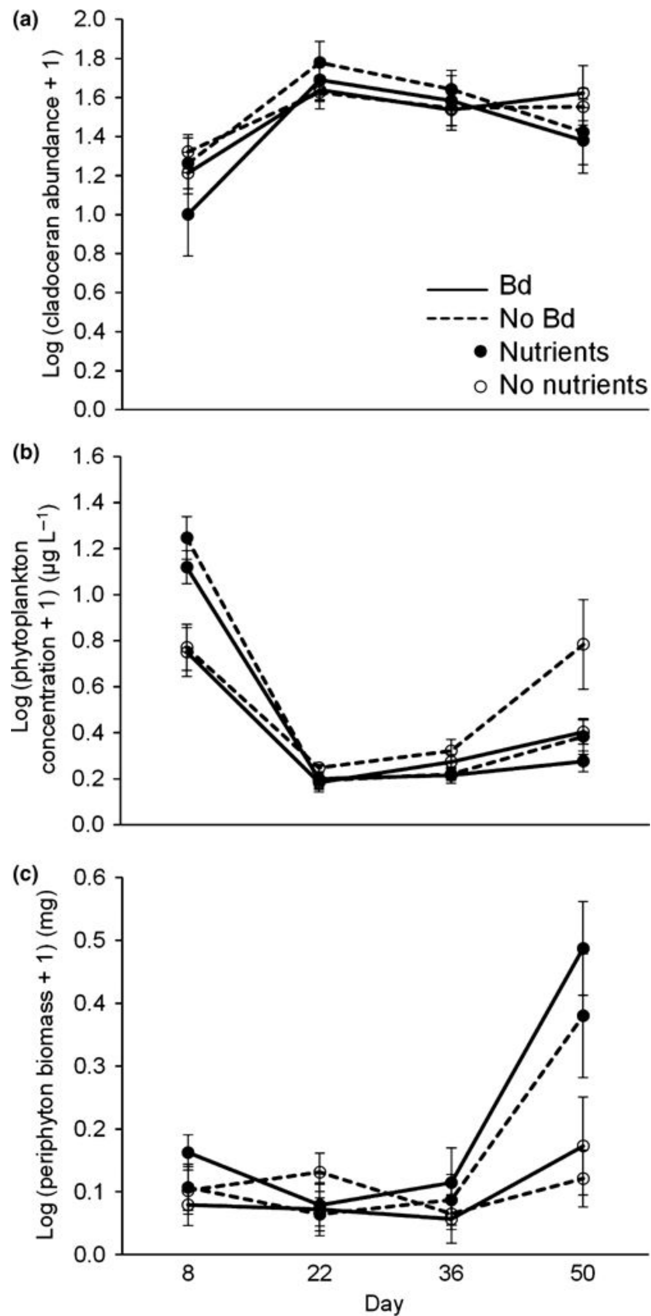

Fig. 3.

Community response variables: (a) log cladoceran abundance, (b) log phytoplankton concentration and (c) log periphyton biomass in the absence (open symbols) and presence (filled symbols) of nutrient additions and the absence (dashed lines) and presence (solid lines) of Bd over the four sampling periods. Values plotted are means ± 1 SE (n = 32 tanks).

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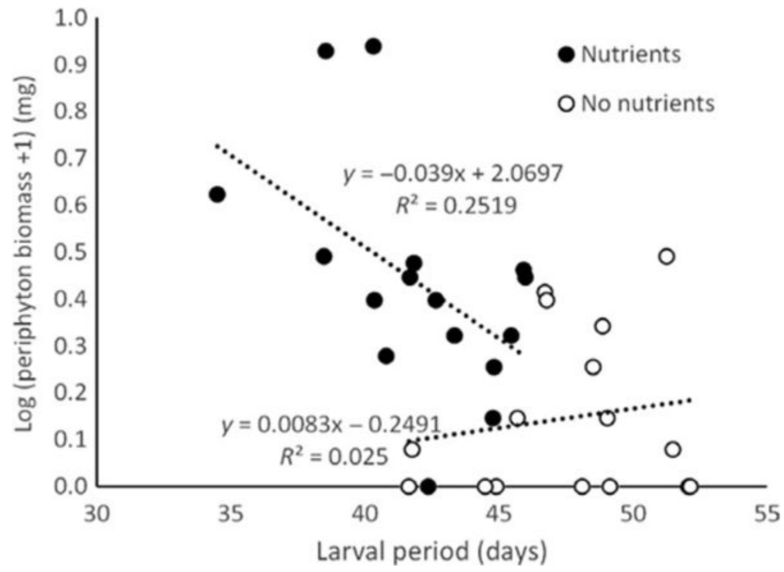

