

Importance of niche partitioning and phenotypic plasticity in mediating species
coexistence in larval anuran communities

(両生類幼生群集において種の共存に果たすニッチ分割と表現可塑性の重要性)

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Abstract

Ecologists have long been interested in understanding how and why many species can simultaneously coexist in the same locality. Although the underlying mechanism has been the subject of long debates (e.g., niche (deterministic) vs. neutral theory), recent evidences suggest that niche partitioning, phenotypic divergences and phenotypic plasticity could be critical mechanisms that allow multiple species coexist in their environment. However, the generality of such an assumption has not been tested yet. Using species rich lotic Madagascan tadpole community and lentic pond dwelling Japanese tadpoles as model systems, I tested the predictions of ecological niche theory and adaptive phenotypic plasticity theory (1) that niche partitioning (habitats and trophic spaces) allow multiple species co-occur in nature (2) that phenotypically divergent species (in oral structure) are not functionally redundant, and (3) that phenotypic plasticity allows ecologically similar species alleviate the strength of competition. I studied tadpole assemblages in three habitats that differ in disturbance history, a primary forest, a previously logged forest and a forest edge in Ranomafana Madagascar. I sampled tadpoles in 3 streams in each habitat and determined whether the environment could structure tadpole communities. I subsequently analyzed trophic spaces combining stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes and gut content analyses to test for signals of resource partitioning in the tadpoles. I conducted field incubation and enclosure experiments to determine the effects of tadpoles on nutrient cycling and leaf litter decomposition. In a second suite of laboratory experiments, I observed changes in behavior and in morphology in tadpoles as potential strategies to increase fitness in the presence of competitors. The results indicate that, in highly diverse Madagascan tadpole communities, microhabitat heterogeneity (i.e., space partitioning) and habitat disturbance strongly structured assemblages. Disturbance influenced species composition through environmental filtering. Trophic spaces revealed by gut content and stable isotope analyses suggested resource partitioning in the tadpoles, even in phenotypically similar species. As expected, morphologically divergent tadpoles had differential effects on ecosystem functioning in nutrient recycling and in litter decomposition, two central processes in stream ecosystems. In examining how do ecologically similar species alleviate the strength of competition, I found that tadpoles are able to shift their resource preference in the presence of competitors. Further, I found that inferior competitors were able to invest in a longer gut, analogous to what an increase in root biomass is for plants under competition, a strategy suggested to help increase assimilation efficiency when resource are limited. My research demonstrates that resource niche partitioning (in spaces and

resources) and phenotypic divergence are important mechanisms of co-occurrence of multiple species of tadpoles in Madagascar. My findings also indicate that phenotypic plasticity may mediate species coexistence by alleviating strength of competitive interactions. These results do not necessarily imply that species stably coexist in nature, but the mechanisms described in this thesis represent some of the necessary conditions for species coexistence. These results are applicable to broader systems and help explain how and why species can stably persist in their environment.

要旨

生態学において、どのように、そしてなぜ多くの種が同じ環境で共存できるかは、長い間、重要な研究課題とされている。そのメカニズムは、ニッチ vs. 中立理論のように長い議論の対象だったが、複数の種が共存する上で、ニッチの分割や表現型の多様化、表現型可塑性が重要なメカニズムになりうることで最近わかってきた。しかし、その仮説は未だ検証されていない。本研究ではモデルシステムとして、マダガスカルが多様性が高く流水域に生息するオタマジャクシや日本の止水域に生息するオタマジャクシを用い、エコロジカルニッチ理論や表現型可塑性理論による以下の三つの仮説を検証する。(1) ニッチの分割（生息場所や栄養段階の位置）があるため、多様な種が同所に共存できる。(2) 口器形状の表現型が分化している種はそれぞれ生態学的な役割が異なっている。(3) 表現型可塑性によって、生態学的に類似した種の競争が緩和されている。結果とし、マダガスカルの高多様性オタマジャクシ群集では、マイクロハビタットの異質性や生息場所の攪乱によって群集構造が決まる。攪乱が生じると、環境フィルタリングを通じて、種組成が変化する。腸の内容や安定同位体分析結果から推定された栄養連鎖上の位置によって、表現型が似ている種でも食物がそれぞれ異なることが明らかとなった。表現型が異なる種が生態系機能や栄養循環にそれぞれ違う影響を与えている。生態学的に近似した種間において、どのように競争からの影響を緩和するか実験によって確認した結果、競争者の存在下で、オタマジャクシの餌嗜好性が変化することがわかった。その上、競争の激しい時、劣位の競争者が腸を伸張することが見られた。その戦略は餌資源に限られる時に、同化効率を高めるために使われている。本研究はニッチ分割（餌資源や生息場所）や表現型の相違が、マダガスカルにおける多様な種が共存するために重要なメカニズムであることを示した。そして表現型可塑性が競争を緩和し、種の共存を容易にさせることが分かった。本結果は必ずしも種が安定的に共存していることを意味するものではないが、本論で記述されたメカニズムは種の共存に必要な条件を示す。本結果はより幅広いシステムにも適用することができ、種の安定共存の理由の説明にも貢献する。

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Chapter 1. Introduction

Ecologists have always been interested in understanding why and how many species can coexist together in the same environment (Chesson 2000; Hutchinson 1959; Kraft and Ackerly 2010; Schoener 1974). There is an ongoing debate on the relative importance of neutral vs. deterministic processes on community structure. On one hand, the concept of niche (deterministic process), which can be defined as a set of abiotic and biotic factors that define the requirements of a species for its population to persist (Levin et al. 2009), postulates that each species should occupy a particular niche in order to coexist (Hutchinson, 1959). Under this hypothesis, niche overlap penalizes inferior competitors and may result in their exclusion from a community. To the converse, the neutral view of community structure suggests that communities are composed of ecologically identical species (Hubbell 2006). Under this assumption, species would have identical parameters that regulate their population. As a result, all ecologically identical species experience the same strength of density-dependence determined by the total abundance of all species. Local species composition and relative abundance are expected to vary unpredictably as a result of demographic stochasticity (Hubbell 2006).

Although the neutral view of community has found support in some systems (Chave 2004; Rosindell et al. 2012; Siepielski et al. 2010), suggesting that species functional differences might be of lesser importance to generate the observed patterns of diversity in nature; it fails, however, to explain why some species perform better under a set of biotic and abiotic axes while others decrease in fitness. In fact, there is increasing evidence suggesting that biodiversity within communities cannot be understood without taking into account deterministic processes such as habitat filtering and niche differentiation (Levine and HilleRisLambers 2009; Valladares et al. 2015; Vanderhaeghe et al. 2016).

Here below, I briefly review some mechanisms of coexistence from the point of view of ecological niche. I also describe a few mechanisms through which coexistence is possible without niche partitioning. In-depth reviews can be found in the seminal articles of Chesson (2000) and Chase and Leibold (2003).

Temporal and spatial partitioning

Competition could be avoided between sympatric species if different species use the same resource at different times, but this is only possible if the resource is not depleted by

one of the species (Albrecht and Gotelli 2001). For example, two species of spiny mice (*Acomys cahirinus* and *A. russatus*) can coexist by being active at different times of the day (Gutman and Dayan 2005), allowing these species with high diet overlap to avoid each other. Spatial partitioning occurs when sympatric species use similar resource but occupy different areas where the resource occurs. This form of resource partitioning is ubiquitous in plants (Fargione and Tilman 2005; Tilman 1982) but is well documented in animals as well (Schoener 1974). Habitat heterogeneity can strongly influence species coexistence, and species can be segregated in space according to their niche preferences (Muko and Iwasa 2000). For example, some chaetodontid fishes that have similar diet tend to be localized in different parts of reefs with little overlap in spatial distribution (Bouchon-Navaro 1986).

Character displacement, resource specialization, and coexistence

Competition and ecological opportunity can have evolutionary effects on species morphology, physiology and behaviour, and on speciation (Grant and Grant 2006; Wagner et al. 2012; Wagner et al. 2009). In ecological character displacement, species evolve to specialize on a single or few resources to avoid overlapping foraging, thus reducing the effects of interspecific competition (Svanbäck and Bolnick 2007). Diet specialization is made possible by a suite of morphological changes (phenotypic divergence) that accommodate novel ways of capturing and processing food resources (Wagner et al. 2009). Popular examples are Darwin's finches that have adaptively evolved different beak sizes, allowing these species to partition resources and coexist in nature (De León et al. 2014; Grant and Grant 2006).

Environmental filter

Environmental filter refers to abiotic factors that prevent the establishment or persistence of species in a particular location (Kraft et al. 2015). Under this concept, species should differ in their ability to perform (e.g., changes in abundances) under various abiotic conditions (Chesson 2000). This concept typically finds its popularity in disturbance ecology. Disturbance often creates new environments that may no longer be suitable for the better performing species, resulting into colonization of species whose niche is better adapted to the novel environmental setting. Disturbance can also impact the demographic parameters of species (e.g., density, reproduction, mortality), allowing for more resistant species to outperform formerly superior competitors in the new environment, ultimately allowing species coexist in the local system.

Life history trade-offs and species coexistence

Another form of coexistence is based on interspecific trade-offs between life history traits that influence competition (e.g. fecundity, growth, resistance to predator) and those that allow species to reduce competition (e.g., dispersal) (Amarasekare 2003). Such trade-offs suggest that life-history traits of an inferior competitor mitigate its exclusion by the superior one. For example, superior competitors that are better foragers may experience higher predation risk (McPeck 2004; Yurewicz 2004), and, ultimately, such a growth/mortality trade-off can mediate species coexistence.

Phenotypic plasticity and species coexistence

Phenotypic plasticity is the ability of an organism to change its phenotype in response to changes in the environment (Schlichting and Pigliucci 1998). Theoretically, ecologically similar species should compete strongly for resources, which might lead to the exclusion of poor competitors (Holt et al. 1994). It is observed, however, that in nature, many ecologically similar species can stably coexist without evidence of exclusion. Phenotypic plasticity may help species coexist in their environment by minimizing competitive imbalances between species (Turcotte and Levine 2016). Although plasticity may harm coexistence if it favors superior competitors (Bret-Harte et al. 2001; Molina-Montenegro et al. 2012), it could help less competitive species minimize their competitive disadvantages and persist in the local system. For example, in plants, inferior competitor can elongate their shoot or increase belowground biomass, allowing them to better compete for light and nutrients (Dudley et al. 2013; Dudley and Schmitt 1996; Lepik et al. 2005; Lipowsky et al. 2015). In animals, examples include modification of behavior, morphology and life-history traits. For example, Relyea (2000) reported that tadpoles of leopard frogs (*Rana pipiens*) can modify the size of their mouthpart in the presence of competitors wood frog (*Lithobates sylvaticus*) tadpoles, allowing them to increase resource intake. Arribas et al. (2015) demonstrated that tadpoles can shift their diet to minimize interference competition.

Study system and hypotheses

The main aim of this thesis is to understand how species can coexist in their environment without apparent exclusion from the viewpoint of ecological niche and phenotypic plasticity theories. Because of their ease to manipulate, diversity, and sensitivity to environmental changes, tadpoles, the larval stage of anurans, represent ideal model

systems to answer important eco- evolutionary questions (Roelants et al. 2011; Semlitsch and Boone 2010; Van Buskirk et al. 2011).

I was interested in how environmental heterogeneity and phenotypic divergence can mediate species coexistence through resource partitioning. I used tadpole community in Ranomafana Madagascar as model system. Ranomafana harbors an exceptional frog community (Vieites et al. 2009) and has the world richest tadpole community in the world (Strauß et al. 2013). Previous studies revealed that, in Ranomafana, more than 22 tadpoles, belonging to at least 7 mouthpart guilds, can coexist within a single stream. However, the mechanism mediating species maintenance remains elusive. I tested whether morphological cluster (i.e., phenotypic divergence) could be an important axis of species co-occurrence. I was further interested in the importance of such diversity on stream ecosystem functioning and determined whether phenotypically divergent species are functionally distinct or merely redundant.

In a second suite of experiments, I tested the predictions of phenotypic plasticity theory to understand how ecologically similar species can stably coexist in their environment. In contrast to tropical tadpoles, which can be highly diverse in their oral structure and in which niche divergence could be expected, temperate tadpoles tend to be homogeneous in their morphology (Altig and Johnston 1989), and many tadpoles fall within one generalized oral cluster. Using pond dwelling Japanese tadpoles I examined how competitors are able to invest in phenotypes (behavior and morphology) that help them alleviate the strength of competition.

In this thesis, I am addressing the following questions:

Question 1: How do multiple tadpole species coexist in streams? What mechanisms mediate their coexistence?

- Hypothesis 1: Environmental heterogeneity and habitat disturbance organize tadpole assemblages (through environmental filtering)
- Hypothesis 2: Phenotypic divergence allows tadpoles exploit different resources and avoid trophic niche overlap

Question 2: Do phenotypically divergent species play distinct roles in the ecosystem?

- Hypothesis 3: By occupying different niches, species are not functionally redundant.

Question 3: How do ecologically similar, generalist species persist in the same environment?

- Hypothesis 4: Coexisting ecologically similar species are phenotypically plastic allowing them alleviate the strength of competition.

Summary of the chapters

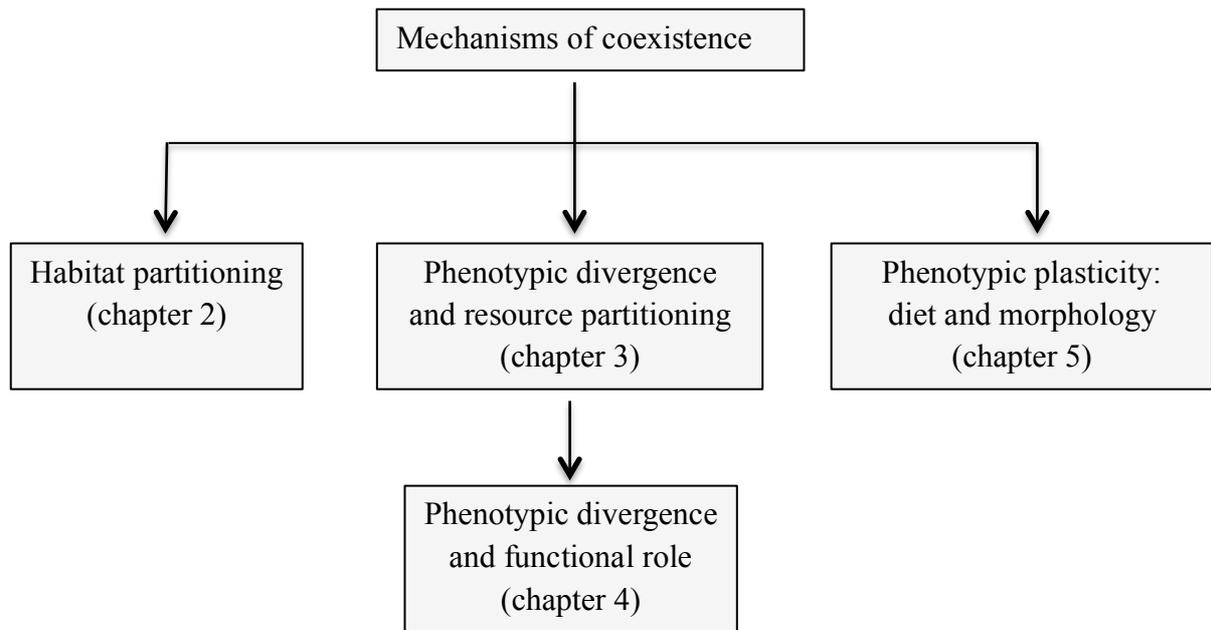
This thesis is comprised of four papers that are either submitted, in review, or in preparation for publication in peer-reviewed journals (Chapters 2 – 5). Chapter 1 provides the framework for this dissertation and Chapter 6 summarizes the findings and the importance of the results in the field of ecology. Below, I briefly summarize each chapter, the primary objectives and the hypotheses to be tested.

In chapter 2, I investigated the factors structuring species rich tadpole community in streams in Ranomafana. Environmental filtering hypothesis predicts that species that perform well under a set of environmental features should be outperformed by other species when these conditions change. I examined how environmental heterogeneity and disturbance structure tadpole assemblages. I compared the abundance and diversity of tadpoles in three habitats differing in disturbance history: undisturbed primary forest, disturbed logged forest, and forest edge. Dissimilarities in species composition were found between the three habitats, suggesting that disturbance influenced species assemblages. In fact, edge specialists replaced forest specialists in open habitats. Environmental microhabitat heterogeneity strongly structured species assemblages. Four major environmental axes influenced assemblages: water depth, water velocity, canopy openness, and substrate type. Collectively, these findings indicate that environmental heterogeneity, which could be intensified by disturbance, organizes species community assembly and may promote coexistence in species rich communities.

While biologists have long understood that phenotypic divergence can maintain diversity by promoting specialization and reducing competition, the generality of this assumption has not been widely tested. Classical theory suggests that species can persist in the same environment if they do not overlap in their feeding habits and/or differ in their trophic space. In chapter 3, I examined how mouthpart differentiation in tadpoles influences their trophic space in streams, combining isotope and gut content analyses. The findings indicate that many tadpoles occupy similar trophic level (as inferred by N isotope values) but exhibited greater differences in carbon isotope signatures. Gut content analysis revealed that tadpoles with apparent similar trophic space indeed differ in their food resource, indicating resource partitioning in these tadpoles.

Biodiversity crisis in the tropic has appealed for the need to investigate the importance of diversity on ecosystem function. Are all species relevant to maintaining ecosystem integrity or are species functionally redundant? In chapter 4, I examined how tadpole oral structure and feeding behavior mediate their impact on the ecosystem. I measured the contribution of tadpoles to nutrient cycling and to leaf litter decomposition, two of the most important stream ecosystem processes. I found that tadpoles differentially recycled nutrients, at least in six ecomorphotypes. The effects of tadpoles on litter decomposition were density-dependent and depended on leaf toughness. Although tadpoles play differential functional role, they did not have additive effects on litter decomposition; that is, the observed effects in mix treatments did not equal the sum of species individual effect. Tadpole abundance appeared to have stronger effect on ecosystem functioning than diversity *per se*, suggesting that a decrease in tadpole biomass could be more detrimental than the loss in species on stream ecosystem functioning. Manuscript under revision (*Freshwater Biology*).

In chapter 5, I report on the ability of pond dwelling tadpoles with generalized morphology to alter their phenotype in the presence of competitors, ultimately allowing them to alleviate the strength of competition. I found that tadpoles can shift their food preference when competition is high. Further, to circumvent the negative effects of competition, inferior competitors invest in a longer digestive tract, a strategy to increase food assimilation efficiency and make the most of limited resources. Phenotypic plasticity may allow ecologically similar species persist and coexist in their environments. Manuscript published in *Herpetologica* and in *Journal of Zoology*.



