ECOSYSTEMS

Disease-Driven Amphibian Declines Alter Ecosystem Processes in a Tropical Stream

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ABSTRACT

Predicting the ecological consequences of declining biodiversity is an urgent challenge, particularly in freshwater habitats where species declines and losses are among the highest. Small-scale experiments suggest potential ecosystem responses to losses of species, but definitive conclusions require verification at larger scales. We measured ecosystem metabolism and used whole-ecosystem stable isotope tracer additions to quantify nitrogen cycling in a tropical headwater stream before and after the sudden loss of amphibians to the fungal pathogen *Batrachochytrium dendrobatidis*. Tadpoles are normally dominant grazers in such streams, where greater than 18 species may co-occur and densities often exceed 50 individuals m^{-2}. Loss of 98% of tadpole biomass corresponded with greater than $2\times$ increases in algae and fine detritus biomass in the stream and a greater than 50% reduction in nitrogen uptake rate. Nitrogen turnover rates in suspended and deposited organic sediments were also significantly lower after the decline. As a consequence, the stream cycled nitrogen less rapidly, and downstream exports of particulate N were reduced. Whole stream respiration was significantly lower following the decline, indicating less biological activity in the stream sediments. Contrary to our predictions, biomass of grazing invertebrates, or any invertebrate functional groups, did not increase over 2 years following loss of tadpoles. Thus, reductions in ecosystem processes linked to the amphibian decline were not compensated for by other, functionally redundant consumers. Declining animal biodiversity has ecosystem-level consequences that may not be offset by ecological redundancy, even in biologically diverse regions such as the Neotropics.

Key words: biodiversity-ecosystem function; extinction; ecological redundancy; nitrogen cycling; emerging infectious disease; isotope tracer; ecosystem metabolism; primary production.

INTRODUCTION

Earth is undergoing one of six mass extinctions in its history (Butchart and others 2010; Barnosky and others 2011). Although some contemporary extinctions involve loss of one or a few species, or wholesale ecosystem destruction (for example, tropical deforestation), many result in losses of entire assemblages of animals (Pauly and others 1998; Barnosky and others 2004). The consequences of these losses are less understood than species additions, for instance, through invasions (Wardle and others 2011). Loss of species from an ecosystem can significantly affect ecosystem properties (Estes and others 2011), although remaining organisms with similar functions could compensate to some degree. Higher biodiversity ecosystems may be more resilient because of greater ecological redundancy (Hooper and others 2005).

Understanding how declining biodiversity affects ecosystems is central to understanding the consequences of extinction (Cardinale and others 2006; Loreau 2010). However, such insight can be difficult to achieve through small-scale manipulations, which may not accurately reflect responses at larger scales (Petchey and others 2004; Duffy 2009). Large-scale field studies can be logistically challenging, expensive, and difficult to replicate, but they have revealed how whole ecosystems respond to a variety of impacts (Wallace and others 1997; Carpenter and others 2001).

Freshwater habitats are particularly relevant for examining the consequences of declining biodiversity because extinction rates and species declines in freshwaters are among the greatest of any habitat (Jenkins 2003; Vaughn 2010). Freshwater animals can significantly influence ecosystem processes such as nutrient cycling through ingestion, egestion, and excretion (Vanni and others 2002). For example, McIntyre and others (2008) estimated fish excretion in a neotropical stream could account for greater than 75% of the ecosystem demand for dissolved inorganic nitrogen. Recent ecosystem-level studies suggest strong effects of losses or reductions of dominant freshwater animal species on ecosystem functioning (for example, Taylor and others 2006). Here, we examine the ecosystem-level consequences of a sudden, massive decline of an entire group of dominant stream animals.

Globally, many amphibian population declines have been attributed to the chytrid fungus, *Batrachochytrium dendrobatidis*. Ongoing infections in Central America, a region rich in amphibian diversity, have created a wave of massive die-offs and extinctions (Lips and others 2006; Crawford

and others 2010). Anuran larvae are the most abundant and diverse group of stream vertebrates in unaffected upland headwaters in this region, with approximately 20 stream-dwelling species and densities often greater than 50 individuals m^{-2} (Ranvestel and others 2004). The predictable advance of the disease front in this region allows for pre- and post-decline studies of streams.

Prior investigations in neotropical streams suggest that amphibian declines may alter stream ecosystem structure and function (Whiles and others 2006). Small-scale manipulations demonstrate that tadpoles can reduce algal biomass on substrata, and tadpoles suspend organic sediments while feeding (bioturbation) (Flecker and others 1999; Ranvestel and others 2004). Comparisons of Central American streams with and without healthy amphibian populations suggest that tadpoles can control primary production, enhance the quality of entrained fine organic particles (seston), and directly and indirectly influence other consumer groups (Colón-Gaud 2008; Connelly and others 2008; Colón-Gaud and others 2009, 2010).

