Tadpole species have variable roles in litter breakdown, sediment removal, and nutrient cycling in a tropical stream

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Abstract: Quantifying the influence of biodiversity on ecosystem function is an increasingly important goal as biodiversity declines. Tadpoles can be important contributors to ecosystem processes in streams, so amphibian declines over recent decades may have far-reaching ecosystem effects. We, therefore, used artificial channels located near an Australian rainforest stream to assess how the tadpoles of 2 frog species affect leaf litter decay, sediment bioturbation, and nutrient cycling in the presence and absence of invertebrates. *Mixophyes coggeri* (Myobatrachidae) tadpoles did not increase leaf mass loss, but were important in sediment removal, which benefits smaller consumers. In contrast, high densities of *Litoria serrata* (Hylidae) tadpoles increased leaf mass loss, possibly because their excretion of nutrients facilitated decomposition, but were not important in sediment removal. However, we found no effect of nutrient excretion by *L. serrata* tadpoles on the nutrient quality of leaves and sediments, or on biofilm growth, even though *L. serrata* tadpoles and invertebrates together appeared to remove significant quantities of nutrients from sediment. Our results show that tadpoles of different species can have different functional roles in the ecosystem, which need to be taken into account when assessing the influence of amphibian declines on ecosystem processes.

Key words: ecosystem processes, *Litoria serrata*, *Mixophyes coggeri*, species interaction, species loss, Australia

The global decline in biodiversity is likely to have major impacts across ecosystems and may affect processes such as production and decomposition of organic matter (Hooper et al. 2012, Naeem et al. 2012). Stream frog biodiversity worldwide has declined substantially over the last 3 decades (IUCN 2015), in part because the fungal disease chytridiomycosis has locally extirpated many species (Berger et al. 1998, Lips et al. 2006, Crawford et al. 2010). In the Australian Wet Tropics biogeographic region (hereafter, the 'Wet Tropics'), all known high elevation populations of 7 endemic rainforest treefrogs declined or disappeared in the late 1980s to the early 1990s, probably because of chytridiomycosis (Richards et al. 1993, McDonald and Alford 1999). Knowledge of the basic ecology, resource use, and trophic status of tropical stream tadpoles is required to understand the impact of species loss on tropical stream communities (Altig et al. 2007). The loss of tadpoles in the New World is considered likely to have large effects on ecosystems, including changes in primary production, biotic communities, and organic matter dynamics (Whiles et al. 2006). For example, experimental exclusion of tadpoles in a Panamanian stream reduced sediments, organic detritus, and abundance of algae and invertebrates largely because of the absence of bioturbation (Ranvestel et al. 2004). However, the ecosystem effects of the reduced tadpole abundance and diversity in tropical streams elsewhere are largely unknown, apart from a recent study that suggested that tadpole declines caused substantial shifts in food-web structure (Schmidt et al. 2017) in the Wet Tropics.

Tadpoles are abundant in many tropical streams, including those in the Wet Tropics (Alford 1999, Richards 2002). Tadpoles have variable ecological roles and can graze on algae and biofilms (Whiles et al. 2006, Iwai et al. 2012) or fine or coarse detritus (Flecker et al. 1999, Iwai et al. 2009). The effects of changes in overall tadpole presence and abundance will, therefore, depend on the particu-

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lar traits and roles of the individual species (Jonsson and Malmqvist 2003). Changes to ecosystem processes after the loss of 1 species potentially can be mitigated by other species with similar traits (Rosenfeld 2002). Such redundancy can allow ecosystems to maintain their functional integrity after species loss (Lawton and Brown 1994). Complete functional redundancy is considered unlikely because species occupying similar niches perform differently under different conditions (Wellnitz and Poff 2001, Loreau 2004), but partial redundancy has been reported. For example, a study in Panama showed that invertebrates increased with tadpole declines, suggesting at least partial redundancy among tadpole and invertebrate grazers (Colon-Gaud et al. 2010). Functional redundancy has not been otherwise investigated in stream tadpoles.

Allochthonous leaf litter decomposition is an important food source in forest stream communities (e.g., Cummins 1974, Wallace et al. 1997, Gessner et al. 2010). Leaf litter decomposition typically involves leaching, abrasion, and processing by microbes and invertebrate shredders (Cummins 1974, Gessner and Chauvet 2002, Cheshire et al. 2005). Tadpoles may contribute to litter processing directly or via facilitation of other stream organisms, thereby contributing to ecosystem processes beyond the direct effect of the individual species (Iwai et al. 2009, Rugenski et al. 2012). Tadpoles may also increase the nutrient content of fine particulate organic matter by excreting nutrients (Whiles et al. 2006, Colon-Gaud et al. 2008). Nutrient excretion may increase microbial production and nutritional quality of the organic material, which can benefit the invertebrates and tadpoles that consume it (Bärlocher and Kendrick 1975, Pearson and Connolly 2000, Iwai and Kagaya 2007). Additionally, primary producers may use the excreted nutrients (Iwai and Kagaya 2007), which increases biofilm growth in the presence of tadpoles in some systems (Iwai et al. 2012), further improving leaf nutritional quality and encouraging consumption by shredders (Abelho et al. 2005). Tadpoles may benefit from their own nutrient excretion by consuming biofilm, but this possibility has not been investigated in streams (Iwai et al. 2012).

