

# Do tadpoles affect leaf decomposition in neotropical streams?

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## SUMMARY

1. Of the relatively few studies that have examined consequences of amphibian declines on stream ecosystems, virtually all have focused on changes in algae (or algal-based food webs) and little is known about the potential effects of tadpoles on leaf decomposition. We compared leaf litter decomposition dynamics in two neotropical streams: one with an intact community of tadpoles (*with frogs*) and one where tadpoles were absent (*frogless*) as a result of a fungal pathogen that had driven amphibians locally extinct. The stream with tadpoles contained a diverse assemblage (23 species) of larval anurans, and we identified five species of glass frog (Centrolenidae) tadpoles that were patchily distributed but commonly associated with leaf detritus and organic sediments in pools. The latter reached total densities of 0–318 tadpoles m<sup>-2</sup>.

2. We experimentally excluded tadpoles from single-species leaf packs incubated over a 40-day period in streams with and without frogs. We predicted that decomposition rates would be higher in control (allowing access of tadpoles) treatments in the study stream *with frogs* than in the *frogless* stream and, in the stream *with frogs*, in the control than in the tadpole exclusion treatment.

3. In the stream *with frogs*, *Centrolene prosoblepon* and *Cochranella albomaculata* tadpoles were patchily distributed in leaf packs (0.0–33.3 m<sup>-2</sup>). In contrast to our predictions, leaf mass loss and temperature-corrected leaf decomposition rates in control treatments were almost identical in our stream *with frogs* (41.01% AFDM lost,  $k_{\text{degree day}} = -0.028 \text{ day}^{-1}$ ) and in the *frogless* stream (41.81% AFDM lost,  $k_{\text{degree day}} = -0.027 \text{ day}^{-1}$ ) and between control and tadpole exclusion treatments within each stream. Similarly, there were no significant differences in leaf pack bacterial biomass, microbial respiration rates or macroinvertebrate abundance between treatments or streams. Invertebrate assemblages on leaf packs were similar between treatments (SIMI = 0.97) and streams (SIMI = 0.95) and were dominated by larval Chironomidae, Simuliidae (Diptera) and larval *Anchytarsus* spp. (Coleoptera).

4. In contrast to dramatic effects of grazing tadpoles on algal communities observed previously, tadpoles had no major effects on decomposition. While centrolenid tadpoles were common in the stream *with frogs*, their patchy distribution in both experimental and natural leaf packs suggests that their effects on detrital dynamics and microbes are probably more localised than those of grazing tadpoles on algae.

*Keywords:* Centrolenidae, decomposition, fungal biomass, glass frogs, neotropical streams, tadpoles

## Introduction

Understanding the ecological roles of amphibians has become increasingly important in the light of the ongoing catastrophic decline of amphibians around the world. Little is known about the natural history or functional role of many taxa of larval frogs in freshwater ecosystems. Consequently, changes to ecosystem processes resulting from their losses are poorly understood (but see Colón-Gaud *et al.*, 2008; Connelly *et al.*, 2008; Iwai, Pearson & Alford, 2009). The few previous studies that have examined the role of tadpoles in freshwater ecosystems have generally focused on herbivorous taxa and their top-down effects on algal communities. However, there is increasing evidence that many taxa of tadpoles have much more diverse diets than previously assumed (Altig, Whiles & Taylor, 2007; Schiesari, Werner & Kling, 2009).

The degree to which the presence or absence of tadpoles may affect stream processes such as leaf litter decomposition dynamics, and their influence on microbial and macroinvertebrate communities, is relatively unknown (but see Iwai & Kagaya, 2007; Iwai *et al.*, 2009). Tadpoles of the glass frog (Centrolenidae) family are particularly understudied, yet they are often common in dense leaf packs and accumulated decaying organic matter in neotropical streams (McDiarmid & Altig, 1999). Species of this fossorial group have characteristic long tails and fusiform bodies and are generally adapted to burrowing into sediments (Villa & Valerio, 1982). Gut content analysis indicates that glass frog tadpoles ingest a high proportion of fine benthic detritus, although recent studies suggest these tadpoles assimilate energy primarily from associated microbes rather than the detritus itself (Hunte-Brown, 2006; Whiles *et al.*, 2006). The tadpoles of many glass frog species have yet to be described formally, however, and species-specific natural history characteristics (i.e. typical densities in streams, feeding behaviour and food requirements) are generally unknown. While many studies have shown that various aquatic macroinvertebrates can influence leaf litter decomposition in headwaters (e.g. Whiles & Wallace, 1997; Teigs *et al.*, 2008), the potential roles of anuran larvae, such as glass frog tadpoles, have not been investigated. Our objectives were to: (i) characterise neotropical tadpole assemblages associated with decaying leaves in Panamanian headwater streams; (ii) compare experimentally leaf

litter decomposition dynamics between a stream with an intact tadpole community (*with frogs*) and one from where tadpoles had been lost (*frogless*); and (iii) assess experimentally how the presence/absence of tadpoles affects leaf decomposition rates, fungal and bacterial biomass and macroinvertebrate communities associated with decaying leaves. Because tadpoles can change their immediate environment physically, via bioturbation of accumulated leaves (*sensu* Ranvestel *et al.*, 2004), and have the potential to stimulate microbial production via excreted ammonia, we hypothesised that significantly higher breakdown rates of leaves would occur in our study stream *with frogs* than in the *frogless* stream. We predicted that experimental exclusion of centrolenid tadpoles from leaf packs in the stream *with frogs* would result in: (i) a reduced decomposition rate (i.e. reduced loss of leaf mass over time); (ii) increased fungal and bacterial biomass on leaves; and (iii) an increased abundance of shredding macroinvertebrates.

## Methods

### Study sites

This study was conducted in two upland Panamanian streams, the Rio Guabal and the Quebrada Chorro, in an area with a distinct dry season (c. January–April) and a pronounced wet season (c. May–December).

Rio Guabal (hereafter referred to as the stream *with frogs*) is a second-order stream 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 heavily forested, high-gradient stream characterised by distinct pool–run–riffle sequences, with a substratum of pebbles and gravel with frequent cobbles, boulders and depositional sandy areas. At the time of our study, 40 species of riparian frogs were found at this stream, 23 of which had stream-dwelling larvae (Lips, Reeve & Witters, 2003). Tadpoles occurred in all stream habitats, including detrital accumulations in pools where glass frog (Centrolenidae) tadpoles are found (Lips *et al.*, 2003).

