Contrasting effects of functionally distinct tadpole species on nutrient cycling and litter decomposition in a tropical rainforest stream

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Summary

1. There is growing interest in predicting how loss of species diversity and abundance affects the structure and functioning of ecosystems. Tadpole diversity and abundance can be exceptionally high in tropical streams, but, compared to other groups, relatively little is known about their functional roles. We assessed the trophic niches and investigated the contribution of tadpoles to nutrient recycling (excretion and egestion) and litter decomposition in streams.

2. We used two ecomorphologically distinct tadpoles belonging to the two most dominant feeding guilds in Ranomafana, Madagascar: *Boophis quasiboehmei* (hereafter BQ, generalized mouthpart, grazer) and *Mantidactylus melanopleura* (hereafter MM, umbelliform funnel mouthpart, suspension feeder). We conducted field incubation experiments to quantify nutrients recycled from excretion and egestion, and set enclosures to analyze the effects of tadpoles on litter decomposition (*Pauridiantha* sp. and *Chrysophyllum* sp.) in 9 treatments: control (no tadpoles), monospecific treatment (BQ or MM) at three densities (low, medium, high: 3, 6, 12 tadpoles/0.0625 m^2, respectively), mix species treatment (3 BQ + 3 MM, 6 BQ + 6 MM).

3. BQ and MM occupied the same trophic level but tended to differ in their carbon isotopic signatures. MM excreted Nitrogen (N) and Phosphorus (P) at higher rates, but the two species had similar N:P molar excretion ratio. Nutrients recycled from fecal pellets (egestion) were not immediately available but needed to be mineralized: N increased with time in the water column and was more than 25 times higher in concentration at the end of the experiment (day 16), whereas P concentration showed a hump-shaped pattern with a maximum value at day 4 of incubation. Effects of tadpoles on litter decomposition depended on tadpole identity, density, and litter

identity. Particularly, no decomposition occurred at low tadpole density, and effects were generally stronger on the softer texture leaves *Pauridiantha* sp. We found additive effects of tadpole diversity on litter decomposition.

4. These findings indicate that: (i) tadpoles with different mouthparts play different roles on the ecosystem (ii) tadpole fecal pellets are important latent sources of nutrients in freshwater environments (iii) tadpole abundance exerted stronger effects than species diversity on litter decomposition, emphasizing the importance of tadpole biomass and communities in tropical streams. Interspecific variation in nutrient recycling and differential effect on litter decomposition illustrate a strong linkage between species identity and ecosystem function.

Introduction

Understanding the role of species diversity and abundance in maintaining ecosystem services is an important goal given high rates of biodiversity loss (Pimm *et al.*, 2014). This is particularly true in freshwater ecosystems where species declines are occurring at higher rates than in most other ecosystems (Dudgeon *et al.*, 2006). Given the current status of many amphibian species, and the likelihood that past and ongoing declines have ecosystem-level consequences, there is urgent need for quantitative information on the diverse ecological roles of larvae (Whiles & Altig, 2010). Anuran larvae, commonly known as tadpoles, can reach high diversity and can represent high biomass in tropical streams, but compared to other aquatic consumer groups, such as fishes and macroinvertebrates, relatively little is known about their functional roles (Altig, Whiles & Taylor, 2007). Earlier studies suggested that tadpoles are ecosystem engineers and can strongly influence community structure (Connelly *et al.*, 2008; Ranvestel *et al.*, 2004), production of aquatic macroinvertebrates (Ranvestel *et al.*, 2004; Colón-Gaud *et al.*, 2010), and organic matter dynamic (Colón-Gaud *et al.*, 2008; Flecker, Feifarek & Taylor, 1999). While their influences on some ecosystem processes have been quantified to some degree, we know far less about their contribution to nutrient recycling (Vanni *et al.*, 2002) along with their ability to decompose litter in tropical streams (Iwai, Pearson & Alford, 2009; Rugenski, Murria & Whiles, 2012), that are two integral parts of ecosystem functioning.

Consumer-mediated nutrient recycling is a key mechanism driving nutrient turnover and primary productivity in many ecosystems (Vanni, 2002). Recycled nutrients can be excreted in dissolved form (excretion) or in the form of fecal pellets (egestion). The contribution of excretion to consumer-mediated nutrient recycling is relatively well studied across many taxa (reviewed by Vanni (2002) and Atkinson et al. (2016)),

but information on the role of egestion is still limited (Wotton & Malmqvist, 2001; Halvorson *et al.*, 2015; Halvorson, Hall & Evans-White, 2017); in fact, egestion could be an important component of nutrient recycling that is often not quantified (Capps, Atkinson & Rugenski, 2015). Scarce evidence suggest that, in a pond-dwelling tadpole, 75 – 92% of nutrients are recycled through egestion and only 8 to 25% in dissolved form (Liess *et al.*, 2015). However, nutrients bound in fecal pellets are not readily available, but must be mineralized (Liess & Haglund, 2007), suggesting that egestion from tadpoles may represent an important but latent source of nutrients in their respective habitats. Tropical tadpoles are often morphologically highly diverse, especially in their oral structures (Strauß *et al.*, 2013). Mouthparts typically influence diet, which may consequently affect the quality and the quantity of recycled nutrients in tadpoles (Liess *et al.*, 2015).