Our objective was to examine the degree to which the sudden loss of an amphibian assemblage from a tropical headwater stream would alter ecosystem processes. We measured whole-stream metabolism and added a $^{15}NH_4Cl$ tracer to a headwater stream in Panama before (2006) and after (2008) a disease-driven amphibian decline. Our prior investigations in similar streams in the region (for example, Ranvestel and others 2004; Whiles and others 2006; Connelly and others 2008) suggested loss of tadpoles could reduce overall N demand and shift the system to a less heterotrophic state by increasing gross primary production (GPP) relative to ecosystem respiration (R). Based on patterns that we observed in these prior studies, we developed 11 a priori hypotheses of specific ecosystem responses (Table 1). Given the predicted increase in epilithon mass, we further hypothesized that grazing insects would respond positively, potentially compensating for the loss of the tadpoles.

MATERIALS AND METHODS

Study Site

Río Maria is a relatively undisturbed wet-forest headwater stream located at approximately 900 m.a.s.l. in the eastern Cordillera Central of Panama. It is a heavily shaded, high gradient, second-order stream with abundant coarse substrata. Average dry season (Jan. to mid-May) discharge was about 23 L s^{-1}, and average wetted width was

Table 1. Means and Statistical Results for Ecosystem Rates and Resource Pool Masses and Turnover Rates in Río Maria Before (2006) and After (2008) the Amphibian Decline

Variable	Prediction	2006 with tadpoles		2008 without tadpoles		P	Hypothesis supported?
		Mean	SD	Mean	SD		
Epilithon							
Mass (g DM m^{-2})	+	39.50	6.49	93.86	31.76	<0.05	Y
Turnover (d^{-1})	−	0.06	0.02	0.05	0.008	NS	N
SPOM							
Concentration (g DM L^{-1})	−	3.59	0.30	0.62	0.34	<0.01	Y
Turnover (d^{-1})	−	0.18	0.03	0.04	0.01	<0.01	Y
CPOM							
Mass (g DM m^{-2})	NC	147.87	78.25	90.32	39.83	NS	NA
Turnover (d^{-1})	NC	0.07	0.026	0.06	0.01	NS	NA
FPOM							
Shallow mass (g DM m^{-2})	+	58.98	58.93	127.44	19.16	<0.05	Y
Shallow turnover (d^{-1})	−	0.02	0.016	0.01	0.007	<0.05	Y
Deep mass (g DM m^{-2})	+	99.79	52.79	255.02	93.83	<0.05	Y
Deep turnover (d^{-1})	NC	0.02	0.015	0.02	0.008	NS	NA
Ecosystem rates							
Respiration (g O$_2$ m^{-2} d^{-1})	−	0.71	0.06	0.32	0.03	<0.05	Y
1° production (g O$_2$ m^{-2} d^{-1})	+	0.001	0.001	0.012	0.014	0.1	Y
NH$_4$ uptake length (m)	+	63.00	7.07	108.00	20.51	<0.05	Y

Values are means of different sampling dates each year (masses, production, and respiration), or different sampling stations (modeled turnover rates) and standard deviations (SD). P values are from permutation tests comparing 2006 to 2008; + or − indicates predicted increases (+) or decreases (−) from 2006 to 2008; Y or N indicates whether hypothesized responses were supported or not, respectively. NC and NA indicate no change was predicted. SPOM suspended particulate organic matter, CPOM coarse particulate organic matter, FPOM fine particulate organic matter, DM dry mass.

3.5 m. Dry season conditions in 2006 and 2008 were nearly identical; temperature was nearly constant at 20°C, and average discharges were 22.4 ± 2.9 (mean ± 1 SD) L s^{-1} in 2006 and 22.9 ± 2.4 L s^{-1} in 2008, with no storm flows during the studies either year. Background concentrations of nutrients were similar for both years; average concentration of ammonium was 2.7 µg N L^{-1}; nitrate = 123 µg N L^{-1}, dissolved organic N = 24 µg N L^{-1}, and soluble reactive P = 4 µg P L^{-1}. Dense canopy cover suggests that primary producers are first limited by light, and N:P ratios indicate that P is the primary limiting nutrient and N is secondarily limiting. Pre-decline tadpole assemblages in Río Maria and similar headwater streams in the region included grazers, filter feeders, and burrowing detritivores, with grazers being most abundant (Ranvestel and others 2004). Only one small insectivorous fish (*Brachyrhaphis roswithae*) was abundant in Río Maria.

Tracer Addition

We added 16 g (Feb. 4–9 2006) and 20 g (Feb. 12–17 2008) of ^{15}NH$_4$Cl solution to the study stream before and after a massive amphibian decline following the arrival of the fungal disease *B. dendrobatidis* in 2006 (~3 months after the 2006 tracer addition). The ^{15}NH$_4$Cl solution was used as a food web tracer that could be followed through all primary uptake components (for example, epilithon, benthic organic matter, and so on) and the animals that consumed them. We pumped the tracer into the upstream end of a 180-m study reach at 5 mL min^{-1} over 5 days during the dry season of both years following the first Lotic Intersite Nitrogen eXperiment (LINX) project procedures (Dodds and others 2000; Peterson and others 2001, http://www.biol.vt.edu/faculty/webster/linx/linx2proto-rev5.pdf). We co-injected NaCl to measure discharge via dilution and to correct uptake length estimates based on 10% groundwater accrual in the reach. Additions raised the δ^{15}N of stream NH$_4$ by 35,000‰, but only raised average concentration of ammonium by 11% and dissolved inorganic N by 1.5%. This level of enrichment was within the range of daily variability in the stream.

