

Initial versus longer-term effects of tadpole declines on algae in a Neotropical stream

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SUMMARY

1. Information about temporal patterns of ecological responses to species losses is integral to our understanding of the ultimate effects of declining biodiversity. As part of the Tropical Amphibian Declines in Streams (TADS) project, we quantified changes in algal biomass and N cycling in algae in upland Panamanian streams following the widespread decline of larval anurans.
2. Reach-scale monitoring during and after a catastrophic, disease-driven amphibian decline showed significant 2.8-fold increases ($P < 0.05$) in algal biomass in pools and 6.3-fold increases in riffles in the 5 months following the decline. 3 years after the decline, the magnitude of this initial change dampened to increases ($P < 0.05$) of 2-fold in pools and 3.5-fold in riffles over pre-decline levels. Similarly, total organic matter of benthic biofilms, measured as ash-free dry mass (AFDM), increased significantly by 2.2-fold in pools and 2.3-fold in riffles in the initial 5-month post-decline period, with the magnitude of these changes dampening slightly to a 2-fold increase in pools and 1.9-fold increase in riffles over pre-decline levels after 3 years ($P < 0.05$ for differences at 5 months and 3 years). There were initial increases ($P < 0.05$) in Chl *a*:AFDM ratios 5 months after the decline, but ratios had returned to pre-decline levels after 3 years.
3. Algal food quality (as C/N) increased slightly, but not significantly, during the initial 5-month post-decline period and remained constant over 3 years. Mean $\delta^{15}\text{N}$ in biofilms in pool habitat (measured over the reach scale) was significantly depleted initially following tadpole declines and remained significantly depleted 3 years after the decline (4.34 ‰ pre- versus 3.24‰ post-; $P < 0.05$), suggesting that the loss of tadpoles reduced N recycling.
4. Increases in abundance and production of some grazing macroinvertebrate taxa after the decline may have contributed to the gradual reduction in the difference between initial and longer-term post-decline algal biomass. However, algal biomass was still 2-fold greater than pre-decline levels after 3 years, indicating that grazing macroinvertebrates did not fully compensate for the loss of tadpoles.

Keywords: amphibian declines, chytrid, emerging infectious disease, functional redundancy, Panama

Introduction

Ecosystems across the planet are experiencing accelerating losses of biota, with largely unknown ecological

consequences. In response to these losses, there has been increasing effort to better characterise and predict ecosystem-level consequences. However, predicting effects of species loss, and interpreting results of

experiments that quantify effects of these losses, can be difficult (Chapin, Schulze & Mooney, 1992; Petchey *et al.*, 2004). In part, this is a result of the inherent challenge in conducting research at ecologically relevant spatial and temporal scales, and the lack of pre-decline baseline data. Ecosystems are dynamic and their responses to a perturbation, such as the loss of an abundant taxon, could become apparent across various levels of biological organisation, including physiological, behavioural and population levels. For logistical reasons, results of short-term studies are often generalised to longer temporal scales, even though the limitations of this approach (e.g. time-lags in responses of indirect effects) are recognised (Sarnelle, 1997). The extent to which ecological experiments and observations performed over relatively short temporal scales are indicative of ecosystem change over more ecologically meaningful time scales remains largely unknown (Kohler & Wiley, 1997; Symstad *et al.*, 2003).

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

In a previous study (Connelly *et al.*, 2008), we documented changes in algal biomass and composition at the reach scale in an upland Panamanian stream over 2 years, encompassing the 19 months preceding and the 5 months following a dramatic, disease-driven decline of amphibians. We found significant increases, relative to a reference stream, in algal standing crop and biofilm total organic matter, as measured by ash-free dry mass (AFDM), in the 5 months following tadpole declines. During this period, the macroinvertebrate community composition also shifted: shredder production declined, and some grazing mayfly taxa responded positively to the increased algal standing crop, suggesting the potential for functional redundancy between grazing tadpoles

and grazing macroinvertebrates (Colón-Gaud *et al.*, 2009, 2010).

Here, we report results from post-decline sampling of biofilms and tadpoles from five to 36 months. We also provide data on longer-term changes in biofilm quality (stoichiometry) and in-stream nitrogen cycling (as indicated by stable isotopes in algae). To our knowledge, no previous studies have documented relatively long-term (i.e. up to 3 years) changes in basal food resources, or compared short-term trends (i.e. on the scale of months) to longer-term patterns, following the loss of an entire group of consumers in a freshwater system. Our objective was to examine longer-term patterns following an amphibian decline and, in doing so, test the following hypotheses: (i) initial high levels of algal standing crop, observed 5 months after tadpole declines, would not be sustained over 3 years; (ii) algal food quality (as measured by biofilm C/N ratios) would decrease following extirpation in both the short term (5 months) and longer term (3 years); and (iii) nutrient cycling would be altered following the decline, as indicated by decreased fractionation of N stable isotopes in biofilm.