Litter decomposition is a central component in aquatic food webs and can strongly affect community structure (Graça *et al.*, 2015). Tadpoles can mediate litter decomposition directly by consumption (Ramamonjisoa, Iwai & Natuhara, 2016), or indirectly by enhancing microbial activities through their excretory products (Rugenski *et al.*, 2012), an important process in tropical streams (Mathuriau & Chauvet, 2002). Yet, empirical studies have yielded conflicting results, from which no generalization emerges. For example, Connelly *et al.* (2011) found that centrolenid tadpoles (*Centrolene prosoblepon* and *Cochranella albomaculata,* Centrolenidae) have no effect on bacterial biomass, respiration rates, and on litter decomposition. In contrast, Rugenski *et al*. (2012) found that common grazing tadpoles of *Smilisca sila* (Hylidae）can substantially facilitate the process by enhancing microbial activities, making litter more palatable to shredder macroinvertebrates. In a tropical Australian stream, Iwai *et al*. (2009) found that *Litoria gemimaculata* (Hylidae) tadpoles could

100 decompose litter only once it has been pre-processed by shredders. These studies
101 suggest that specific traits of tadpoles may influence their impact on litter
102 decomposition.

103 The abundance and diversity of tadpoles can vary greatly in time and space in tropical
104 streams (Strauß *et al.*, 2016), and different tadpole species may have different effects
105 on the ecosystem. Yet, so far, studies that investigated the contribution of tadpoles to
106 litter decomposition in tropical streams have typically focused on one species (i.e.,
107 one functional trait), and, usually, at one density treatment. Furthermore, most studies
108 have used one type of litter although many different types of litter are present in the
109 stream. Depending on litter quality and the ability of tadpoles to decompose them,
110 different results may be found. Studies that examine density and diversity of species
111 (i.e., functional diversity) would allow for more accurate appreciation of the
112 functional roles of tadpoles and the ecological consequences of their loss on
113 ecosystem functioning.

114 In this study, we examine the trophic niches and the functional roles of two
115 ecomorphologically distinct tadpoles in a rainforest stream in Madagascar.
116 Specifically, we quantified the contribution of the tadpoles to nutrient recycling
117 through excretion and egestion, and subsequently tested the effects of tadpole density
118 and diversity on litter decomposition. We asked the following questions: do
119 ecomorphologically distinct tadpoles occupy the same trophic niche? Do they
120 similarly recycle nutrients? What is the contribution of tadpole egesta to nutrient
121 recycling? Do tadpoles affect litter decomposition? Specifically, how do litter identity,
122 tadpole density and diversity influence litter decomposition? We hypothesized that
123 (H1) tadpoles occupy different trophic niches due to their difference in mouthparts;
124 (H2) different tadpoles have different N and P excretion rates; (H3) egesta are

important latent sources of nutrients, and (H4) effect of tadpoles on litter decomposition depends on litter identity, and increases with tadpole density. We expect that tadpoles are not functionally redundant and would have additive effects on litter decomposition, i.e., effect on decomposition is specific to tadpole identity and effects of mixed species is equal to the sum of their respective independent effects.

Methods

We conducted our study in Ranomafana National Park Madagascar in October - November 2015. The park consists of 43,500 ha of continuous moist humid forest (midaltitude montane rainforest), with annual rainfall ranging from 1700 to 4300 mm. The high-rainfall season is from December to March, with the driest periods in September and October. Temperatures range from lows in June – September (4 – 12 ºC) to highs in December – February (36 – 40 ºC) (Wright & Andriamihaja, 2003). Ranomafana harbors the richest lotic tadpole community in the world with more than 44 species (Strauß *et al.*, 2013). Tadpoles are abundant in streams and can reach more than 200 ind.m^{-2} in pools (Ramamonjisoa and Natuhara, unpublished data, November 2014). Because of poor fish fauna, it is assumed that tadpoles represent the largest biomass in Ranomafana streams (Strauß *et al.*, 2010); thus, they may be of paramount importance in stream ecosystem functioning.

We recorded 27 tadpole species (Ramamonjisoa, 2017) belonging to four genera in the Mantellidae family: *Mantidactylus*, *Boophis*, *Spinomantis, Gephyromantis,* and *Guibemantis*, and seven mouthpart groups (following the classification proposed by Strauß *et al.* (2013)): sand eater, reduced teeth, generalized, podgy generalized, fossorial, suctorial, funnel mouthed). Among these, generalized and funnel-mouthed groups account for more than 70% and 10% in abundance, respectively, of all

tadpoles. Each one of the other feeding groups represent less than 5% of tadpole abundance (Ramamonjisoa, 2017).

Boophis quasiboehmei (Mantellidae, hereafter BQ, generalized mouthpart) and *Mantidactylus melanopleura* (Mantellidae, hereafter MM, funnel-mouthed mouthpart,) are two dominant tadpoles in Ranomafana streams and account for 40% and 9% of tadpole species abundance, respectively, in Ranomafana streams in October – November (Ramamonjisoa, 2017). Little is known about the feeding ecology of these tadpoles, but field observations and their mouthparts suggest no feeding interference between the two species. Tadpoles with generalized mouthpart, such as *Boophis quasiboehmei*, are generally grazers that feed on a variety of animal and plant resources (Whiles and Altig, 2010); feeding usually operates by anchoring their oral disk to a substrate and rasping materials off of it (Venesky *et al.*, 2011). Ramamonjisoa (2017) noted that loams, diatoms, and fungi occupied a large proportion of the gut volume. In contrast, funnel-mouthed tadpoles are suspension feeders that hang from the water surface and suck in suspended fine materials (Nachtigall, 1974). The larvae are characterized by umbelliform oral discs and absence of well-developed keratodonts (Grosjean *et al.*, 2011). The tadpoles of MM are suggested to be omnivorous feeders and gut volume is dominated by algae, insect fragments, diatoms, plant matters as well as organic particles (Vences *et al.*, 2016).