The second stream, Quebrada Chorro (*frogless* stream), is approximately 200 km from the stream *with frogs*. This *frogless* stream drains the Reserva Forestal Fortuna, Chiriquí, Panama (8°42'N, 82°14'W). It is similar to the tadpole-dominated stream in terms of order, geology, canopy cover, substratum and nutrient

concentrations, and it is characterised by riffle and run sequences with isolated pools (Colón-Gaud *et al.*, 2008). One *Macrobrachium* shrimp species is found in Rio Guabal and Quebrada Chorro, and larvae of the beetle *Anchytarsus* dominate the invertebrate biomass (Colón-Gaud *et al.*, 2009) in both streams. Past studies have treated the *frogless* stream as a control relative to the stream *with frogs* (Whiles *et al.*, 2006; Connelly *et al.*, 2008; Colón-Gaud *et al.*, 2009).

The *frogless* stream and surrounding region suffered a catastrophic extinction event associated with the fungal pathogen *Batrachochytrium dendrobatidis* (Longcore) in 1996 (Lips, 1999). Fifty-seven anuran species were previously found at the site, including the centrolenid anurans found at the stream *with frogs* (Lips, 1999). Virtually no tadpoles (average during our study  $\leq 0.01$  tadpoles  $\text{m}^{-2}$ ) have been seen since 2000. More detailed descriptions of these two study sites are found in the studies of Connelly *et al.* (2008) and Colón-Gaud *et al.* (2009).

#### *Centrolenid tadpole densities and ammonium excretion rates*

To estimate natural tadpole densities associated with detrital accumulations in our stream *with frogs*, surveys were conducted in July, August and September 2003 (wet season), in February, March and April 2004 (dry season), and in May, June, July and August 2004 (wet season). Tadpoles in three randomly chosen depositional habitats (sites), approximately 30 m apart, were quantified with a stove-pipe benthic corer (22 cm diameter) that was modified with external rubber flaps at the base to help seal the bottom of the sampler when the substratum was irregular. The core sampler was pushed approximately 3 cm into the substratum, and tadpoles were removed with a dip net (15 × 10 × 10 cm), counted, identified to species and released, following methods described in the study of Connelly *et al.* (2008). Three samples were obtained from each of the three sites.

Mass-specific ammonium excretion rates were estimated from a mixed species assemblage of seven centrolenid tadpoles, ranging in mass from 1.4 to 78.5 g, collected from leaf packs in the stream *with frogs*. Individual tadpoles were placed in 60-mL centrifuge vials filled with stream water and incubated on the stream bank for 1 h. After the removal of tadpoles,  $\text{NH}_4^+$  concentrations in the water

in the centrifuge vials were immediately measured following the method of Holmes *et al.* (1999), as modified by Taylor *et al.* (2007). Ammonium concentrations after 1 h were corrected for background concentrations from tubes without tadpoles and then standardised for the dry mass of each tadpole. Tadpole body lengths were measured at the end of excretion incubations, and tadpole biomass was estimated using length–mass relationships following methods of Benke *et al.* (1999). All fluorometric measurements of ammonium were taken within 5 h of the end of each incubation trial using a Turner Designs 10-AU fluorometer (Turner Designs, Inc., Sunnyvale, CA, U.S.A.).

Water samples were taken during base flow once during June 2004 from the stream *with frogs* and once during July 2004 from the *frogless* stream and analysed for  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and soluble reactive phosphorus (SRP). Samples (two replicates per stream) were filtered through 0.45- $\mu\text{m}$  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). Water temperature was recorded hourly at each site with HOBO temperature data loggers (Onset Corporation, Pocasset, MA, U.S.A.), and discharge at the downstream end of each study reach was recorded daily during the course of the experiment (May–August 2004).

#### *Tadpole exclusion experiment*

Tadpole exclusion experiments were run consecutively in reaches of each of the two study streams from 26 May to 4 July 2004 (in the stream *with frogs*) and from 6 July to 15 August 2004 (in the *frogless* stream). Electric exclusion devices (0.5- $\text{m}^2$  frames constructed of PVC tubing and concentric copper wire loops), modified from the study of Pringle & Hamazaki (1997), were used to exclude tadpoles from experimental leaf packs placed on the stream bottom. Similar devices have been used to exclude tadpoles from artificial substrata at this study site (Ranvestel *et al.*, 2004; Connelly *et al.*, 2008), and exclusion devices were tested prior to this experiment by placing several individual glass frog tadpoles within each replicate and confirming that the tadpoles promptly left electrified quadrats. Ten treatment pairs

(one electrified device and one control device, i.e. without electricity) were placed under naturally accumulated leaf litter in pools and runs of two comparable 400-m reaches, one from each stream. Locations chosen were based on similar accumulations of organic sediments, current velocity (near zero at base flow), depth (19–29 cm) and per cent canopy cover (75–85%). Current velocity was measured with a Marsh McBirney current meter, and canopy cover was measured with a spherical densiometer. Most suitable naturally occurring sediment accumulations within the 400-m study reaches of both streams had quadrats placed in them.

Freshly fallen leaves were collected from several individual trees of *Trichospermum galeotti* (Turcz.) Kosterm. (Tiliaceae), a common riparian species, found growing within 5 m of each stream bank. Leaves were air-dried for 2 days, and 6 g of leaves was placed in coarse mesh (1 × 1 cm) plastic bags (15 × 20 cm). The large mesh size was used to allow access to the largest centrolenid tadpoles in the stream, which ranged in body length from 4.1 to 7.2 mm. Eight leaf packs were fastened horizontally with plastic cable ties within each of the 20 PVC frames, for a total of 80 bags per treatment per stream. Replicates of each pair of frames (electrified and non-electrified) were situated at least 0.5 m apart, were buried under about 2–10 cm of leafy detritus in pools and runs, representative of habitats where glass frog tadpoles are found and were anchored with tent stakes to ensure the leaf packs were in contact with the stream bed and to prevent movement during spates. Each replicate was observed for 3 min on each of 20 days and 20 nights (for a total of 40 h for all treatments in each stream) during the experiment to ensure that electric exclusion devices were functioning properly and excluding all tadpoles and to make observations of any other macroconsumers (e.g. fishes and shrimps) that might enter control treatments.