Methods

Study site

Our study stream, Rió Guabal, is located in the Parque Nacional G. D. Omar Torrijos Herrera, El Copé, Coclé, Panama (8°40'N, 80°35'W). Our study reach is part of a high-gradient stream characterised by distinct pool–riffle sequences, with substrata consisting of pebbles and gravel, with frequent cobbles, boulders and depositional sandy areas. At the onset of our study, the stream drainage supported 74 species of amphibians, 40 of which were found in the riparian zone. Of these, 23 had stream-dwelling larvae (Lips, Reeve & Witters, 2003). Tadpoles occurred in all stream habitats. Biofilm-grazing tadpoles of *Lithobates warszewitschii*, *Hyloscirtus palmeri* and *H. colymba* were numerous and prominent, achieving densities of up to 50 m⁻² in pools; *Ateopus varius* grazed biofilms in riffles, and tadpoles were the only vertebrate biofilm grazers in this stream at this elevation (Ranvestel *et al.*, 2004).

Dead and dying frogs infected with the pathogenic fungus *Batrachochytrium dendrobatidis* were first observed at the stream during September 2004. Subsequent frog mortality was high through January 2005, resulting in vastly reduced riparian amphibian abundances (Lips *et al.*, 2006). A dramatic reduction in tadpole density, compared with previously documented numbers,

occurred concurrently. Between September 2004 and January 2005, overall tadpole densities declined from a mean of 8.2 tadpoles m^{-2} to 1.3 tadpoles m^{-2} ($P = 0.042$, randomised intervention analysis, Connelly *et al.*, 2008) and have since remained at dramatically reduced densities (S. Connelly, pers. obs.). We characterise the periods of June 2003–September 2004 as ‘pre-decline’, February 2005–June 2005 as ‘initial post-decline’ and July 2005–February 2008 as ‘longer-term post-decline’. The transitional period during tadpole decline (October 2004–January 2005) was not used in our analyses.

Data collection and analyses

To characterise algal standing crop, biofilm AFDM, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and percentage change of C, N, and P in biofilm over time, stream rocks were sampled monthly beginning in June 2003. A benthic sampler, modified after Loeb (1981), was used to sample biofilm quantitatively from a known area of these natural substrata. Five subsamples of benthic biofilm were collected from rock surfaces, during base flow, in each of five pools and riffles along a 200-m reach. The five subsamples were pooled resulting in five riffle and five pool samples on each sampling date ($n = 10$ samples per date per reach). Samples were placed in a cooler and transported to the laboratory where the homogenate was transferred to a beaker and diluted to a known volume. While stirring, three 40–100 mL subsamples were removed and filtered through Whatman glass fibre filters (0.7 μm nominal pore size). Filters were frozen and transported to either Drexel University (for isotope analysis preparation) or the University of Georgia.

The first filter of each sample was dried at 60 °C for 24 h, weighed to the nearest 0.0001 g, ashed at 500 °C for 2 h and reweighed to determine ash-free dry mass (AFDM) and inorganic sediments. The second filter was analysed for chlorophyll *a* with a Turner Designs model 10-AU fluorometer (Turner Designs, Inc., Sunnyvale, CA, USA) using standard methods (APHA, 1998). A subset of the third filters ($n = 4$ per month, from two pools and two riffles) were placed in a drying oven at 65 °C for 24 h. The dried biofilm homogenate was processed either by scraping the material from the filter and weighing into 5 x 9 mm pressed tin capsules (when possible because of sufficient amount of dried material) or by removing two punches of 0.9 cm diameter from the filters and loading into 5 x 9 mm tin capsules (when material was of insufficient amount to be separated from the filter). Tin capsules were placed in 96-well plates, with duplicate samples included for every fifth sample. The

samples were analysed for carbon and nitrogen content and for C and N stable isotope ratios at the Odum School of Ecology Analytical Chemistry Laboratory, University of Georgia, Athens, GA, using a Carlo Erba NA 1500 CHN analyzer coupled to a Finnigan Delta C mass spectrometer. Poplar and bovine standards were inserted after 12 samples. Isotope ratios are expressed as $\delta^{13}\text{C} \text{‰}$ or $\delta^{15}\text{N} \text{‰}$ according to the equation:

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

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

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

Monthly tadpole abundance surveys began in June 2003 and continued until June 2005: from June 2005 until February 2008, tadpole surveys were conducted quarterly. Tadpoles in three randomly chosen pools were quantified with a stove pipe benthic corer (22 cm in diameter) that was modified with external rubber flaps at the base, which helped seal the bottom of the sampler when substrata were irregular. The core sampler was pushed approximately 3 cm into the substrata, and tadpoles were removed with a dip net (15 x 10 x 10 cm), counted, identified and released. Kick net sampling was used to quantify tadpoles in three randomly chosen riffles, according to the methods described by Heyer *et al.* (1994) and Hauer & Resh (1996).