We estimated the ^{15}N uptake rate as the downstream decline in δ^{15}N assimilated in actively growing biofilm. We placed unglazed clay tiles in the stream approximately 2 weeks before the addition at one upstream reference station and

each of six stations spaced downstream from the $\delta^{15}N$ release point along the 180-m study reach. A tile was collected from each station at days 2 and 4 (2006) and 2, 4, and 5 (2008) during the addition and was processed as described below. Tiles with new growth had minimal confounding influence of detrital N. We calculated the per meter removal rate (k) using least squares regression on distance (x) versus natural log of $\delta^{15}N$:

$$\ln \delta^{15}N_x = \ln \delta^{15}N_0 - kx \qquad (1)$$

The inverse of k is the uptake length of ammonium (S_{NH4}), which is the average distance NH_4^+ travels before biotic uptake.

Food Web Sampling for Isotopes

We collected samples of all major food web components at the upstream reference station and the six stations below the $\delta^{15}N$ release point on day 0 (immediately pre-release during both years), and days 2, 4, and 5 (during release) and 8, 12, 20, 33, and 46 (post release) during 2006, and days 2 and 5 (during release) and 8, 13, 20, 35, 48, 54, and 59 (post release) during 2008 for analyses of $\delta^{15}N$ following LINX project procedures (Dodds and others 2000; Hall and others 2009). We scrubbed epilithon from rocks and filtered samples through glass fiber filters (GFF; particle retention size = 1.6 μm). Coarse particulate organic matter (CPOM, >1 mm) samples were collected by hand. Shallow (water–sediment interface) and deep fine particulate organic matter (FPOM, <1 mm >250 μm) were collected using a 20-cm-diameter coring device. For shallow FPOM, we placed the corer on the substrata, gently agitated water and materials within it by hand, and collected a sample of the suspension. We then lifted the corer, allowed water to remove the suspended materials, replaced the corer at the same place, agitated materials physically by disrupting the substrata over the top few cm and constantly stirring the contents inside the corer, and collected a sample of the suspension for deep FPOM; both shallow and deep samples were filtered on to GFFs. We collected suspended particulate organic matter (SPOM) by filtering 0.5–3 L water samples from each station through GFFs. We collected invertebrates, tadpoles, and fish by hand (picking individuals from rocks with forceps) or net. Samples were dried to constant mass at 50°C, ground to a fine powder, and analyzed for C and N content and isotopic composition at the University of Georgia Ecology Analytical Chemistry Laboratory (2006) and Kansas State University Stable Isotope Mass Spectrometry Laboratory (2008).

Food Web Sampling for Biomass

We sampled invertebrates in the study reach using a benthic corer (20-cm diameter, three samples each date) in pools and a Surber sampler (250-μm mesh, four samples each date) in riffles and runs, following procedures of Colón-Gaud and others (2009). Invertebrate sampling took place twice in 2006 (days 0 and 48 of the experiment) and three times in 2008 (days 0, 20, and 48). We split samples into coarse (>1 mm) and fine (<1 mm >250 μm) fractions. All invertebrates were removed from coarse fractions. We occasionally subsampled fine fractions (up to 1/8 of total) using a Folsom plankton splitter. Invertebrates were identified to the lowest practical taxonomic level (usually genus), measured (total body length), and assigned to functional groups (Merritt and others 2008). Freshwater crabs in these streams are omnivorous, and we thus divided their biomass evenly among collector-gatherers, shredders, and predators based on our observations of gut contents. We estimated biomass with length–mass regressions (Benke and others 1999).

We obtained estimates of CPOM dry mass (DM) and ash-free dry mass (AFDM) from benthic samples by drying (50°C to constant mass), weighing, ashing (500°C for ~2 h), and re-weighing organic components of coarse fractions after invertebrates were removed. For FPOM, we collected an additional set of core samples in addition to invertebrate samples in pool (three samples per date) and riffle/run habitats (four samples per date); sampling for shallow and deep fractions was performed as described above for isotope sampling, except that volume of material in the corer was recorded and a known volume subsample of material was collected from within the corer. We processed GFFs in the same manner as CPOM to obtain DM and AFDM, and corrected subsample volume to estimate g m^{-2}. We habitat-weighted invertebrate and organic matter values based on proportions of each habitat type in the stream during baseflow conditions (64% riffle/run and 36% pool for both years). We sampled SPOM on 8 (2006) and 9 (2008) dates using procedures described for SPOM isotope sampling above, except that filters were processed for mass and values converted to concentration based on volume filtered.

We sampled epilithon in pools and riffles on days 0, 20, and 48 of the experiment in 2006 and days 0, 20, and 54 of the experiment in 2008 using a Loeb sampler (Loeb 1981). On each sample date, we collected 5–7 samples in randomly selected pools and 5–7 in randomly selected riffles/runs. Samples

were filtered on to GFFs and processed and habitat-weighted as for other particulate samples.