On day 0, one leaf pack was taken to each stream site and returned to the laboratory to control for handling losses. Subsequently, one randomly selected leaf pack was removed from each replicate every 5 day over the 40-day experiment (20 leaf packs on each sampling date: 10 control and 10 tadpole exclusion). The leaf pack was cut from each PVC frame, placed within a fine mesh dip net to prevent the potential escape of tadpoles and invertebrates and placed in a Ziploc® (SC Johnson, Racine, WI, U.S.A.) bag along with the contents of the

dip net. Leaf packs were then transported in a cooler to the laboratory, where the leaves were rinsed (along with the contents of the bag), over a 250- $\mu$ m mesh sieve to remove tadpoles, invertebrates and sediments. Tadpoles were measured, identified to species and returned to the stream. Tadpole dry mass was estimated using mass–length regression according to the equation: tadpole mass =  $(6.61 \times 10^{-5}) \times (\text{length}^{3.6132})$ . Macroinvertebrates were identified and preserved in 8% formalin solution. Forty leaf discs (8 mm) were punched from randomly selected leaves from each pack. Twenty discs were dried and ashed to determine mean disc ash-free dry mass (AFDM), and the remaining 20 were used to determine microbial respiration and fungal and bacterial biomass. Remaining leaf material was dried for 24 h at 40 °C and weighed, and a approximately 1-g subsample was then ashed at 500 °C and reweighed to determine AFDM. Day 0 leaf packs were used to estimate initial AFDM.

*Fungi.* Fungal biomass associated with leaf packs was estimated by measuring ergosterol concentration, following methods described by Suberkropp & Weyers (1996). On each sampling date, 10 leaf discs from each leaf pack were placed in 5 mL of methanol and transported to the laboratory. Ergosterol was extracted from leaf discs in alkaline methanol by refluxing for 30 min, partitioning into pentane, drying and redissolving in methanol. Ergosterol concentration was determined by comparing absorbance at 282 nm after separation from other lipids by high-performance liquid chromatography (HPLC; Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) with a standard concentration of ergosterol (Fluka, St. Louis, MO, U.S.A.). To convert ergosterol to fungal dry mass, we used an ergosterol concentration of 5.5  $\mu\text{g mg}^{-1}$  of mycelial dry mass, following methods of Gessner & Chauvet (1993).

*Bacteria.* Bacteria were counted using epifluorescence microscopy after staining cells with DAPI (Porter & Feig, 1980; Velji & Albright, 1993). Five discs from each leaf pack were preserved in a 0.2- $\mu$ m filtered solution of 5% formaldehyde and stored at 4 °C. In the laboratory, bacterial cells were separated from leaf material by sonication (HT 150 Sonicator; VWR Scientific Inc., West Chester, PA, U.S.A.) (Weyers & Suberkropp, 1996), and 2-mL subsamples were placed in a Millipore vacuum filter manifold and stained with

10  $\mu\text{g mL}^{-1}$  of 4',6-diamidino-2-phenylindole (DAPI) solution for 10 min in the dark. Samples were filtered through black polycarbonate membrane filters (0.22  $\mu\text{m}$ ; Poretics, Gorinchem, The Netherlands) backed with a 0.45  $\mu\text{m}$  Millipore cellulose nitrate filter and mounted on glass microscope slides. Slides were refrigerated in the dark until counted (Velji & Albright, 1986). Ten random fields per slide were counted using 1000 $\times$  epifluorescent microscopy. Cell biovolumes were estimated using geometric shapes (Psenner, 1993; Wetzel & Likens, 2000), and bacterial carbon was calculated by multiplying biovolumes by  $5.6 \times 10^{-13}$  g C  $\mu\text{m}^{-3}$  (Bratback, 1985).

**Respiration.** Microbial respiration was measured as oxygen uptake by decomposing leaves at ambient stream water temperatures. On each collection date, five leaf discs from each leaf pack were placed in 30-mL glass respiration chambers containing unfiltered stream water and incubated in shallow stream margins. Initial oxygen concentration was obtained using a YSI Model 58 Dissolved Oxygen Meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, U.S.A.) with a self-stirring probe. Water was recirculated over a period of 20 min in darkness, and a final oxygen reading was obtained. Four additional chambers containing only stream water were used as controls. Oxygen consumption was expressed as  $\text{O}_2$  uptake per leaf AFDM per hour.

#### Statistical methods

We calculated leaf decay rate ( $k_{\text{day}}$ ) for each leaf pack by regressing the natural log of percentage leaf mass remaining against days. To correct for temperature differences between the two streams, processing coefficients (the slope of the regression line when the natural log of percentage leaf mass remaining is plotted against accumulated heat) were computed using accumulated heat ( $k_{\text{degree day}}$ ). Daily water temperature was recorded over the course of each 40-day experiment for the determination of accumulated degree days above 0 °C. We used repeated measures ANOVA to test for potential differences in temperature-corrected leaf pack decomposition rates (i.e. remaining ash-free dry mass), fungal and bacterial biomass, respiration and invertebrate abundance through time. We conducted the analyses using all control and tadpole exclusion replicates (i.e. 10 pairs

of replicate control and tadpole exclusion treatments). To assess more effectively tadpole effects in controls in the stream *with frogs*, we tested (using repeated measures ANOVA) for differences in mass loss, fungal and bacterial biomass, respiration and macroinvertebrates between replicates using only pairs where controls were colonised by more than one tadpole. Response values were log-transformed prior to analysis to correct for non-normality of variances. Analyses were conducted on SAS System for Windows, version 8.0 (SAS Institute, Cary, NC, U.S.A.) using  $\alpha = 0.05$ . Since results of both types of analyses were similar (i.e. non-significant  $P$ -values;  $P > 0.05$ ), we present results including all 10 control and tadpole exclusion pairs. To test in addition for differences in response variables, in those replicate pairs where controls were colonised by more than one tadpole, we constructed 90% confidence intervals around mean leaf mass loss, fungal and bacterial biomass, respiration and macroinvertebrate abundance. These results are significant at  $\alpha < 0.10$ .