Water samples were collected monthly (two replicates/sampling date) from the study stream between August 2003 and May 2006 and analysed for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and soluble reactive phosphorus (SRP). Samples were filtered through 0.45- μm Millipore filters, frozen and transported to the Analytical Chemistry Laboratory at the University of Georgia. SRP was measured spectrophotometrically using the ascorbic acid method (APHA, 1998). Nitrate and $\text{NH}_4\text{-N}$ were measured using the cadmium reduction and phenate methods, respectively (APHA, 1998).

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

Results

Pre- and post-decline comparisons of chlorophyll *a*, AFDM and Chl *a*:AFDM in biofilm

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

decrease ($P < 0.05$) to $5.70 \pm 1.63 \text{ mg m}^{-2}$ ($n = 20$) during the longer-term post-decline period (i.e. 3 years) (Fig. 1). Chlorophyll *a* in riffles followed a similar trend, increasing ($P < 0.05$) 6.3-fold from a pre-decline mean of $1.07 \pm 1.27 \text{ mg m}^{-2}$ ($n = 16$) to $6.74 \pm 0.62 \text{ mg m}^{-2}$ ($n = 5$) initial post-decline, but decreasing ($P < 0.05$) to $3.74 \pm 1.73 \text{ mg m}^{-2}$ ($n = 20$) during the longer-term post-decline period (Fig. 1). Biofilm total organic matter (as measured by AFDM) in pools increased ($P < 0.05$) 2.2-fold from a pre-decline mean of $19.14 \pm 5.04 \text{ g m}^{-2}$ ($n = 16$) to $41.95 \pm 4.98 \text{ g m}^{-2}$ ($n = 5$) during the initial post-decline, followed by a decrease ($P < 0.05$) to $37.90 \pm 2.27 \text{ g m}^{-2}$ ($n = 20$) during the longer-term post-decline period. Biofilm total organic matter in riffles increased ($P = 0.05$) 2.3-fold from a pre-decline mean of $10.74 \pm 7.32 \text{ g m}^{-2}$ ($n = 16$) to $24.25 \pm 2.14 \text{ g m}^{-2}$ ($n = 5$) initial post-decline, but decreased ($P = 0.05$) to $20.84 \pm 4.24 \text{ g m}^{-2}$ ($n = 20$) during the longer-term post-decline period (Fig. 2).

Chl *a*:AFDM of riffle biofilm increased ($P = 0.05$) from 0.16 ± 0.01 to 0.28 ± 0.01 initially, but then decreased to near pre-decline levels of 0.18 ± 0.01 over the 3-year