Chapter 2: The importance of microhabitat heterogeneity, spatial distance, and disturbance history in structuring tadpole stream community in Ranomafana, Madagascar

Abstract

Communities often contain a wide diversity of species that respond to changes in their environment depending in part on their niche requirements. Anuran larvae can be highly diverse and can represent the largest biomass in tropical stream, but compared to adult frog communities, relatively little is known about what structure communities or if there is any assembly rule. Here, I took advantage of an established field experiment in Ranomafana Madagascar, which harbors the richest tadpole community in the world, to investigate tadpole assemblages in habitats that differ in disturbance history: primary forest, disturbed forest (mainly from selective logging), and forest edge (interface between agricultural areas and forest habitats). I then examined the effect of stream microhabitat heterogeneity on structuring larval frog communities. The three habitats harbored different assemblages. This difference in assemblage was explained by the presence of edge specialist species, and also because forest specialists declined outside forest. The presence of specialists allowed species richness to be maintained between primary forest and edge habitats. Disturbed forest was the least diverse community. Cross products correlation indicated that both microhabitat environmental and spatial factors determined tadpole assemblages, but the environment exerted larger effect. Detailed analysis of community structure revealed that four major axes determined tadpole assemblages: water depth, water velocity, canopy openness, and substrate type. I distinguished four guilds based on ordination: species adapted to pool environments, generalist species, species adapted to open habitats, and species adapted to riffle environments with predominance of gravel substrate. Habitat partitioning and environmental filter can mediate tadpole co-occurrence but whether or not species can stably coexist remains unclear. Though I cannot infer a straight causal relationship between habitat disturbance and tadpole assemblages, this study underlines the importance of microhabitat heterogeneity, spatial distance and habitat features in structuring tadpole community.

2.1. Introduction

Ecological communities characteristically contain a wide diversity of species, but how many species can simultaneously co-occur within a habitat and what forces structure communities remain elusive. A number of factors have been proposed to explain the pattern of species composition. Among these are assembly history (Fukami 2004), competition and predation (Chesson and Kuang 2008), spatial effects (Parris 2004), stochastic events (Hubbell 2006), environmental features (Keller et al. 2009) as well as disturbance (Dornelas 2010). Disturbance, in particular, is thought to greatly influence community organizations (Dornelas 2010), but its effect can vary with the type and the level of intensity (Fahrig 2003).

The negative effects of disturbance is relatively well documented and can result in species decline directly or indirectly by altering dispersal, genetic exchange, food webs, habitats, vulnerability to predators (Burger et al. 2007; Fahrig 2003; Frid and Dill 2002; Neckel-Oliveira and Lannoo 2007). To the converse, disturbance can positively impact communities by increasing habitat quality and by creating favorable foraging opportunities (Dornelas 2010). Disturbance can mediate species coexistence by influencing biomass, frequency, and relative abundance of species (Crawley 2004). By inducing changes in the environment, disturbance can shape the coexistence of specialists and generalists (Büchi and Vuilleumier 2014). When local extinctions occur following disturbance, compensatory colonization events can maintain species richness (Brown et al. 2001) by providing new species with niches better suited to the new environmental conditions. Compensatory mechanisms allow distributions of abundance to be maintained, as declines in biomass by some species are compensated for by increases in others (Morgan Ernest and Brown 2001; Ramamonjisoa et al. 2013), which can ultimately stabilize communities.

Because of their sensitivity to environmental changes, amphibians are model systems in disturbance ecology. With alarming population declines, mainly due to rapid habitat loss, information about the effects of disturbance on amphibians is urgently needed (Cushman 2006; Irwin et al. 2010). In tropical environments, logging and land conversion are still the major threats to biodiversity. Small changes in vegetation structure can create significant alterations to amphibian communities (Cortés-Gómez et al. 2014). Although some cases reported increase in species richness following forest disturbance (Hillers et al. 2008), it has been observed that logging, even when conducted selectively, can bear dramatic effects on amphibians, especially on forest specialists (Burivalova et al. 2014; Ferreira et al. 2016). However, most of what we know about amphibian disturbance ecology stem from studies that

were conducted on the adult stage, to which life history strongly depends on the quality of terrestrial environments (Ernst and Rodel 2005; Ernst and Rödel 2008; Ferreira et al. 2016; Gardner et al. 2007; Riemann et al. 2015). Next to nothing is known about the potential effects of forest disturbance on anuran larvae despite their paramount importance in tropical stream functioning (Ranvestel et al. 2004; Rugenski et al. 2012).

This difference in research pace between adult and larval communities, however, should not be surprising. In fact, compared to adult frogs, still very little information is available about the larval stage of many tropical amphibians (Altig et al. 2007). Also, there has been an idiosyncratic assumption that tadpole communities would simply match the adult community present at a site. Although reciprocal influences on communities of adult and larval stages have been documented (Inger et al. 1986), there should not be obligate link between these two communities. Indeed, adult males can call from water bodies where no breeding takes place. Larvae represent concrete evidence of breeding and larval surveys are less likely to overestimate breeding distribution (Skelly and Richardson 2010). Studying larval cohorts can provide critical information regarding the trajectory of a population and the factors that may affect abundance, distribution, and assemblages (Skelly and Richardson 2010).

Tropical tadpoles represent a fascinating model for community ecology because of their high diversity, especially in their oral structures (Strauß et al. 2013). The high species richness is an important key to reveal environmental filters that might be otherwise overseen in less species rich assemblages. Still, studies on tropical tadpole community ecology are scarce (see review in Borges Júnior and Rocha (2013)). The few available studies suggest that stream habitat heterogeneity can strongly structure assemblages (Eterovick and Barata 2006; Inger et al. 1986). Stream size, canopy cover, marginal vegetation, water flow, water volume, dissolved oxygen and conductivity have distinct effects on tadpole assemblages (Eterovick and Barata 2006; Strauß et al. 2010). Recently, Strauß et al. (2013) revealed that morphological traits can influence species-habitat associations. While we have started to develop an understanding of the factors that structure anuran larvae in relatively stable environments, we still know little about how disturbance can mediate the organization of tadpole community (but see Moreira et al. 2016) about the effect of pasture on tadpole occupancy in wetlands in Brazil. In this study, I examined the variation in tadpole assemblages along microhabitat heterogeneity in a primary forest, a disturbed forest (selectively logged forest), and at forest edge. I hypothesized that:

- (1) Dissimilarities in tadpole assemblages exist between disturbed and undisturbed habitats.
- (2) Generalist species dominate assemblages in all habitats. However, outside forest, edge specialists replace forest specialists.
- (3) Tadpole assemblages are structured by the environment.
- (4) Tadpoles are clustered by their oral structure; that is, tadpoles within the same oral cluster are influenced by same environmental parameters.

2.2. Materials and methods

Ranomafana National Park is located in the southeastern part of Madagascar (47°29' E, 21°16' S, Fig. 1) comprises 43,500 ha of continuous mid-altitude montane rainforest (500–1300 m a.s.l.) with an annual precipitation between 1700 and 4300 mm. Ranomafana is characterized by high amphibian diversity with 112 candidate species (Vieites et al. 2009) and harbors the richest tadpole community in the world (Strauß et al. 2010). Precipitations are high, with alternating periods of low and heavy rains. Mean water temperature was 18 °C during the study. Water temperature was slightly higher in matrix habitats, outside forest ~19 °C.

Ranomafana is a model system to investigate the effects of anthropogenic disturbance on biological communities. Previous studies have examined such effects on mammals (Gerber et al. 2012; Herrera et al. 2011; Tecot 2008), insects (Rainio and Niemelä 2006), birds (Razafimahaimodison 2004), plants (Ramaharitra 2006), and amphibians (Ramamonjisoa et al. 2013; Riemann et al. 2015)). One part of Ranomafana National park was selectively logged (Talatakely forest) approximately 30 years ago (logging activities during 1986–1989) and has become a protected area ever since. Although it is difficult to separate the effects of logging from other disturbances (e.g., tourism, invasive species), in this study, I refer this forest as “disturbed forest” though selective logging was the major known disturbance recorded in that habitat. Selective logging negatively impacted forest structure by reducing basal area by 53% and mean crown volume by 17% (Ramaharitra 2006; Tecot 2008). The other parts of the park have remained relatively undisturbed (Tecot 2008). Adjacent to the park are agricultural areas dominated by rice paddy fields and rainfed crops. I took advantage of this naturally established field experiment to study the changes in tadpole community along habitat heterogeneity and disturbance history. The differences in ecological conditions over short distances in Ranomafana also makes it an ideal study site as small-scale

contrasts are more sensitive at detecting ecological determinants than comparisons made on larger scales often carried out in previous amphibian studies (Ernst et al. 2006; Parris 2004).

Sampling procedures

I conducted survey in streams located at three sites: primary forest (“control”), disturbed forest (previously logged forest), and at “forest edge” (degraded areas, habitats at the interface between forest habitats and agricultural areas). Shrubs and remnant trees surround streams at edge. In the rest of this document, I use “primary forest”, “disturbed forest”, and “edge” to designate the three habitats.

I sampled tadpoles in 9 streams, 3 in each habitat. These replicates were realistically the maximum number of streams that met the objectives of the study and were within the same range of altitude (the streams were nearly on equal elevation 900–1020 m a.s.l.).

Samplings were conducted in October–November 2014 and in March–April 2015. The streams were second-order streams and were 2.1–3.05 m width (microhabitat). Each stream was distant of at least 200 m with no direct connection between them. I sampled tadpoles once at the beginning (Oct–Nov) and once in the later part of breeding season (March–April). In contrast to adult frogs, that are often cryptic, arboreal, and for which detection considerably depends on sampling efforts, climate, and calling activity (Vonesh et al. 2010), tadpoles’ detection rate can be relatively high in a relatively defined small area (Skelly and Richardson 2010).

The general sampling procedure followed that of (Keller et al. 2009), in which microhabitat (stream portion), not stream, represented the sampling units. Indeed, amphibian assemblage can strongly vary with microhabitat heterogeneity within few meters in streams (Keller et al. 2009). As for tadpoles, microhabitats can strongly influence species assemblages (Afonso and Eterovick 2007; Borges Júnior and Rocha 2013; Inger et al. 1986), and our field observations along with previous studies suggested that many tadpoles can specialize into specific microenvironment. Sampling longer stream portion would not be appropriate because it crosses multiple microhabitats, rendering characterization of relationship between species occurrence and environmental features difficult.

At each stream, I sampled 8 distinct microhabitats, each representing portions of 2.5 meters. The “microhabitat-sites” were not chosen systematically (i.e., regular distance between microhabitats) but rather at random with irregular intervals to cover the heterogeneity (substrate, water velocity, canopy openness, water depth, characteristics of the

surrounding vegetation). 10 m stream-distance, at least, separated between two consecutive “microhabitat-site”. I sampled tadpoles using dipnets of different size, adjusted to obtain optimal sampling results for each microhabitat. An important component of the fieldwork was to standardize sampling method that would allow estimating tadpole relative abundance. Sampling consisted of sampling tadpoles in microhabitats until no tadpoles were caught (Skelly and Richardson 2010), and I restricted sampling to 4 minutes (time was stopped during sample processing), to provide per-unit-time density estimates (Werner et al. 2007). Samplings were always conducted in the morning. Because tadpoles are mobile and can escape from the microhabitat, I started sampling downstream and ensured that tadpoles within the microhabitat were sampled.

The tadpoles were kept alive and were brought back to the laboratory in buckets filled with stream water. The tadpoles were sorted visually into series based on morphological differentiation. Because of the high number of species and my limited ability to distinguish each species, we assigned series of provisional numbers. I took specimen of each series and after anesthetization by Tricaine Methanesulfonate (MS-222), took a fragment of tadpole tail that was used in DNA analysis for species identification. DNA barcoding was based on a fragment of the mitochondrial 16SrRNA gene (modified 16Sar (550bp) [5'-CGCCTGTTTAYCAAAAACAT-3'] and modified 16Sbr (550bp) [5'-CCGGTYTGAACTCAGATCAYGT-3']). PCR and sequencing methods followed (Bossuyt and Milinkovitch 2000). PCR products were prepared for sequencing using BigDye Terminator sequencing chemistry (Applied Biosystems, CA, USA). These analyses were conducted at Nagoya University (NU Gene Center and Graduate School of Bioagricultural Science).

I must, however, acknowledge the limitations of the sampling design, especially if individuals moved from one microhabitat to one another, then these sampling units would not be independent. However, such event did not occur during our sampling and under the constraints imposed by the objectives, I focused on the bio-ecological meaning of the data (Davies and Gray 2015). If “true” replicates were sought, then multiple streams should be investigated, in which a microhabitat-site would be replicated on separate streams; but this is realistically not possible given the objectives of the study.

Environmental characterization

Two groups of habitat variables were recorded (Table 1): (1) characteristics of the microhabitat-site, representing habitat relevant for tadpoles, (2) characteristics of the adjacent forest and riparian vegetation, representing habitat relevant for frogs such as for breeding activities. These measurements were conducted in 2014.

Aquatic and stream microhabitat

At each microhabitat, I recorded stream width, depth (from 3 measurements then averaged) and canopy openness (using a fish-eye lens mounted on a digital camera, picture was taken at 1 m above stream level). Canopy openness was estimated using the CanopOn2 software (<http://takenaka-akio.org/etc/canopon2/index.html>). I recorded water velocity (measured with a digital electro-magnetic water velocity meter, Kenek CO., LTD, VE20) and estimated the relative proportion of each ground substrate type (modified from (Strauß et al. 2010)): leaves (organic material), sediment (silt), sand (very finely grained up to ~3 mm grain size), gravel (grain size 3 mm to 5 cm), and rock (5 to 20 cm) and boulder (>20 cm) in each microhabitat. Field estimations were cross-checked in the laboratory by examining pictures of each microhabitat. The number of predators was counted (mainly dragonfly larvae and crustaceans) in each microhabitat. At the stream level, dissolved oxygen (mgO/L), water conductivity (Siemens), and water temperature (°C) were recorded.

Terrestrial habitat

Two 5x10 m plots, with the longer side parallel to the stream, were randomly established on each side of a stream (then 4 plots at each stream). I recorded Diameter at Breast Height (DBH) of trees >5 cm to estimate basal area of riparian vegetation. Within each 5x10 m plot, I had a 5x5m subplot, in which the number of trees DBH<5cm (shrubs) was counted. Canopy openness of the habitat was estimated at the center of each plot. Two random 1x1 quadrats were set in each 5x5 plot to measure understory height (3 measurements) and litter depth (3 measurements). Measurements were averaged within each 1m² quadrat. Heights of hanging vegetation were also recorded at 2 m interval along a 5x10 m plot.

Data analysis

Species richness

I compared species richness (absolute number of species within a stream) and abundances between the three habitats (primary forest, logged forest and matrix habitat) using linear mixed models with the function `lmer` in the `lme4` package (Bates et al. 2012) in R. I entered “year” as random factor and either “species richness” or “abundance” as response variable. Statistical tests were performed following the same procedures as above (i.e., for computing *P*-values and conducting post-hoc tests). All data were log-transformed before analysis.

Species diversity

I used Shannon H' index as a measure of diversity and compared it between sites using a diversity ‘t’ test between two samples of abundance data (Seshadri 2014). This test was conducted in PAST (Hammer et al. 2001). Shannon H' is computed by $H' = -\sum p_i \log(p_i)$, where p_i is the proportion of individuals belonging to the i^{th} species in the habitat.

Beta diversity, which represents the extent of change in community composition or degree of community differentiation, was computed by the formula $\beta = S/\alpha - 1$, where S is the total number of species, and α is the average number of species per site (Whittaker 1960).

Environmental differences between sites

I used linear mixed models to analyze the changes in riparian vegetation (understorey height, litter cover, vegetation cover, canopy cover, basal area, shrub density, riparian vegetation height) and in water conductivity between the three habitats. I entered “plot” nested in “stream” as random factors and computed “environmental parameters” as response variables. *P*-values were obtained by likelihood ratio test of the full model with the effect in question against the model without the effect of explanatory variables using the function “`anova`”. Upon significance, I ran pair-wise differences with the function “`lsmeans`” (Lenth et al. 2015) with Tukey adjustment after bootstrapping method using the package “`pbkrtest`” (Halekoh and Højsgaard 2014). All data were log-transformed before analysis.

Spatial autocorrelation and community analysis

These analyses were conducted on 2014 data that had richer species.

Spatial autocorrelation

A reliable identification of the effects of disturbance on species composition requires an understanding of spatial effects, i.e., the spatial structuring of assemblages. Species assemblages can be spatially structured even without a spatial environmental signal (Ernst and Rödel 2008). I checked for cross-product correlations between distance matrices (species: Bray–Curtis distance, environmental parameters: Euclidean distance and spatial distance: aerial distance) with Mantel tests. This test allows testing the importance of environmental and spatial distance on species assemblages. To test whether the pattern of species assemblages is attributable to disturbance, I compared transect matrices of species compositional similarity and geographical distance between transect pairs within each habitat and across the whole data set (following the recommendation of Ghazoul (2002); Ramage et al. (2013)). If correlations within forest types and across forest types are different, this would support the hypothesis that disturbance effects exist and compositional differences are not caused by spatial distance alone (Ghazoul 2002). *P*-values were based on 999 permutations. These tests were conducted on R with the package *vegan* (Oksanen et al. 2016).

Community analysis

I used non-metric multidimensional scaling (NMDS) to visualize and evaluate patterns of dissimilarity among microhabitats based on their species composition. NMDS can handle data with many zeros, ranked and non-normal data (both are common in species data) better than classical ordination methods (e.g., PCA, CCA), and is well suited for ecological data (Riemann et al. 2015). Unlike PCA, the order of the axes in the resultant ordination does not imply the order of the variation explained. Unlike methods that attempt to maximise the variance or correspondence between objects in an ordination, NMDS represents, as closely as possible, the pairwise dissimilarity between objects in a low-dimensional space. The number of axis was selected based on the lowest stress. As a rule of thumb, a stress value lower than 0.2 represents a good fit of the data. The ordination was constructed from a Bray–Curtis (i.e., Sorensen) dissimilarity matrix using species presence/absence data (Riemann et al. 2015; Strauß et al. 2013). NMDS was performed with function “*metaMDS*” from R package *vegan* (Oksanen et al. 2016). I used *perMANOVA* (Anderson 2001) to test the hypothesis of differences in species composition between primary forest, logged forest and matrix habitat. *perMANOVA* was conducted on *PAST* (Hammer et al. 2001), based on Bray–Curtis dissimilarities using species presence/absence data and 9999 permutations. I conducted *SIMPER* analysis to break down the contribution of each species to the observed dissimilarity

between samples. It allows identifying the species that are most important in creating the observed pattern of dissimilarity. The function performs pairwise comparisons of groups of sampling units and finds the average contributions of each species to the average overall Bray-Curtis dissimilarity.

Species-microhabitat associations

To examine species habitat preference, I related species abundance to variables of the microhabitat using NMDS with Bray-Curtis distance. I used abundance rather than incidence because it better reflects how species respond to changes in environment parameters (environmental filter effect). I excluded rare species (species where number of individuals were less than 10 across all sites) to reduce their influence on ordination.

2.3. Results

Species diversity

4444 individuals of 27 species belonging to the family Mantellidae (2014) were recorded. Tadpoles belong to nine mouthpart guilds (Table 2, Fig. 2–3). The pattern of species abundance shows that by far the most abundant species were *Boophis quasiboehmei*, *Boophis madagascariensis*, and *Boophis reticulatus* (Fig. 3). These species were ubiquitous in all streams. *Boophis quasiboehmei* was the dominant species among all sites (Fig. 4). At edge, *B. andohahela*, *B. tasymana*, and *B. sp37* that are adapted to open habitats became more common while forest specialists, *B. picturatus*, *S. perraccae* and *S. aglavei*, became rare (Fig. 4). These forest specialists are species to which life histories are associated with large trees (Glaw and Vences 2007).

Species richness

Species richness (absolute number of species found in stream) differed between the three habitats (Fig. 4, Chi-square (2) = 11.72, $P = 0.002$). Primary forest harbored the highest number of species found in a stream (range = 7–14 species). Disturbed forest harbored lesser SR (range = 4–8 species) compared to primary forest (Tukey, $P = 0.004$) and edge habitats (Tukey, $P = 0.041$). Edge habitats (range = 8–12 species) and primary forest (Tukey, $P = 0.629$) harbored relatively the same number of species, but species composition differed (Fig. 5).