Tadpoles can further facilitate invertebrate feeding by removing sediments while they forage ("bioturbation"), thereby uncovering periphyton, which small invertebrates can more readily consume (Ranvestel et al. 2004). This mechanism was indicated in studies in Panama, where loss of stream tadpoles led to a decline in grazer and detritivore abundance (Whiles et al. 2006, Colon-Gaud et al. 2009). Shredding invertebrates, a subset of detritivores, may reciprocally facilitate tadpole feeding via leaf breakdown that allows tadpoles to feed on smaller leaf fragments (Iwai et al. 2009).

We measured the effects on litter breakdown, sediment removal, and nutrient cycling of tadpoles of two species (*Litoria serrata* and *Mixophyes coggeri*) from a headwater rainforest stream in the Wet Tropics. These species are pool dwellers that feed on organic material (Trenerry 1988) and were the only frog species present in the study stream. A previous study found that *L. serrata* tadpole feeding increased in the presence of invertebrates, but not vice-versa (Iwai et al. 2009). Here, we built on that study with a series of experiments conducted in streamside artificial channels that approximate natural stream conditions (Pearson and Connolly 2000, Connolly and Pearson 2013). We included a second tadpole species, *M. coggeri*, and invertebrates from 2 feeding groups (shredders and grazers) instead of only *L. serrata* (then, *L. genimaculata*). We also used leaves of several plant species because invertebrates consume different plant species at different rates (Bastian et al. 2007). Finally, we also measured the influence of the tadpoles on sediment accumulation, nutrient cycling, and biofilm growth.

We hypothesized that 1) the 2 tadpole species would not preferentially consume the leaf litter of any plant species; 2) facilitation between tadpoles and invertebrates would cause greater leaf mass loss when they occurred together than when either occurred alone; 3) the 2 tadpole species would remove similar quantities of sediments (with biofilm), and the species would therefore be functionally redundant; 4) tadpoles would maintain condition (mass) during the course of the experiments; 5) leaf breakdown and sediment accumulation would be positively correlated with tadpole density, whereas tadpole mass would be negatively correlated with density; and 6) tadpole presence would increase nutrient content in water and sediments via nutrient recycling and thereby enhance biofilm growth.

METHODS

Artificial stream mesocosms

We conducted 3 experiments in artificial stream channels beside Birthday Creek, a 2nd-order stream in Paluma Range National Park, in the Australian Wet Tropics (lat 18°59′49″S, long 146°10′59″E). We did experiments 1 and 2 in summer 2012, and experiment 3 in summer 2013 to 2014. We fed water into a header tank that supplied 20 open PVC channels from above a small waterfall in Birthday Creek (Pearson and Connolly 2000). We controlled water flow into each channel with adjustable taps set at about 1.0 L/min, resulting in a surface current velocity of 0.0 to 10 cm/s, similar to stream pools. The inlet to the header tank was covered with 1-mm mesh to prevent input of coarse plant material. We put 63-μm mesh screens at the up- and downstream ends of each channel to prevent suspended material from washing in and out of the channels. Each artificial stream channel was 2.4 m long and 15 cm wide and divided into upper, middle and lower chambers with V-notched separators. These separators allowed us to keep the channel depth at 5 cm and to set up multiple experiments per chan-

nel by fitting the separators with 1-mm or 63-μm mesh, depending on the experiment. Both meshes prevented movement of animals, whereas the 63-μm mesh also prevented movement of suspended material between chambers. We further eliminated extra sediment input by not performing experiments in the upper chambers, where small amounts of fine sediment accumulated. We covered the channels with 1-mm-mesh netting to exclude falling plant material, which had a minor shading effect. Mean water temperature in the channels was similar to stream temperatures (±SD): 18.1 ± 1.3°C for experiment 1 (October–November 2012), 20.6 ± 1.2°C for experiment 2 (December 2012), and 19.7 ± 1.1°C for experiment 3 (November 2013–January 2014), as measured hourly by temperature dataloggers (Thermochron® iButtons, Baulkham Hills, Australia). We collected tadpoles and invertebrates used in experiments from Birthday Creek by sweeping a triangular dip net (0.9- × 0.3-mm mesh size) through the water column and along the substrate. This sweeping motion dislodged loose rocks to expose sheltering animals. We inspected the channels at least weekly during the experiments to ensure that flow was maintained, that mesh was not clogged, and that there was no loss of animals. We replaced occasional missing animals with similar specimens as indicated below.