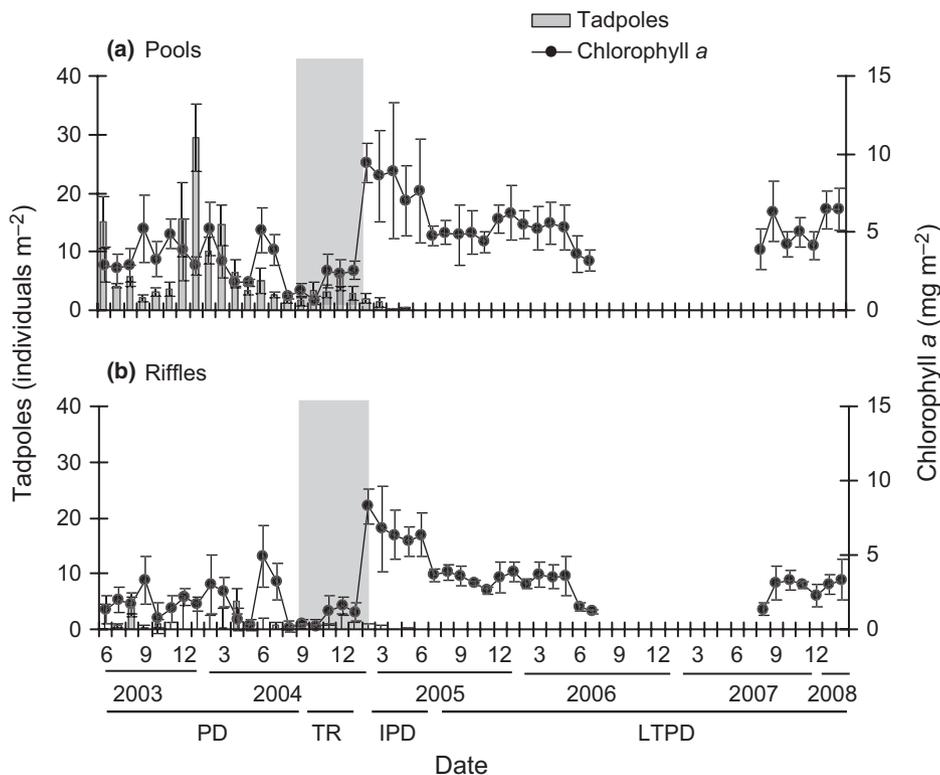


Fig. 1 Mean ($\pm 1\text{SE}$) tadpole density (shaded bars) and chlorophyll *a* (black dots) sampled monthly from: (a) pools and (b) riffles over 45 months. The amphibian decline began in September 2004. The transitional period of tadpole decline is shaded. PD indicates the pre-decline period, TR indicates the transitional period, IPD indicates the initial post-decline period, and LTPD indicates the longer-term post-decline period.

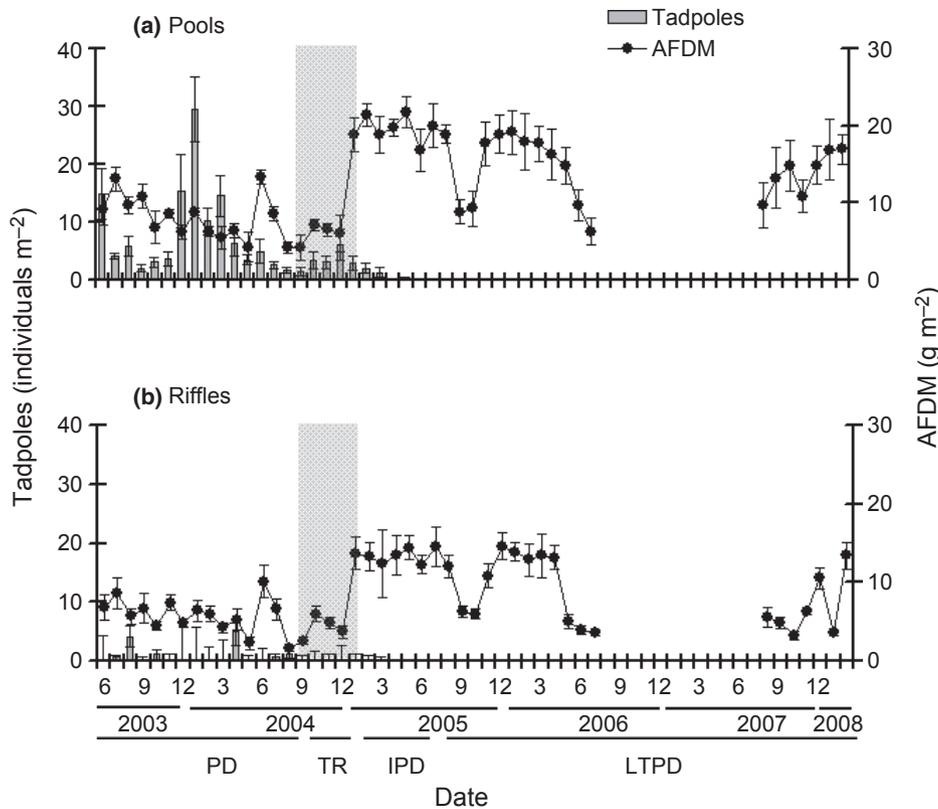


Fig. 2 Mean ($\pm 1SE$) tadpole density (shaded bars) and AFDM (black dots) sampled monthly from: (a) pools and (b) riffles over 45 months. The amphibian decline began in September 2004. The transitional period of tadpole decline is shaded. PD indicates the pre-decline period, TR indicates the transitional period, IPD indicates the initial post-decline period, and LTPD indicates the longer-term post-decline period.

post-decline period (Fig. 3). The ratio of Chl *a* to AFDM of pool biofilm did not change significantly over the sampling periods.