Although the two tadpole species only represent a minor fraction of the tadpole species richness present in the study stream, they represent an ideal system for a biodiversity-ecosystem functioning because: (i) they allow exploring diversity effects in different ecological situations, (ii) they are the dominant species in the community occurring in the study stream, (iii) these species are functionally different but using the same resource pool, and (iv) using two species facilitates interpretation of mixing

effects in cases in which species-specific contributions to the performance of the mixture are challenging to measure.

Isotope analysis for determining trophic niches of the tadpoles

We used stable isotopes of carbon (C) and nitrogen (N) to determine the trophic niches of the tadpoles (Fry, 2007). Typically, consumers with different diets are likely to occupy different trophic niches. C isotope signatures are generally similar to those of their food source or sources (i.e., "you are what you eat") while N signature informs about the trophic position of consumers (Whiles and Altig, 2010). We followed standard methods described in Whiles & Altig (2010). We collected five individuals of each species from three second-order streams in pools using D-frame net (N = 15 per species). The tadpoles were at the same developmental stage (< Gosner 32; Gosner, 1960) to avoid possible biases associated with ontogenic changes in diet, as reported in some tadpoles (Wickramasinghe, Oseen & Wassersug, 2007). Gosner 32 corresponds to the tadpole life stage where hind limb buds appear, but the toes are not yet differentiated (Gosner, 1960). Tadpoles were transported to the laboratory and euthanized by immersion in MS-222 solution. The use of MS-222 does not affect stable isotope measurements (e.g., Arribas *et al.*, (2015)). We determined carbon and nitrogen isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of tail muscle of tadpoles. The muscle tissue samples were oven-dried at 60 °C and ground to a fine and homogenous powder using mortar and pestle. We removed lipids from samples prior to analysis following Logan & Lutcavage (2008). Dried homogenized samples were immersed in a 2:1 ratio of chloroform/methanol with solvent volume 5-10 times greater than the sample volume. Samples were mixed and left undisturbed for approximately 5 min, and centrifuged at 15000 rpm, and the supernatant containing solvent and lipids was then removed. The process was repeated until the supernatant was clear. The samples

were re-dried for 24 h at 60 °C to remove any remaining solvent. Isotope analyses were performed using a continuous-flow isotope-ratio mass spectrometer (Thermo Scientific™ *DELTA V Advantage*). In addition to the sample isotopic values, body elemental composition % C and % N were obtained from the analysis, which allowed to calculate C:N.

Nutrient recycling from excretion

We quantified nutrient excretion rates of BQ and MM in the field. We followed standard techniques that consist of incubating tadpoles in bags and measuring changes in nutrient concentrations in the surrounding water (Vanni, 2002; Whiles et al., 2009). Tadpoles were collected with D-frame nets in streams; upon capture, animals were quickly sorted and placed in distilled water to "wash" the tadpoles (removing nutrients and particles attached to tadpoles). The tadpoles were subsequently placed in 6 transparent plastic bags (~10 individuals of similar size per bag) containing 500 mL of stream water previously filtered through Whatman GF/A glass microfiber filters (pore = 0.7 μm). The bags were placed in the stream we collected the tadpoles to minimize variation in temperature.

After 1h, the contents of bags were filtered through Whatman GF/A glass filters to remove feces and other particles, and then through Gelman AE filters (pore < 0.45 μm) to remove particles that might absorb nutrients (e.g., microorganisms). Animals were removed, weighed (wet) to the nearest 0.1 g and released. Filtrate samples were collected in acid-washed bottles, frozen and transported on ice to Nagoya University. Colorimetric analyses (using spectrophotometer, HACH DR2800) determined N concentration as ammonia NH3-N by salicylate method (HACH Ammonia-N TNTplus 830) and total phosphorus by ascorbic acid method and persulfate digestion (HACH Phosphorus Reactive and Total TNTplus 843). Ammonia excretion has been

typically used in fish studies, but the method has also been successfully applied in tadpole excretion studies (Vanni *et al.*, 2002; Guariento *et al.*, 2015). Furthermore, ammonia can account for the largest percentage of total nitrogen excretion in tadpoles (Tattersall & Wright, 1996). The HACH method does not use blanks, and we tested for accuracy using standards (0.1 $mg.L^{-1}$, 0.5 $mg.L^{-1}$, and 1 $mg.L^{-1}$). Rate of N and P excretions were calculated as the amount of nutrients N and P released (in µmol) in the surrounding water per hour per g wet mass tadpoles (Vanni *et al.*, 2002). The values were corrected for the background water before analysis.

Nutrient recycling from egestion

Nutrient recycling from egestion was evaluated by incubating a known amount of egesta and by quantifying the concentration of nutrients released in the water column (Liess & Haglund, 2007). We collected groups of tadpoles, usually more than 100 individuals for each species; the tadpoles were briefly "washed" in distilled water to remove attached materials and placed them in plastic trays containing filtered stream water. We collected egesta using pipettes as they deposited in the trays. The collected egesta were shaken and divided into two equal parts, and filtered through Whatman GF/A glass microfiber filters. One filter with egested material was incubated in 8-liter buckets containing 2.5 L of filtered stream water, and the second filter was dried to estimate weight. We replicated the procedure two times for each species (N = 2). The buckets were covered with transparent plastic film (to avoid any materials falling in the buckets), and were firmly fixed in pair in shallow pools. Placing the buckets in the field allowed exposing the experiment to natural variation in temperature. We sampled 100 mL water at a time per bucket on days 0, 1, 4, 8, 12 and 16 following Liess & Haglund (2007). The sampling syringe was used to gently mix the water carefully before sampling (we sampled water column at half depth). Samples were

filtered following standard procedures and were analyzed for ammonia and TDP as described above. Concentrations of nutrients (N and P) released in the water column are expressed by μmol nutrient per g of dry fecal pellet per liter of incubating water.