Experiment 1: leaf breakdown and sediment accumulation

In the 1st experiment we assessed how tadpoles and invertebrates directly affected the leaf breakdown of 3 plant species (hypotheses 1 and 2) and removal of sediment and biofilm (hypothesis 3), and whether the 2 tadpole species were functionally redundant. We also tested whether tadpoles maintained mass during the experiment (hypothesis 4). We measured changes in mass of leaf material, sediment and biofilm in 5 treatments with different combinations of tadpoles, invertebrates, and plants, and compared them to a control treatment with no consumers. We replicated each experiment 3× and ran the experiment for 42 d. We did this experiment in the middle chambers of the artificial streams (Fig. S1), separated from upper and lower chambers by 1-mm mesh. We used a block treatment design with 1 replicate of each treatment and control spread across 3 sets of 6 adjacent channels. We randomized the treatment locations within each set, but never placed the same treatment in adjacent channels. We also measured downstream effects of the treatments on leaf mass loss, sediment deposition, and biofilm growth in the lower (animal-free) chambers.

We used tadpoles of *L. serrata* and *M. coggeri*, and 4 invertebrate larvae: 1 larva each of 3 caddisfly species, *Anisocentropus kirramus* (Calamoceratidae), *Lectrides varians*, and *Triplectides gonetalus* (Leptoceridae), the most common shredders in the stream, and 1 mayfly species, *Atalophlebia* sp. (Leptophlebiidae), a grazer and generalist shredder (Cheshire et al. 2005, Boyero et al. 2006). The treatments and controls included: (i) 8 *L. serrata* tadpoles, (ii) 8 *L. serrata* tadpoles plus invertebrates, (iii) 2 *M. coggeri* tadpoles, (iv) 2 *M. coggeri* tadpoles plus invertebrates, (v) invertebrates only, and (vi) no animals (control). The densities and biomass of tadpoles and invertebrates were similar to in-stream densities in Birthday Creek (Schmidt 2016) and all tadpoles were at developmental stages 25 to 30 (Gosner 1960) (*L. serrata* average mass = 0.29 g ± 0.16, *M. coggeri* average mass = 2.37 g ± 0.87). We replaced the occasional missing or metamorphosed animal (8 and 1 animals, respectively, of a total of 48) with animals of similar mass and stage to maintain the number of animals in each treatment. We measured tadpole body lengths from photographs of the animals against a scale and weighed them with a digital balance (to 0.1 g, wet mass).

Within each treatment, we provided leaves and sediment as potential food sources for the animals and we provided surfaces for biofilm growth. We used leaves of 3 common riparian species, *Apodytes brachystylis*, *Endiandra bessaphila*, and *Cryptocarya leucophylla*, which commonly occur in stream litter packs and are consumed by shredders. We collected green leaves to ensure correct identification. We made separate 10-mm-mesh leaf bags for each plant species by oven-drying the leaves for 48 h at 60°C and then placing approximately 2 g of leaf material into each bag (6 g total per treatment). This procedure allowed tadpoles and invertebrates to readily access plant material and allowed us to compare breakdown of each plant species (Boyero et al. 2011). Prior to beginning the experiment, we conditioned the leaf bags in the middle chambers of the channels for 16 d to allow leaching and microbial colonization (Connolly and Pearson 2013). We then cleared the channels of any accumulated sediments and placed 1 leaf bag of each plant species into each middle and lower chamber. We collected sediment from Birthday Creek by agitating the substratum and filtering the slurry through a 1-mm sieve. We placed a petri dish filled with wet filtrate (approximately 25 g dry mass) in each chamber as a 2nd food source and as a way to quantify sediment removal by tadpoles and invertebrates. Finally, we put 1 new 10- ×10-cm unglazed terracotta tile in each chamber to quantify biofilm growth. All middle and lower chambers thus contained 3 litter bags (1 of each plant species), a dish with sediment, and a tile. Only the middle chambers contained animals.

At the end of the experiment, we rephotographed the tadpoles to measure their length, weighed them, and released them and the invertebrates into the stream. We removed the leaf bags and placed them into separate Ziploc® bags. We scrubbed biofilm and other organic material from the tiles and rinsed it into plastic jars with stream water. We collected accumulated sediment from the chambers with 63-μm-mesh nets and rinsed it into separate jars. We stored all samples on ice and froze them later the same day. In the

laboratory, we removed any remaining invertebrates from the biofilm, sediment samples, and leaves. We oven-dried the leaves at 60°C until dry, weighed them, then ashed them in a muffle furnace at 550°C, and finally reweighed them to obtain ash-free dry mass (AFDM) and to quantify the % leaf mass loss during the experiment.

Experiment 2: tadpole density effects on leaf breakdown and sediment accumulation

In the 2nd experiment we measured the effect of tadpole density on leaf breakdown and sediment accumulation (hypothesis 5) over 25 d in the presence of invertebrates. We placed *L. serrata* tadpoles (developmental stages 25–30, Gosner 1960) (mean mass = 0.17 g ± 0.14) in the middle chambers at densities of 0 (control, 2 replicates) and 2, 4, 8, 12, 16, and 20, with 3 replicates of each. A single tadpole was lost from an 8-tadpole treatment early in the experiment, resulting in 1 replicate with 7 tadpoles (Fig. S2). We also added 2 *A. kirramus*, 2 *L. varians*, and 1 *T. gonetalus* invertebrate larvae to each chamber. We dried *C. leucophylla* leaves as described above and added ~6 g of leaves (weighed as above) enclosed in 10-mm-mesh bags to each chamber. We conditioned the leaves for 2 weeks in the middle chambers of the artificial stream channels, then cleaned the channels and placed the leaf bags haphazardly in the treatment chambers, along with a petri dish containing stream sediment (approximately 25 g dry mass), as above.