Diversity indices

Primary forest and edge habitats were similarly diverse (Shannon $H' = 1.87$ and $H' = 1.92$, respectively; diversity t-test $t = -1.27$, $df = 2377$, $P = 0.201$). Disturbed forest was dominated by few species and was the least diverse (Shannon $H' = 1.45$; all pairwise comparisons $P < 0.001$). Beta-diversity was lower between primary and disturbed forest ($\beta = 0.24$) than it was between disturbed forest and edge habitat ($\beta = 0.45$). Beta diversity was 0.36 between edge and primary habitats.

Abundance

Primary forest tended to harbor higher tadpole abundance (mean \pm SD, 480 ± 175 individuals) than the other habitats (disturbed forest, mean \pm SD, 397 ± 121 individuals; edge habitats, mean \pm SD, 376 ± 86 individuals) but the difference was not statistically significant (Chi-square = 3.32, $df = 2$, $P = 0.189$) (Fig. 4). This was because tadpole abundance was highly variable across year. Indeed, in 2014, 1842, 1442, and 1178 individuals were sampled in the primary forest, disturbed forest, and edge habitat, respectively (all streams combined for each habitat). However, in 2015, 1059, 943, and 1057 individuals were found at these habitats.

Habitat characteristics

Primary forest and disturbed forest did not differ in any of the environmental parameters I measured. Forest habitats differed from forest edge habitats in canopy cover, basal area, water conductivity, water temperature, and the number of shrubs (Table 3).

Spatial and environmental correlation

Species composition was influenced by both microhabitat environmental (Mantel test, $r = 0.479$, $P = 0.001$) and spatial distance (Mantel test, $r = 0.321$, $P = 0.001$). Species composition was independent of spatial distance in disturbed forest (Mantel test, $r = -0.01$, $P = 0.495$); however, assemblage was correlated with spatial distance in primary forest (Mantel test, $r = 0.12$, $P = 0.03$) and edge habitats (Mantel test, $r = 0.331$, $P = 0.002$). These results indicate (although not necessarily proving) that compositional differences were not caused by spatial distance alone, but disturbance affected assemblage in disturbed forest. However, in edge, species compositions cannot be attributed to habitat disturbance alone but also to spatial distance.

Community analysis

Species assemblages

The general stress of NMDS model was 0.11, indicating a quite good preservation of ordering relationships of the multidimensional among-microhabitat dissimilarities. Tadpole assemblages differed between the three habitats (perMANOVA test, $F(5.03, 8.41) = 22.9$, $P < 0.001$) (Fig. 6). Pairwise differences indicate that assemblages differed from one habitat to another (for all tests $P < 0.001$, following Bonferoni P -value corrections). Overall dissimilarity between primary forest and disturbed forest was 35.43% (mainly because of *M. sp47* and *B. reticulatus*, *M. sp28*, and *Spinomantis* species; Table 4). Dissimilarities between primary forest and edge habitat were 61.25% (mainly because of *B. picturatus*, *B. andohahela*, *B. elenae*; Table 5) and between logged forest and edge habitats was 54.19% (*B. andohahela*, *B. picturatus*, *B. elenae*; Table 6).

Species-habitat associations

Environmental variables associated with NMDS1 were stream velocity, microhabitat water depth, percentage of boulder, and canopy openness (Table 7). Species positively correlated with NMDS1 were *B. marojezensis*, *B. andohahela*, *B. sp37*, *B. tasymena*, and *B. elenae* (Table 8). Species that are negatively correlated with NMDS1 are the forest specialist species *B. picturatus*, *S. aglavei* and *S. peraccae*. NMDS1 represents microhabitats with open canopy, deep and relatively fast water, and the presence of boulder.

Environmental variables positively associated with NMDS2 were litter, sediment, and sand. Variables negatively associated with NMDS2 were rock and gravel. Species that are positively correlated with NMDS2 are *B. madagascariensis*, *B. quasiboehmei*, *B. reticulatus*, *M. melanopleura*, *M. sp47*, *M. sp28*, *M. majori*. *B. luteus* was weakly correlated with NMDS2 but its ordination scores suggest that this species prefers open habitat with slow flowing water with substrates composed of litter, sand and sediment. NMDS2 represents microhabitats such as pools with slowing water and composed of litter. Common species (e.g., *B. quasiboehmei*, *B. madagascariensis*) aggregated around the ordination origin; this is because these species were found both in forest and open habitats, but they were more abundant in forest habitats.

Oral structure-habitat associations: species guild

Based on NMDS results (Fig. 7), the tadpoles can be categorized into four groups: species with preference to pool environments, generalist species that do not show clear

microhabitat preference, species adapted to open habitats, and species adapted to riffle environments with predominance of gravel substrate. Except the *Boophis* sand-eater cluster, most forest species aggregated around the litter-sand-sediment environmental axis. *Boophis*-sand eater tadpoles were positively correlated with gravel and negatively correlated with canopy openness. *B. picturatus*, which is the only species in this cluster, was recorded in riffle habitats with relatively fast flowing current and in which, gravel is the dominant substrate. Suctorial tadpoles prefer microhabitats with relatively open canopy, deep water and relatively fast flowing stream section, with dominance of boulders. Some *Boophis* with generalized morphology (*B. luteus*, *B. elenae*, *B. tasymena*) found in open habitat are positively correlated with both NMDS1 and NMDS2. These species, indeed, can be found in stream portions that are deep, moderately fast flowing with litter, sediment and sand substrate (personal observations).

2.4. Discussion

Primary forest, disturbed forest, and forest edge harbored different tadpole assemblages. At edge, species better adapted to open habitats replaced forest specialists; this compensatory mechanism allowed the maintenance of species richness outside forest (Fig. 4). Species-habitat associations revealed that both spatial and environmental parameters determined the composition of tadpoles' assemblage, but the effects of the environment on assemblage were larger. Detailed analysis with NMDS indicated that community structure was determined by canopy openness and microhabitat substrate composition.

27 species were encountered, which was far less the number recorded by Strauß et al. (2013) who identified 44 species in the study area. Further, I recorded a maximum of 14 species (in primary forest) while Strauß et al. (2013) recorded up to 22 in a single stream. Although this difference could stem from difference in sampling sites, sampling protocols may also explain these results. Strauß et al. (2013) systematically sampled tadpoles in 30 m stream sections of 33 streams. I did not follow similar methodology because amphibian assemblages can change with microhabitats within few meters (Keller et al. 2009), and tadpole assemblages are suggested to follow similar pattern (Afonso and Eterovick 2007; Inger et al. 1986); therefore, the methodology of Strauß et al. (2013) would not be appropriate for this study. Undoubtedly, species richness would have been higher had we surveyed more sites and larger stream, but the objectives of the study limited the number of available candidate streams. Annual variation in tadpole assemblage may explain differences in species richness here (Strauß et al. 2016), but I do not think that seasonality would have much

influenced species detection because having repeated sampling in March–April 2015, similar assemblages as in October–November were found.

The fact that abundances and species richness did not differ between forest edge and primary forest suggests that compensatory mechanism may occur at the forest edge. Following local extinctions, colonization by new species with niches better suited to the new environmental conditions helps maintain species richness (Brown et al. 2001; Dornelas 2010), and allow distributions of abundance to be maintained, as declines in biomass by some species are compensated for by increases in others. Riemann et al. (2015) found similar pattern in amphibian diversity in fragmented forest in the study site. Following the predictions, edge specialists replaced forest specialists at the edge. Forest specialists are represented by Spinomantis clade (*S. aglavei*, *S. fimbriatus*, *S. peraccae*) and the sand-eater *B. picturatus*, which is known to occur only in relatively undisturbed habitats (Glaw and Vences 2007). Spinomantis are associated with forest habitats, where they often call from the canopy of large trees (Glaw and Vences 2007). Species that were typical to open habitats include *Boophis andohahela*, *B. tasymena* and *B. sp37*. *B. andohahela* was thought to require pristine habitats (Glaw and Vences 2007), but Strauß et al. (2013) along with our study suggest that this species can adapt to degraded habitats as well. Forest edges are characterized by higher temperatures, increased wind speed and decreased relative humidity (Lehtinen et al. 2003), and although such environmental traits can affect amphibians, the persistence of amphibians in open habitat remains unclear, but hypothesis holds that frequent natural disturbances, such as cyclones, by modifying forest structure, could have favored amphibian adaptations to such habitats (Glaw and Vences 2007).

The results of this study show that the environmental difference predicted tadpole assemblages by both spatial distance and environmental parameters. This means that proximate streams and similar habitats bear similar assemblages (Ernst and Rodel 2005; Parris 2004). The environment explained a larger part of variation in assemblage composition (hypothesis 1), consistent with the results of Strauß et al. (2013). The high environmental predictability of tadpole assemblages emphasizes the importance of niche-based processes in structuring tadpole assemblages. Spatial effects can be of primary importance in structuring terrestrial frog assemblages (Ernst et al. 2006), but environmental filter can play greater effect on stream biological communities (Parris 2004). This indicates that stream habitats could be heterogeneous enough to influence biological assemblages by environmental parameters, whereas those of the forest leaf litter and canopies may not.

Constraints on the design of the sampling regime limit the inferences that might be drawn on the general impact of disturbance on tadpole communities. However, while it is not possible to prove that differences in tadpole composition across habitats are a direct consequence of their disturbance histories, difference in habitat structure and Mantel tests revealed inherent differences between sites, suggesting that disturbance might still be good a candidate explanation for these differences. Mantel test suggests that disturbance influenced community dissimilarities between disturbed and primary forest. However, at edge, correlation between species composition and spatial distance indicate that not only disturbance but also spatial distance, explain variation in species composition (Ghazoul 2002). Here, disturbance could be more important than spatial effect because it induces changes in vegetation structure, i.e., the quality of breeding habitats (Bickford et al. 2010; Hillers et al. 2008). Riparian habitat did not influence species organization in forest habitats, but this result was rather expected because, in the study site, logging mostly targeted upper parts of the forest. Logging activities reduced canopy cover and basal area (Ramaharitra 2006; Tecot 2008), to which forest specialists are the most sensitive (Burivalova et al. 2014). My results are consistent with such an assumption and, indeed, forest specialists *Spinomantis* species explained a considerable part of community dissimilarity between the two forest habitats (Table 4).

Ordination revealed that species composition changed with changes in microhabitat canopy cover, water depth, water velocity, substrate, and velocity. Based on microhabitat environmental features, we are able to define four groups, namely species adapted to pool environments, generalist species, species adapted to open habitats, species adapted to riffle environments with predominance of gravel substrate (Fig. 7). Microhabitat differences were strong enough to result in distinct assemblages at specific habitats even within the same stream. As it could be expected, canopy openness (i.e., less tree cover) exerted strong effect on species assemblages, as this variable was clearly the most distinct trait between forest and edge habitats. In general, tadpoles were more diverse and abundant in microhabitats with deeper and slower running water, and containing higher proportion of leaf litter (“pool” habitat), which tadpoles can use as refuge, e.g., against predator or simply alleviating the strength of current. These observations are consistent with previous studies (Eterovick and Barata 2006; Strauß et al. 2013) that reported preference for slow moving water in tadpoles. Not all species prefer pool habitats, however. Tadpoles with suctorial mouthpart specialize in stream with open canopy and deep water level. Using their mouthpart, these species can

firmly attach to substrates, allowing them to circumvent strong current. Sand-eater *B. picturatus* specializes in microhabitats dominated by gravel such as riffles. Previous studies (Grosjean et al. 2011; Strauß et al. 2013) were not able to associate this species to any environmental variables. These lacks of association between environmental parameters and species occurrence could be because of difference in sampling protocol as we examined assemblages at the microhabitat level whereas Strauß et al. (2013) studied tadpoles in longer stream portion, which could have crossed multiple microhabitats.

NMDS shows that there was a tendency for tadpoles with the same oral structure to aggregate along similar axes. This suggests that the pronounced heterogeneity at the microhabitat level has probably led to differing habitat-specific morphological adaptations. In line with (Strauß et al. 2013), I found that tadpoles with the same mouthpart cluster do not necessarily follow similar environmental axes. For example, though *B. elenae*, *B. tasymana*, *B. luteus*, and *B. marojezensis* have the same mouthpart, they differed from *B. quasiboehmei*, *B. reticulatus*, and *B. madagascariensis* in their environmental determinants; but this was probably because the later three species were common species, thus they could not strongly be assigned to any axes.

Conclusions and implications

The factors structuring tropical tadpole assemblages are still poorly known (Borges Júnior and Rocha 2013), I show here that microhabitat heterogeneity and stream surrounding vegetation can strongly influence the organization of tropical tadpoles. While previous studies examined tadpole assemblages at the stream level, I suggest that more focus should be put on the microhabitat level. It appears that tadpoles can occupy different microhabitats even within the same stream.

Habitat partitioning represents a fundamental condition in maintaining biodiversity (Chase and Leibold 2003), and the fact that environment variables structured tadpole community does not necessarily imply that tadpole species can stably coexist. One crucial condition is whether intraspecific competition is stronger than interspecific interaction (invasibility criteria) (Siepielski and McPeck 2010), and testing this hypothesis could be a venue for future research.

While environmental axes allowed species to occupy different habitat spaces, it remains unclear how generalist species (species that were recorded in all microhabitats *B.*

quasiboehmei, *B. reticulatus*, *B. madagascariensis*) can stably coexist. Trophic space analysis (chapter 3) may clarify how these species partition their resources.

From conservation perspective, this study and others like it, could be relevant only to the areas in which they were conducted (Ghazoul 2002). In fact, the response of amphibian community diversity to anthropogenic disturbance is dependent on the type of forest formation and on the nature and history of the disturbance, and is a function of the amphibian community itself. Further, habitat structure can greatly vary among streams in degraded habitats and such variability could be an important source of discrepancies among studies on disturbance ecology (Burivalova et al. 2014). Generalization from any single study is therefore inappropriate (Ghazoul 2002), but there is, nevertheless, an urgent need for reliable generalizations in planning conservation strategies.

This study highlights the importance of matrix habitats in the dynamics and composition of amphibian communities, and has important implications for the management of disturbed habitats. Ongoing habitat loss due to habitat disturbance and land conversion is threatening amphibian diversity in Madagascar. It might not be sufficient to limit conservation activities to current established protected areas that mainly focused on forest blocks. It is important to understand the importance of degraded habitats in conserving amphibian diversity (Irwin et al. 2010; Riemann et al. 2015), as a considerable number of threatened species are currently outside protected areas and for many of them, distribution is limited to very small areas (Ramamonjisoa et al. 2013).

2.5. Figures

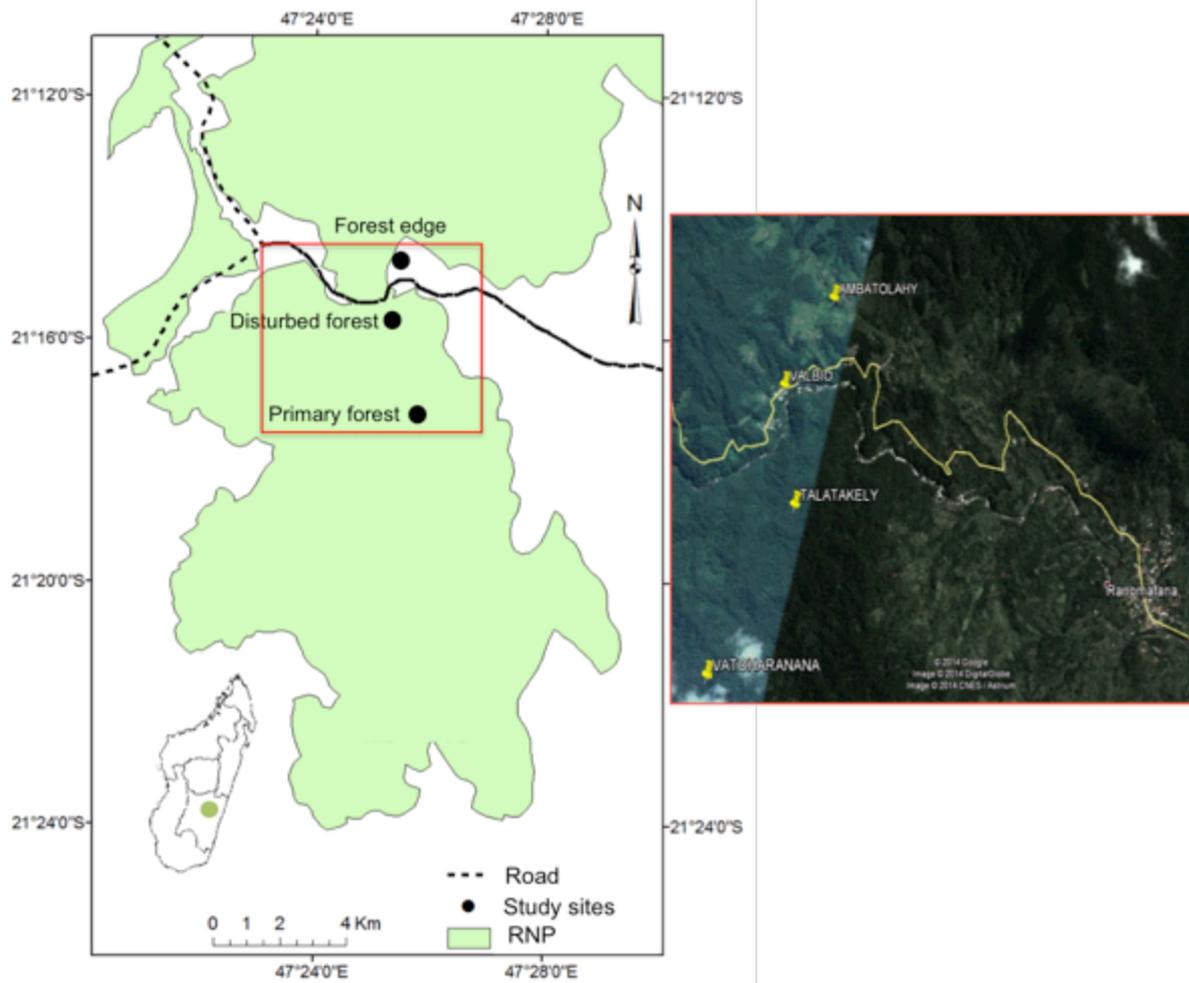


Figure 1: Localization of Ranomafana National Park (RNP) and study sites (Sources: GoogleEarth, Rio Riddish)