Effects of tadpoles on litter decomposition

We used field enclosures to examine the effects of tadpole density and diversity on litter decomposition. The experiment was conducted in a second-order, 2.5 – 3.5 m wide stream. The stream runs on granite bedrock; typically, such streams are characterized by low water calcium ion concentration. Substrata ranged from boulders and cobbles in riffles, to silt, leaf litter and sand in pools. Stream flow was relatively stable during the experiment. Background nutrient concentrations were 0.023 $mg.L^{-1}$ in total dissolved phosphorus and 0.036 $mg.L^{-1}$ in ammonia. Dissolved oxygen was 7.27 $mg.L^{-1}$ and average temperature during the experiment was 18.2°C (range 17.5 – 19 °C).

Enclosures dimensions were 0.25 m × 0.25 m × 0.35 m (length × width × height), made of double 1 mm mesh polyethylene cloth (modified from Kupferberg, 1997). The bottom was closed with nylon fabric. Enclosures were set in pools where water velocity was relatively low (< 1.03 $cm.s^{-1}$) (measured with an electro-magnetic water velocity meter, Kenek CO., LTD, VE20). Each enclosure was firmly fixed in the substrate using bamboo poles and covered with 2 mm mesh to prevent colonization from external materials (e.g., fallen leaves, insects). Enclosures were distant at least 1.6 m from one another. The experiment was carried over 40 days (October 12^{th} – November 23^{rd}).

We manipulated tadpole abundance and diversity in 9 treatments: control (no tadpoles), monospecific treatment (BQ or MM) at three densities (low, medium,

high: 3, 6, 12 tadpoles/enclosure (0.0625 m^2), respectively), and the combination of the two (3BQ + 3MM, 6BQ + 6MM). These densities are within the natural density of tadpoles recorded in stream pools in October - November (Ramamonjisoa & Natuhara, unpublished data). Each treatment was replicated five times for a total of 45 experimental units. Tadpoles for one species were of the same size and less than Gosner 32 (Gosner, 1960). Initial tadpole total lengths and body masses (mean ± SD, N = 30) were 33.1 ± 3.8 mm (0.37 ± 0.06 g) and 40.5 ± 5.7 mm (0.35 ± 0.08 g) for QB and MM, respectively.

We used leaves of *Pauridiantha* sp. (Rubiaceae) and *Chrysophyllum* sp. (Sapotaceae), two common riparian tree species in Ranomafana. Both litter species have similar C/N, but *Pauridiantha* sp. leaves are softer and contain higher concentration of calcium, potassium and magnesium (Table 1). Litter toughness (in Newton) was measured with a tension gauge with 1 mm head probe (Ouba Instrument Ltd, Osaka, Japan). Nutrient concentrations (K, P, Mg, Ca, in % litter dry mass) were measured using mass spectrometry ICP-MS (Agilent 7700x). C and N measurements were conducted on a CN analyzer (CN Corder MT 700, Yanaco). Leaves that were approximately the same size, from the same position on the tree and showing no sign of herbivory were selected. Leaves were dried at 60 °C for 48 hours. Bundles of approximately 0.8 g of each type were weighed to the nearest 10 mg, secured in labeled 1 mm mesh bags and were put into the stream for microbial pre-conditioning (following Rugenski *et al*., 2012). After 10 days, the bags were retrieved and the leaves were randomly assigned to enclosures. Each enclosure contained a bundle of each litter with the respective number of tadpoles. The control only had litter but no tadpoles.

297 Because of the number of replicates, we used different sections of the stream. These
298 sections were relatively similar in environmental conditions (e.g., light intensity,
299 general structure of the surrounding vegetation). Five sections were used; we had one
300 replicate of each treatment at each section (which was later treated as random factor
301 in the statistical analysis). We checked enclosures every 4 days for mortality and
302 damages. We removed dead tadpoles and replaced them with ones of similar size. A
303 heavy rain occurred in the later part of the experiment but did not destroy the
304 enclosures. We terminated the experiment one week after this event. After 40 days,
305 the tadpoles were removed from their enclosures, weighed, photographed and
306 released. BQ tadpoles grew faster than MM tadpoles in the enclosures (monospecific
307 medium density treatment, mean ± SD, 20.01 ± 6.41 mg.40 $days^{-1}$ vs. 3.07 ± 2.02
308 $mg.40days^{-1}$, respectively). Litter bundles were retrieved from enclosures, gently
309 washed to remove attached sediments. We did not record any invertebrates attached to
310 the litter. Leaves were dried at 60 °C for 48 hours, and weighed to estimate litter mass
311 loss, which was used as indicator of litter decomposition (Lecerf *et al.*, 2007).

312 Data analysis

313 We compared mean isotopic values ($\delta^{13}C$ and $\delta^{15}N$), elemental body (C, N, and C:N)
314 excretion rates (N, P, and N:P) ratio between the two species (BQ and MM) using t-
315 tests. In the egesta incubation experiment, we analyzed the variation in nutrient
316 concentrations over time in the water column using repeated measures ANOVAs. We
317 used "time of sampling" as factor and "N or P concentration" as response variables.
318 Because of sample size (replicate = 2 for each species), we combined the data for BQ
319 and MM to analyze the effect of time on nutrient concentrations in the water column.
320 We argue that this was appropriate because the pattern of nutrient release from fecal
321 pellets was similar for BQ and MM (Cf. Fig. 3). The assumptions of sphericity had

been violated; therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.214$, $\varepsilon = 0.316$ for N and P tests, respectively).