We separated the middle (treatment) chambers from the upper and lower chambers with 63-μm-mesh screens to prevent the input and loss of sediment from the treatment chambers. We weighed and measured the tadpoles for this experiment as above. One channel (density of 4 tadpoles) dried out due to a blocked inlet pipe, and two other channels lost ≥50% of the tadpoles (densities of 4 and 16 tadpoles), so we excluded these channels from analyses. At the end of the experiment, we weighed and measured the tadpoles and released them and the invertebrates. We collected leaves and sediment from the chambers and carried out laboratory analysis to determine change in leaf and sediment mass as described above.

Experiment 3: tadpole-driven nutrient cycling

In this experiment we measured the effects of tadpole nutrient excretion on sediment and leaf litter nutrient quality and biofilm growth (hypothesis 6). We used the middle chambers for the main treatments and tested for their downstream effects in the lower chambers over a period of 76 d. We separated the upper and lower chambers with 1-mm mesh to allow sediment to move from the middle chamber to the lower chamber. We used only *L. serrata* tadpoles (Gosner stages 25–30, Gosner 1960) (avg mass = 0.19 g ± 0.08), and larvae of *A. kirramus*, *L. varians*, *T. gonetalus*, *Atalophlebia* sp., and other small Leptophlebiidae. We included 4 treatments and 1 control, each with 4 replicates. The treatments were: i) 8 tadpoles only (high density), ii) 8 tadpoles plus invertebrates, iii) invertebrates only, iv) 4 tadpoles only (low density), and v) no animals (control) (Fig. S3). We placed 11 invertebrates into each chamber (details in Table S1). We measured and weighed tadpoles before we placed them into the chambers to ensure similar size. Throughout the experiment, we replaced insects and tadpoles that emerged or metamorphosed (including 11 missing and 8 metamorphosed tadpoles of the starting total of 104).

We placed unglazed terracotta tiles (5 × 5 cm) in the middle and lower chambers to measure biofilm growth. To determine direct and indirect effects of tadpole presence on biofilm growth, we placed 2 'enclosed' (indirect effect) and 2 'exposed' (direct effect) tiles in each treatment chamber. We put the enclosed tiles in plastic containers with 1-mm-mesh sides to prevent animal access. We put the exposed tiles into the same plastic containers with open sides to allow animal colonization. We placed 3 leaf bags with approximately 2 g (weighed) of *C. leucophylla* leaves each into each chamber. We allowed animal colonization of 2 leaf bags by leaving them free in the chambers (between the plastic containers), and prevented animal colonization of 1 leaf bag by placing it in the enclosed plastic container with the tiles. The enclosed containers allowed water through-flow, so flow around all leaf bags was similar. We left the leaves and tiles in the middle chambers of the channels to condition for a week before we haphazardly distributed them among the chambers. During this time, small amounts of fine sediment entered the channels from the header tank through the mesh dividers. In contrast with the previous experiments, we did not remove sediment accumulations prior to the experiment because they appeared to be similar across the channels.

At the end of the experiment we weighed, photographed, and released the tadpoles. We collected the leaves, tiles, sediment, and biofilm and processed them as described above. We also analyzed the phosphorus, nitrogen, and carbon content of the dried sediment samples from the downstream chambers and the leaves from the enclosed treatment at the Waite Analytical Lab and CSIRO, Adelaide, Australia.

Statistical analyses

Our analyses included ANOVA, Tukey's tests, *t*-tests, linear regression (S-Plus Version 8.2 [TIBCO Software Inc., Somerville, Massachusetts]), split-plot ANOVA, and Tukey tests (*lme4* [Bates et al. 2014], *lmerTest* [Kuznetsova et al. 2016] in R version 3.2.3, R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

Experiment 1 To test hypotheses 1 and 2, we used split-plot ANOVAs to test if the percentage of leaf mass remain-

ing from each plant species differed among animal treatments and controls and Tukey tests to identify treatments that were different. Most data were homogeneous (Levene's test), so we used untransformed data unless stated otherwise.

We tested hypothesis 3, the effect of tadpoles and invertebrates separately and together on combined sediment + biofilm mass, with a 1-way ANOVA followed by Tukey's tests ($\alpha = 0.05$). We analyzed the biofilm and sediment together because we were unable to separate them. We used changes in sediment mass in middle and lower (downstream) chambers to determine whether sediment had been displaced. We addressed hypothesis 4, that tadpoles would maintain condition during the experiment, by testing for differences in tadpole wet mass in the presence or absence of invertebrates separately for each species with 2-tailed *t*-tests.

Experiment 2 We used linear regression analysis to test hypothesis 5, that leaf breakdown, sediment accumulation, and tadpole wet mass varied as a function of tadpole abundance.

Experiment 3 We used a split-plot ANOVA followed by Tukey's tests to test hypothesis 6, that tadpole presence would affect nutrient content and biofilm growth on tiles. These data were not homogeneous so we arcsin$\sqrt{(x)}$-transformed them prior to analysis. We used a 1-way ANOVA followed by Tukey's tests to test if animal treatments affected nutrient quality of leaves and sediment (comparison of C:N and C:P ratios) and if changes in tadpole biomass differed among the tadpole and tadpole + invertebrate treatments.