We sampled tadpoles and fish at randomly selected 1–3 m reaches of the study reach using block nets and depletion sampling. We placed 0.5-cm mesh block nets at the upstream and downstream end of each selected 1–3 m reach and collected fish and tadpoles with dip nets until repeated sweeps produced no animals. This generated six habitat-weighted density estimates for each year. We converted densities to biomass using length–mass relationships following procedures of Benke and others (1999).

We calculated a mass balance for tracer ^{15}N following Hall and others (2009). For each N pool (including epilithon, CPOM, shallow, and deep FPOM), we calculated N standing stock using mass estimates described above and measured C:N. The ^{15}N in each pool was extrapolated to a ^{15}N standing stock (g ^{15}N m^{-2}) by multiplying the atomic fraction of ^{15}N excess (^{15}N$_{xs}$ which is background-corrected atomic fraction of ^{15}N) by the standing stock of N. We multiplied standing stock by wetted area to extrapolate to the reach for each date.

Food Web Models

We used data on N content of ecosystem compartments through the experiment (g N m^{-2}) and temporal trends of ^{15}N in those compartments and the N sources for those compartments to calculate N flux through each of the major compartments (Hall and others 1998; Wollheim and others 1999; Dodds and others 2000). Each dominant "compartment" was modeled. For primary uptake compartments, these included epilithon, FPOM, CPOM, and SPOM. Dominant genera from each of the major invertebrate functional feeding groups (insect grazers, shredders, gatherers and filterers), tadpoles, and crabs were modeled separately (that is, modeled as its own "compartment") for turnover. For the primary uptake compartments where N influx was from the water (organic matter and epilithon), we calculated turnover rate (k_x, d^{-1}) of the compartment at each station downstream (x) as the exponential decline in label in the compartment immediately after cessation of the ^{15}N addition:

$$\ln \delta^{15}N_{t,x} = \ln \delta^{15}N_0 - k_x t, \tag{2}$$

where $\delta^{15}N_{t,x}$ is the isotope label of a compartment at time t and station x downstream. We averaged among stations to calculate an aggregate turnover rate, k.

We calculated the overall N flux (F g N m^{-2} d^{-1}) through detrital and epilithic compartments (Nmass, g N m^{-2}) as:

$$F = k \times \text{Nmass} \tag{3}$$

For compartments that depended on food that retained label after the ^{15}N addition was stopped (invertebrates, fishes, and tadpoles), the temporal pattern of ^{15}N in the pool was modeled based on the observed patterns in the food compartments. In cases where more than one compartment was used as food, such as omnivores and predators that relied upon more than one prey taxon, each food compartment was weighted by its relative availability. We assumed predators fed on the most available appropriately sized prey in the stream. The change in label over time in the consumer compartment was a function of uptake rate of label from the food compartment, with the ^{15}N/^{14}N set by measurements of the $\delta^{15}N_{t,x}$ in food compartment and source-averaging between the two sampling dates (Laws 1984). Losses of ^{15}N and ^{14}N were assumed proportional to the $\delta^{15}N_{t,x}$ of the consumer. The ratio of uptake to loss was set by the measured difference in the N mass values of the modeled consumer between the measured values at the beginning and end of the experiment. The model used the "solver" option in Excel to minimize the mean square sum of error between the observed and modeled ^{15}N in the compartment by changing the rate of uptake from the food pool into the consumer pool, and the ratio of uptake and loss subject to the additional constraint that calculated and measured biomass values at the end of the experiment were equal.

We estimated turnover rates for each compartment at each sampling station where data were available (not all stations had all large consumers). Values from each station were used to statistically compare turnover rates in 2006 and 2008 using the randomization–permutation test described below.

Tadpole Excretion

We estimated tadpole excretion by developing taxon- and mass-specific ammonium excretion rate estimates following procedures of Whiles and others (2009). We used tadpoles collected from Río Maria and other streams in the region. Numbers of individuals used and body size ranges (mg DM) where $n = 83$ *Lithobates warszewitschii* (0.45–87.06 mg), $n = 73$ *Hyloscirtus* spp. (0.5–82.8 mg), and $n = 25$ *Colostethus* spp. (0.63–19.5 mg).

Reach Scale Metabolism

We modeled reach-scale metabolism using a single station method and diel dissolved O$_2$ patterns at each of two sites along the reach over several

sunny days each year during the period of the tracer additions (days 0–4 of the experiment in 2006 and days 1–5 in 2008) (Bott 2006). The basic modeling and equations for this procedure are described by Holtgrieve and others (2010), though we used least squares minimization rather than a Bayesian implementation. We used measured discharge, re-aeration rate [from propane releases (Bott 2006)], light, and mean width to parameterize the metabolism model. Our model used a hyperbolic relationship between light and photosynthetic rate (Jassby and Platt 1976) and varied gross photosynthetic rate and whole stream respiration to match observed temporal trends of dissolved O_2. The model used the ''solver'' option in Excel to minimize the mean square sum of error between the observed and the modeled dissolved O_2 every 10 min over each day of measurement. Two and three estimates of reach scale R and GPP were generated for 2006 and 2008, respectively.