To analyze the effect of tadpoles on litter decomposition, we used linear mixed model with the function "lmer" in the package "lme4" (Bates, Maechler & Bolker, 2012) on R 3.2 (2015, R Foundation for Statistical Computing). In the model, "litter type" (two level: *Pauridiantha* sp. and *Chrysophyllum* sp.), "density" (low, medium and high), and "composition effect" (tadpole identity and mixture effect) were the fixed factors. "Litter mass loss" was the response variable and we used "stream section" as random factor. *P*-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect of explanatory variables using the function "anova." We considered a fixed effect as significant when the difference between the likelihood of two models (with and without the effect in question) was significant. We ran pairwise differences among treatments with the function "lsmeans" (Lenth, Herv & Lenth, 2015) with Tukey adjustment after bootstrapping method using the package "pbkrtest" (Halekoh & Højsgaard, 2014).

To test additivity effect of tadpole species mixture on litter decomposition, we compared the expected and the observed litter mass loss, using one-sample t-tests. Expected litter mass loss is the sum of weighted effect of each species from the monospecific treatments while observed value is the measured litter mass loss of the mixture treatments (Kominoski et al, 2007; Lecerf et al, 2007). Expected values were computed as follows:

$$(n_{BQ} \times L_{BQ} + n_{MM} \times L_{MM})/ (n_{BQ} + n_{MM})$$

where n_{BQ} and n_{MM} are the number of BQ and MM individuals, respectively, in the mixture treatment. L_{BQ} and L_{MM} are the average litter mass loss in the presence of BQ

and MM, respectively, in monospecific treatment. A significant difference (expected vs. observed values) indicates non-additivity and species have interacting effects on litter decomposition. In contrast, an absence of difference suggests that tadpole mixture has additive effect on litter decomposition i.e., no interaction between mixed tadpole species.

T-tests and repeated measures ANOVA were conducted on SPSS 17. Data were log-transformed when necessary to meet test assumptions.

Results

The tadpoles of BQ and MM occupied a similar trophic position (as shown by their N isotopic signature values), but tended to differ in C isotopic values (Fig.1, Table 2), suggesting potential differences in the food sources of the tadpoles. We did not find differences in body elemental C, N, nor C:N between the two tadpoles (Table 2)

The funnel-mouthed tadpole MM excreted nutrients at higher rates than BQ for N and P, but we did not find differences in N:P excretion ratio between the two species (Fig. 2, Table 2).

N was mineralized at higher rate than P in tadpole fecal pellets (Fig. 3). P concentration in the water column reached a maximum at day 4 (average for the two species = 0.18 μmol.g dry feces^{-1}.L^{-1}) and decreased towards the end of the experiment (F (df_{time} = 1.582, df_{error} = 4.745) = 13.659, P = 0.012). Concentration in N, however, increased with time in the water column (F (df_{time} = 1.068, df_{error} = 3.203) = 64.463, P = 0.003) (Fig. 3), and was more than 25 times higher on Day 16 (average for the two species = 92 μmol.g dry feces^{-1}.L^{-1}) compared to Day 1 of the experiment.

Tadpoles influenced litter decomposition, but effects depended on tadpole density and litter identity (Table 1). No litter mass loss occurred at low tadpole density (i.e., compared to control) (Fig. 4). Effects were higher on *Pauridiantha* sp. than on *Chrysophyllum* sp. (significant effect of litter identity on leaf mass loss, Table 4). MM influenced decomposition only at high density irrespective of litter identity. BQ effects were observed at medium and high densities, and only on the softer litter (Fig 4). Effects of tadpole mixtures on litter decomposition were additive (Fig. 4; Table 3) i.e., observed litter mass losses did not differ from expected values ($P > 0.05$ for all pairwise tests). Additive effects suggest an absence or a very weak complementary effect between the two tadpole species. There were interactive effects of "tadpole density" and "litter identity", "density" and "composition effect". These interactions indicate that the effect of tadpole density on litter decomposition further depends on litter identity and the tadpole species present in enclosures (Fig. 4, Table 4). The interaction between "litter identity" and "composition effect" could be explained by the absence of BQ effect on decomposition of *Chrysophyllum* sp. (Fig. 4, Table 4).

Discussion

Trophic niches and nutrient recycling

The tadpoles of *Boophis quasiboehmei* (BQ, generalized mouthpart, grazer) and *Mantidactylus melanopleura* (MM, funnel-mouthed, suspension feeder) occupied a similar trophic position but tended to differ in their carbon isotopic signatures (partly supporting hypothesis 1). Typically, carbon isotope signatures of consumers are similar to those of their food sources. Prior studies on gut contents in these tadpoles revealed that plant, insect and algal materials make an important part of the gut volume in MM (Vences *et al.*, 2016), while diatoms and fungi account for a larger proportion of the gut in BQ (Ramamonjisoa, 2017). Despite this apparent difference

394 in the food materials ingested by these tadpoles, gut content analysis only represents a
395 snapshot of the diet, and does not indicate what is assimilated (Whiles & Altig, 2010).
396 Relative importance of sources can be difficult to assess because we do not have
397 estimate of the quantity of each type of material that was assimilated. The marginal
398 difference in carbon isotope signatures suggests some degree of diet similarity
399 between the two species at least at the developmental stage we sampled these tadpoles.
400 Depending on their level of diet specialization, however, these tadpoles might evolve
401 to occupy different trophic niches along ontogeny as previously reported for some
402 other species of tadpoles (Hocking & Babbitt, 2014).