RESULTS

Experiment 1: leaf breakdown and sediment accumulation

Hypotheses 1 and 2 Both plant species and animal treatments influenced leaf litter breakdown. Leaf mass loss differed among the 3 plant species within each treatment and control ($F_{2,24} = 119.83$, $p < 0.001$; Fig. 1) and was greater in the invertebrate (Inv)-only and tadpole + invertebrate (Tad + Inv) treatments than in the Tad-only treatments or the controls, regardless of tadpole species present, by up to 27 (*A. brachystylis*), 22 (*C. leucophylla*) and 45% (*A. brachystylis*, *C. leucophylla*, and *E. bessaphila*, respectively) ($F_{5,12} = 91.59$, $p < 0.001$; Fig. 1). However, invertebrates broke down plant leaves of different species at significantly different rates 66, 50, and 79% (for *A. brachystylis*, *C. leucophylla*, and *E. bessaphila*, respectively; $F_{10,24} = 10.90$, $p < 0.001$). Tadpoles did not differ in their effects as none of the Tad + Inv treatments differed from the Inv-only treatments, and none of the Tad-only treatments differed from the controls.

Hypothesis 3 Sediment and biofilm AFDM differed between tadpole treatments with or without invertebrates. Less sediment remained in the treatment chambers when *M. coggeri* tadpoles (MC) were present (0.5–0.6 g; Mc and Mc + Inv) than in the other treatments or controls (1.5–2.6 g), regardless of whether invertebrates were present or not (1-way ANOVA, $F_{5,12} = 45.01$, $p < 0.001$; Fig. 2). Conversely, more sediment remained in the Inv-only treatments (2.6 g) than in the other treatments (0.5–1.9 g). More sediment also remained in the chambers downstream of the Mc-only (2.6 g) and Mc + Inv (3.2 g) treatments than for other treatments or controls, indicating that *M. coggeri* tad-

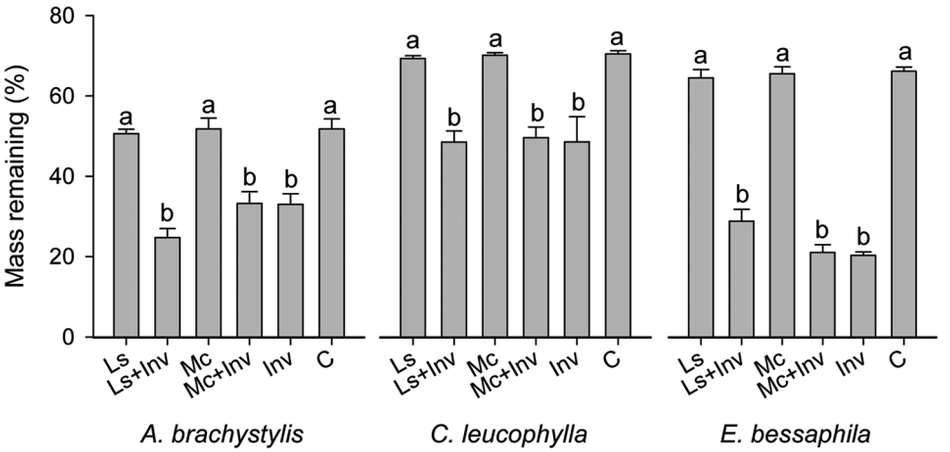

Figure 1. Mean (±SE) % leaf mass remaining from 3 plant species in the treatment (middle) chambers in experiment 1. Treatments with the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$). Ls = *Litoria serrata*; Mc = *Mixophyes coggeri*; Inv = invertebrates; and C = control. Plant species: *Apodytes brachystylis*, *Cryptocarya leucophylla*, and *Endiandra bessaphila*.

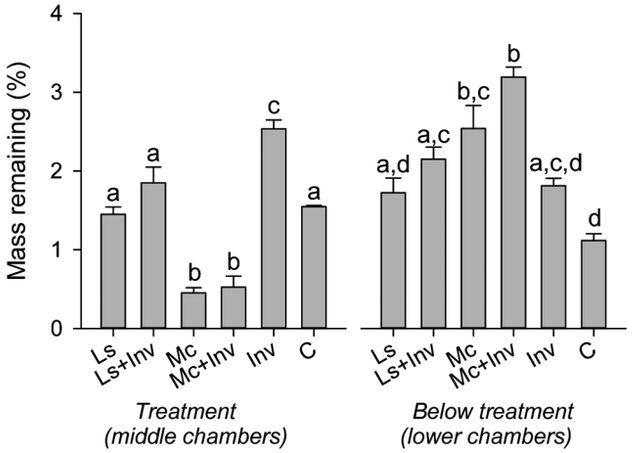

Figure 2. Mean (±SE) sediment and biofilm AFDM accumulation in the treatment (middle) chambers and downstream (lower) chambers in experiment 1. Treatments with the same letter are not significantly different (Tukey's tests, $\alpha = 0.05$). Ls = *Litoria serrata*; Mc = *Mixophyes coggeri*; Inv = invertebrates; and C = control.

poles remove more material than *L. serrata* (1-way ANOVA, $F_{5,12} = 17.47$, $p < 0.001$; Fig. 2).