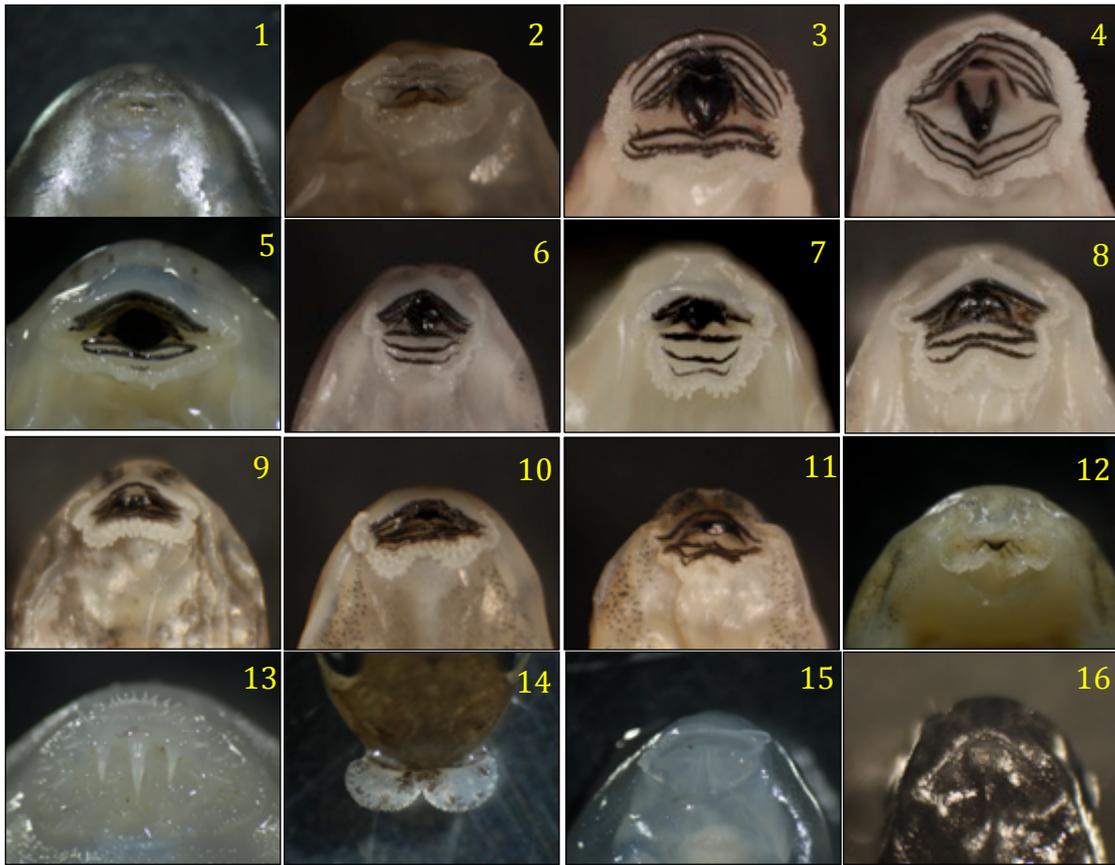


Figure 2. Tadpole oral structures found in Ranomafana streams. (1). Spinomantis-generalized (*Spinomantis aglavei*), (2). *Spinomantis*-generalized (*S. fimbriatus*), (3). Suctorial (*B. andohahela*), (4). Suctorial (*B. marojezensis*), (5). *Boophis*-generalized (*B. quasiboehmei*), (6). *Boophis*-generalized (*B. albilabris*), (7). *Boophis*-generalized (*B. reticulatus*), (8). *Boophis*-generalized (*B. periegetes*), (9). *Boophis*-generalized (*B. tasymena*), (10). *Guibemantis*-Podgy-generalized (*Guibemantis liber*), (11). *Mantidactylus*-generalized (*M. sp28*), (12) *Mantidactylus*-reduced teeth type 1 (*M. sp47*), (13). *Mantidactylus*-reduced teeth type 2 (*M. majori*), (14). *Mantidactylus*-funnel mouth (*M. melanopleura*), (15). *Boophis*-sand eater (*B. picturatus*), (16). *Gephyromantis* non-feeding (*G. ventrimaculatus*).

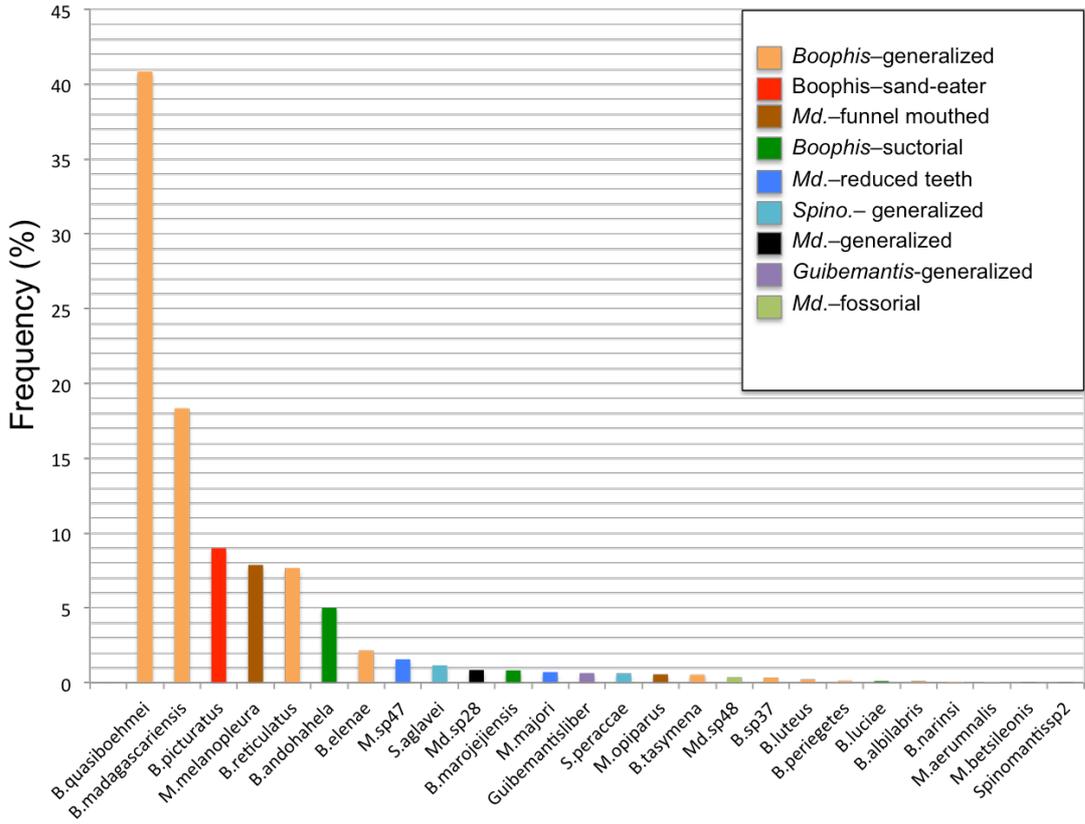


Figure 3. Tadpole frequencies, all habitats combined (2014 samples).

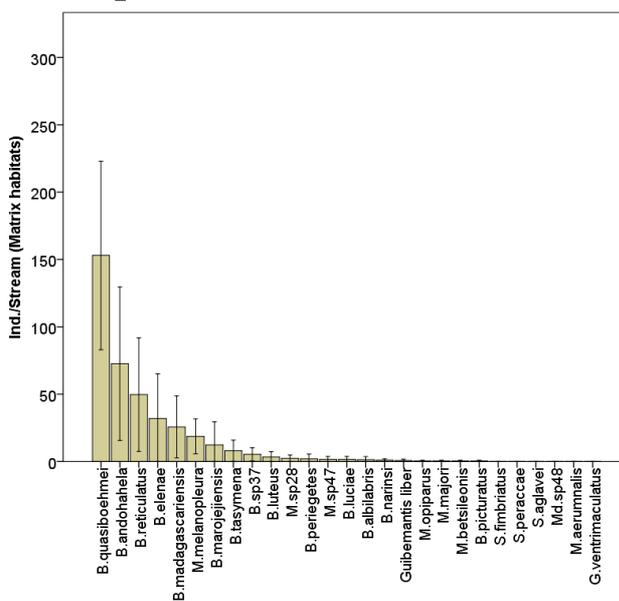
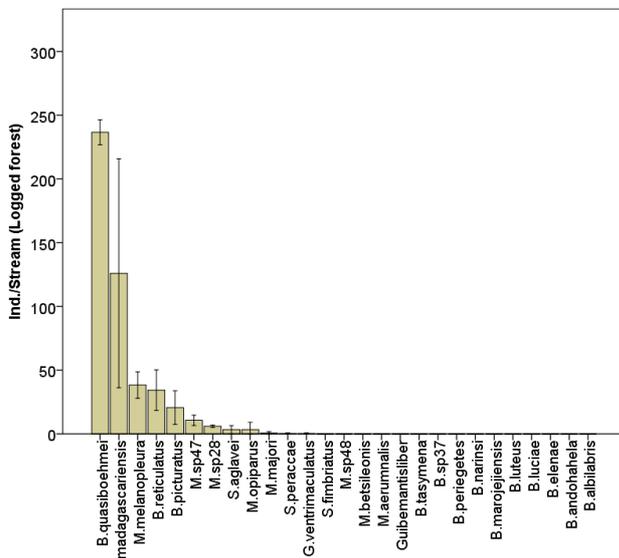
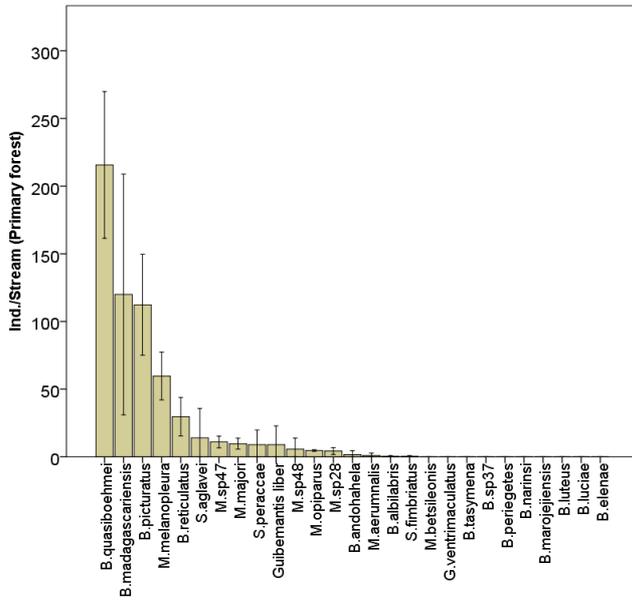


Figure 4. Tadpole frequencies in primary forest, disturbed forest, and in streams at forest edges (2014 data, values represent mean abundance of species in streams, mean \pm SD).

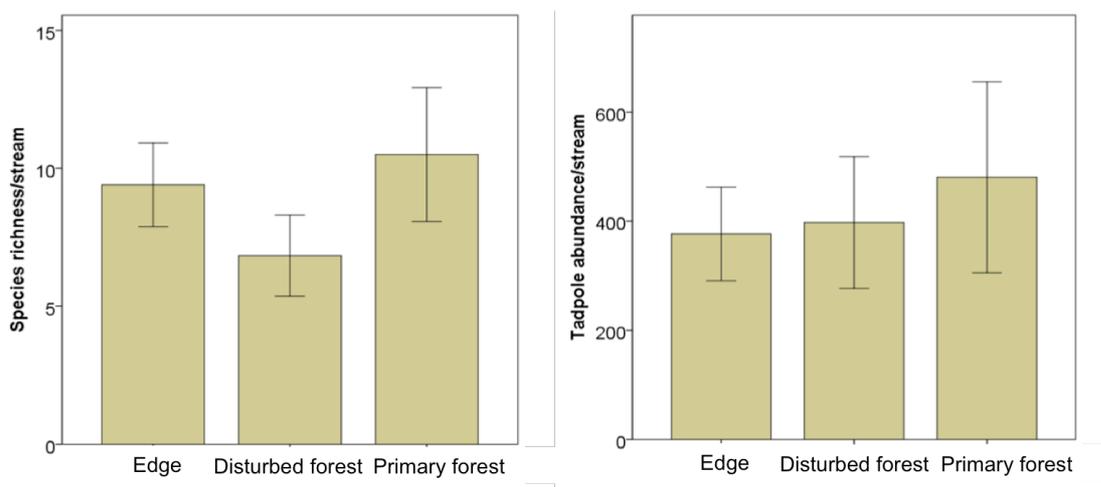


Figure 5. Species richness and relative abundance of tadpole in streams at forest edge, in disturbed forest, and in primary forest. mean \pm SD.

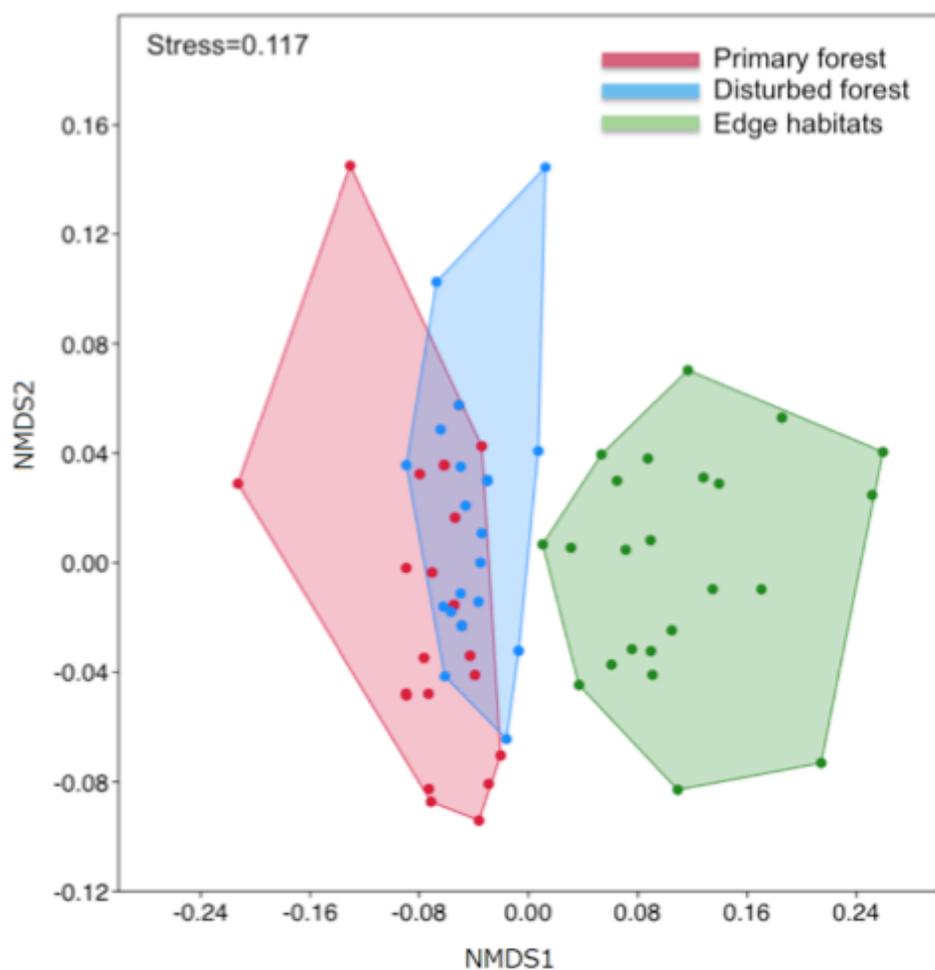


Figure 6. Dissimilarities in tadpole community recorded in streams in primary forest (red), in disturbed forest (blue), and at forest edge (green)

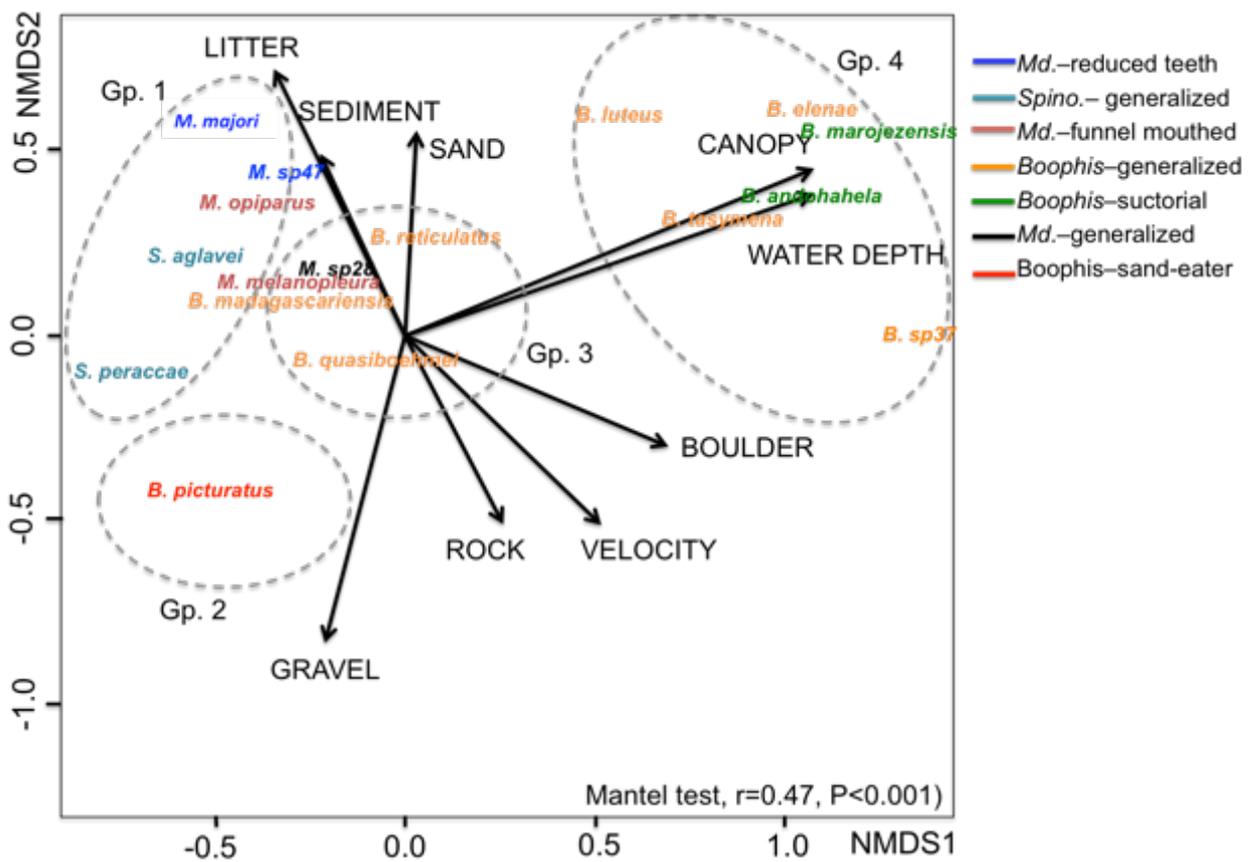


Figure 7. Non-metric multidimensional scaling of species in community space (first two axes). Arrows are significant correlations of environmental parameters with axes. Lengths of arrows increase with correlation coefficients. Species could be grouped into four guilds: Gp. 1 for pool specialist species, Gp. 2 for species adapted to gravel substrate, Gp. 3 for generalist species (ubiquitous), Gp. 4 for open habitat species.