403 Tadpole excretion rates were within the range of previous studies (Cree, 1985; Vanni
404 *et al*., 2002). Our values were slightly lower than those reported for *Rana palmipes*
405 and *Bufo marinus* in tropical streams in Venezuela (Vanni *et al.*, 2002), but
406 interspecific differences in body stoichiometry, tadpole life stages, and time of
407 sampling among others (Vanni *et al.*, 2002) are factors that might explain these results.
408 Corroborating our hypothesis 2, we found that the two tadpoles differentially recycled
409 nutrients; MM excreted dissolved nutrients (N-ammonia and P-TDP) at higher rates
410 than BQ (almost two times faster for P), but the two species had similar N:P molar
411 excretion ratios. The quality and quantity of nutrients recycled by an animal could be
412 a result of complex interactions between diet, resource assimilation efficiency (Liess
413 *et al.*, 2015), metabolic rate, and body elemental composition (Vanni & McIntyre,
414 2016). It is suggested that consumers with relatively high content of a nutrient in their
415 body should minimize the excretion of this nutrient, but we do not think that this
416 principle may account for the difference in excretion, at least for N, in these tadpoles
417 because first they have similar body N content and second, correlation between N
418 body content and N excretion is generally weak (Vanni *et al*., 2002). Alternatively,

we suspect, but cannot confirm, that differences in diet and life history-traits such as growth rate between MM and BQ are possible explanations. Tadpoles consuming low quality resource tend to excrete lower amounts of nutrients (Liess et al., 2015). The quantity of insect fragments in the gut of MM (Vences *et al.*, 2016; Ramamonjisoa, 2017), assuming that these high-nutrient materials were actually assimilated, may contribute to the higher N excretion rates in this species. Further, animals with high gross growth efficiency are generally better at assimilating limiting nutrients and thus should recycle fewer nutrients (Liess *et al.*, 2015). Although the tadpoles in enclosures may not have access to all materials they naturally feed on in their natural environment, BQ grew faster than MM (body mass increments 20.01 ± 6.41 mg.40 $days^{-1}$ vs. 3.07 ± 2.02 mg.40 $days^{-1}$ for BQ and MM, respectively), suggesting that this species might have higher nutrient requirements in order to maintain body elemental balance (stoichiometrically balanced growth), which resulted in lower excretion rates in this species.

We recognize that handling stress could possibly bias excretion rates. Estimations from unstressed animals are difficult to obtain, but we argue that difference in excretion rates in this study was not caused by interspecific difference in handling stress alone. First, our incubation times should have been sufficient enough to reduce elevated concentrations as a result of stress (Whiles *et al.*, 2009); second, interspecific differences in excretion rates among sympatric tadpoles have been reported in many studies (Vanni *et al.*, 2002; Kupferberg, 1997; Cree, 1985; Greene, 2015), suggesting that the pattern we found could be rather common.

Egestion in tadpoles represented an important latent source of nutrients (supporting hypothesis 3), especially in N (Fig. 3). Nutrients mineralized from egesta exhibited temporal variation in concentration; N concentration increased in the water column

while P showed a hump shaped pattern with a maximum at day 4. Typically, N is released mostly in excretion while P in the form of egesta (André, Hecky & Duthie, 2003; Liess *et al.*, 2015), thus we expected higher concentration of P at the end of the experiment. Although the results did not fully follow our predictions, a recent study revealed that egesta could be long-term sinks and sources of dissolved inorganic nutrients, shifting from net release to net uptake of dissolved nutrients (Halvorson *et al*., 2017). Our egesta incubation experiment was conducted in the short term (16 days), but dynamics of N and P concentrations in the water column are consistent with the patterns reported in Halvorson *et al*. (2017). However, this was only the case for one of their three macroinvertebrate species on a low-P diet, suggesting that diet and species identity control properties of egesta and nutrient release. In their study, P concentration peaked in the water column at day 9 while it did at day 4 in our experiment. In a similar fashion, N started to increase from day 51 while, in our experiment, this burst occurred at day 4. One explanation could be that we measured ammonia-N (which include NH3 and NH4+), while they measured only NH4+, and it is possible that these nutrients have different turnover rates. Further, difference in incubation temperature (~18°C this study vs. 10°C) may play a large role by influencing microbial mineralization (Wotton & Malmqvist, 2001). We still know relatively little about the contribution of egesta to consumer-driven nutrient dynamics (Atkinson et al., 2016). Given that 75 – 92% of nutrients could be recycled through egestion in some tadpole species (Liess et al., 2015), egesta could be a relatively important source of nutrients in freshwater environments. In tropical streams, tadpoles can represent the largest biomass and can produce high quantities of egesta through their continuous feeding. Egesta can be deposited near the location of animal feeding or transported to other sites where they can generate sites of enhanced microbial

469 activity (Halvorson et al., 2015).

470 **Litter decomposition**

471 Results from our enclosure experiment demonstrate that tadpole identity, density, and
472 litter identity influenced litter decomposition (supporting hypothesis 4). Tadpoles had
473 no influence on litter decomposition at low density, irrespective of litter type.
474 However, for the softer leaves *Pauridiantha* sp., BQ influenced litter decomposition
475 at medium and high densities while MM effects were observed only at high density.
476 We found additive effect in the mix-species treatments (i.e., the observed effects were
477 not different from the expected values), indicating that combination of tadpole species
478 did not increase or decreases their effects on decomposition rate (Fig. 4). These
479 results might be explained by their contrasting feeding behavior; MM is a suspension
480 feeder while BQ is a grazer, and each species occupy different levels in the same
481 water column.