Hypothesis 4 Tadpoles lost wet mass over the duration of the experiment (for *L. serrata*, 20% in the Tad-only treatment and 42% in the Tad + Inv treatment; *M. coggeri*, 0% in the Tad-only treatment and 9.5% in the Tad + Inv treatment; Table S2). The difference between treatments was significant for *M. coggeri* tadpoles (2-tailed *t*-test, $t = 5.762$, $p = 0.0045$), but not for *L. serrata* tadpoles ($t = 1.768$, $p = 0.152$).

Experiment 2: tadpole density effects

Hypothesis 5 Leaf mass loss increased with density of *L. serrata* tadpoles by about 8% ($F_{1,15} = 22.26$, $p < 0.001$; Fig. 3A), but tadpole density had no effect on sediment accumulation ($F_{1,15} = 0.193$, $p = 0.666$; Fig. 3B). Greater tadpole density led to lower individual tadpole biomass from a 130% increase in the 2-tadpole treatment to a 10% decline in the 20-tadpole treatment ($F_{1,123} = 168.6$, $p < 0.001$; Fig. 4).

Experiment 3: nutrient cycling

Hypothesis 6 Tadpoles had variable effects on nutrient content in sediment and biofilm growth. As expected, Inv-only and Tad + Inv treatments caused more leaf mass loss of exposed *C. leucophylla* leaves than control or Tad-only treatments (by about 10–25%; $F_{4,15} = 5.69$, $p = 0.006$), and leaf breakdown rates differed significantly between exposed and enclosed treatments (by about 7–25%; $F_{1,15} = 66.40$, $p < 0.001$). C:N and C:P ratios (by mass) in remaining leaf masses did not differ among animal or exposure treatments and controls (respectively: $F_{4,15} = 0.93$, $p = 0.475$; and $F_{4,15} = 0.33$, $p = 0.851$; Fig. 5). However, the sediment in the control treatment had a lower C:N ratio than the Tad- or Inv-only treatments (by about 15–23%; $F_{4,15} = 5.00$, $p = 0.009$; Fig. 5), but there was no such effect for the C:P ratio ($F_{4,15} = 2.87$, $p = 0.060$). No treatment, tile enclosure, or interaction effects on biofilm growth were significant (treatment $F_{4,15} = 0.21$, $p = 0.930$; exposure $F_{1,15} = 2.84$, $p = 0.113$; interaction $F_{4,15} = 0.50$, $p = 0.733$), and there was no treatment effect on biofilm accumulation on the exposed tiles ($F_{4,15} = 2.70$, $p = 0.07$). In the downstream chambers, there was no animal-treatment or tile-enclosure effect on biofilm AFDM (treatment $F_{4,30} = 0.30$, $p = 0.873$; exposure $F_{1,30} = 0.002$, $p = 0.964$), or an animal-tile interaction ($F_{4,30} = 0.62$, $p = 0.652$). The gain in tadpole biomass was greater in the low- than in the high-density

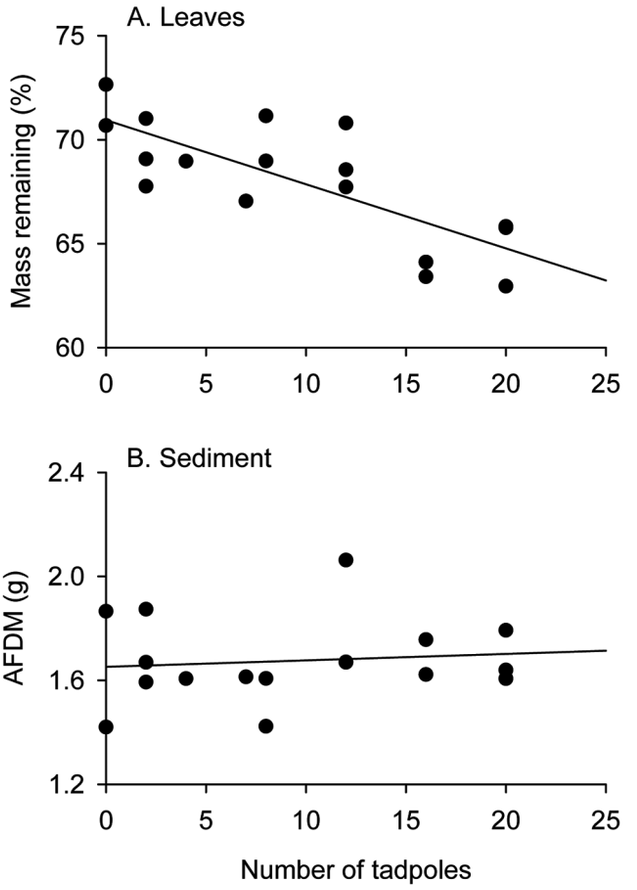

Figure 3. Percentage leaf mass (A) and sediment AFDM (B) remaining in 17 channels in experiment 2, plotted against numbers of *Litoria serrata* tadpoles present at the start of the experiment. Linear regression lines are shown: (A) $r^2 = 0.60$, $p < 0.001$, and (B) $r^2 = 0.01$, $p = 0.666$.