Statistical Analyses

Inference for this study was different from the one if we had conducted a randomized, replicated field experiment. The disease wave extirpated amphibians from all upland streams in the region, eliminating potential spatial controls, and logistics precluded simultaneous ^{15}N additions in several streams. However, based on our prior small-scale experimental studies in nearby streams (for example, Ranvestel and others 2004; Whiles and others 2006; Connelly and others 2008; Colón-Gaud and others 2009), we developed informed predictions for potential ecosystem responses to tadpole extirpation. We compared means of response variables between years and compared responses to 11 a priori hypothesized changes (Hebblewhite and others 2005). We used randomization and re-sampling to develop variance structures and standard deviations for pre- and post-decline means of compartment biomass, metabolism, and ammonium uptake. We used values from each station to develop means for N turnover rates in food web compartments because these estimates are based on patterns over time at stations. Any one statistical test could only test for a difference in means between 2006 and 2008, and not test whether loss of tadpoles caused these differences. However, if most of our predictions were met, we inferred that tadpoles caused the changes using a balance of evidence approach, following Hebblewhite and others (2005).

We performed one-tailed nonparametric permutation tests (Fisher exact test), which do not assume an underlying distribution. Test statistics were differences between 2006 and 2008 means. We compared these to the distribution of permuted means based on re-sampling of the pooled 2006 and 2008 data 10,000 times and taking the difference in the means. P values were the fraction of permuted differences in means that lay outside the test statistic. We considered differences significant at $P < 0.05$, and marginally significant at $P < 0.1$. Randomization and re-sampling and all statistical analyses were performed using the statistical package R (R Development Core Team 2010).

RESULTS

Tadpole populations plummeted in Río Maria following the arrival of the fungal pathogen; total habitat-weighted tadpole biomass in 2008 was 2% that of 2006 ($P < 0.001$, Figure 1C). The appearance of the stream substrata changed after the decline. In 2006, rock substrata were relatively clean, with little fine sediment and patches of prostrate periphyton (Figure 1A). Substrata in the same reach in 2008 were covered with dense organic sediments composed primarily of senescent diatoms (Figure 1B). Accordingly, mass of epilithon, which includes living and senescent periphyton, organic sediments, and microbial components, increased by 73% from 2006 to 2008 (Table 1, $P < 0.01$). Despite increased epilithon biomass after the decline, grazing invertebrates, and all other functional groups, did not exhibit a significant positive biomass response (Figure 1D, $P > 0.1$ for all groups).

Fine particulate organic matter (FPOM) in the stream channel more than doubled from 2006 to 2008 ($P < 0.05$ for both shallow and deep FPOM, Table 1). In contrast, SPOM concentrations in 2008 were 1/5 of those in 2006 ($P < 0.01$, Table 1). There was no change in CPOM mass during 2006-2008.

Contrary to our prediction, N turnover in epilithon did not change from 2006 to 2008. However, post-decline N turnover in SPOM in 2008 dropped to one-third that of pre-decline, and shallow FPOM (uppermost layer) N turnover in 2008 was half that of pre-decline rates ($P < 0.01$ and $P < 0.05$, respectively, Table 1). There was no change in N turnover in CPOM.

The overall N turnover rate in the stream was slower following tadpole extirpation. Nitrogen tracer inventoried in the channel depurated from the stream at a lower rate in 2008 compared to 2006 (Figure 2). Average residence time for N in 2008 (22 days) was twice that of 2006 (11 days). Ammonium cycling rates also declined following
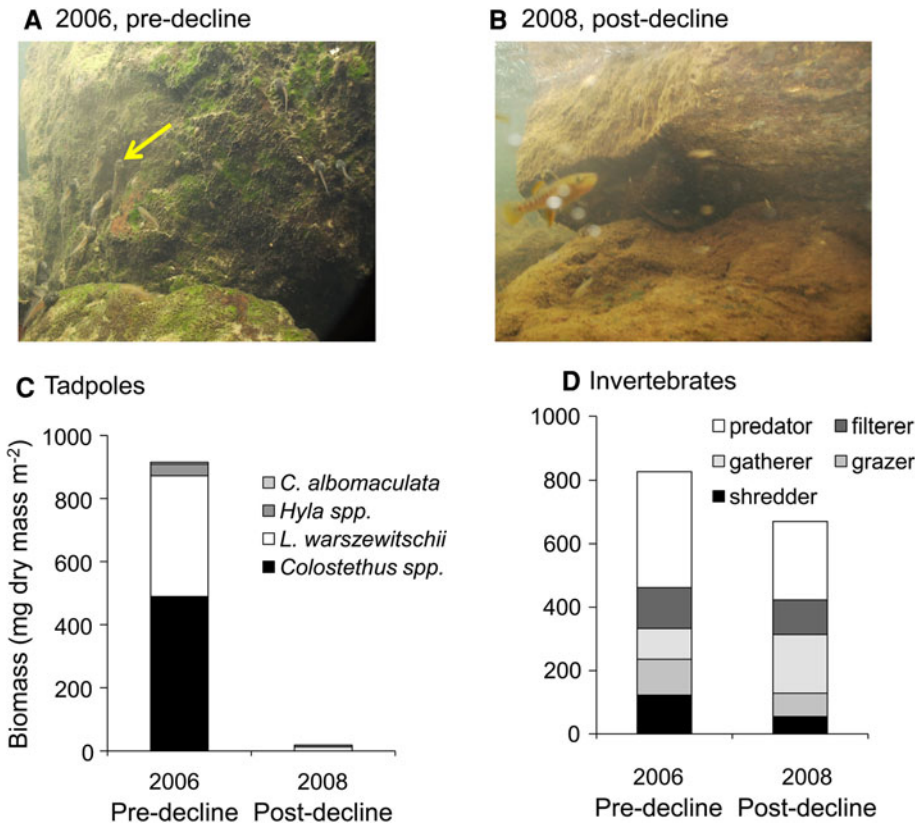
Figure 1. Photographs of the same rock pile approximately 20 cm deep in the study stream before (**A** 2006) and after (**B** 2008) the amphibian decline and pre- and post-decline average habitat-weighted biomass of dominant tadpole taxa (**C**) (*C. albomaculata Cochranella albomaculata* and *L. warszewitschii Lithobates warszewitschii*) and invertebrate functional groups (**D**) in Río Maria. Note the grazing tadpoles on the rock in (**A**), one is indicated by the *arrow*. Tadpole biomass declined by 98% from 2006 to 2008 ($P < 0.001$); biomass of invertebrate functional groups did not differ significantly between years.