As a comparison of the degree of similarity in macroinvertebrate assemblages between treatments and streams, Stander's similarity index (SIMI) was used. SIMI is computed using the equation:

$$\text{SIMI} = \sum_{i=1}^s \left[ a_i \times b_i \left( \sum_{i=1}^s a_i^2 \times \sum_{i=1}^s b_i^2 \right)^{-1/2} \right]$$

where  $a_i$  = ratio of the number of individuals of species  $i$  to the total number of individuals  $N$  in the sample A;  $b_i$  = ratio of the number of individuals of species  $i$  to the total number of individuals  $N$  in the sample B; and  $s$  = total number of species in both samples (McIntire & Moore, 1977).

#### Results

Mean discharge at the stream *with frogs* (over the 40-day experiment) was  $48.93 \pm 2.25$  L  $\text{s}^{-1}$ . Mean rainfall was 6.0 mm  $\text{day}^{-1}$ , mean daily water temperature  $21.07 \pm 0.04$  °C, pH 8.2,  $\text{NO}_3\text{-N}$  169  $\mu\text{g L}^{-1}$ ,  $\text{NH}_4\text{-N}$  4  $\mu\text{g L}^{-1}$  and  $\text{PO}_4\text{-P}$  8  $\mu\text{g L}^{-1}$ . At the *frogless* stream, there was higher and more variable mean discharge ( $93.29 \pm 5.40$  L  $\text{s}^{-1}$ ), higher mean daily rainfall (12.5 mm  $\text{day}^{-1}$ ) and lower mean water temperature ( $18.20 \pm 0.04$  °C). Stream pH was 8.1,  $\text{NO}_3\text{-N}$  153  $\mu\text{g L}^{-1}$ ,  $\text{NH}_4\text{-N}$  5  $\mu\text{g L}^{-1}$  and  $\text{PO}_4\text{-P}$  20  $\mu\text{g L}^{-1}$ .

*Background tadpole densities*

We found wide variation in the ambient density of centrolenid tadpoles during periodic sampling of detritus in the stream *with frogs* (sampled between July 2003 and August 2004), ranging from 0.0 to 318.5 m<sup>-2</sup>. Four species of centrolenid tadpoles were found during the dry season (February–April). *Centrolene prosoblepon* (Boettger) was the most abundant, with a density of 18.96 ± 6.87 m<sup>-2</sup>. *Cochranella albomaculata* (Taylor), *Hyalinobatrachium colymbiophyllum* (Taylor) and an unknown centrolenid tadpole were also found though at much lower densities (Table 1). Overall tadpole density during this period was 31.09 ± 11.01 m<sup>-2</sup>. Only two species of tadpoles were found during the wet season (May–September), and densities were greatly reduced (Table 1). Mean mass-specific tadpole excretion rate was 0.063 ± 0.018 µg N mg tadpole<sup>-1</sup> h<sup>-1</sup>.

*Colonisation of experimental leaf packs*

We found patchy distribution of tadpoles in control treatments of the stream *with frogs* in our experimental exclusion study, with densities ranging widely from 0.00 to 33.33 m<sup>-2</sup>. Thirty-one tadpole individuals colonised the leaf packs over the course of the experiment, of which 29 were in the control treatments and two were in electric exclusion treatments (Table 2). All species of tadpoles colonising leaf packs in the stream *with frogs* were glass frogs (Centrolenidae). *C. prosoblepon* and *C. albomaculata* were encountered most frequently (22 and seven individuals, respectively), and there was one individual each of

*H. colymbiophyllum* and an unknown species of Centrolenidae. No tadpoles were found in experimental leaf packs in the *frogless* stream.

Of the 10 controls in the stream *with frogs*, four were uncolonised by tadpoles during the 40-day experimental period. Leaf packs of those six replicates that were colonised were colonised by one (two replicates), five, six, six and 10 tadpoles over all sampling dates of the experiment. Mean centrolenid tadpole density in the control treatment was 9.99 ± 13.00 individuals m<sup>-2</sup>. In control replicates colonised by more than one tadpole during the 40 days, densities ranged from 16.65 to 33.33 individuals m<sup>-2</sup> (mean = 23.31 ± 8.75).

Tadpole biomass in control replicates in the stream *with frogs* ranged from 0 to 55.92 g m<sup>-2</sup> (mean = 15.24 ± 22.58). In control replicates that were colonised by more than one tadpole during the experiment, tadpole biomass ranged from 13.50 to 55.92 g m<sup>-2</sup> (mean = 34.97 ± 23.01). Electric exclusion devices were effective in deterring tadpoles. Only two small tadpoles were found in exclusion treatments, and mean tadpole density and biomass in the exclusion treatments were more than an order of magnitude smaller than in control treatments, ranging from 0.00 to 3.33 individuals m<sup>-2</sup> (mean = 0.67 ± 1.47) and 0 to 0.70 g m<sup>-2</sup> (mean = 0.02 ± 0.10), respectively. No fish or shrimps were ever observed in control or tadpole exclusion replicates in either the *frog* or the *frogless* stream.

*Leaf pack mass loss*

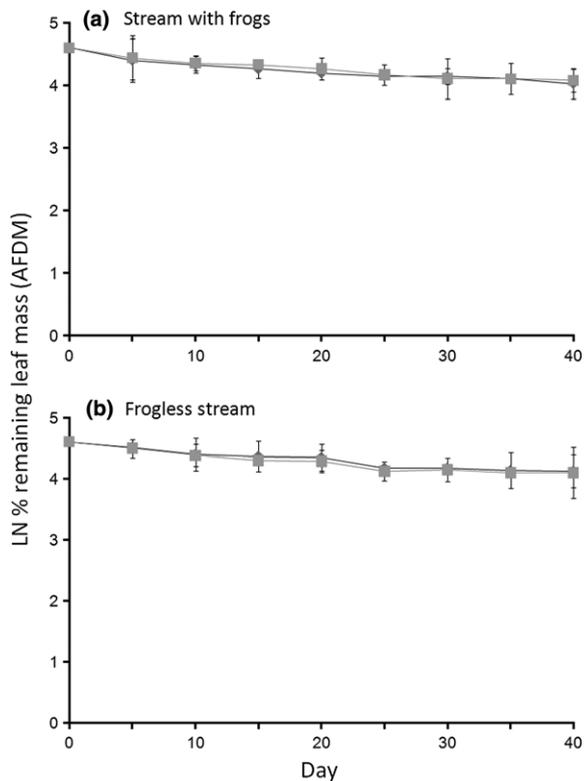
There were no differences in leaf mass loss between control and tadpole exclusion treatments ( $F_{1,8} = 0.00$ ,