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

The $\delta^{15}N$ biofilm signature in pool habitat sampled over a 200-m reach decreased ($P < 0.05$) from $4.34 \pm 1.14\text{‰}$ pre-decline to $3.12 \pm 0.68\text{‰}$ and $3.42 \pm 0.77\text{‰}$ during initial (5 months) and longer-term (3 years) post-decline periods (Table 1). There were no significant changes in the $\delta^{15}N$ signature in riffle habitat among periods ($3.02 \pm 0.55\text{‰}$ pre-decline; $2.48 \pm 0.21\text{‰}$ initial post-period; and $2.49 \pm 0.71\text{‰}$ longer-term post-decline), nor were there differences in the $\delta^{13}C$ biofilm signature during the periods following tadpole decline (Table 1).

Changes in C, N and P in biofilms and water

There were no significant changes in biofilm %C, %N or %P between pre- and post-decline periods, nor were

there significant changes in biofilm C:N, C:P or N:P ratios between periods (Fig. 4). There were no significant differences in stream water nutrient concentrations between pre- and post-decline periods. Background concentration of water nutrients remained similar between pre- and post-decline periods; average concentration of NO_3-N was $250.26 (\pm 27.26, \text{pre-})$ and $238.20 (\pm 29.05; \text{post-}) \mu\text{g L}^{-1}$; that of NH_4-N was $4.79 (\pm 1.60; \text{pre-})$ and $4.76 (\pm 2.41; \text{post-}) \mu\text{g L}^{-1}$; and that of SRP was $7.23 (\pm 2.56; \text{pre-})$ and $6.69 (\pm 1.06; \text{post-}) \mu\text{g L}^{-1}$.

Discussion

As hypothesised, we did not observe sustained elevated algal standing crop over the 3-year study period in response to the continued (3-year) loss of tadpoles. Our results suggest that long-term monitoring of an ecosystem is necessary to assess the full consequences of a decline of a functionally dominant group. The loss of amphibians from our study stream impacted several, but not all, ecosystem properties, and the magnitude of the impact varied with the property and time span. For

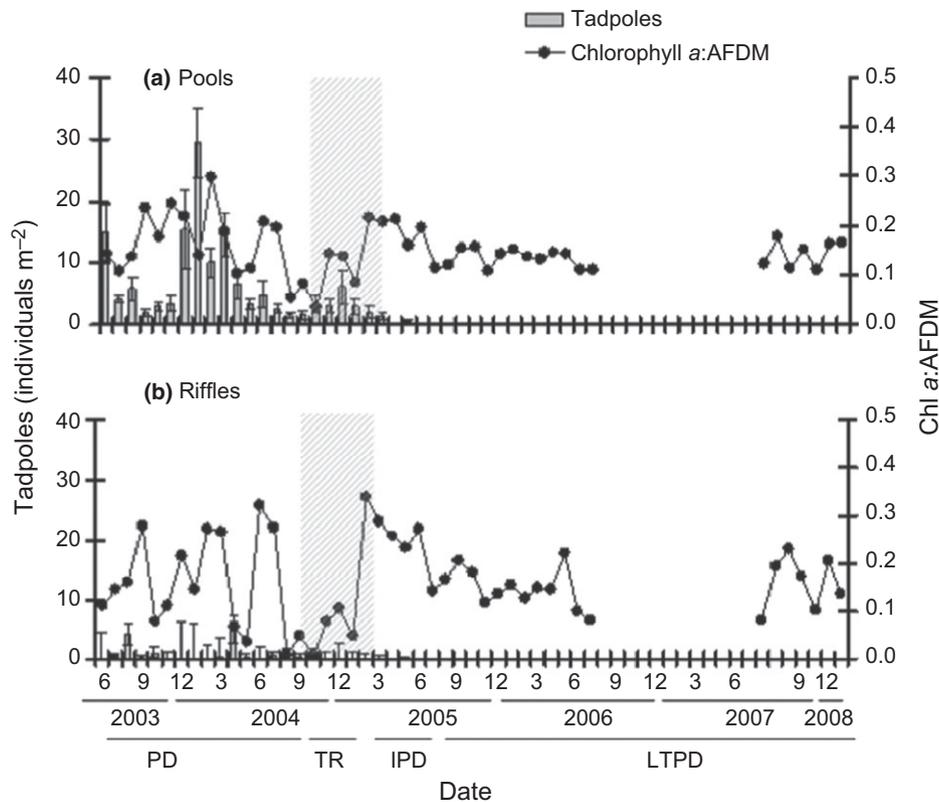


Fig. 3 Mean (\pm SE) tadpole density (shaded bars) and Chl *a*:AFDM (black dots) sampled monthly from: (a) pools and (b) riffles of the study stream over 45 months. The amphibian decline began in September 2004. The transitional period of tadpole decline is shaded. PD indicates the pre-decline period, TR indicates the transitional period, IPD indicates the initial post-decline period, and LTPD indicates the longer-term post-decline period.