tadpole extirpation. Ammonium uptake length nearly doubled from 63 ± 7 m in 2006 when tadpoles were present to 108 ± 15 m in 2008 ($P < 0.05$), indicating lower demand for NH_4^+ despite the more than doubled epilithon biomass in the stream.

Mineralization of NH_4^+–N via animal excretion was substantially reduced after the decline. Excretion rates for the three dominant tadpole taxa in the stream were *Lithobates warszewitschii* = 0.28 µg mg^{-1} h^{-1}, *Hyloscirtus* spp. = 0.27 µg mg^{-1} h^{-1}, and *Colostethus* spp. = 0.48 µg mg^{-1} h^{-1}. Based on these estimates, mineralization by tadpoles in 2008 (0.005 mg m^{-2} h^{-1}) was 1.4% of that of 2006 (0.35 mg N m^{-2} h^{-1}).

The heterotrophic state of Río Maria decreased following the decline. Reach-scale R in 2008 was less than 50% of that observed in 2006 ($P < 0.05$, Table 1). Reach-scale GPP was low and variable, but did increase from 2006 to 2008 ($P = 0.1$, Table 1). Overall, the degree of heterotrophy in Río Maria decreased tenfold after the amphibian decline ($P/R = 0.001$ in 2006 and 0.038 in 2008).

Fluxes of N from basal resources to animals decreased after the decline (Figure 3). Coarse particulate organic matter (CPOM) dominated N uptake by basal resources during both years and FPOM accounted for the second largest percentage
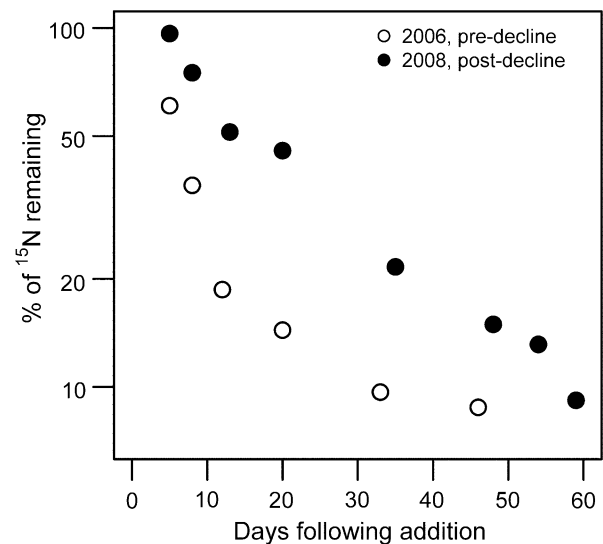

Figure 2. Percent of added ^{15}N tracer inventoried in all ecosystem compartments of the study stream (benthic and suspended organic matter, epilithon, invertebrates, and vertebrates) over time following the 2006 and 2008 additions (day 0 is last day of ^{15}N release). Note log scale on vertical axis. Slower decline in 2008 indicates the tracer cycled more slowly through the food web.

of uptake in 2006, followed by epilithon (Figure 3A). However, the greatest flux from basal resource to animals in 2006 was from epilithon to

grazers, 92% of which was accounted for by tadpoles (Figure 3A, B). Before the decline, tadpoles consumed approximately 100% of N input to epilithon and about 6% of epilithon mass per day. After the decline, a 566% increase in epilithon N mass resulted in greater flux of N to the epilithon compartment, but without tadpoles, a little of this N was directly taken up by animals (Figure 3C, D); N flux from epilithon to grazers after the decline was 3.5% of that observed in 2006.

DISCUSSION

The amphibian decline we studied strongly altered the structure and function of Río Maria, resulting in overall decreased uptake and cycling of N, a vital ecosystem process in headwater streams (Mulholland and others 2009). Overall, nine of our 11 a priori hypotheses were supported in terms of direction of change following amphibian loss, and three predictions of no change were also

supported (Table 1). Only two of our predictions were not supported, but one of them (compensatory responses by other grazers) was ultimately consistent with our overall conclusion that amphibian declines significantly alter ecosystem functions, as we found no evidence for ecological redundancy.