482 Our results appear to contrast with prior studies that reported either no effect
483 (Connelly *et al.*, 2011) or minor effects of tadpoles on litter decomposition in the
484 absence of macroinvertebrate shredders (Iwai et *al.*, 2009; Rugenski *et al*., 2012).
485 This contradiction is most likely due to differences in the experimental design. Had
486 we only used the leaves of *Chrysophyllum* sp. and conducted the experiment at low
487 tadpole density, we would have indeed concluded absence of effects (Fig. 4).

488 Previous studies found that tadpoles can influence litter decomposition either directly
489 by consuming leaves or indirectly by stimulating decomposer microbial activities
490 through their excretory products (Rugenski *et al*., 2012). We could not disentangle
491 these mechanisms (consumption and nutrient-mediated decomposition) in our
492 experiment, thus asserting which one played greater role on litter processing is

493 difficult. Though it is unlikely that MM tadpoles with its umbelliform, funnel
494 mouthpart (Grosjean *et al.*, 2011) would directly feed on the litter given their feeding
495 behavior, the grazer BQ tadpoles could possibly exert physical activities and enhance
496 microbial breakdown on the litter, as previously reported in a grazer tadpole
497 (Rugenski *et al*., 2012). Grazing tadpoles were thought to be ineffective at consuming
498 litter because their jaw structure and teeth are not strong enough to fragment leaves
499 (Iwai *et al*., 2009). However, the ability of tadpoles to feed on relatively hard
500 materials (Ramamonjisoa *et al*., 2016), suggests that they might still be able to
501 facilitate litter breakdown, for example, by opening frays in the leaf surface, allowing
502 them to gradually feed on the inner part of the leaves (Iwai *et al*., 2009). Although
503 MM and BQ are often associated with litter accumulations in streams (Ramamonjisoa,
504 personal observations), the question is whether these tadpoles, especially BQ, would
505 indeed incorporate litter in their diet, given that these materials often have low
506 nutritional values (Bowen, 1987). Detritus is assumed to be important resources for a
507 species of tadpole in Australian tropical streams (Iwai *et al*., 2009), but it is
508 commonly thought that these materials only represent a secondary resource as
509 tadpoles may receive more energy from associated bacteria rather than from the litter
510 material itself (Altig *et al*., 2007).

511 The two litter species have similar C/N, but *Pauridiantha* sp. are relatively softer,
512 richer in calcium, potassium, and in magnesium, which may have facilitated
513 decomposition in this species (Fig. 4). N and C/N are thought to influence
514 decomposition (Ostrofsky, 1997), based on the assumption that fungi growing on
515 leaves may obtain N from the leaf itself. However, these parameters do not
516 substantially vary between the two litter species, and fungi growing on litter
517 alternatively may obtain N and P from the water column, rather than the litter

substrate (Suberkropp & Chauvet, 1995). Softer texture of *Pauridiantha* sp could have been partly responsible of the faster decomposition (Ostrofsky, 1997). Other litter characteristics that control litter decomposition are condensed tannins (Ardon, Pringle & Eggert, 2009) and lignin (Gessner & Chauvet, 1994). We did not measure these variables, but they often correlate with toughness (Ostrofsky, 1997). These traits may have affected leaf breakdown due to their influence on the activity of decomposing microorganisms and the tadpoles.

Microbial processes play important roles in litter decomposition in tropical streams (Mathuriau & Chauvet, 2002), but whether the tadpoles enhanced microbial activities in our enclosures is unclear though a previous study supports this assumption (Rugenski *et al.*, 2012). Several studies have indicated that the effects of dissolved nutrients on litter decomposition and microbes colonizing the litter strongly depend on the water background (e.g. concentrations in nitrogen and phosphorus). In some streams with relatively high background nutrient concentration, neither litter decomposition rates (Royer & Minshall, 2001) nor fungal biomass were affected by nutrient enrichment (Abelho & Graça, 2006; Abelho *et al.*, 2010). In contrast, decomposition rate, microbial respiration, fungal and bacterial biomass, and the sporulation rate of aquatic hyphomycetes associated with decomposing leaf material were significantly higher in nutrient-enriched streams than in relatively oligotrophic streams (Gulis & Suberkropp, 2003; Suberkropp & Chauvet, 1995). Therefore, in streams with low background concentrations, a fertilizing effect of tadpoles on microbes was more likely than in streams with higher nutrient concentrations. Ambient nutrient concentrations in our stream were relatively low with 36 $\mu g.L^{-1}$ and 23 $\mu g.L^{-1}$ for ammonia-N and TDP, respectively. Thus litter decomposition was possibly enhanced by nutrient addition (excretion and egestion) from the tadpoles

543 (Rugenski *et al.*, 2012). Though it is difficult to estimate nutrient concentrations in the
544 enclosures from the excretion data, prior studies found that even a slight nutrient
545 enrichment significantly enhances litter decomposition in streams. For example,
546 Elwood *et al.* (1981) reported that P enrichment accelerated processing of deciduous
547 leaves in streams where nutrient concentration was brought from 10 $\mu g.L^{-1}$ to 60 $\mu g.L^{-1}$
548 SRP. In similar fashion, Gulis & Suberkropp (2003) reported similar pattern in
549 microbial activities when increasing SRP from 4 $\mu g.L^{-1}$ to 34 $\mu g.L^{-1}$ in a headwater
550 stream. Further, Ferreira, Gulis & Graça (2006) found that even minor increases in
551 nitrate (33 $\mu g\ NO_3\text{-}N.\ L^{-1}$) significantly influenced microbial dynamics and litter
552 decomposition. We did not measure nitrate background but it should be noted that
553 nitrate could become more important than ammonia for microbial activities in well-
554 oxygenated streams (Ferreira *et al.*, 2006); more research on this topic needs to be
555 undertaken to clarify the association between tadpole nutrient recycling and litter
556 decomposition.