2.6. Tables

Table 1: Environmental parameters (at the microhabitat level and at the stream level) recorded in the study

Microhabitat level		Stream level	
Litter (%)	Predator (density)	Understory height (cm)	Canopy cover (%)
Boulder (%)	Water velocity (cm/s)	Water temperature (°C)	Basal area (m ² /ha)
Gravel (%)	stream width (cm)	Litter cover (%)	Shrub density (N/ha)
Rock (%)	DO (mg/L)	Vegetation cover (%)	Water conductivity (Siemens)
Gravel (%)	Canopy openness (%)	Soil cover (%)	Riparian vegetation height (cm)
Sand (%)	Water depth (cm)	Canopy height (m)	Density of epiphyte plants
Sediment (%)			

Table 2. Species of tadpoles recorded in streams in primary forest, disturbed forest (selectively logged forest), and at forest edge in Ranomafana. Species mouthparts are illustrated in Fig. 2

	Mouthpart cluster	IUCN Redlist status
<i>Boophis albilabris</i>	<i>Boophis</i> –Generalized	LC
<i>Boophis andohahela</i>	Suctorial	VU
<i>Boophis elenae</i>	<i>Boophis</i> –Generalized	NT
<i>Boophis luciae</i>	Suctorial	LC
<i>Boophis luteus</i>	<i>Boophis</i> –Generalized	LC
<i>Boophis madagascariensis</i>	<i>Boophis</i> –Generalized	LC
<i>Boophis marojezensis</i>	Suctorial	LC
<i>Boophis narinsi</i>	<i>Boophis</i> –Generalized	EN
<i>Boophis periegetes</i>	<i>Boophis</i> –Generalized	NT
<i>Boophis picturatus</i>	Sand-eater	LC
<i>Boophis quasiboehmei</i>	<i>Boophis</i> –Generalized	NA
<i>Boophis reticulatus</i>	<i>Boophis</i> –Generalized	LC
<i>Boophis sp. 37</i> (aff. <i>elenae</i>)	<i>Boophis</i> –Generalized	DD
<i>Boophis tasymena</i>	<i>Boophis</i> –Generalized	LC
<i>Guibemantis liber</i>	<i>Gu.</i> –Podgy	LC
<i>Gephyromantis ventrimatulatus</i>	<i>Ge.</i> –non-feeding	LC
<i>Mantidactylus aerumnalis</i>	Funnel mouthed	LC
<i>Mantidactylus betsileonis</i>	<i>Md.</i> Generalized	LC
<i>Mantidactylus majori</i>	Reduced teeth	LC
<i>Mantidactylus melanopleura</i>	Funnel mouthed	LC
<i>Mantidactylus opiparus</i>	<i>Md.</i> –Funnel mouthed	NA
<i>Mantidactylus sp. 47</i> (aff. <i>mocquardi</i>)	<i>Md.</i> –Reduced teeth	NA
<i>Mantidactylus sp. 28</i> (aff. <i>betsileanus</i>)	<i>Md.</i> –Generalized	NA
<i>Mantidactylus sp. 48</i> (aff. <i>cowani small</i>)	<i>Md.</i> –fossorial	NA
<i>Spinomantis aglavei</i>	<i>Spinomantis</i> –Generalized	LC
<i>Spinomantis peraccae</i>	<i>Spinomantis</i> –Generalized	LC

D: data deficient; NT: Near threatened; LC: Least concern; VU: Vulnerable; EN: Endangered

Table 3. Characteristics of riparian habitats and stream environments in primary forest, disturbed forest, and at forest edge.

Habitat parameters	Mean \pm SD			Pairwise differences <i>P</i> -values		
	Disturbed forest (Dist)	Edge habitats (Edge)	Primary forest (Prim)	Dist vs. Edge	Prim vs. Edge	Prim vs. Dist
Basal area (m ² /50m ²)	0.165 \pm 0.10	0.03 \pm 0.05	0.17 \pm 0.14	<0.001	<0.001	0.999
Shrubs (# in 100m ²)	44.5 \pm 31.89	25.06 \pm 20.4	29.75 \pm 16.67	0.004	0.347	0.132
Riparian plants (cm)	110.73 \pm 17.64	118.14 \pm 30.68	100.46 \pm 24.84	0.891	0.175	0.356
Understory height (cm)	59.22 \pm 21.39	63.45 \pm 33.4	53.89 \pm 14.67	0.959	0.621	0.78
Litter depth (cm)	4.54 \pm 1.37	2.88 \pm 1.93	3.55 \pm 1.01	0.001	0.057*	0.304
Canopy openness (%)	5.86 \pm 0.81	62.52 \pm 20.83	5.85 \pm 2.56	<0.001	<0.001	0.757
Conductivity (siemens)	1.21 \pm 0.08	1.13 \pm 0.07	1.32 \pm 0.19	0.313	0.014	0.27
Temperature (°C)	18.03 \pm 0.4	18.88 \pm 0.77	17.93 \pm 0.29	0.008	0.003	0.906

Table 4. Simper analysis detailing the relative contribution of species to community dissimilarity between primary forest and disturbed forest

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Primary forest	Mean Disturbed forest
<i>M.sp47</i>	3.89	10.97	10.97	0.50	0.42
<i>B.reticulatus</i>	3.70	10.45	21.42	0.58	0.88
<i>Md.sp28</i>	3.63	10.24	31.66	0.29	0.46
<i>Spinomantisaglavei</i>	3.39	9.58	41.24	0.38	0.33
<i>M.majori</i>	3.21	9.06	50.30	0.46	0.04
<i>Spinomantisperaccaea</i>	3.10	8.76	59.06	0.42	0.04
<i>Guibemantisliber</i>	2.40	6.78	65.83	0.33	0.00
<i>M.opiparus</i>	2.32	6.55	72.38	0.33	0.04
<i>M.melanopleura</i>	2.19	6.17	78.56	0.83	0.92
<i>B.madagascariensis</i>	2.12	5.99	84.55	0.88	0.88
<i>B.picturatus</i>	1.79	5.06	89.61	1.00	0.79
<i>Md.sp48</i>	1.02	2.87	92.48	0.17	0.00
<i>M.aerumnalis</i>	0.97	2.74	95.22	0.13	0.00
<i>B.andohahela</i>	0.71	2.01	97.23	0.13	0.00
<i>Spinomantissp2</i>	0.44	1.24	98.47	0.04	0.00
<i>B.albilabris</i>	0.28	0.80	99.27	0.04	0.00
<i>G.ventrimaculatus</i>	0.26	0.73	100.00	0.00	0.04

Table 5: Simper analysis detailing the relative contribution of species to community dissimilarity between primary forest and forest edge habitat

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Primary forest	Mean Edge
<i>B.picturatus</i>	7.04	11.49	11.49	1.00	0.04
<i>B.andohahela</i>	5.84	9.53	21.03	0.13	0.87
<i>B.elenae</i>	4.78	7.80	28.82	0.00	0.65
<i>M.sp47</i>	3.51	5.74	34.56	0.50	0.17
<i>B.reticulatus</i>	3.49	5.69	40.25	0.58	0.74
<i>M.melanopleura</i>	3.45	5.63	45.88	0.83	0.57
<i>B.tasymena</i>	3.24	5.30	51.18	0.00	0.48
<i>B.madagascariensis</i>	3.01	4.91	56.08	0.88	0.65
<i>M.majori</i>	2.99	4.88	60.96	0.46	0.04
<i>Spinomantisperaccae</i>	2.82	4.61	65.57	0.42	0.00
<i>Md.sp28</i>	2.58	4.21	69.78	0.29	0.22
<i>Guibemantisliber</i>	2.46	4.02	73.80	0.33	0.09
<i>Spinomantisaglavei</i>	2.46	4.01	77.80	0.38	0.00
<i>M.opiparus</i>	2.27	3.70	81.51	0.33	0.04
<i>B.sp37</i>	2.25	3.67	85.18	0.00	0.30
<i>B.marojejiensis</i>	1.79	2.93	88.10	0.00	0.26
<i>B.luteus</i>	1.74	2.84	90.94	0.00	0.26
<i>Md.sp48</i>	0.96	1.56	92.50	0.17	0.00
<i>M.aerumnalis</i>	0.90	1.47	93.96	0.13	0.00
<i>B.periegetes</i>	0.89	1.45	95.41	0.00	0.13
<i>B.albilabris</i>	0.82	1.34	96.75	0.04	0.09
<i>B.luciae</i>	0.67	1.09	97.84	0.00	0.09
<i>B.narinsi</i>	0.62	1.02	98.85	0.00	0.09
<i>Spinomantissp2</i>	0.39	0.64	99.50	0.04	0.00
<i>M.betsileonis</i>	0.31	0.50	100.00	0.00	0.04

Table 6. Simper analysis detailing the relative contribution of species to community dissimilarity between disturbed forest and edge habitat

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Logged foerest	Mean Matrix
<i>B.andohahela</i>	7.10	13.09	13.09	0.00	0.87
<i>B.picturatus</i>	6.18	11.40	24.49	0.79	0.04
<i>B.elenae</i>	5.35	9.87	34.37	0.00	0.65
<i>M.melanopleura</i>	3.81	7.03	41.40	0.92	0.57
<i>Md.sp28</i>	3.69	6.81	48.21	0.46	0.22
<i>B.tasymena</i>	3.60	6.65	54.86	0.00	0.48
<i>B.madagascariensis</i>	3.37	6.23	61.09	0.88	0.65
<i>M.sp47</i>	3.36	6.19	67.28	0.42	0.17
<i>B.reticulatus</i>	2.90	5.35	72.63	0.88	0.74
<i>B.sp37</i>	2.52	4.65	77.27	0.00	0.30
<i>Spinomantisaglavei</i>	2.40	4.42	81.70	0.33	0.00
<i>B.marojejiensis</i>	1.99	3.68	85.37	0.00	0.26
<i>B.luteus</i>	1.93	3.56	88.93	0.00	0.26
<i>B.periegetes</i>	0.98	1.82	90.74	0.00	0.13
<i>M.opiparus</i>	0.76	1.41	92.15	0.04	0.04
<i>B.luciae</i>	0.75	1.39	93.55	0.00	0.09
<i>B.narinsi</i>	0.69	1.28	94.83	0.00	0.09
<i>B.albilabris</i>	0.66	1.23	96.05	0.00	0.09
<i>Guibemantisliber</i>	0.66	1.23	97.28	0.00	0.09
<i>M.majori</i>	0.58	1.07	98.35	0.04	0.04
<i>M.betsileonis</i>	0.35	0.64	98.98	0.00	0.04
<i>Spinomantisperaccae</i>	0.28	0.52	99.51	0.04	0.00
<i>G.ventrimaculatus</i>	0.27	0.49	100.00	0.04	0.00

Table 7. Correlation of environmental parameters with the tadpole community ordination and ordination projection for the two first axes. P-values were based on 999 permutations.

	NMDS1	NMDS2	R ²	P	
Velocity	0.708	-0.706	0.258	0.001	***
Depth	0.942	0.335	0.624	0.001	***
Width	0.882	0.472	0.048	0.148	
Predators	0.949	0.316	0.048	0.202	
DO	0.450	0.893	0.043	0.244	
Litter	0.436	0.900	0.309	0.001	***
Sediment	0.418	0.908	0.141	0.007	**
Boulder	0.916	0.401	0.275	0.001	***
Rock	0.452	0.892	0.157	0.002	**
Gravel	0.246	0.969	0.353	0.001	***
Sand	0.054	0.999	0.148	0.005	**
Canopy	0.923	0.385	0.657	0.001	***

Table 8. Correlation of species with ordination of anuran community structure and vectors of ordination projections. P-values are based on 1000 permutations.

	NMDS1	NMDS2	R ²	P	
<i>B.madagascariensis</i>	-0.668	0.744	0.563	0.001	***
<i>B.picturatus</i>	-0.935	-0.354	0.603	0.001	***
<i>B.quasiboehmei</i>	-0.535	0.845	0.574	0.001	***
<i>B.reticulatus</i>	-0.054	0.999	0.329	0.001	***
<i>M.melanopleura</i>	-0.479	0.878	0.436	0.001	***
<i>M.opiparus</i>	-0.544	0.839	0.081	0.071	.
<i>Md.sp28</i>	-0.433	0.901	0.086	0.051	.
<i>M.sp47</i>	-0.396	0.918	0.239	0.001	***
<i>Spinomantisaglavei</i>	-0.716	0.698	0.153	0.007	**
<i>M.majori</i>	-0.482	0.876	0.192	0.002	**
<i>Spinomantisperaccae</i>	-0.976	0.217	0.136	0.009	**
<i>B.marojejiensis</i>	0.783	0.622	0.200	0.003	**
<i>B.andohahela</i>	0.811	0.585	0.697	0.001	***
<i>B.sp37</i>	0.980	0.198	0.287	0.001	***
<i>B.tasymena</i>	0.776	0.631	0.196	0.002	**
<i>B.eleuae</i>	0.733	0.680	0.488	0.001	***
<i>B.luteus</i>	0.471	0.882	0.085	0.047	*

Chapter 3: Phenotypic divergence and diet specialization in species rich tadpole community: mechanism of coexistence?

Abstract

Phenotypic diversification is thought to promote coexistence in species rich communities, but the generality of this assumption has not yet been tested. Since not all species undergo morphological differentiation during character displacement, it is intriguing how generalists and specialists can coexist in a same habitat. Using species rich amphibian community in Madagascar, I investigated the feeding niche of 17 tadpoles by combining gut content and stable isotope analyses. The tadpoles differ in their feeding mode and could be grouped into seven feeding guilds: one generalized and six specialized mouthparts. I hypothesized (1) that tadpoles occupy different trophic spaces, a prerequisite assumption for coexistence, (2) that tadpoles with similar mouthpart would occupy similar feeding niche and (3) that tadpoles with generalized mouthparts would exhibit higher intraspecific niche variation than species with specialized oral structure. The tadpoles mainly differed in their carbon signatures and values ranged from -19‰ to -32‰ . Most species were primary consumers (N isotopic values $3\text{‰} - 6\text{‰}$) except for one species, sand-eater tadpole, that occupied higher trophic position. Though some species occupied similar trophic space as suggested by stable isotope analysis, gut content examination revealed different feeding habits in these tadpoles. In contrast to prediction, species with similar mouthpart did not necessarily occupy similar trophic position indicating that mouthpart could be a poor predictor of feeding niche. Further, I did not find any differences in intraspecific trophic space variation between tadpoles with generalist and specialist mouthparts, suggesting that diet breadth was not related to phenotypic specialization. Coupled with environmental filter and microhabitat space partitioning, resource partitioning between these tadpoles might further explain why many species can co-occur in this system, corroborating predictions of classical niche theory.

3.1. Introduction

There is still ongoing debate on how and why multiple, potentially competing species can coexist in nature (Levine and HilleRisLambers 2009). Resource partitioning, in which different members of a community use different component of the environment, is suggested to be a major mechanism allowing species reduce strength of competition (Chase and Leibold 2003; Chesson 2000; Levine and HilleRisLambers 2009). Resource partitioning could be

facilitated by a suite of morphological changes through adaptive radiation (i.e., phenotypic divergence) that accommodate novel ways of exploiting food resources (Wagner et al. 2012). However, because not all species undergo phenotypic divergence during character displacement (Pfennig and Pfennig 2010), it is intriguing how and why generalists and specialists can stably coexist in the local system.

Tropical tadpoles offer a unique system for understanding the evolution of phenotype and niche specialization. First, tadpoles can represent the largest biomass in tropical streams (Altig et al. 2007), and can be of paramount importance for ecosystem functioning in these systems (Colón-Gaud et al. 2010a; Ranvestel et al. 2004; Whiles et al. 2006). Surprisingly, almost next to nothing is known about the trophic ecology of tropical tadpoles. Most information comes from studies that were conducted in temperate areas, where one ecomorphotype, pond-dwelling tadpoles with generalized morphology, dominates lentic environments (Altig et al. 2007; Schiesari et al. 2009). Still, despite decades of research on those tadpoles, there is still large uncertainty about what tadpoles really eat (Altig et al. 2007). Further, because of their feeding behavioral and diet plasticity, our knowledge about their trophic position, even within one feeding group, has been put into question (Arribas et al. 2015; Caut et al. 2012).

Second, tropical tadpoles often exhibit an exceptional diversity especially in their external morphology, ranging from generalized mouthpart to highly specialized oral structure (Altig and Johnston 1989; Strauß et al. 2013; Wells 2010). For example, in Ranomafana Madagascar, at least 44 species of tadpoles and 11 feeding guilds were recorded in streams (Strauß et al. 2013). Compared to other tropical areas, this diversity is remarkable. More than 22 species of tadpoles can be found in a single stream (Strauß et al. 2013), almost double than the species richness recorded in Brazilian streams with 10 species (Eterovick 2003). But, how such a diversity can be maintained and coexist remained unclear. Earlier studies suggested that, in tropical streams, space partitioning and environmental filter (Cf. previous chapter) could be mechanisms allowing species reduce species interaction in rich communities (Borges Júnior and Rocha 2013; Eterovick 2003; Eterovick and Barata 2006; Strauß et al. 2013; Strauß et al. 2010). Although these mechanisms have considerably helped understand the presence/absence of species at a given microhabitat, it is still intriguing why many species with apparent little niche space differentiation can stably co-occur in streams. The diversity of feeding mode may represent an important axe of niche partitioning allowing species avoid or, at least, alleviate the strength of interspecific competition. However, there is still a lack of

empirical evidence supporting such an assumption. This study investigated the trophic spaces of tadpoles in Ranomafana and examined how generalized and specialist feeders partition their trophic niche in streams.

Traditionally, the trophic ecology of tadpoles has been approached by numerous gut content studies (Altig et al. 2007, Whiles and Altig 2010). However, gut content analysis can be tedious and is limited to provide only a snapshot of the diet. Further, it has limited potential to distinguish between different feeding modes if food items are similar in appearance; gut content may not represent a consumer's range of potential food items (Whiles and Altig 2010). Where contents of the gut are identifiable, it can be unclear if the ingested items would eventually have been assimilated into biomass or excreted undigested. It is also doubtful that the snapshot nature of gut content surveys reflect longer-term dietary preferences, which could be further complicated if species exhibit ontogenic shift in their diet (Whiles and Altig 2010). Isotopes ^{13}C and ^{15}N allow for tracking transfers of organic carbon and nitrogen from living plants and detrital sources to primary consumers and predators. Stable isotope approach can provide an indirect evidence of a species trophic niche, niche overlap, and specialization vs. generalized feeding mode, and identification of percentage contributions of potential food items (Layman et al. 2012). Carbon isotope signatures are generally similar to those of their food sources (i.e., you are what you eat) (Whiles and Altig 2010). Nitrogen is used to assess trophic position, as ^{15}N shows a fairly consistent change with each trophic step (= 3.4 % increase in ^{15}N with each trophic set up from primary consumer to predator) (Whiles and Altig 2010). Because of the limitations of each method, combining gut content and stable isotope analysis has the potential to yield a better characterization of consumers' diet (Layman et al. 2012); but, such combination has rarely been applied in tadpole studies (Huckembeck et al. 2014). In this study, I tested the following hypotheses:

- (1) Tadpoles with generalized and specialized mouthparts occupy different trophic spaces
- (2) Tadpoles with similar mouthparts occupy the same trophic space, that is, the use of food resources is similar among tadpoles of the same ecomorphotype (Altig and Johnston 1989),
- (3) Tadpoles with generalized mouthpart, generalists, use a wide diversity of resources (Hoff et al. 1999). As a result, coefficients of variation in trophic space are expected to be higher in tadpoles with generalized mouthpart than in tadpoles with specialized oral structure.

3.2. Materials and methods

This study was carried out in Ranomafana National Park. The Park comprises 43,500 ha of continuous mid-altitude montane rainforest (500–1300 m a.s.l.) with an annual precipitation between 1700 and 4300 mm. Ranomafana is characterized by high amphibian diversity with 112 candidate species (Vieites et al. 2009) and harbors the richest tadpole community in the world (Strauß et al. 2010). Precipitations are high, with alternating periods of low and heavy rains. Mean water temperature was 18°C during the study. Among the 44 species and the 11 feeding guilds, the generalized mouthpart cluster is the most common and is recorded in 45% of all species (20 species). The other 24 species are clustered into 10 “specialized” feeding groups (Strauß et al. 2013)(Cf. chapter 2, Fig. 2 for details).