Fig. 4.

Correlation between larval period and periphyton biomass at the end of the experiment in the absence (open symbols) and presence (filled symbols) of nutrient additions.

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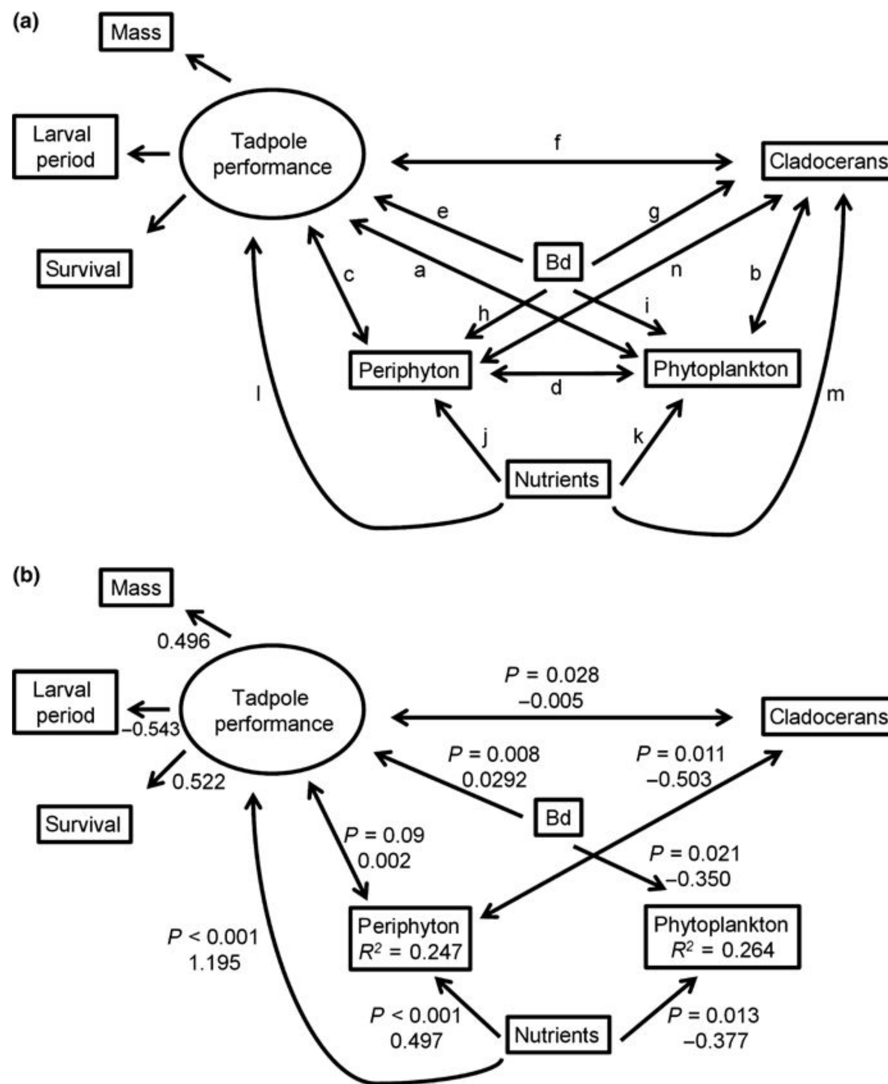

Fig. 5.