Table 1 Carbon and nitrogen stable isotope composition of biofilms in the study stream (mean \pm SD). Values were collected monthly from pool ($n = 3$) and riffle ($n = 3$) habitats over 45 months. Units are per mil (‰)

Pre-decline	Initial post-decline	Long-term post-decline
$\delta^{13}\text{C}$		
Pool habitats $-29.34 (\pm 1.13)$	$-28.84 (\pm 0.57)$	$-30.20 (\pm 1.30)$
Riffle habitats $-29.53 (\pm 1.09)$	$-29.00 (\pm 0.39)$	$-30.62 (\pm 1.16)$
Combined pools and riffles $-29.46 (\pm 3.94)$	$-28.90 (\pm 0.52)$	$-30.51 (\pm 1.23)$
$\delta^{15}\text{N}$		
Pool habitats $4.34 (\pm 1.14)$	$3.12 (\pm 0.68)$	$3.24 (\pm 0.77)$
Riffle habitats $3.02 (\pm 0.55)$	$2.48 (\pm 0.21)$	$2.49 (\pm 0.71)$
Combined pools and riffles $3.55 (\pm 1.10)$	$2.67 (\pm 0.51)$	$2.69 (\pm 0.68)$

example, biofilm $\delta^{15}\text{N}$ in pool habitat declined in the 5 months immediately following amphibian declines, but did not decline further during the next 3 years. Conversely, the long-term impact of amphibian declines on algal standing stock and benthic organic matter was

less pronounced than in the 5 months immediately following the amphibian decline. However, not all ecosystem properties were affected. The percentage of biofilm C, N and P did not change during the 3 years of post-decline monitoring. Together, our results emphasise the importance of monitoring multiple ecosystem properties and that ecosystem properties may respond to the loss of a functionally dominant species over different time spans.

The total organic content (AFDM) in pools remained at elevated levels for the duration of our monitoring, suggesting that tadpoles in pools had previously limited sediment accumulation through bioturbation (e.g. Ranvestel *et al.*, 2004). The long-term increased sediment accumulation in pools may be one factor that prevented a strong compensatory population increase by macroinvertebrate algal grazers following amphibian declines (Colón-Gaud *et al.*, 2010). We hypothesise that this pattern may be related to increased abundance of some functionally similar grazing invertebrate taxa such as mayflies, which we observed in Rio Guabal over the 5 months immediately following the tadpole decline