557 **Conclusions and implications**

558 Based on our results, and other similar studies, tadpoles can play important roles in
559 tropical streams. Their high abundance and the virtual absence of fishes in
560 Ranomafana streams suggest that tadpoles could be critical to these ecosystems
561 (Strauß *et al.*, 2010). Tadpoles can produce substantial amounts of egesta due to their
562 continuous feeding (Jenssen, 1967). Egested particles are still understudied (Capps *et*
563 *al.*, 2015; Halvorson *et al.*, 2017) but could be very important in animal-driven
564 nutrient dynamics. Our incubation experiment suggests that, at least in the short-term,
565 tadpole egesta are important sources of nutrients in streams. Contribution of tadpoles
566 to litter decomposition depended on tadpole identity, tadpole density and litter
567 identity, suggesting that their effects may vary with the type of ecosystem and the

tadpole community therein. Biodiversity-ecosystem functioning experiments suggested that ecosystem functions typically increase with phenotypic diversity (reviewed by Tilman, Isbell & Cowles (2014)). Our study supports the assumption that species identity (dominant species) and abundance (biomass) could play more significant roles than diversity (Winfree *et al.*, 2015). We found that species mixture had additive effects, suggesting that the tadpoles had distinctive yet neither facilitative nor antagonistic effects on litter decomposition. It may be fruitful to further explore the relative contribution of species membership and understand which ones can deliver significant parts of the ecosystem services in highly diversified systems (Winfree *et al.*, 2015).

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793 Table 1 Litter toughness (in Newton) and nutrient concentrations (C, N, K, P, Mg, Ca,
794 in % litter dry mass) of the litter species used in the experiment. Values are mean ±
795 SD.

	N	*Pauridiantha* sp.	*Chrysophyllum* sp.
Toughness	31	0.86 ± 0.21	1.63 ± 0.33
Calcium	3	0.876 ± 0.03	0.542 ± 0.02
Potassium	3	0.163 ± 0.02	0.077 ± 0.01
Phosphorus	3	0.078 ± 0.01	0.066 ± 0.01
Magnesium	3	0.177 ± 0.02	0.078 ± 0.01
Carbon	1	40.40	47.07
Nitrogen	1	1.51	1.76
C:N	1	26.75	26.74

796 Table 2 Student's t-tests comparing isotope signatures, body elemental compositions,
797 and excretion rates between the tadpoles of *Mantidactylus melanopleura* and *Boophis*
798 *quasiboehmei.*

	M. melanopleura	*B. quasiboehmei*			
	Mean		t	df	*P*
Isotope signatures					
$\delta^{13}C$	-25.697	-26.225	-2.027	28	0.052
$\delta^{15}N$	4.445	4.255	-1.172	28	0.251
Body elemental composition (%)					
C	0.410	0.415	0.538	28	0.595
N	0.129	0.131	0.586	28	0.562
C/N	3.175	3.173	-0.203	28	0.841
Excretion rates (μmol.g wet mass^{-1}.h^{-1})					
N	1.060	0.760	-2.713	10	0.022
P	0.210	0.120	-6.283	10	<0.001
N:P	5.047	6.333	1.399	10	0.192

799 Table 3 Results of one sample t-tests comparing mean litter mass loss in each mixture
800 treatment (observed values) to expected (computed) values to test additivity effects.
801 BQ: *Boophis quasiboehmei*, MM: *Mantidactylus melanopleura*

	Pauridiantha sp.			*Chrysophyllum* sp.		
	t	df	P-value	*t*	df	P-value
3BQ+3MM	1.439	4	0.223	0.869	4	0.433
6BQ+6MM	0.070	4	0.947	0.352	4	0.742

802 Table 4 Results of mixed-effect models analyzing the effects of tadpole density, litter
803 type, and species composition (tadpole identity and mixture), *Boophis Quasiboehmei*
804 and *Mantidactylus melanopleura*), and their interactive effects on leaf litter
805 decomposition. *P*-values were obtained likelihood-ratio tests.

	Chi-square	d.f.	*P*-values
Tadpole density (dens)	20.515	2	<0.001
Litter identity (lit)	61.896	1	<0.001
Species composition (comp)	4.143	2	0.125
Dens × Lit	17.741	2	<0.001
Dens × Comp	8.688	3	0.033
Lit × Comp	9.774	2	0.007
Dens × Lit × Comp	5.712	3	0.126

Fig. 1 Carbon and nitrogen isotope signatures of *Boophis Quasiboehmei* (triangle) and *Mantidactylus melanopleura* (square). Values are mean ± SD. *N* = 15

Fig. 2 Excretion rates in N and in P (molar) of *B. quasiboehmei* (generalized mouthpart) and *M. melanopleura* (funnel-mouthed). Values are mean ± SD. *N* = 6. "*" means significant at 0.05, "***" significant level at 0.001, "ns" not significant. N and P were calculated from ammonia-N and total dissolved P, respectively.

Fig. 3 Mean concentrations of N and P in the water column released by egesta (fecal pellets) of *B. quasiboehmei* (generalized mouthpart) and *M. melanopleura* (funnel-mouthed). Pellets were incubated in buckets containing stream water, and samplings were conducted every 4 days. Values are mean ± SD. *N* = 2

Fig. 4 Effects of tadpoles of *B. quasiboehmei* (generalized mouthpart) and *M. melanopleura* (funnel-mouthed) when alone or together on litter mass loss of *Pauridiantha* sp (A) and *Chrysophyllum* sp. (B) in enclosures. The control had only litter but no tadpoles. Values are mean ± SD. *N* = 5