(a) Full model depicting the latent variable tadpole predation and competition pressure and paths a-m to be tested. (b) Best model for the end of the experiment (based on corrected Akaike information criterion) showing positive effects of nutrients on periphyton and amphibians and a negative effect of Bd-infected tadpoles on phytoplankton. *P*-values and standardised coefficients are shown next to each path. R^2 values indicate total variance explained by predictor variables.

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Table 1

Output of linear mixed-effects models on mass and developmental stage of tadpoles

	Coefficient	SE	d.f.	*P*-value
Mass				
Nutrients	0.16574	0.0410	28	<0.001
Bd	0.01604	0.0410	28	0.70
Nutrients*Bd	−0.02175	0.0580	28	0.71
Development (Gosner stage)				
Nutrients	0.03891	0.0138	28	0.0088
Bd	0.01481	0.0138	28	0.31
Nutrients*Bd	0.01152	0.0195	28	0.56

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Table 2

Output of linear mixed-effects models and a Cox mixed-effects model on mass, development and survival to metamorphosis

	Coefficient	**SE**	**d.f.**	***P*-value**
Mass				
Nutrients	0.13947	0.02822	28	<0.001
Bd	0.00308	0.02892	28	0.91
Nutrients*Bd	–0.04757	0.03970	28	0.24
Development (larval period)				
Nutrients	–0.05530	0.0144	28	<0.001
Bd	–0.03836	0.0148	28	0.015
Nutrients*Bd	0.00305	0.0202	28	0.88
	Hazard ratio	SE	Z	*P*-value
Survival to metamorphosis				
Nutrients	1.61	0.1830	2.59	0.0095
Bd	1.02	0.1866	0.11	0.91
Nutrients*Bd	1.17	0.2577	0.61	0.54

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Table 3

Output of repeated-measures ANOVAs on cladoceran abundance, phytoplankton concentration and periphyton biomass. Included are all main effects and any significant interactions

	DFn	DFd	*F*	*P*-value
Cladoceran abundance				
Nutrients	1	28	0.32	0.58
Bd	1	28	0.86	0.36
Time	3	84	11.71	<0.001
Phytoplankton concentration				
Nutrients	1	28	0.10	0.76
Bd	1	28	3.86	0.06
Time	3	84	100.48	<0.001
Nutrients*Time	3	84	17.36	<0.001
Periphyton biomass				
Nutrients	1	28	8.02	0.008
Bd	1	28	0.48	0.49
Time	3	84	19.19	<0.001
Nutrients*Time	3	84	9.31	<0.001

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Table 4

Comparison of the 19 competing models used to explain interactions among *Pseudacris regilla* tadpoles, *Batrachochytrium dendrobatidis*, zooplankton, periphyton, phytoplankton and nutrients. See Fig. 5a to match letters with paths

Model	Paths	AICc	AIC	ω
1	c,e-f,i-l,n	−90.44	0.00	0.75
2	a,e-f,i-l,n	−87.03	3.41	0.14
3	a,c,e-f,i-l,n	−85.32	5.12	0.06
4	a,c,e,i-l,n	−85.18	5.26	0.05
5	a,c-f,i-l,n	−79.71	10.73	0.00
6	a-c,e-f,i-l,n	−79.54	10.90	0.00
7	a-f,i-l,n	−73.23	17.21	0.00
8	a-f,i-l	−70.81	19.63	0.00
9	a-f,i-n	−67.74	22.70	0.00
10	a-f,h-l,n	−67.70	22.74	0.00
11	a-f,h-n	−61.23	29.21	0.00
12	a-g,i-n	−60.03	30.42	0.00
13	a-l,n	−59.44	31.00	0.00
14	a-h,j-n	−56.31	34.13	0.00
15	a-d,f-n	−55.96	34.48	0.00
16	a-j,l-n	−55.59	34.85	0.00
17	a-n	−51.79	38.65	0.00
18	a-i,k-n	−48.24	42.20	0.00
19	a-k,m-n	−14.30	76.14	0.00