Unlike many studies that manipulate species presence (for example, Ranvestel and others 2004; Taylor and others 2006; Hall and others 2011), our study documented an actual, ''natural'' loss of an entire animal assemblage. The pattern of disease wave movement, and lack of comparable accessible streams nearby did not allow for establishment of a spatial control in the region. However, unlike some experiments that test what could happen if a species declines, we quantified an actual decline and associated changes in ecosystem processes. Thus, although our study lacked the power of a controlled experiment, it provided the realism of a regional decline of an animal assemblage.
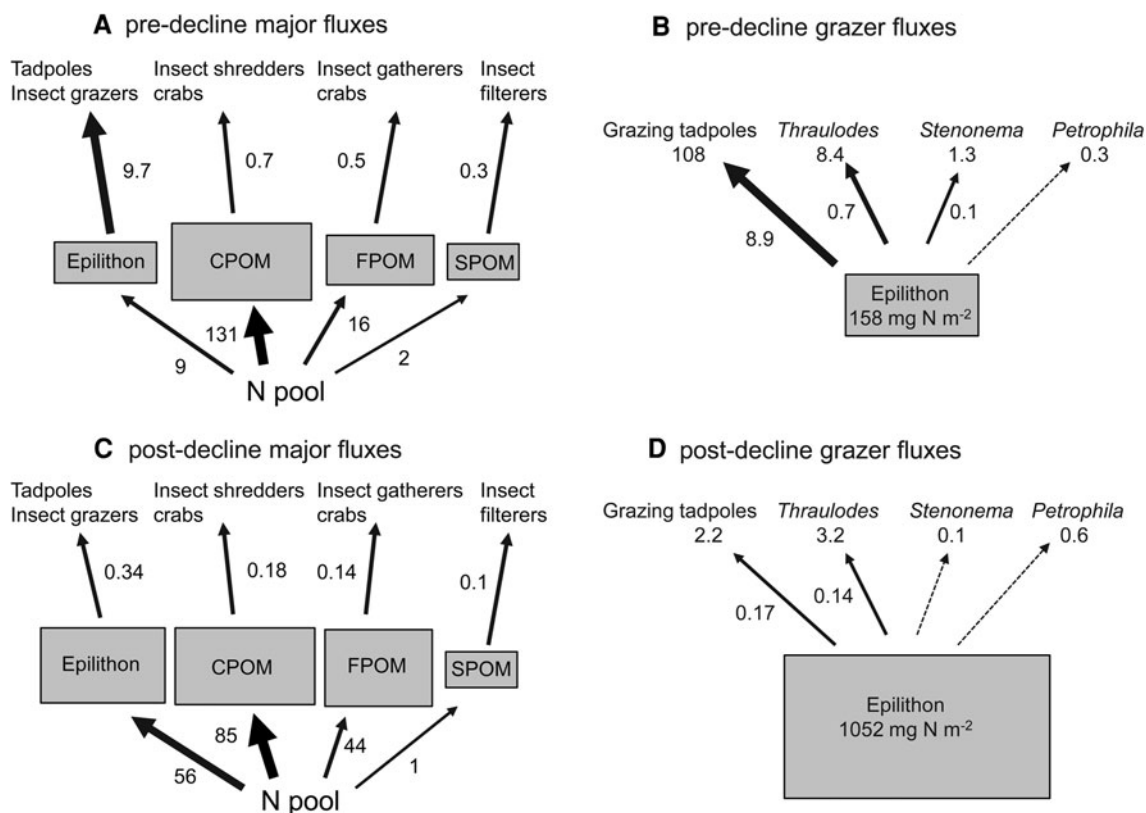

Figure 3. Food web fluxes (mg N m^{-2} day^{-1}) and biomass standing stocks (mg N m^{-2}) in Río Maria before (2006) and after (2008) the amphibian decline. **A** and **C** show major pathways from basal resources to animal functional groups; **B** and **D** show fluxes from epilithon to dominant grazer taxa. Fluxes are calculated as the product of habitat-weighted N mass and modeled turnover rates of each food web component. Values underneath grazer taxa and epilithon in **B** and **D** are mg N m^{-2} in those compartments. *Dashed arrows* are fluxes less than 0.1 mg N m^{-2} day^{-1}. *Thraulodes* (Leptophlebiidae) and *Stenonema* (Heptageniidae) are mayflies (Ephemeroptera); *Petrophila* (Crambidae) are aquatic moth larvae (Lepidoptera).

Ecological Redundancy and Compensatory Responses

Tropical habitats are generally more speciose by nature than their temperate counterparts, and thus higher degrees of ecological redundancy might be expected. However, alpha diversity of stream invertebrates does not increase at lower latitudes (Pearson and Boyero 2009), and there are no other vertebrate grazers in these streams. Freshwater shrimps, which are important grazers in some Neotropical streams (Greathouse and others 2006), are scarce in Río Maria, presumably due to migratory barriers in downstream areas.