**Table 1** Mean tadpole density (±1SE), by season, of centrolenid-tadpoles sampled in depositional habitat of the study stream *with frogs*. Wet-season estimates are based on monthly sampling from May to September, and dry-season estimates are from February to April

	<i>Centrolene prosoblepon</i>	<i>Centrolene ilex</i>	<i>Cochranella albomaculata</i>	<i>Hyalinobatrachium colymbiophyllum</i>	Centrolenidae sp.	Combined species
<b>Wet Season</b>						
Total tadpoles	0	0	0	1	2	3
Tadpoles m <sup>-2</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.54 ± 0.54	1.08 ± 1.07	1.62 ± 1.19
Habitat-weighted tadpoles m <sup>-2</sup>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.34	0.68 ± 0.68	1.02 ± 0.76
<b>Dry Season</b>						
Total tadpoles	25	0	8	6	2	41
Tadpoles m <sup>-2</sup>	18.96 ± 6.87	0.00 ± 0.00	6.07 ± 5.99	4.55 ± 3.14	1.52 ± 1.05	31.09 ± 11.01
Habitat-weighted tadpoles m <sup>-2</sup>	5.54 ± 1.96	0.00 ± 0.00	1.84 ± 1.84	1.81 ± 1.41	0.53 ± 0.39	9.72 ± 3.76

**Table 2** Total number of tadpoles and mean tadpole density ( $\pm 1$ SE) colonising experimental leaf packs over 40-day experiment in the stream *with frogs* and the *frogless* stream

	<i>Centrolene prosoblepon</i>	<i>Cochranella albomaculata</i>	<i>Hyalinobatrachium colymbiphylum</i>	Centrolenidae sp.	Combined species
<i>Stream with frogs</i>					
Tadpole access					
Total tadpoles	20	7	1	1	29
Tadpoles m <sup>-2</sup>	8.33 $\pm$ 0.00	2.91 $\pm$ 0.00	0.41 $\pm$ 0.54	0.41 $\pm$ 1.07	9.99 $\pm$ 13.00
Tadpole exclusion					
Total tadpoles	2	0	0	0	2
Tadpoles m <sup>-2</sup>	0.83 $\pm$ 0.02	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.83 $\pm$ 0.02
<i>Frogless stream</i>					
Tadpole access					
Total tadpoles	0	0	0	0	0
Tadpoles m <sup>-2</sup>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Tadpole exclusion					
Total tadpoles	0	0	0	0	0
Tadpoles m <sup>-2</sup>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

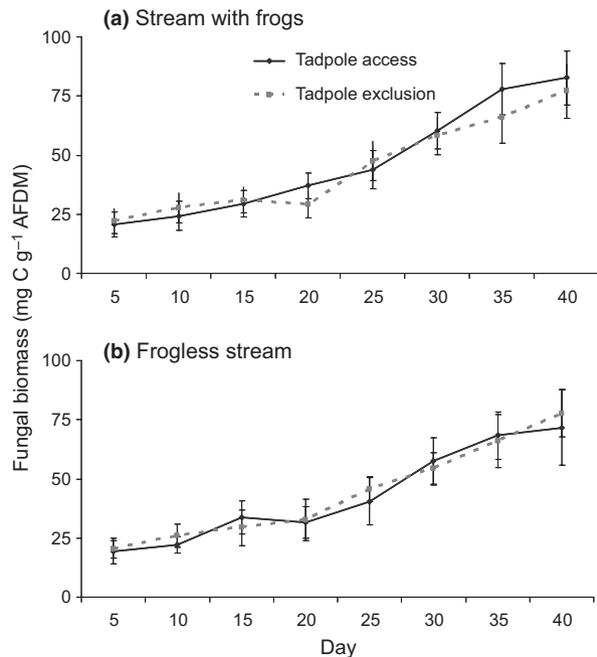
**Fig. 1** Mean leaf mass remaining (% AFDM;  $\pm 1$ SE) over 40 days in the (a) stream *with frogs* and (b) *frogless* stream in control ( $n = 10$  per date) and tadpole exclusion ( $n = 10$  per date) treatments.

$P = 0.99$ ) (Fig. 1) in the stream *with frogs*. On the final day of the experiment (day 40) in the stream *with frogs*, 58.99% of leaf mass remained in the control treatment ( $k_{\text{day}} = -0.028 \text{ day}^{-1}$ ) and 58.12% remained in the tadpole exclusion treatment ( $k_{\text{day}} = -0.028$

$\text{day}^{-1}$ ). Similarly, there were no differences in mass loss between treatments in the *frogless* stream ( $F_{1,8} = 1.2$ ,  $P = 0.20$ ), with 58.19% ( $k_{\text{day}} = -0.027 \text{ day}^{-1}$ ) in the control treatment and 60.43% leaf mass remaining ( $k_{\text{day}} = -0.027 \text{ day}^{-1}$ ) in the exclusion treatment. After correcting for temperature differences between streams, there were no statistical differences in rates of mass loss in either control ( $k_{\text{degree day}} = -0.0013$  versus  $k_{\text{degree day}} = -0.0015 \text{ day}^{-1}$ ) or tadpole exclusion ( $k_{\text{degree day}} = -0.0014$  versus  $k_{\text{degree day}} = -0.0015 \text{ day}^{-1}$ ) treatments. Leaf mass loss was generally constant (linear) over the course of the experiment.

#### Fungal biomass

Fungal biomass, as indicated by ergosterol, was similar between control and tadpole exclusion treatments in the stream *with frogs* ( $P > 0.05$ ) and between treatments in the *frogless* stream (Fig. 2). Fungal biomass was highest on day 40, when values reached  $82.65 \pm 13.87 \text{ mg C g}^{-1}$  AFDM (control) and  $77.11 \pm 11.34$  (tadpole exclusion) in the stream *with frogs*, and  $71.71 \pm 15.97 \text{ mg C g}^{-1}$  AFDM (control) and  $77.62 \pm 10.03$  (tadpole exclusion) in the *frogless* stream. There were no statistical differences in fungal biomass between streams. When replicates were grouped by the presence or absence of tadpoles (i.e. uncolonised control leaf packs and tadpole exclusion leaf packs compared to tadpole-colonised control leaf packs), we found greater fungal biomass, averaged over the 40-day experiment, on the control leaf packs



**Fig. 2** Fungal biomass ( $\pm 1\text{SE}$ ) on submerged leaf packs in the (a) stream *with frogs* and (b) *frogless* stream. Diamonds represent control ( $n = 10$  per date) and squares represent tadpole exclusion ( $n = 10$  per date) treatments.