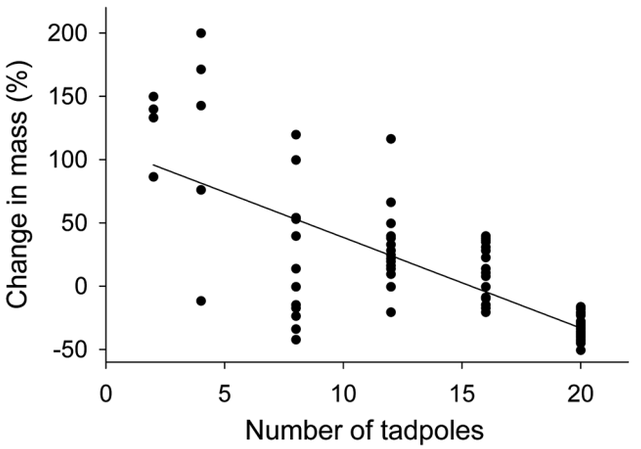

Figure 4. Percentage biomass change for *Litoria serrata* tadpoles at different tadpole densities in experiment 2. Linear regression line is shown: $r^2 = 0.58$, $p < 0.001$.

treatments or tad + Inv treatments (by about 42–54%; $F_{2,8} = 11.10$, $p = 0.005$) (Table S3).

DISCUSSION

We aimed to determine how 2 tadpole species influence stream ecosystem processes. Our results demonstrate that (1) tadpoles did not consume leaves, but at high densities one species increased leaf mass loss, probably by facilitation resulting from nutrient recycling, (2) nutrients were removed from sediments by tadpoles, and (3) tadpoles caused sediment removal by bioturbation, with one species having a much greater effect than the other. Below, we put our results in context of relevant literature and discuss their implications.

Leaf breakdown and nutrient cycling

Invertebrate treatments had the most leaf mass loss (experiment 1, Fig. 1). The highest leaf mass loss for *A. brachystylis* occurred in the *L. serrata* tadpole and invertebrates treatment, but in contrast with Iwai et al. (2009), this difference was not significant. Our results therefore did not support hypothesis 1, that tadpoles would consume leaf litter of the plant species offered, as there was no direct evidence of consumption by tadpoles. The presence of tadpoles or invertebrates upstream did not facilitate shredder activity downstream (experiment 3; hypothesis 2). However, *C. leucophylla* leaf breakdown by invertebrates increased as tadpole density increased (experiment 2; Fig. 3A), suggesting that tadpoles at high densities facilitated leaf processing. This response may have occurred because of nutrient excretion and recycling (Iwai et al. 2009, Rugenski et al. 2012), which partly supports hypothesis 2 (that facilitation between tadpoles and invertebrates increases leaf consumption of certain plant species) and hypothesis 6 (that tadpoles would enhance the nutrient content of sediment by nutrient recycling). Iwai et al. (2009) found no such effect. The difference is probably due to the effect of nutrient enhancement being detectable only at the higher tadpole densities of the current study, and partly supports hypothesis 5, with regard to leaf breakdown being positively related to tadpole density. Low densities of *L. serrata* tadpoles, however, did not increase leaf breakdown or sediment accumulation. This lack of response may be a result of *C. leucophylla* leaves having low nutrient content and, thus, not being consumed by the tadpoles (Iwai and Kagaya 2007). The N:P ratio of a consumer's body tissue also affects nutrient release, as animals retain greater proportions of nutrients that are scarce in their food (Vanni 2002). The presence of tadpoles and invertebrates reduced the nutrient quality of the sediment downstream, probably because the animals preferentially assimilated N and P, leaving carbon-rich fecal matter with a high C:N ratio (i.e., lower N content) in the sediment (Rugenski et al. 2012). Physical activity by the tadpoles (see below) would facilitate delivery of this high C:N ratio sediment downstream.

Algae may use N and P excreted by tadpoles (Iwai and Kagaya 2007), and nutrient supply promotes microbial production (Pearson and Connolly 2000, Connolly and Pearson 2013). Therefore, we hypothesized that tadpole presence would increase nutrient content in sediment and enhance biofilm growth (hypothesis 6). However, biofilm growth was not greater on the enclosed tiles in the tadpole treatments, so we found no support for this hypothesis.