Sampling

I sampled tadpoles in 4 streams, two in primary forest and two at forest edge. Specimens were collected in Oct–Nov 2014 and March–April, Oct–Nov 2015 using nets of varying size dependent on the structure of the substrate. These replicates were within the same altitude range (the streams were nearly on equal elevation 900-1020 m a.s.l.). The streams were second-order streams and were 2.5–4 m width. I sampled tadpoles using nets in microhabitats that varied in structure (see previous chapter for details on sampling design).

The tadpoles were kept alive and were brought back to the laboratory in buckets filled with stream water. The tadpoles were sorted visually into series based on morphological differentiation. Because of the high number of species, we assigned series provisional numbers. I took specimen of each series after anesthetization by MS–222 (Tricaine methane-sulfonate), and took a fragment of tadpole tail that was used in DNA analysis for species identification. DNA barcoding was based on a fragment of the mitochondrial 16SrRNA gene (modified 16Sar [5'-CGCCTGTTTAYCAAAAACAT-3'] and modified 16Sbr [5'-CCGGTYTGAACTCAGATCAYGT-3']). PCR and sequencing methods followed Bossuyt and Milinkovitch (2000) and PCR products were prepared for sequencing using BigDye Terminator sequencing chemistry (Applied Biosystems, CA, USA). These analyses were conducted at Nagoya University (NU Gene Center and Graduate School of Bioagricultural Science).

Diet analysis

Gut content analysis

Tadpole individuals, from which a fragment of tail was taken, were preserved in formalin (10 %). Two to three tadpoles of each species were dissected and gut lengths were measured with a digital caliper (care was taken not to stretch the guts). Tadpole gut contents from 10-mm sections of the gut were flushed, and their contents mixed. The flushed liquid containing small particles were sonicated and then mounted on gridded plankton counter slide. Using a compound microscope at 100–200–400x magnification, I identified and measured the area of all visible items in 20 randomly chosen grids. Percentage contribution of for each item is quantified by dividing the area occupied by the item in the 20 grids by the summed area of all food items (Whiles and Altig 2010). Measurements of individual particles were done on ImageJ (<http://imagej.nih.gov/ij/>). Food items were categorized as detritus (plant particles, debris), unidentifiable items, insect fragments, diatoms, sediments, loams, fungi, rotifer, protozoa, and algal materials.

Stable isotope analysis

I followed standard methods described in Whiles and Altig (2010). I collected between 3 to 30 individuals of each species for stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes (Cf. Table 1). Sample sizes depended on rarity, as some species were not as abundant than others. One crucial component of sampling was to use tadpoles of the same developmental stage ($< \text{Gosner } 32$) to minimize possible biases associated with ontogenic diet shift, as reported in some tadpoles (Wickramasinghe et al. 2007). This allowed approximating the trophic space of species irrespective of their development stage.

The tadpoles were euthanized by immersion in MS–222 solution. The anesthetic MS–222 is commonly used for euthanizing aquatic organisms and no effects have been reported on stable isotope measurements (e.g., Arribas et al. 2015). I determined carbon and nitrogen isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of tail muscle of tadpoles. Comparisons of C and N isotope values allow estimating the relative trophic position of individuals within a food web. It should be noted, however, that although N gives the relative position of the tadpoles, it does not imply that the species studied represent predator-prey system.

Collection of food resources

Potential food resources of the tadpoles were collected in streams. I sampled detritus, fine particulate organic matter (FPOM), periphyton, leaf litter, sediments, and macrophytes.

Periphyton samples were scraped from rocks. Detritus and sediments were collected by gently sucking deposited materials using a turkey baster. Because identification is challenging for tropical dead leaves, we took the two most abundant leaf litter species found in stream. FPOM was collected with plankton net that was set in riffle for one hour. Macrophytes represented plant materials that grew on boulders. The number of samples is given in Table 1.

Sample processing and data analysis

Tadpole tissue samples and food resources were oven-dried at 60 °C, ground to a fine and homogenous powder using mortar and pestle. Lipid was removed from tadpole tissue samples prior to analysis (Logan and Lutcavage 2008). I weighed 0.5 ± 0.01 (mean \pm SD) mg of amphibian muscle, 2.00 ± 0.1 mg of periphyton algae, 2.00 ± 0.1 mg of detritus, 1.5 ± 0.04 mg of macrophytes, and 2.00 ± 0.1 mg leaf litter, and placed them into tin capsules for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations. Analyses were performed using a continuous-flow isotope-ratio mass spectrometer (Thermo Scientific™ *DELTA V Advantage*) at the University of California Davis (California, USA) and at the Research Institute for Humanity and Nature (Kyoto, Japan). Results of the analysis were expressed in standard delta denotation, which is parts per thousand deviation from a standard, calculated as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000 \text{ where } R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}$$

A positive or less negative C value indicates the sample is enriched with the heavy isotope.

I conducted linear mixed effect models to investigate whether species occupy similar trophic space with “location” as random factor and C-N isotope values as response variables. “Species” was the dependent variable. *P*-values were obtained by likelihood ratio test of the full model with the effect in question against the model without the effect of explanatory variables using the function “anova”. Upon significance, I ran pair-wise differences with the function “lsmeans” (Lenth et al. 2015) with Tukey adjustment after bootstrapping method using the package “pbkrtest” (Halekoh and Højsgaard 2014). Species were assumed to represent different trophic groups if they had significantly different mean $\delta^{13}\text{C}$ and/or mean $\delta^{15}\text{N}$ values. Data were log transformed before analysis (carbon isotope values were turned into positive before log-transformation).

To test whether generalists would exhibit higher trophic space variation than specialists, I computed the coefficient of variation (CV) of five species with generalized mouthpart (*B.madagascariensis*, *B. quasiboehmei*, *B. reticulatus*, *B.tasymena*, *M. sp28*) and

five species with “specialized mouthparts” (*M.majori*, *B. picturatus*, *S. aglavei*, *M. sp47*, *M. melanopleura*) for which I could have a reasonable sample size ($N > 9$ individuals). A higher CV indicates that a species cohort (thus a mouthpart type) show considerable individual variation in diet. To the converse, a lower CV indicates that the individuals within a cohort have nearly similar trophic space, thus less variation in carbon and nitrogen isotope values. I conducted independent samples Welch t-tests to test difference in CV between “generalists” and “specialists”.

I conducted Non Metric Multidimensional Scaling to evaluate similarities between the diets of tadpoles, using a matrix of relative frequency (in percentage) of food items. The ordination was constructed from a Bray–Curtis dissimilarity. In NMDS, distances indicate degree of similarity between elements; that is, closer elements share more similarities than more distant ones (see previous chapter for details on this method). NMDS was performed with function “metaMDS” from R package *vegan* (Oksanen et al. 2016). All statistical tests were performed in R 3.12. Figures were made on SPSS 17 and PAST 3.

3.3. Results

I obtained the isotopic values of 18 species belonging to eight mouthpart clusters, and analyzed gut contents of 16 species of tadpoles (Table 1).

Species trophic spaces

Isotope values differed between food resources in carbon $\delta^{13}\text{C}$ (Chi-square = 132.53, $df = 7$, $P < 0.001$) and in nitrogen $\delta^{15}\text{N}$ (Chi-square = 40.43, $df = 7$, $P < 0.001$). Periphyton, FPOM and sediment were similar in carbon $\delta^{13}\text{C}$ (all pairwise comparisons, $P > 0.05$). Carbon isotope values of macrophytes were highly variables and ranged from -31.78 ‰ to -20.99 ‰ in forest and in open habitats, respectively.

The tadpoles differed in trophic space in carbon $\delta^{13}\text{C}$ (Chi-square = 303.53, $df = 17$, $P < 0.001$) and nitrogen isotope $\delta^{15}\text{N}$ signatures (Chi-square = 143.29, $df = 17$, $P < 0.001$) (Fig. 1). Major differences were observed in $\delta^{13}\text{C}$ (Fig. 2) values as many species had nearly similar $\delta^{15}\text{N}$ values, except for one distinct species, *Boophis picturatus* sand-eater that occupy higher trophic level (Fig. 3, all pairwise differences in $\delta^{15}\text{N}$ between this species and other species were significant, $P < 0.001$). Interestingly, species within the same mouthpart cluster did not necessarily occupy similar trophic space. For example, tadpoles with generalized morphology (*Boophis*-generalized) *B. luteus*, *B. quasiboehmei*, *B. tasymena*, *B. madagascariensis* occupied similar N position but all differed in their carbon trophic spaces

(Fig. 2). However, this was not the case for the two *Spinomantis* species (*S. aglavei* and *S. peracca*, *Spinomantis*-generalized) that occupied similar trophic C and N spaces (Fig. 1–3).

Individual diet variation in generalist and specialists

There were no differences in coefficient of variation neither in $\delta^{13}\text{C}$ ($t = 0.79$, $df = 6.19$, $P = 0.45$) nor in $\delta^{15}\text{N}$ ($t = -0.02$, $df = 7.43$, $P = 0.97$) trophic spaces between tadpoles with generalized mouthpart and tadpoles with specialized mouthpart. However, for both clusters, there was more variation in N space than there was in C signatures among individuals of the same species ($t = -6.36$, $df = 17.77$, $P < 0.001$) (Fig. 4). This suggests that individuals of a species could feed on resources that are more variable in N than in C contents.

Gut contents

The general stress of NMDS was 0.082 indicating a good fit of the data. Consistent with stable isotope results, mouthpart did not necessarily predict the type of ingested food resources (Fig. 5). Sediment was ubiquitous in all tadpoles but was ingested at different proportions (Fig. 5). Although there was a tendency for species to aggregate together, three species with specialized mouthpart that shared less commonality in diet with the other species could be distinguished (Fig. 6). These species are *M. majori* (reduced teeth), *M. melanopleura* (funnel mouthed), *B. picturatus* (sand-eater). *M. melanopleura* is a suspension feeder that specialize in floating particles in streams and, thus the absence of minerals in its gut was not surprising. The foregut of *B. picturatus* (sand-eater), to the converse, is dominated by sand particles (58%). The other food items consisted of algal materials, sediment and unidentified materials. The gut of *M. majori* contained an important fraction protozoa (32%) and minerals (44%). For three tadpoles of generalized mouthpart (*B. quasiboehmei*, *B. elenae*, *B. reticulatus*), diatoms represented an important part of the gut content at no less than 23% (Fig. 5).

3.4. Discussion

Understanding how and why so many species can coexist in the same habitat has been a long-term interest in ecology. Despite some reservations (Hubbell 2006), resource partitioning still represents a central tenet that explains community structure (Levine and HilleRisLambers 2009). I found that tadpoles differed in their resource use, as revealed by stable isotope and gut content analyses, suggesting resource partitioning among sympatric species, one prerequisite for species coexistence (Levine and HilleRisLambers 2009). Although some species occupied similar trophic space as suggested by stable isotope analyses,

gut content examination revealed that these species, indeed, differ either in their diet or extract resources at different proportions (Fig. 5–6). In this respect, combining the two methods (gut content and stable isotope analysis) yields better results than performing one method alone to examine the trophic ecology of tadpoles.

The results indicate that most tadpoles are primary consumers, for which, in general, $\delta^{15}\text{N}$ values range from 3 to 6 (Whiles and Altig 2010). Trophic spaces greatly varied along the carbon axis (range: -19‰ to -32‰) but did not show much difference along the nitrogen axis (range 3.14‰ – 8.05‰). That many species occupied similar trophic position in N was rather expected for two reasons. First, there could be less variation in N in the basal resources consumed by the tadpoles (Kupferberg 1997b). Second, many tadpoles are thought to be primarily herbivorous resulting in lower N variation, but such an assumption has been put into question lately (Altig et al. 2007). Gut content analysis indicates that tadpoles feed on protozoa, fungi, insect fragments, algae, detritus and sediments; and, the trophic position of tadpoles may be influenced by the respective contribution of these items in their diet. Because gut content does not allow making inference of what tadpoles eat in the long term (and what materials were indeed assimilated), it is possible that these tadpoles feed on resources that were not recorded here, all the more so tadpoles may exhibit spatio-temporal changes in their diet (Altig et al. 2007). Gut content analysis allowed, however, having a snapshot of what tadpoles eat allowing a quick diet comparison between species (Whiles and Altig 2010).

Tadpoles occupied nearly similar trophic levels (as indicated by N isotope values) except for the sand eater tadpoles, *B. picturatus* (highest value). These results are consistent with a recent study of Vences et al. (2016) that reported on the trophic position of *B. marojezensis*, *B. narinsi*, *B. picturatus*, *B. reticulatus*, and *M. melanopleura*. In this study, however, *B. narinsi* had the lowest N isotope values among the tadpoles while Vences et al. (2016) suggested that the species could occupy the same trophic level as *M. melanopleura*. Knowing the development stage of the tadpoles in Vences et al.'s experiment may clarify such a discrepancy because trophic level in tadpole can increase with development stage (Wickramasinghe et al. 2007).

The tadpole of *B. picturatus* has a peculiar oral morphology that is unique amongst anuran larvae (Grosjean et al. 2011). High nitrogen isotope value suggests potential carnivory in *B. picturatus*. But, such an assumption is not consistent with gut content analysis. Sand accounted for a large part of the gut, and only a short section of the foregut (~1 cm anterior

portion) contained organic matters that were composed of mixture of algae and unidentified items. *B. picturatus* has been suggested to feed on micro organisms (e.g., miniature metazoans living in between and attached to sand grains) living in the sand by passive filtering, and they seem to ingest sand for aiding digestion (Grosjean et al. 2011). I did not record any traces of animal materials in the gut, but it is possible that either animal materials could have already been digested within the few cm of gut or the unidentified items could be highly nutritious materials that may explain the high N value in this species. As Grosjean et al. (2011) noted, sand particles are mixed with organic matter in the anterior but not in the posterior part of the gut, suggesting that the organic matter is largely digested during its passage through the intestine (personal observation). Another hypothesis holds that food organic materials could have increased in $\delta^{15}\text{N}$ during microbial decomposition under oxic condition (Lehman et al. 2002), which could have been the case in riffles where the species mostly occurs. But, I could not test such a pathway in this study. Interestingly, diatoms were little represented, which was surprising because in other tadpoles diatoms constituted a considerable fraction of gut content.

A second noticeable feeding guild was the group of suctorial tadpoles (*B. marojezensis* and *B. andohahela*) that are characterized by large oral disk in a nozzle-shape, which is adapted to stream with relatively strong current (Wells 2010). Along with two tadpoles with generalized morphology (*B. sp37* and *B. narinsi*), that are typically found in deep streams in open canopy habitats (Cf. Chapter 2), these species had relatively higher C isotope values. Carbon isotopes of primary producers can greatly vary in lotic environments and typically increase with light intensity (Hill et al. 2008). Changes in resource isotope values could have influenced the carbon values of these tadpoles (“you are what you eat”). This mechanism may not operate in all species, however. It is particularly intriguing to observe that *B. luteus*, which is sympatric with the afore-mentioned tadpoles, had low carbon signature values (-32‰). Gut content analysis suggests that *B. luteus* has little commonality in diet with the two species, and not only this difference in food habits but also dissimilarity in microhabitat preference might explain these differences. Two microhabitat properties, in particular, can lower $\delta^{13}\text{C}$ in basal resources: low light environment (Hill et al. 2008) and water velocity (Finlay et al. 1999; Fry 2007). It was not clear, however, whether *B. luteus* would have affinity for the two afore-mentioned microhabitat variables.

Gut content analysis in *B. marojezensis* in this study indicates that sediments occupy a considerable volume of their gut. This finding is not consistent with the results of Vences et

al. (2016) who suggested that this species specializes on diatoms (99 %). I could not find any explanations for this contrast because in my analysis, I found that all tadpoles ingested sediments, even at low proportion.

M. majori (reduced teeth mouthpart) contained considerable amount of protozoan in its gut indicating some degree of specialization in this species. I expected that this species would occupy higher trophic level because of the amount of protozoa in their gut but it is possible that these items were poorly assimilated. Protozoans are typically poor resources that contribute very little to growth (DeBiase et al. 1990).

In contrast to expectations and to a previous assumption (Altig and Johnston 1989), species within the same cluster did not necessarily occupy similar trophic spaces, suggesting that diet could not simply be predicted by mouthpart. This pattern was observed not only in tadpoles with generalized mouthparts (e.g., *B. luteus*, *B. periegetes*, *B. quasiboehmei*, *B. tasymena*, *B. sp37*) but also in species with specialized oral structure (suctorial mouthpart, *B. andohahela*, *B. marojezensis*). This result is not consistent with the pattern observed in other systems either in which close relationship often exists between ecomorphotype and diet (e.g. Grant and Grant 2006, Pessanha et al. 2015, Sampaio et al. 2013). Three possibilities may explain this weak relationship between mouthpart and the corresponding diet in these tadpoles. First, it is possible that there are simply inherent differences in food preference between species of the same mouthpart (Fig. 5–6). The second possibility could be related to interspecific differences in microhabitat choice (Cf. previous chapter), thus in the foraging environment and in the available resources (Altig et al. 2007). A third hypothesis holds that tadpoles are phenotypically plastic and are able to shift their trophic space in the presence of competitors (Arribas et al. 2015; Caut et al. 2012).

There was no difference in coefficients of variation in trophic space among generalists and specialists. Typically, tadpoles with generalized morphology, which are omnivorous generalist feeders (this study; Altig et al. 2007; Hoff et al. 1999; Schiesari et al. 2009), are suggested to be heterogeneous collections of relatively specialized individuals (Bolnick et al. 2002). Thus, I expected a larger coefficient of variation in tadpoles with generalized mouthparts; to the converse, I expected specialists to feed on a narrow set of resources (according to definition of specialist) that would result into low coefficient of variation in their N and C trophic spaces. Whether specialists are as plastic as generalists is still poorly understood, but the findings here and a previous study (Costa et al. 2015) suggest that even specialists can exhibit a high level of diet plasticity and generality in diet. It should be noted,

however, that resource variation in carbon and in nitrogen isotope values could have influenced these results. Although gut content analysis only provides snapshots of a consumer's diet, it can give insights into the degree of diet breadth in these tadpoles. Indeed, except for sand-eater tadpoles, the other specialist clusters appear to feed on a wide variety of resources (Fig. 5). A higher diet breadth should increase species resilience to competition and should allow ecologically more similar species coexist (Forister et al. 2015).

Conclusions

Coupled with environmental filter (Chapter 2), resource partitioning can help understand how and why members of species rich communities can stably coexist. Competition and ecological opportunity are thought to represent the driving forces of phenotypic diversification in species rich ecosystem (Wagner et al. 2012). Such an assumption has found support in this system (Strauß et al. 2016), suggesting that interspecific competition drove and maintains difference in mouthpart between these species of tadpoles. The ability of tadpoles to shift their diet in the presence of competitors has been documented and could represent an interesting pathway allowing species with considerable overlapping ecological traits to coexist in the nature. I could not isolate the effects of plasticity on tadpoles' trophic space, but, at least, these results indicate that the tadpoles differed in feeding niche in streams.

Broadly, niche partitioning can help understand the impact of species extinction on ecological processes. If species show great differences in resource partitioning, when one species is lost, so too is the ability of other groups to exploit the resources that the extinct species used to use. Increasing lines of evidence suggest that species loss reduces the levels of ecosystem processes. Resource partitioning is suggested to play an important role in causing such effects (through complementary effects), although ecologists are only just beginning to directly test this. We will explore the functional role of tadpoles in the next chapter.