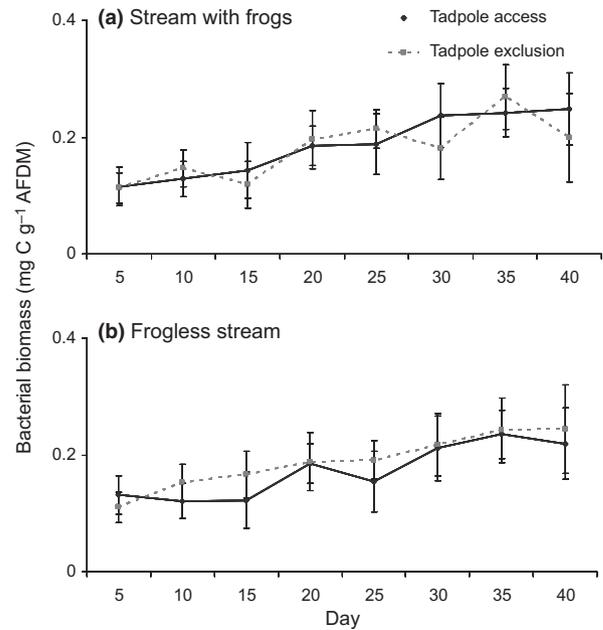
colonised by tadpoles ( $56.9$  versus  $42.7$   $\text{mg C g}^{-1}$  AFDM).

#### Bacterial biomass

Bacterial biomass did not differ between control and tadpole exclusion treatments in the stream *with frogs* ( $F_{1,8} = 0.20$ ,  $P = 0.40$ ) or in the *frogless* stream ( $F_{1,8} = 0.15$ ,  $P = 0.35$ ) (Fig. 3). Bacterial biomass tended to level off by day 30, at which time there was  $0.23 \pm 0.06$   $\text{mg C g}^{-1}$  AFDM in the control treatment and  $0.18 \pm 0.05$   $\text{mg C g}^{-1}$  AFDM in the tadpole exclusion treatment in the stream *with frogs*. In the *frogless* stream, there was  $0.21 \pm 0.05$   $\text{mg C g}^{-1}$  AFDM in the control treatment and  $0.21 \pm 0.04$   $\text{mg C g}^{-1}$  AFDM in the tadpole exclusion treatment. There were no statistical differences in bacterial biomass between streams.

#### Respiration

Microbial respiration did not differ between control and exclusion treatments in the stream *with frogs* ( $F_{1,8} = 0.30$ ,  $P = 0.35$ ) or in the *frogless* stream ( $F_{1,8} = 0.65$ ,  $P = 0.60$ ). On day 40 of the experiment,



**Fig. 3** Bacterial biomass ( $\pm 1\text{SE}$ ) on submerged leaf packs in (a) stream *with frogs* and (b) the *frogless* stream. Diamonds represent control and squares represent tadpole exclusion treatments.

mean respiration was  $2.55 \pm 0.61$   $\text{mg O}_2$  AFDM $^{-1}$  h $^{-1}$  (control) and  $2.61 \pm 1.01$  (tadpole exclusion) in the stream *with frogs*. Mean respiration in the *frogless* stream was  $2.08 \pm 0.86$   $\text{mg O}_2$  AFDM $^{-1}$  h $^{-1}$  in the control treatments and  $2.19 \pm 0.95$  in the tadpole exclusion treatment.

#### Macroinvertebrates

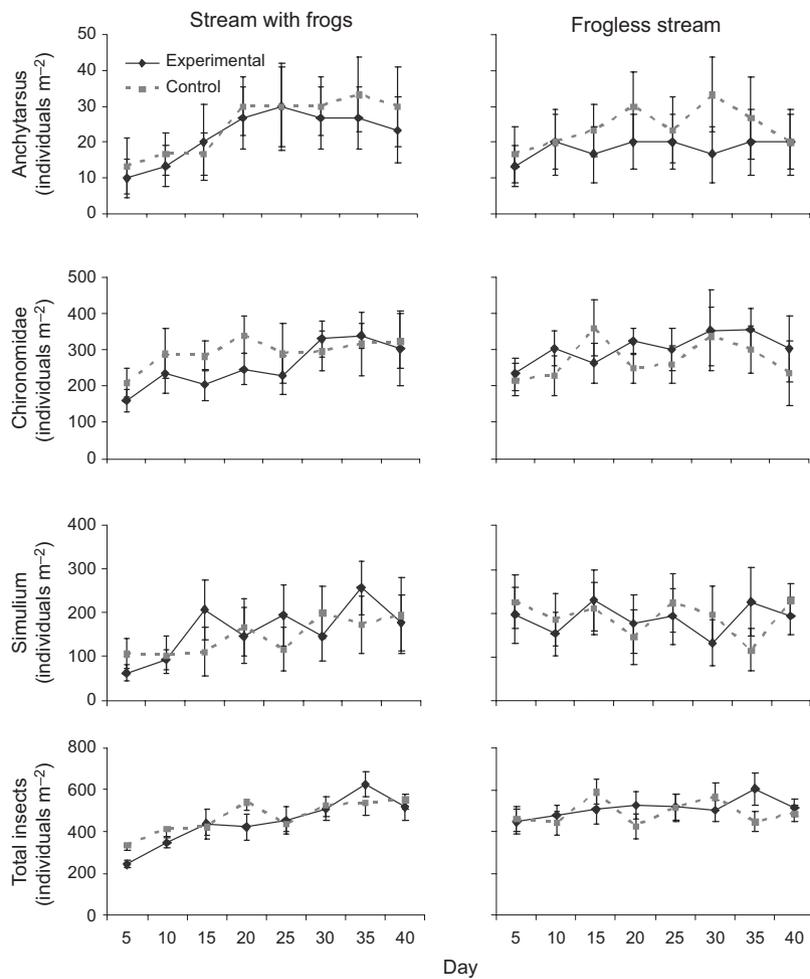
The most common macroinvertebrates colonising leaf packs in both streams were dipterans in the families Chironomidae and Simuliidae (*Simulium* sp.) and *Anchyrtarsus* sp. (Coleoptera) (Table 3; Fig. 4). Leaf packs were colonised much less frequently by *Phanocerus* spp. (Coleoptera), *Baetis* spp. and *Tricorythodes* spp. (Ephemeroptera), and oligochaetes (Table 3). There were no statistical differences between control and exclusion treatments within streams (SIMI = 0.97) or between streams (SIMI = 0.95).