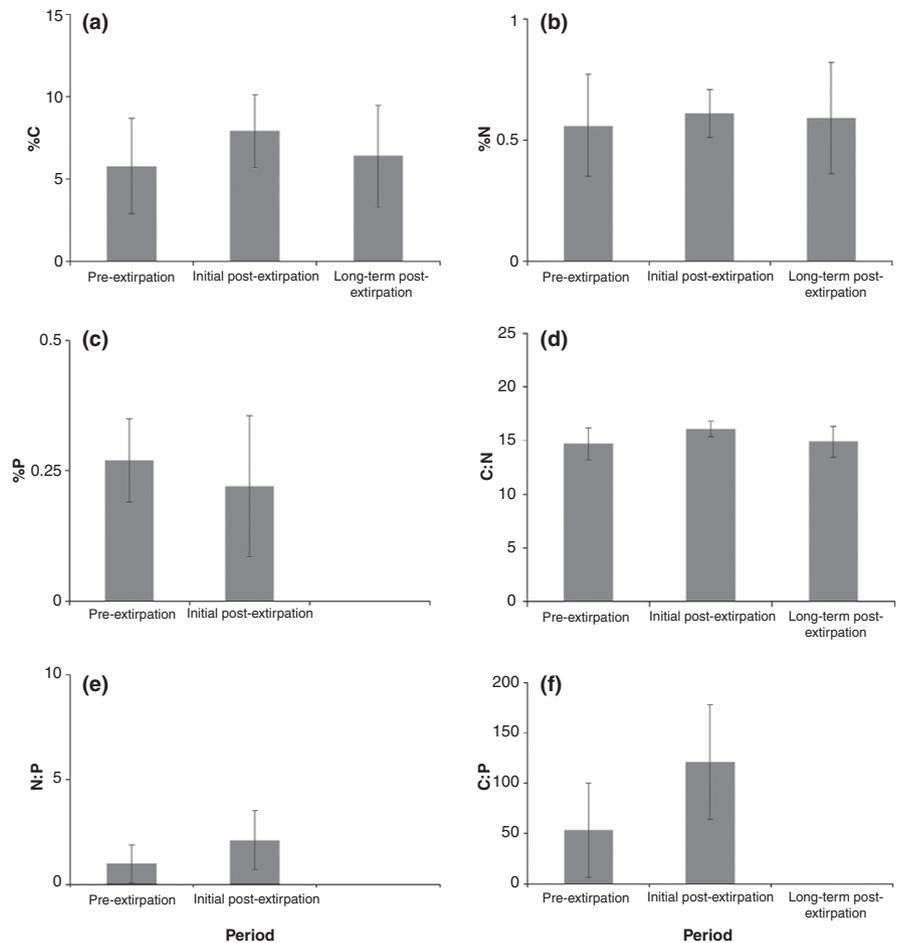


Fig. 4 Mean ($\pm 1SE$) %C, %N and %P and ratios of C:N, N:P and C:P from biofilm in pools in the study stream. Values of C and N were collected over 45 months. P was sampled over 5 months during 2004 (pre-decline) and over 5 months in 2005 (initial post-decline).

(Colón-Gaud *et al.*, 2010). However, post-decline increases in these grazing macroinvertebrates did not fully replace the top-down effect of tadpoles in terms of overall effects on algal standing crop or total organic matter, which was still almost 2-fold greater than pre-decline levels 3 years later, and the decrease in algal-grazing tadpoles may have reduced functional trait diversity in Rio Guabal. The long-term algal standing stock and benthic organic matter data suggest that, even with time for multiple generations, these macroinvertebrates are not entirely replacing the functional role of tadpoles in Rio Guabal. This is an important result, suggesting that tadpoles had at least one functional trait that was absent from the algal-grazing macroinvertebrates that affected the resiliency of the community to tadpole loss.

Tadpoles were the largest algal consumers in the stream and differences in body size between macroinvertebrates and tadpoles could explain why macroinvertebrates could not compensate for tadpole declines. Large-bodied herbivores are known to reduce sediment accumulation through bioturbation in other stream systems (McIntyre *et al.*, 2007), including Rio Guabal

(Ranvestel *et al.*, 2004; Connelly *et al.*, 2008), and can have significant effects on community structure (Estes *et al.*, 2011). However, the loss of tadpoles resulted in changes to the diatom community with a shift towards larger-bodied diatoms (Connelly *et al.*, 2008), potentially reducing the availability of edible diatoms to macroinvertebrates. The mechanism that prevents macroinvertebrates from compensating for the loss of tadpoles warrants further investigation to understand better the consequences of species loss on a community.

Previous work in our study stream had indicated that algal periphyton was a limiting resource, particularly when tadpoles were present, potentially resulting in a more generalist feeding strategy by invertebrate scrapers (Colón-Gaud *et al.*, 2009). We speculate that the initial increases in algal standing crop during the months following tadpole decline could have provided the opportunity for more selective feeding on the more nutritious components of the algae community or reduced levels of omnivory by macroinvertebrates, as algae previously consumed by tadpoles became available to invertebrate grazers.

Biologically relevant time scales must be considered when assessing the potential for functional compensation by grazing macroinvertebrates following tadpole declines. The speed at which these remaining functionally similar groups respond may be driving some of the differences in responses of algal basal resources that we found between the initial and longer-term post-decline periods. The positive response of some grazing macroinvertebrates in our study stream found by Colón-Gaud *et al.* (2009) may have increased over the longer term (relative to their initial, 5-month response) due to reproductive delays. Developmental times for invertebrates in Neotropical streams like our study sites vary from as fast as 17 days for some Chironomidae to several months for many Trichoptera (Jackson & Sweeney, 1995; Colón-Gaud *et al.*, 2010). The longer-term amelioration of initial dramatic increases in biofilm in our study stream could reflect the time necessary for shifts in abundance and community structure of grazing macroinvertebrates over multiple generations in response to a newly available food resource.

The differences in long-term patterns we observed in chlorophyll *a* in riffles and pools may be related to habitat preferences of grazing invertebrates. Colón-Gaud (2008) found significant increases in initial post-decline (5-month) production and abundance in the mayfly families Baetidae and Leptophlebiidae, which were much more abundant in riffle versus pool habitats. A stronger response to the increase in algal food by grazing mayflies in riffle habitats, relative to the weaker response of macroinvertebrate grazers in pool habitats, may be responsible for ameliorating the difference in chlorophyll *a* increases between habitat types over the longer term.