3.5. Figures

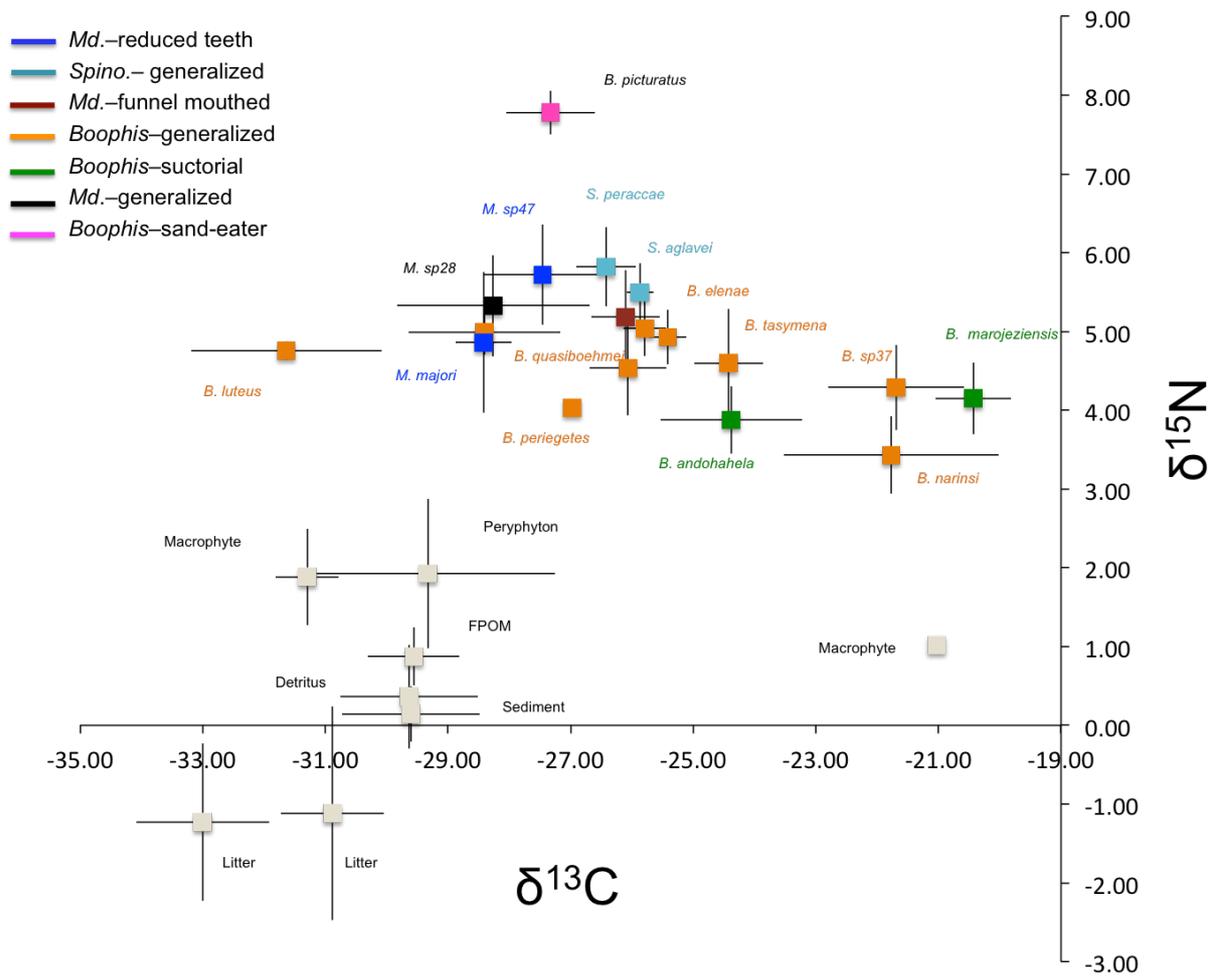


Figure 1. Trophic spaces of tadpoles. Different colors mean different mouthpart cluster. In grey are basal resources. Values are mean \pm SD of stable C and N isotope.

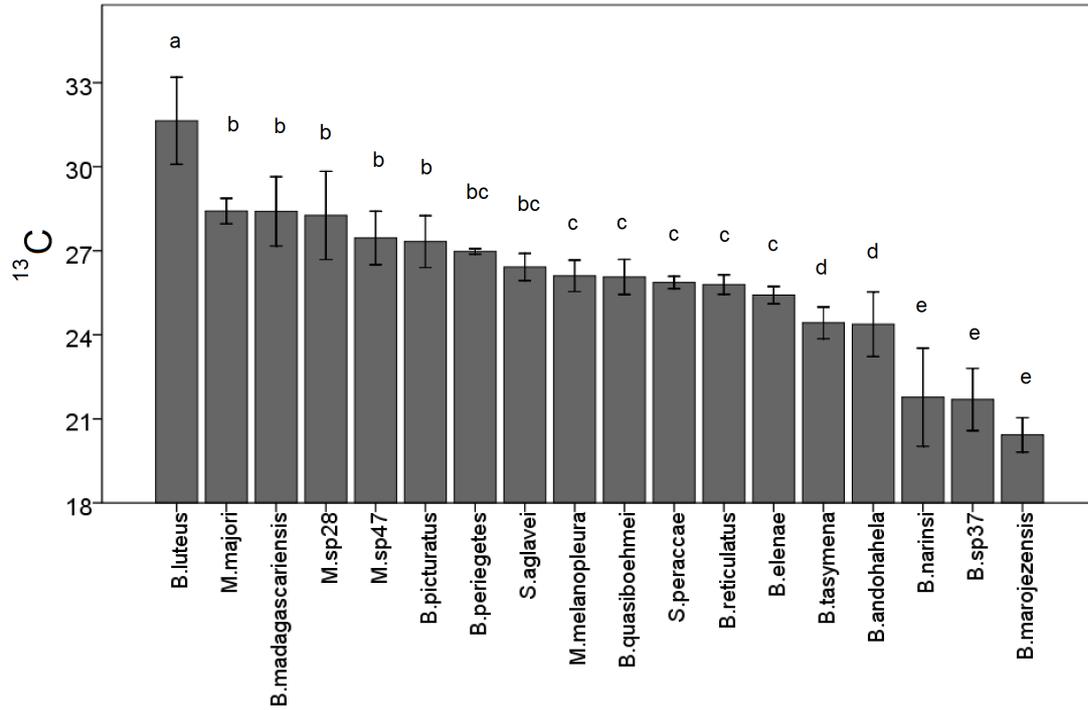


Figure 2. Carbon isotope values of 18 tadpoles in Ranomafana streams. Different letters mean significant differences between species. Values represent mean \pm SD

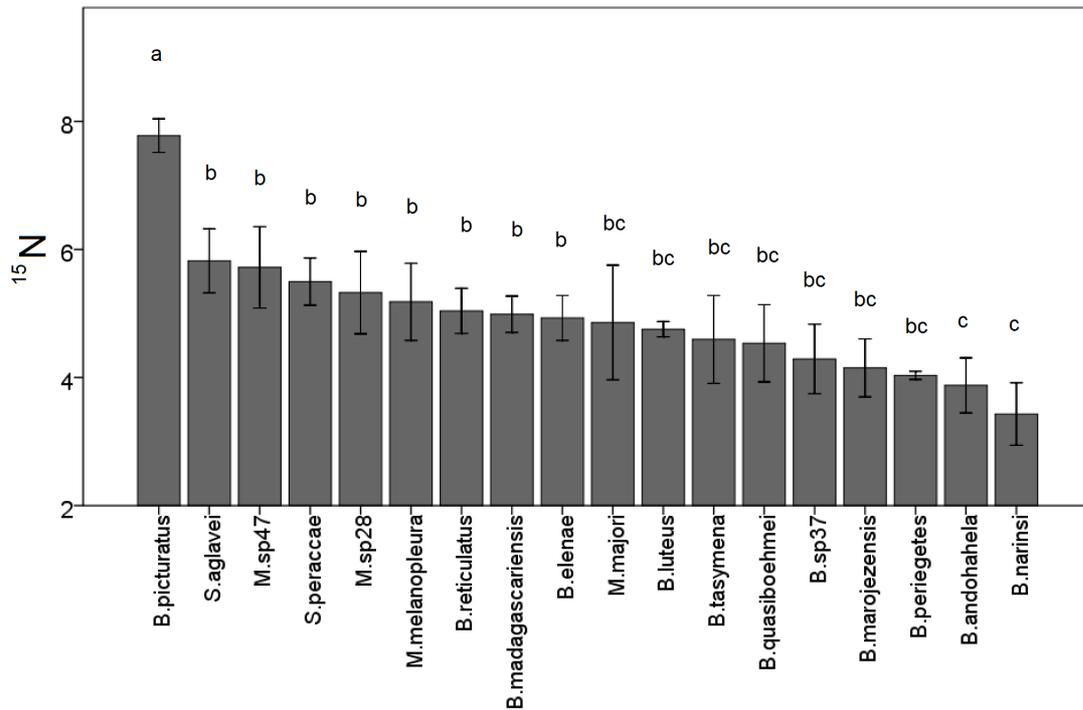


Figure 3. Nitrogen isotope values of 18 tadpoles in Ranomafana streams. Different letters mean significant differences between species. Values represent mean \pm SD

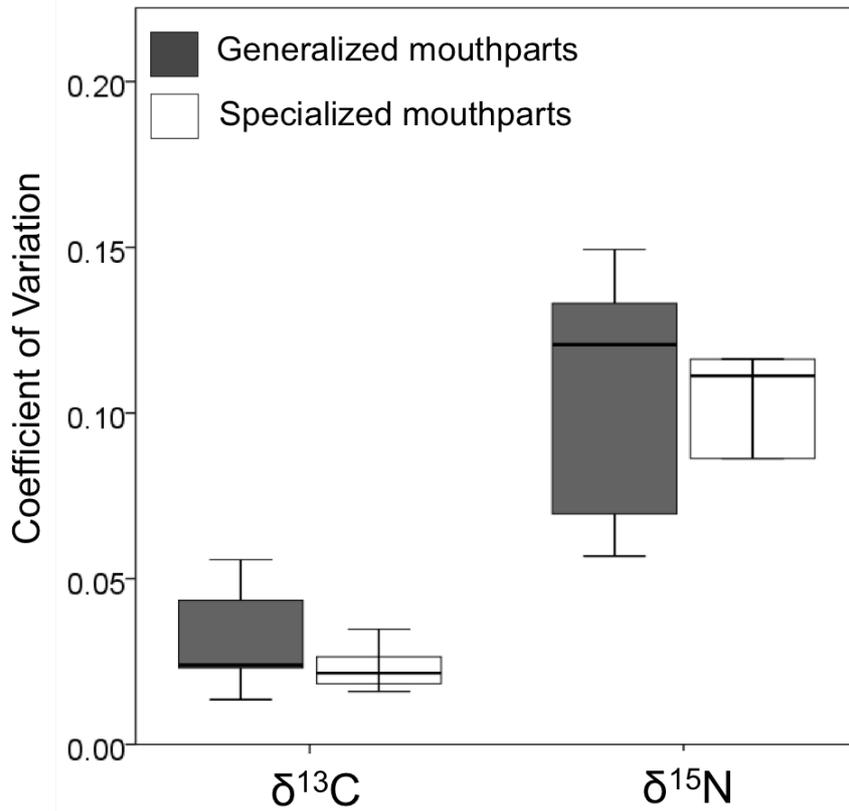


Figure 4. Coefficient of variation in carbon and nitrogen isotopes (trophic spaces) in tadpoles of generalized and specialized mouthparts. N=5

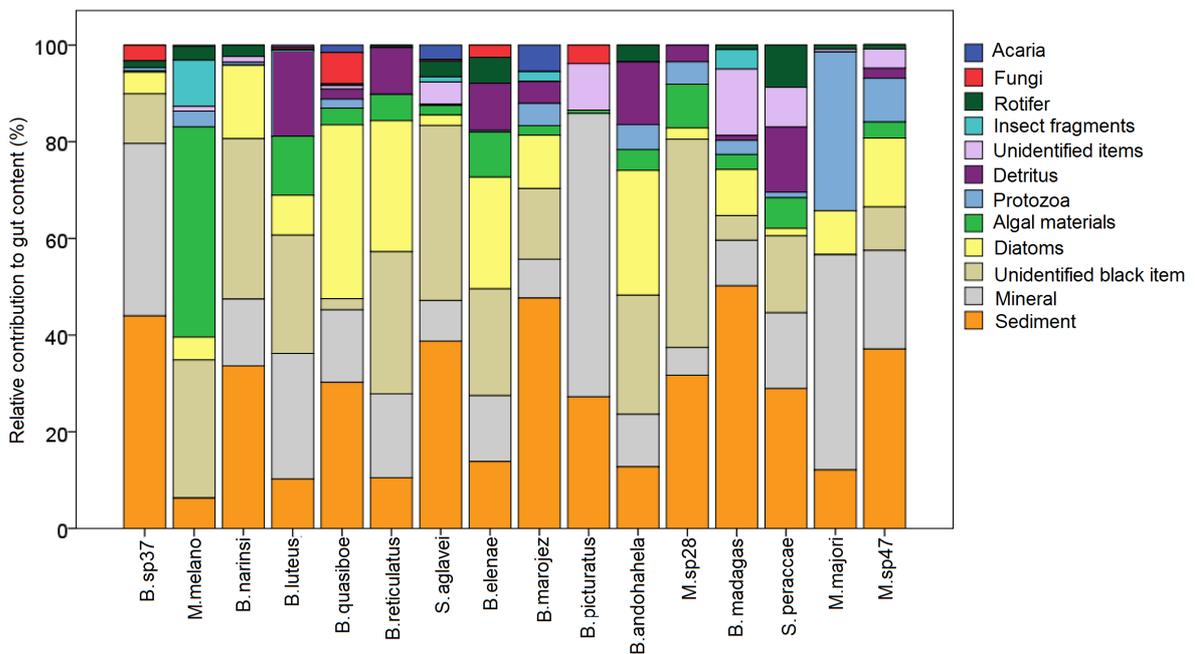


Figure 5. Gut content analysis of 16 species of tadpoles. Numbers represent the relative contribution of each food item in tadpoles' gut.

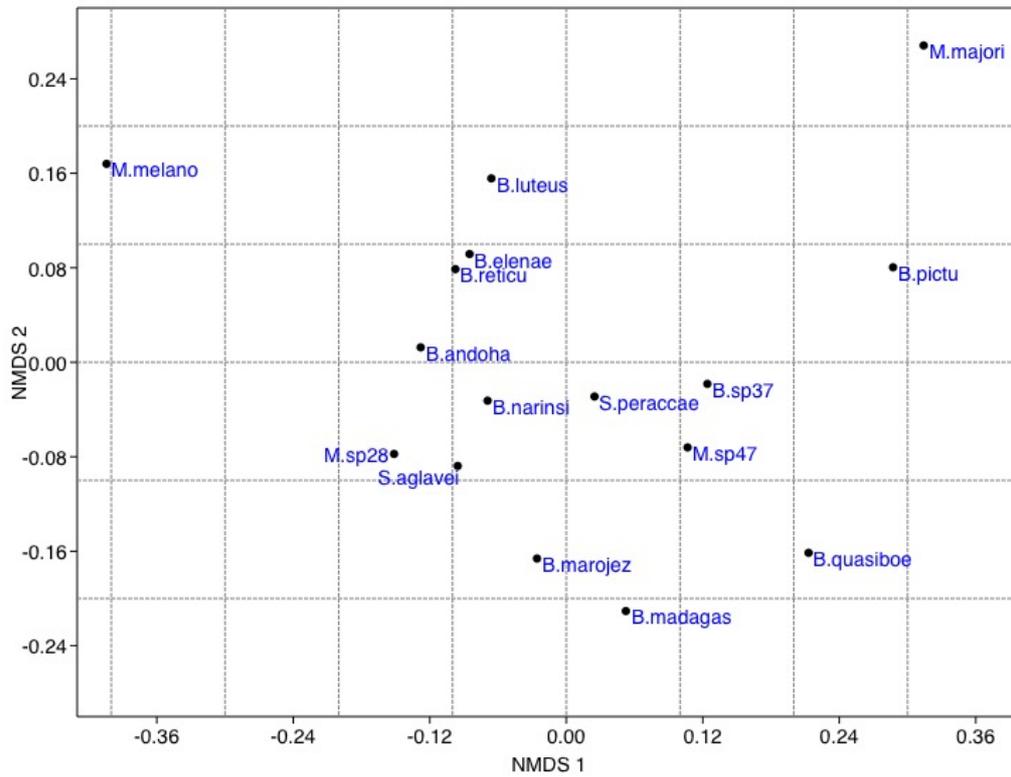


Figure 6. (Dis-)Similarity in gut content of tadpoles found in Ranomafana streams. Closer elements are more similar than more distant ones. Ordination is based on NMDS with Bray-Curtis distance.

3.6. Tables

Table 1. Tadpole species analyzed for stable ^{13}C and ^{15}N isotopes and gut content. Lower part of the table represents tadpole food resources analyzed for stable isotopes. Numbers represent sample size.

Tadpole species	N	Gut analysis	Mouthpart cluster
<i>Boophis andohahela</i>	9	Yes	Suctorial
<i>Boophis elenae</i>	6	Yes	Boophis–Generalized
<i>Boophis luteus</i>	3	Yes	Boophis–Generalized
<i>Boophis madagascariensis</i>	11	Yes	Boophis–Generalized
<i>Boophis marojezensis</i>	3	Yes	Suctorial
<i>Boophis narinsi</i>	3	Yes	Boophis–Generalized
<i>Boophis periegetes</i>	3	No	Boophis–Generalized
<i>Boophis picturatus</i>	12	Yes	Sand-eater
<i>Boophis quasiboehmei</i>	26	Yes	Boophis–Generalized
<i>Boophis reticulatus</i>	15	Yes	Boophis–Generalized
<i>Boophis sp. 37</i>	6	Yes	Boophis–Generalized
<i>Boophis tasymena</i>	13	No	Boophis–Generalized
<i>Mantidactylus majori</i>	11	Yes	Reduced teeth
<i>Mantidactylus melanopleura</i>	30	Yes	Funnel mouthed
<i>Mantidactylus sp. 28</i>	9	Yes	Md.–Generalized
<i>Mantidactylus sp. 47</i>	14	Yes	Md.–Reduced teeth
<i>Spinomantis aglavei</i>	11	Yes	Spinomantis–Generalized
<i>Spinomantis peraccae</i>	6	Yes	Spinomantis–Generalized
Basal resources			
Detritus	21		
FPOM	16		
Periphyton	21		
Sediment	8		
Leaf 1	20		
Leaf 2	22		
Macrophyte 1 (forest habitat)	3		
Macrophyte 2 (edge habitat)	3		

Chapter 4: Phenotypic divergence and functional roles: differential effects of tadpoles on nutrient recycling and litter decomposition in a tropical rainforest stream

Abstract

There is growing interest in predicting how loss of species diversity and abundance affects the structure and functioning of ecosystems. Tadpoles can be highly diverse in their morphology and reach important biomass in tropical streams, but little is known about their functional roles. Whether tadpoles are functionally distinct or merely redundant remains elusive. I evaluated the contribution of morphologically distinct tadpoles to nutrient recycling (excretion and egestion) and to litter decomposition, two of the most important processes in stream ecosystems. I first assessed nutrient recycling in ammonia and in total dissolved phosphorus of six ecomorphotypes (suctorial, sand-eater, *Boophis*-generalized, fossorial, *Spinomantis*-generalized, funnel mouthed) in Ranomafana, Madagascar. I subsequently analyzed the effect of tadpole diversity and abundance on litter decomposition using two tadpoles belonging to the most common feeding groups in streams: *Boophis quasiboehmei* (hereafter BQ, generalized mouthpart, grazer) and *Mantidactylus melanopleura* (hereafter MM, funnel mouth, suspension feeder). I conducted field incubation and enclosure experiments to test the hypotheses 1) that because of difference in mouthparts, species differentially recycle nutrients; 2) that effects of tadpoles on litter decomposition depend on leaf toughness, and are commensurate with tadpole density and diversity. I used leaf mass loss as indicator of leaf litter decomposition in 9 treatments: control (no tadpoles), monospecific treatment (BQ or MM) at three densities (low, medium, high: 3, 6, 12 tadpoles/0.0625 m², respectively), mix species treatment (3 BQ + 3 MM, 6 BQ + 6 MM). Species differentially recycled nutrients in ammonia and in total dissolved phosphorus. MM excreted nutrients (ammonia-N and total dissolved phosphorus (TDP)) at higher rates than BQ. Nutrients recycled from feces (egestion) were not directly available to the ecosystem but needed to be mineralized: dissolved ammonia increased with time in the water column, whereas TDP showed hump-shaped pattern with a maximum value at day 4 of incubation. Effects of tadpoles on leaf decomposition depended on tadpole density and on leaf toughness. No decomposition occurred at low density and effects were stronger on soft leaves. Effects of MM were observed only at high density, whereas BQ influenced litter decomposition at

medium and at high densities. There were no additive effects of tadpole diversity on litter decomposition, indicating no facilitation, which could be because BQ reduced the effect of MM when together. The results indicate that 1) tadpoles are not functionally redundant in streams 2) fecal pellets are important latent sources of nutrients in freshwater environments 3) species abundance exerted stronger effects than species diversity on litter decomposition, contrasting classical predictions of biodiversity-ecosystem functioning experiments.