#### Discussion

There was no support for our hypothesis of a higher decomposition rate in the stream *with frogs* than in the *frogless* stream. These results are in marked contrast to

**Table 3** Mean abundance (individuals m<sup>-2</sup>) of macroinvertebrate taxa colonising leaf packs in experimental and control treatments over 40 days in the stream *with frogs* and *frogless* stream. Means are  $\pm 1$ SE

Taxa	Stream <i>with frogs</i>		Frogless Stream	
	Experimental	Control	Experimental	Control
<i>Anchyrtarsus</i>	22.08 $\pm$ 2.79	25.00 $\pm$ 2.89	18.33 $\pm$ 2.92	24.16 $\pm$ 2.98
<i>Baetis</i>	0.83 $\pm$ 0.59	0.83 $\pm$ 0.59	1.25 $\pm$ 0.72	0.42 $\pm$ 0.42
Chironomidae	255.58 $\pm$ 18.32	292.62 $\pm$ 22.47	304.28 $\pm$ 21.77	273.48 $\pm$ 22.37
Oligochaeta	2.08 $\pm$ 0.91	0.83 $\pm$ 0.59	0.42 $\pm$ 0.42	1.67 $\pm$ 0.82
<i>Phanocerus</i>	1.67 $\pm$ 0.20	0.83 $\pm$ 0.59	0.83 $\pm$ 0.17	0.42 $\pm$ 0.42
Simulium	160.26 $\pm$ 19.12	146.10 $\pm$ 18.78	187.73 $\pm$ 20.17	191.89 $\pm$ 19.33
<i>Tricorythodes</i>	2.50 $\pm$ 1.16	3.75 $\pm$ 1.19	1.25 $\pm$ 0.71	0.83 $\pm$ 0.59

**Fig. 4** Mean densities ( $\pm 1$ SE) of most abundant macroinvertebrate taxa colonising submerged leaf packs in the stream *with frogs* and the *frogless* stream over 40 days. Squares represent controls ( $n = 10$  per date) and diamonds represent tadpole exclusion ( $n = 10$  per date) treatments.

the dramatic effects of grazing tadpoles on algal primary producers in previous studies conducted in the same study streams (e.g. Connelly *et al.*, 2008). Control treatments in the study stream *with frogs* exhibited surprisingly similar decomposition rates as the *frogless* stream. Accordingly, experimental exclusion of centrolenid tadpoles from leaf packs in the

stream *with frogs* also had no significant effect on decomposition rates. We attribute these findings, in part, to the low densities and patchy distribution of glass frog tadpoles associated with both natural and experimental leaf packs. Moreover, glass frog tadpoles did not directly shred decomposing leaf material in experimental leaf packs; rather, evidence from our

study and others suggests that they grazed the surfaces of leaves for microbes and used decomposing leaf packs as habitat and/or refugia. However, our findings provide some evidence that centrolenid tadpoles may stimulate fungal activity in litter and therefore may be important at certain spatiotemporal scales.

#### *Leaf litter breakdown*

Since the lower temperatures in the *frogless* stream could slow decomposition processes, we did not expect the similarity in decomposition rates between streams. There were, however, abiotic differences between streams that may also mask any effect of tadpoles on decomposition rates. We acknowledge that our study was also limited by low replication and sample sizes, limitations that are frequent in ecosystem level investigations. Overall, leaf pack decomposition rates in our study streams ( $k_{\text{day}} = -0.027$  to  $-0.028$ ) were in the general range of other published decomposition rates reported for upland tropical systems in Brazil ( $k_{\text{day}} = -0.014$ ; Goncalves & Callisto, 2006), Puerto Rico ( $k_{\text{day}} = -0.037$  to  $-0.039$ ; Wright & Covich, 2005) and Colombia ( $k_{\text{day}} = -0.024$  to  $-0.065$ ; Mathuria & Chauvet, 2002).

Lack of effects of centrolenid tadpole exclusion on leaf decomposition rates shown in our study, combined with a previous study in our focal stream (Hunte-Brown, 2006), suggests that centrolenid tadpoles do not directly break down decomposing leaves but instead feed on associated microbes. Using carbon and nitrogen stable isotopes, Hunte-Brown (2006) found that centrolenid tadpoles primarily assimilated microbes associated with detritus.

Previous analyses of tadpole gut contents found that larval centrolenids do ingest detritus (McDiarmid & Altig, 1999), of which leaf litter can be a large component, which might suggest that tadpoles act functionally as shredders. However, the rasping mouthpart morphology of centrolenid larvae suggests that they are not particularly well adapted to shredding leaf litter. Rather, centrolenids possibly scrape the biofilm from leaf surfaces into suspension and ingest the suspended biofilm, along with any other fine particulate leaf material that had been previously broken down through other biotic and abiotic mechanisms. Although it has long been thought that tadpoles are unable to break down ingested cellulose (Savage, 1952), the digestive physiology of tadpoles is

poorly understood. Recent work has demonstrated that bullfrog tadpoles, using gastrointestinal microbial fermentation, achieve levels of short-chain fatty acids in the hind gut exceeding those found in some plant-cellulose-based diets of some herbivorous mammals (Pryor & Bjorndahl, 2005).