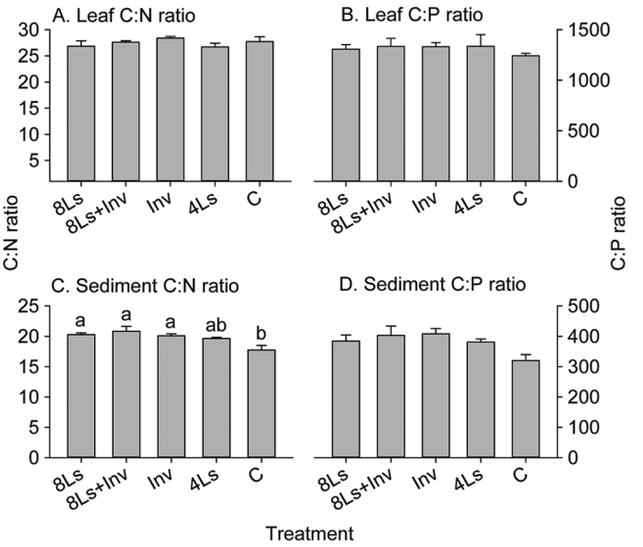

Figure 5. Mean (±SE) C:N and C:P ratios in *Cryptocarya leucophylla* leaves from the treatment chambers (panels A and B, respectively) and in sediment from the downstream chambers (panels C and D, respectively) in experiment 3. 8Ls = 8 *Litoria serrata* tadpoles; 4Ls = 4 *L. serrata* tadpoles; Inv = invertebrates; and C = control. Treatments with the same letter are not significantly different (Tukey's tests, $\alpha = 0.05$).

Sediment accumulation

Only *M. coggeri* tadpoles removed sediment from the treatment chambers (experiment 1, Fig. 2), not supporting hypothesis 3, that both species caused bioturbation that increased the amount of sediment washing downstream. In this regard, therefore, the species are not functionally redundant. Sediment accumulation in the present study was highest in the invertebrate treatments, probably from leaf breakdown and feces, and was lower in the presence of tadpoles. *Mixophyes coggeri* tadpoles, in particular, actively removed sediment and appeared to consume it. These results differ from a study in Panama in which the grazing tadpole *Smilisca sila* did not remove sediment (Rugenski et al. 2012). The difference in results may be due to feeding or behavioral differences in the Panamanian species. Alternatively, the difference could have occurred because the flow velocity in our study was 10 cm/s, but probably ~0 cm/s in Panama because the study was done in a stream pool.

Sediment removal may benefit invertebrate consumers by exposing underlying food resources for smaller grazers (Ranvestel et al. 2004). It can also encourage algal growth by maximizing nutrient and light availability (Connelly et al. 2008). *Mixophyes coggeri* tadpoles displaced sediment by stirring it up, causing it to wash downstream, but *L. serrata* tadpoles did not. The difference may be because *M. coggeri* tadpoles are larger and are strong swimmers (Anstis 2013). Both tadpoles probably consumed sediment, including organic detritus previously recorded in the diets (Trenerry 1988, Schmidt et al. 2017).

Regardless of the mechanism, tadpoles removed or displaced sediment, as reported elsewhere (Flecker et al. 1999, Ranvestel et al. 2004). Invertebrates, on the other hand, added more fine particulate organic material through feeding and egestion than they removed, as reported in Panama (Rugenski et al. 2012). Shredders may input substantial amounts of fine particulate organic material through egestion and, thereby, contribute significant amounts of dissolved inorganic nutrients (Halvorson et al. 2017), but we found no increase in nutrient content in sediments downstream of invertebrate treatments. Accumulation of fine material in the treatment chambers indicates that, in a particular stream section, invertebrates create fine particulate organic material, whereas tadpoles facilitate its removal. However, tadpole density did not affect sediment accumulation (Fig. 3B), possibly because higher densities resulted in lower per capita consumption, reducing differences between treatments, as has been reported for invertebrate shredders (Boyero and Pearson 2006).

Tadpole condition

Tadpoles and invertebrates may benefit from interactions during leaf litter breakdown or sediment removal, but they may also compete with each other (Morin et al. 1988). The loss of tadpole biomass in the presence of invertebrates did not support hypothesis 4, that tadpoles would maintain condition throughout the experiments, and indicated possible competition for resources such as biofilm and other organic material. High tadpole densities may also result in intraspecific competition. Here, individual *L. serrata* tadpoles at low densities doubled their original biomass, whereas at high densities they either gained little or lost biomass. Tadpoles of *L. serrata* and *L. dayi*, which co-occurred in Birthday Creek until the early 1990s, competed when placed together experimentally (Trenerry 1988), and our results suggest that *L. serrata* and *M. coggeri* may also compete for resources in the stream.

Conclusion

Tadpoles contribute to materials processing and bioturbation in Australian Wet Tropics streams when they are present at high densities, typically during the summer months (Schmidt 2016). Tadpoles and invertebrates may benefit each other, but they may also compete for space or food. Therefore, the relationship between tadpoles and invertebrates may change during periods of naturally high tadpole or invertebrate densities, influencing their effects on stream functioning. Our results indicate that only at high densities did *L. serrata* tadpoles directly affect litter breakdown (experiment 2, hypothesis 5, Fig. 3A), and that 2 species of tadpoles differed in their effects on bioturbation (experiment 1, hypothesis 3, Fig. 2), indicating that species were not functionally redundant. The potential effects of amphibian declines, therefore, will depend on species identity, and it is important to consider individual roles of species when assessing possible effects of species declines in streams. Future studies should verify our microcosm results with in situ studies to address issues of realism and scale imposed by use of artificial channels (Connelly et al. 2008).

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