Our results contrast with findings of Kohler and Wiley (1997), who observed strong positive responses within months by multiple grazing insect taxa to disease-driven declines of a dominant grazing insect. They attributed these positive responses to strong competition among grazers in their study streams. Lack of positive responses by grazing insects in Río Maria suggests that they do not compete with tadpoles, or other factors such as predation and/or frequent, natural hydrologic disturbances such as wet-season floods control their populations; the streams Kohler and Wiley (1997) studied were very stable groundwater-fed streams. A prior investigation in a stream in an adjacent region of Panama also found no overall compensatory response by grazing invertebrates 1 year after an amphibian decline, although production of some individual grazer taxa did increase after the decline (Colón-Gaud and others 2009). Our study allowed nearly 2 years for potential compensatory responses from other animals, and none replaced tadpoles as dominant grazers. Two years is ample time for demographic responses by grazing invertebrates in streams in this region, many of which have life cycles of less than 3 months (Colón-Gaud 2008).

Ecological redundancy and the degree of compensatory responses in a given ecosystem may depend on factors such as the traits of the species removed, the remaining species pool, and order and timing of species losses (Solan and others 2004; Wardle and others 2011). Relatively large-bodied grazers other than tadpoles are scarce in these streams, and many of the ecosystem effects of tadpoles can be ascribed to physical processes linked to body size (for example, bioturbation, Flecker and others 1999). Even if tadpole biomass had been replaced by an equal biomass of smaller-bodied animals, such as invertebrates, compensation may not have occurred. Recent observations of responses of insects to a reach-scale removal of fish from a lowland tropical river support this concept; despite a three-fold increase in their production, insects did not compensate for the loss of fish in processing organic matter (Hall and others 2011).

Specific Outcomes of Tadpole Losses

Tadpoles are important bioturbators in tropical streams, and their loss increased sediment accumulation and decreased export of organic particles. Results of our study are consistent with small-scale experiments in the region showing that tadpole feeding reduced sediment accumulation on stream substrata (Ranvestel and others 2004; Whiles and others 2006). The decrease in biological activity (N turnover) of FPOM and SPOM associated with amphibian declines, combined with reduced concentrations and export of SPOM, could adversely affect downstream communities that rely on particulate materials exported from headwaters (Wallace and Webster 1996). Although N flux into the SPOM pool was small in these headwater streams with low turbidity, production of fine organic particles in headwaters and subsequent export as SPOM can dominate N export from streams (Hall and others 2009).

The SPOM and shallow FPOM compartments in streams interact closely, as particulate materials alternate between entrainment and deposition in the stream, and thus the decrease in turnover rate of shallow FPOM is consistent with SPOM patterns. We attribute lower turnover rates of both of these pools to decreased processing and microbial activity associated with tadpole feeding and egestion. Studies in similar streams in Panama indicate the dominant tadpole taxa found in Río Maria before the decline ingest significant quantities of fine organic particles (Ranvestel and others 2004). Turnover of deeper layers of FPOM did not change, as most tadpoles could not access these materials.

Reduced overall N turnover in the stream after the amphibian decline was likely due to lower mineralization rates (less ingestion and excretion by tadpoles) and lower export as fine particulates (reduced bioturbation). Based on our excretion estimates, tadpole excretion was 36% of the ammonium uptake flux in 2006 (0.987 mg N m^{-2} h^{-1}), and this decreased to 0.8% of uptake flux (0.59 mg N m^{-2} h^{-1}) in 2008. Fish excretion in a lowland tropical stream accounted for greater than 75% of ecosystem demand for dissolved inorganic N (McIntyre and others 2008). Tadpoles replace fish as dominant contributors to N mineralization in upland headwaters, and thus their declines shift N cycling away from animal-driven mineralization to microbial pathways such as uptake

and mineralization in biofilms, which did not compensate for the loss of tadpoles.

Turnover rates of N in epilithon did not decrease with the loss of amphibians, contrary to our hypothesis. Small-scale experiments indicated that tadpole grazing stimulates biomass-specific algal production (Connelly and others 2008), which would suggest higher epilithon turnover with tadpoles present. However, GPP in Río Maria was so low relative to epilithon biomass that the increase in GPP after the decline did not result in overall increased epilithon turnover. Rather, heterotrophic microbial activity likely drove epilithon biomass and turnover following the decline, and unconsumed epilithon was subject to bacterial decomposition and/or lost to other compartments such as FPOM.

The amphibian decline we witnessed during this study was, unfortunately, not an unusual or isolated event. The degree of the decline in Río Maria was similar to that observed in 2003–2004 in a nearby site (Lips and others 2006), and documented disease-driven declines on other continents (for example, Swei and others 2011; Becker and Zamudio 2011). Streams in other regions that are experiencing amphibian declines may undergo changes similar to those we observed, as Río Maria is a fairly typical tropical forested headwater stream.

Chytridiomycosis, a disease caused by an invasive fungal pathogen, was the ultimate cause of the decline and associated ecological responses we observed, and similar results can be expected for other tropical streams where amphibians have declined. As emerging infectious diseases like chytridiomycosis increase in number and distribution, we can expect more species declines and extinctions (Daszak and others 2000; Crowl and others 2008). Such drastic losses of entire large animal groups are particularly likely in freshwater habitats, which are already stressed by myriad threats, and we suggest that the remaining species may not compensate for associated losses of ecosystem functions.

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