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

The Chl *a*:AFDM ratio that initially increased after 5 months post-decline returned to pre-decline levels after 3 years. Biofilm is a complex mix of organic matter, including fungi and microbes, of which algae is only one component. The initial increase in proportion of algae, relative to other organic matter, in the biofilm most likely reflected the dramatic increase in algal standing crop resulting from the loss of tadpole grazing and the lack of immediate functional response by macroinvertebrate grazers. However, the proportion of other organic matter, relative to algae, increased in the biofilm over the longer term (3 years). This relative increase in the accumulation of non-algal organic matter may be an indication of the lack of sediment bioturbation by tad-

poles; prior to the decline, tadpoles in this same stream played an important role in recycling fine depositional particulate matter into the water column (Ranvestel *et al.*, 2004; Whiles *et al.*, 2006; Connelly *et al.*, 2008). The longer-term increased accumulation of sediments probably offsets potential increases in algal growth because of light and nutrient blocking.

Potential changes in biofilm stoichiometry and nutrient concentration resulting from tadpole declines would be attributable not only to the direct effects of the missing tadpole community, but also to the subsequent shift in community composition, physiology and body stoichiometry of the remaining aquatic insects. It is known that not only top-down (grazing) but also bottom-up (nutrient availability) factors influence primary producer communities. Ecological stoichiometry provides a framework for understanding how organisms differ in their nutrient recycling roles: different groups of organisms are predicted to recycle nutrients at dissimilar rates due to different elemental composition, physiological demands and growth rates (*sensu* Evans-White & Lamberti, 2006). The loss of grazing tadpoles, followed by the initial shift in the community structure of grazing invertebrates (Colón-Gaud, 2008), could result in altered nutrient availability to the biofilm if the organisms in the new grazing community differed (relative to the tadpoles) in their body and excretion stoichiometry (*sensu* Sterner, Elser & Turner, 2002). In fact, our finding of no significant changes in biofilm stoichiometry or nutrient concentrations between pre- and post-decline periods suggests that the post-decline macroinvertebrate community is not functionally different than the pre-decline grazer community in this respect.

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

Our findings support our hypothesis that tadpoles play a role in processing nitrogen in the study stream. Mean monthly $\delta^{15}\text{N}$ in biofilm in pool habitats (habitat which supported higher tadpole densities than did riffles) decreased by 1.22‰ between the pre-decline and initial post-decline period and remained relatively depleted over 3 years. Stable isotopes of nitrogen are useful tools for identifying linkages within food webs and providing information about trophic nutrient transfers (Peterson & Fry, 1987; O'Reilly *et al.*, 2002; Woodward & Hildrew, 2002), with comparatively higher $\delta^{15}\text{N}$ signals indicative of nitrogen that has been biologically processed. The decrease in benthic $\delta^{15}\text{N}$ after the tadpole decline

suggests that the source of nitrogen may have changed following the amphibian decline.

We hypothesise that tadpole-processed nitrogen may be made available to algae and other microbes through egestion and excretion. One line of evidence supporting this hypothesis is that the rate of tadpole egestion in our study stream during the dry season was considerable, at approximately $10 \text{ mg m}^{-2} \text{ h}^{-1}$ AFDM, and organic seston, partially comprised of tadpole faeces, transported in the study stream has been shown to be significantly higher in N than in a stream without tadpoles (Whiles *et al.*, 2006; Colón-Gaud, 2008). Biofilm-grazing tadpoles (*Lithobates warszewitschii*) in a nearby Panamanian study stream were also found to have faeces that are N-depleted relative to the tadpoles (5.01‰ and 5.50‰, respectively; Piet Verburg, unpublished). Therefore, as tadpole faeces enter the stream, nitrogen (depleted in ^{15}N relative to tadpoles, but enriched relative to biofilm) becomes biologically available to algae and other microbes. Based on excretion estimates, Whiles *et al.* (2013) found tadpole excretion to account for 36% of the ammonium uptake flux in a similar nearby stream, which decreased to 0.8% of uptake flux after tadpoles were extirpated 2 years later. Results of our study suggest that microbial uptake of tadpole-processed ^{15}N -enriched nitrogen is reflected in the enriched $\delta^{15}\text{N}$ biofilm value we found pre-decline and that tadpoles may influence nitrogen mineralisation rates.

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

Despite widespread declines of biodiversity, little is known about how ecosystems will respond to these losses over ecologically meaningful time scales. This study is the first to characterise 'longer-term' changes in basal resources and to compare initial trends to relatively long-term patterns, following the loss of a dominant consumer group. Our 5-year study, which documented changes occurring in a natural field setting, indicates that the loss of tadpoles significantly influenced algal basal resources. However, the magnitude of the effects changed through time, and other grazers had not fully replaced the role of tadpoles 3 years after their decline. Given that there is no indication of

amphibian communities recovering at sites where they have previously declined drastically (Lips, 1999), our results suggest that stream food webs in regions where amphibian declines have occurred may be permanently altered.

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