#### *Centrolenid tadpole distribution and abundance*

Tadpoles have been categorised as stream ecosystem engineers and can alter the spatial heterogeneity of their habitat through a number of mechanisms. For example, concentrating waste deposition (i.e. excretion and egestion) in certain areas can significantly influence localised nutrient cycling (McClain *et al.*, 2003). Whiles *et al.* (2006) provided evidence that a significant portion of the nutrient-rich sediments in our study stream is comprised of tadpole faeces. This resource is likely to be quickly colonised by microbes and becomes a potential resource for reingestion by tadpoles. Potential attraction by centrolenid tadpoles to this patchily accumulated faecal matter within pools may, at least partially, drive observed patchiness in tadpole habitat use. Therefore, habitat heterogeneity within our study stream probably played a role in the patchy distribution of the centrolenid tadpole community.

Centrolenid breeding tends peak during the rainy season (S. Connelly, pers. obs.), with the tadpole stage lasting several months to a year; however, we found tadpole densities to be highest during the dry season. We attribute this in part to the probability that frequent high-discharge events during the rainy season wash tadpoles downstream and also to the fact that the stream-wetted area is reduced during the dry season, thereby crowding tadpoles into a small area.

#### *Macroinvertebrates*

We did not find increased abundances of detritivorous macroinvertebrates in response to tadpole exclusion, as we had hypothesised. The most abundant taxon colonising experimental leaf packs was larval Chironomidae (Diptera), which often feed on particulate organic material. However, chironomid populations were not reduced in the presence of tadpoles, suggesting that they are not competing with tadpoles for food or refugia. Because of their small size relative to tadpoles, chironomids may be ingesting smaller

detrital particles than those consumed by centrolepidids. Additionally, they may be feeding from areas of the leaf packs uncolonised by tadpoles (e.g. the outside of leaf packs), in contrast to centrolepidid tadpoles that tended to burrow into experimental leaf packs (S. Connelly, pers. obs.). Larval simuliids (Simuliidae: Diptera) are filter feeders, and they also did not appear to be influenced by tadpoles and occurred patchily (0–533 m<sup>-2</sup>). Within both streams, we found several individuals of the beetle larvae, *Anchytarsus*, in leaf packs throughout the experiment, although there were no significant differences in their abundance between control and exclusion treatments or between streams. Because of their very low densities, they probably did not affect the decomposer community. Other macroinvertebrate groups associated with leaf packs [i.e. *Baetis* mayflies (Ephemeroptera), *Phanocerus* beetle larvae (Elmidae) and oligochaetes] were at extremely low densities, and these groups similarly did not appear to influence or be influenced by the presence or absence of centrolepidid tadpole.

#### Microbes

Our hypothesis that experimental exclusion of tadpoles from leaf packs in the stream *with frogs* would result in increased fungal biomass was not supported. There are several possible explanations for this finding. Foraging larval centrolepidids may be responding to microbes as an energy-rich food resource; that is, they may be attracted to the leaf packs that have greater fungal biomass. Conversely, the presence of tadpoles may be directly responsible for increased fungal biomass. Although it is possible that tadpoles had a small negative effect on fungal biomass owing to ingestion, most fungi associated with submerged leaves are not on the leaf surface, but instead within the interior of the leaf, and thereby protected from browsing.

Ambient nutrients were similar between streams. However, in the stream *with frogs*, localised effects of tadpole excretion may be responsible for increased fungal biomass in tadpole-colonised leaf packs. Rates of nutrient excretion by tadpoles are higher than those of other stream-dwelling vertebrates, such as fish (Vanni, 2002), and based on tadpole densities measured during the 2004 dry season, overall tadpole community excretion represented approximately 7% of the dry-season bulk uptake estimates of ammonium within the stream *with frogs* (Whiles *et al.*, 2006). This

finding, combined with results from our study, suggests that centrolepidid tadpoles could play an important role in nutrient cycling.

Our experiment was conducted during the beginning of the rainy season, and effects of nutrient recycling by tadpoles could be more enhanced in the dry season when their densities are highest and when reduced stream flow provides less dilution of their excreted ammonium. The centrolepidid excretion rates that we measured were somewhat lower than expected, based on previous estimates of ammonium excretion by *Rana warszewitschii* (O. Schmidt) and *Hyloscirtus* sp., ranging from 0.15 to 3.6 µg h<sup>-1</sup> (Whiles *et al.*, 2006). This difference in excretion rate may be attributable, in part, to the relatively low activity of centrolepidid tadpoles (as compared to other stream-dwelling tadpole species associated with more lotic habitats).

Few data are available that quantify changes in fungal biomass throughout the process of leaf decomposition in upland tropical streams. In a study that examined leaf decomposition rates of two tree species in a fourth-order stream in the Andean Mountains of Colombia, Mathuria & Chauvet (2002) found that fungal biomass peaked at approximately 83.6 and 93.4 mg C g<sup>-1</sup> AFDM, similar to values we found in control treatments (82.65 and 71.71 mg C g<sup>-1</sup> AFDM) here. However, unlike the results of Mathuria & Chauvet (2002), who found a peak in ergosterol on days 10 and 16 of their experiment, ergosterol on leaf packs in our study streams began to peak after day 35.

To our knowledge, we present the first data assessing changes in bacterial biomass during the initial stages of leaf decomposition in upland tropical streams. Peak of bacterial biomass associated with leaf packs in our study streams after 30 days was slightly lower (0.18–0.23 mg C g<sup>-1</sup> AFDM) than those documented by Ardon & Pringle (2007), who found biomass on five leaf species between 0.25 and 0.40 mg C g<sup>-1</sup> AFDM after 30 days in a lowland tropical stream. Our findings did not support our hypothesis that tadpole exclusion would result in increased leaf pack bacterial biomass. Similarities in bacterial biomass between control and tadpole exclusion treatments may be the net result of negative effects on bacterial biomass through grazing, combined with positive effects on bacterial biomass owing to reduction in the boundary layer and increased N availability to bacteria through nutrient recycling by

tadpoles. In summary, larval glass frogs (Centrolenidae) were patchily distributed within both the natural leaf packs that we sampled during reach-scale density monitoring and experimental leaf packs. They were the only group of tadpoles that colonised our experimental leaf packs. The general lack of effects of these detrital-dwelling tadpoles on leaf decomposition dynamics contrasts markedly with the dramatic effects of grazing tadpoles on algal primary producers reported in previous investigations in the same study stream. However, we did find evidence that centrolenid tadpoles may have localised, subtle effects in stimulating growth of stream fungal communities. We suggest that future studies investigating the ecological role of glass frog tadpoles be conducted during the dry season, when their effects may be most apparent.

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