4.1. Introduction

Biodiversity-ecosystem functioning experiments have established that ecosystem functions typically increase with species richness (reviewed by Tilman et al. 2014). Hypothetically, increased diversity of phenotypic traits in an assemblage is thought to improve overall mixture performance through complementarity effects (Cardinale et al. 2007). Some models, however, have highlighted that more than diversity species identity (dominant and/or common species) and abundance (biomass) may play more significant roles in delivering real-world ecosystem services (Winfree et al. 2015). 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 unprecedented rates (Dudgeon et al. 2006).

Tadpoles can reach high diversity and represent the largest 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 et al. 2007). Earlier studies have established that tadpoles are ecosystem engineers and can strongly influence community structure, production of aquatic macroinvertebrates, and organic matter dynamic (Colón-Gaud et al. 2008; Colón-Gaud et al. 2010a; Colón-Gaud et al. 2010b; Flecker et al. 1999; Mallory and Richardson 2005; Ranvestel et al. 2004; Wood and Richardson 2010). While their influences on some ecosystem processes have been quantified to some degree, we know far less about the role of tadpoles in nutrient recycling (Vanni et al. 2002) along with their ability to decompose leaf litter in tropical streams (Iwai et al. 2009; Rugenski et al. 2012), that are two integral parts of ecosystem functioning.

Consumer-mediated nutrient cycling is a key mechanism driving nutrient turnover and primary productivity in many ecosystems (Vanni 2002). In general, the quality of nutrients recycled by an animal should depend on the quality of diet (trophic level), resource

assimilation efficiency and body elemental composition, or the combination of these (Vanni and McIntyre 2016). Recycled nutrients can be excreted in dissolved form (excretion) or in the form of fecal pellets (egestion). Ecologists have begun to appreciate the contribution of consumer-mediated nutrient recycling through excretion in aquatic ecosystems (reviewed by Vanni 2002), but the contribution of egestion has remained relatively less explored (Wotton and Malmqvist 2001). Tadpoles potentially produce high amount of feces through their continual feeding (Jenssen 1967), and their egestion could greatly contribute to ecosystem functioning through nutrient recycling. Scarce evidence suggests that, in a pond-dwelling tadpole, 75–92 percent of nutrients (mostly P) are recycled in the form of fecal pellets and only 8 to 25% in dissolved form (i.e., excretion, mostly N) (Liess et al. 2015). But, nutrients from fecal pellets are not readily available to the ecosystem and must be mineralized (Liess and Haglund 2007). This suggests that animal egestion could represent an important, but latent, source of nutrients in freshwater environments.

Litter decomposition is a central component in food webs (reviewed by Graça et al. 2015) and can strongly influence the structure of freshwater communities (Stoler and Relyea 2016). Tadpoles may directly affect litter decomposition by consuming them (Ramamonjisoa et al. 2016a), or indirectly by enhancing microbial communities through their excretory products (nutrient recycling) (Rugenski et al. 2012). Previous studies revealed that, when alone, tadpoles only play minor effects on litter decomposition (Connelly et al. 2011; Rugenski et al. 2012), but they can considerably facilitate the process by enhancing microbial activities, making leaves more palatable to shredder macroinvertebrates (tadpole-shredder system) (Iwai et al. 2009; Rugenski et al. 2012). Decomposition through consumption has been thought to be negligible because of the limitation of tadpole jaws to penetrate leaf litter (Iwai et al. 2009). However, studies testing relationships between food choice and food toughness (Ramamonjisoa et al. 2016b; Taylor et al. 1995) have suggested that the ability of tadpoles to feed on and decompose leaf materials may strongly depend on leaf toughness. Although leaf decomposition itself is habitually the result of interactions between various traits, such as leaf chemistry and cellulose concentration (reviewed by Bakker et al. (2011) and Stoler et al. (2016)), toughness still represents one of the most important leaf traits determining decomposition and availability of nutrients to decomposers (Pan et al. 2015).

The abundance and diversity of tadpoles can vary greatly in time and space in tropical streams (Strauß et al. 2016). Yet, so far, most knowledge about their functional roles stems from studies that manipulated one species (i.e., one functional trait) and, usually, at one

density treatment. Anuran larvae are often highly diverse in their morphology, especially in their oral structures (Strauß et al. 2013). Because mouthpart may influence feeding behavior and trophic position (Chapter 3), it is possible that tadpoles are not functionally redundant. Thus, only examining one functional group may not fully capture the effects of tadpoles on freshwater ecosystem. Studies that examine density and diversity of species (i.e., functional diversity) would allow for more accurate appreciation of the functional roles of tadpoles and the potential ecological consequences of their loss on ecosystem functioning.

In this study, I examined the functional roles of tadpoles in a rainforest stream in Madagascar. I quantified the contribution of tadpoles to nutrient recycling through excretion and egestion. I subsequently tested the effects of tadpole density and diversity on litter decomposition. We tested the following hypotheses:

(H1) Tadpoles (with dissimilar mouthparts) differentially recycle nutrients in N and P.

(H2) Phosphorus is mostly recycled in pellets; nutrients from feces are not readily available to the ecosystem and must be mineralized.

(H3) Leaf decomposition depends on its toughness, and increases with tadpole density and diversity (i.e., tadpoles are not functionally redundant).

4.2. Materials and methods

This study was conducted in Ranomafana National Park Madagascar in October - November 2015. The designated park consists of 43,500 ha of continuous moist humid forest (mid-altitude 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 °C) to highs in December – February (36–40 °C) (Wright and 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 (personal observation, November 2014). Because of poor fish fauna, tadpoles represent the largest biomass in Ranomafana streams and hypothetically play crucial roles in ecosystem functioning (Strauß et al. 2010).

I recorded 27 tadpole species (Cf. chapter 2) belonging to five genera: *Mantidactylus*, *Boophis*, *Guibemantsi*, *Gephyromantis*, and *Spinomantis*, and seven mouthpart groups (following the classification proposed by (Strauß et al. 2013): sand-eater, reduced teeth,

generalized, podgy generalized, fossorial, suctorial, and funnel mouthed. Among these, generalized and funnel-mouthed groups account for more than 70 % and 10 % in abundance, respectively of all tadpoles. The other feeding groups represent less than 5 % of tadpole abundance (Ramamonjisoa, unpublished data).

Nutrient recycling from excretion

I quantified nutrient recycling rates (excretion in ammonia-N and total dissolved phosphorus) of six species belong to six morphotypes. These are *Boophis andohahela* (suctorial mouthpart), *Boophis quasiboehmei* (generalized mouthpart), *Boophis picturatus* (sand-eater), *Mantidactylus melanopleura* (funnel mouthed), *M. sp47* (fossorial), *Spinomantis aglavei* (*Spinomantis*-generalized). I 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 nets in streams; upon capture, animals were quickly sorted and placed in distilled water to “wash” the tadpoles (removing attached nutrients and particles). The tadpoles were subsequently placed in plastic transparent containers (N = 4–6 replications depending on tadpole rarity) (~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 streams to reduce 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 small bottles, frozen and transported on ice to the University of Nagoya. Colorimetric analyses determined N concentration as ammonia $\text{NH}_3\text{-N}$ and total dissolved phosphorus (TDP) by ascorbic acid method following Vanni (2002) and Vanni et al. (2002). TDP was preferred to the common soluble reactive phosphorus (SRP) because of low detection of SRP in our samples (Vanni et al. 2002). Rate of ammonia and TDP excretions were calculated as the amount of nutrients released in the surrounding water per g wet mass tadpoles per hour.

Nutrient recycling from egestion

Nutrient recycling from egestion was evaluated by incubating a known amount of pellets and by quantifying the concentration of nutrients released in the water column (Liess and Haglund 2007). I used two of the most abundant species in streams, from which I could

collect adequate amount of pellets to incubate. *Boophis quasiboehmei* (hereafter BQ, generalized mouthpart) and *Mantidactylus melanopleura* (hereafter MM, funnel-mouthed mouthpart,) are two dominant tadpoles in Ranomafana streams and account for 40 % and 9 % of tadpole species, respectively, in October - November (Ramamonjisoa and Natuhara, unpublished). Little is known about the feeding ecology of these tadpoles. Data from temperate tadpoles suggest that tadpoles with generalized mouthpart are generally grazers, omnivores that feed on a variety of animal and plant resources (Whiles and Altig, 2010); feeding usually operates by anchoring oral disk to a substrate and rasping materials off of it (Venesky et al. 2011). In contrast, funnel-mouthed tadpoles are suspension feeders that hang from the water surface and suck in suspending fine materials (Nachtigall 1974). The larvae are characterized by umbelliform oral discs and absence of well-developed keratodonts (Grosjean et al. 2011). Field observations suggest that the two species have no feeding interference and occupy different levels of the stream column.

I collected tadpoles of the two species (generally more than 100 individuals), incubated them in plastic trays, and collected feces using pipettes. The collected feces were shaken and divided into two equal parts, and filtered through Whatman GF/A glass microfiber filters. One part (filter loaded with pellets) was incubated in 8-liter buckets containing 2.5 L of filtered stream water. The second part 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 rain 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. I sampled 100 mL water at a time per bucket on days 0, 1, 4, 8, 12 and 16 following (Liess and Haglund 2007). The sampling syringe was used to 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. Nutrient concentration was corrected for water volume prior to analysis. Rates of ammonia and TDP excretion were expressed by amount of nutrient released per g of dry fecal pellet.

Effects of tadpoles on litter decomposition

To examine the effects of tadpole abundance and diversity on litter decomposition, I manipulated the density and the diversity of tadpoles of BQ and MM in field enclosures. The experiment was conducted in a second-order stream. Substrata ranged from boulders and cobbles in riffles, to silt, leaf litter and sand in pools. Stream flow was relatively stable during the experiment. The stream was 2.5 – 3.5 m wide with mean temperature of 18.2°C (range

17.5 – 19 °C) during the experiment. Enclosures dimensions were 0.25 m x 0.25 m x 0.35 m (length x width x height), made of 1 mm mesh polyethylene cloth (modified from (Kupferberg 1997a)). The bottom was closed with nylon fabric. Enclosures were set in pools where water velocity was relatively low ($< 1.03 \text{ cm.s}^{-1}$) (measured with a 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 12th - November 23rd).

I had 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 were of the same size and less than Gosner 32 (Gosner 1960).

I used leaf mass loss as indicator of leaf litter decomposition (Cornelissen 1996). I used leaves that differ in toughness. Leaves of *Pauridiantha* sp. were considered as “soft” and leaves of *Chrysophyllum* sp., which are relatively tougher, were considered as “hard”. Leaf toughness values, as measured with a tension gauge (1 mm head probe, Ouba Instrument Ltd, Osaka, Japan), were $0.86 \pm 0.21 \text{ N}$ and $1.63 \pm 0.33 \text{ N}$ (mean \pm 1 SD, $n = 31$) for the soft and the hard leaves, respectively. Leaves were collected from a riparian tree species by removing them directly from vegetation over-hanging the stream. Leaves that are approximately the same size, same position on the tree and have no signs 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 each of soft and hard leaves with the respective number of tadpoles. The control only had leaves but no tadpoles.

Because of the number of replicates, we used the different portions of the stream. These portions were similar in environmental conditions (e.g., light intensity, general structure of the surrounding vegetation). Five portions were used; we had one replicate of each treatment at each portion (treated as random factor in the analysis). We checked enclosures every 4 days for mortality and damages. We replaced dead tadpoles by new alive

ones. A heavy rain occurred in the later part of the experiment but did not destroy the enclosures. We terminated the experiment one week after this event. After 40 days, the tadpoles were removed from their enclosures, weighed, photographed and released. Leaf bundles were retrieved from enclosures, gently washed to remove any attached materials. Leaves were dried at 60 °C for 48 hours, and weighed to estimate leaf mass loss.

Data analysis

All statistical analyses were conducted in R. 3.2 (R Development Core Team 2011). The figures were made on SPSS 17. Data were log-transformed upon necessity to meet test assumptions.

I compared mean excretion rates in dissolved ammonia and total dissolved phosphorus between the six ecomorphotypes using ANOVA. Posthoc Tukey tests were conducted for pairwise differences. In the fecal pellet incubation experiment, I analyzed the variation in nutrient concentrations over time in the water column with a repeated ANOVA. I took “time of sampling” as factor and “nutrient concentration in ammonia and total dissolved phosphorus” as response variables. Because of low sample size (replicate = 2 for each species), I combined the data for BQ and MM to analyze the effect of time on nutrient concentrations in the water column. This was appropriate because the pattern of nutrient release from fecal pellets was similar for BQ and MM (see Fig. 3).

To analyze the effect of tadpoles on litter decomposition, we used linear mixed model (function “lmer” in the package “lme4” (Bates et al. 2012)) in which “leaf type” (two levels: soft or hard), “density” (low, medium and high), and “diversity treatment” (monospecific or mixture) were the factors. “Leaf mass loss” was the response variable and we entered “stream portion” 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 et al. 2015) with Tukey adjustment after bootstrapping method using the package “pbkrtest” (Halekoh and Højsgaard 2014). Alpha was set at 0.05.

4.3. Results

The six species (that differ in ecomorphotypes) differentially recycled nutrients in ammonia-N ($F(5, 36) = 17.57, P < 0.001$) and in total dissolved phosphorus ($F(5, 27) = 24.42,$

$P < 0.001$) (Fig. 1–2). All species tended to excrete ammonia at similar rate, except for *Spinomantis aglavei* (*Spinomantis*-generalized mouthpart cluster) for which rate was the lowest. We could distinguish two groups based on species TDP excretion rates: *B. andohahela*, *M. melanopleura*, and *M. sp47* excreted nutrient almost two times faster than *B. picturatus*, *B. quasiboehmei*, and *S. aglavei* (Fig. 2). Three species (*B. andohahela*, *M. melanopleura*, and *M. sp47*) excreted nutrients at high rates both in ammonia and in TDP. *Boophis picturatus* and *B. quasiboehmei*, however, only excreted ammonia at high rates.

Incubation experiment indicated that nutrients bound in fecal pellets were not readily available to the ecosystem but were released over time. The pattern of concentration in phosphate in the water column showed a hump-bell shape with a maximum value at day 4 ($F(1.514, 4.541) = 12.831, P = 0.015$) while concentration in nitrogen increased with time ($F(1.062, 3.185), P = 0.003$) (Fig. 3). In contrast to expectations, nitrogen concentration was higher than phosphate concentration in the water column (Fig. 3).

Tadpoles influenced litter decomposition, but effects depended on tadpole density and litter toughness (Table 1). No leaf mass loss occurred at low tadpole density (i.e., in contrast to control) (Fig. 4). Effects were higher on soft leaves than on hard leaves (significant effect of toughness on leaf mass loss). MM influenced decomposition only at high density irrespective of litter toughness. BQ effects were observed at medium and high densities, and only on soft leaves (Fig 4). The tadpoles did not have additive effects on litter decomposition: effects of mix species did not differ from effects of monospecific (Fig. 4, Table 1, “Treatment” effect), suggesting absence or very weak complementary effects between the two species. There were interactive effects of “tadpole density” and “leaf toughness”, “density” and “treatment”. These effects indicate that the effect of density on litter decomposition further depends on leaf toughness and the species present in enclosures (Fig. 4). The interaction between “leaf toughness” and “treatment” could be explained by the absence of BQ effect on decomposition of tough leaves (Fig. 4).

4.4. Discussion

I found that species with different mouthparts recycled nutrients at different rates. In principle, the quality of excretion should depend on the quality of food resources (Liess et al. 2015). Animals fed a lower quality food tend to excrete lower amount of nutrients and vice versa. Most tadpoles used in the experiment occupied different trophic spaces and apparently use different food resources (Chapter 3). However, the relationship between food quality and

the corresponding nutrient recycling appears to be weak in this system. In fact, some tadpoles that occupy similar trophic level (therefore species that use resources of the same quality in N) recycled nutrients at different rates (e.g., *B. quasiboehmei* and *M. melanopleura*). Further, *B. picturatus* that occupy the highest position (Chapter 3) did not recycle nutrient at higher rates than the other species. Together, these results suggest that trophic position alone is not enough to predict the quality and the rate of nutrient recycling in consumers.

Rather, two other pathways may explain these differences in quality of excretion. First, tadpoles typically differ in assimilation efficiency even for ecologically similar species (Altig and McDearman 1975). Interspecific differences in assimilation efficiency could be mediated either by difference in gut length, metabolic rate, or both (Vanni and McIntyre 2016). I did not measure gut length but studies suggested that tadpoles with longer guts are better at assimilating nutrient from poor quality food, thus excreting a lesser amount of nutrients (Liess et al. 2015). A second mechanism lies in the field of ecological stoichiometry, which suggests that the rates and ratios by which animals recycle nutrients reflect the nutrient imbalance between their bodies and their foods (Sterner and Elser 2002). Two species that differ in body elemental compositions C:N:P but feed on similar resources are predicted to recycle nutrients at different rates and ratios (Vanni et al. 2002). Further, the theory predicts that fast growing animals typically have higher N and P requirements, thus release nutrients at lesser rate than slow-growing species (Liess et al. 2015). We did not quantify growth rate in these species, but field observations (enclosure experiment) suggest that BQ (lower excretion rate) tadpoles tended to develop faster than MM ones (higher excretion rate).

Excretory products are soluble and are usually available for immediate uptake, but nutrients bound in feces must be mineralized before being available (Fig. 3). Tadpoles can produce important amounts of fecal pellets in freshwater environments through their continuous feeding (Jenssen 1967). This study indicates that fecal pellets represent an important latent source of nutrients in freshwater environments. Dynamics of nutrients recycled from fecal pellets were nearly identical for the two species. N availability increased with time in the water column and could have further increased had we continued the experiment for more days. In contrast, P tended to increase around day 4 and decreased in concentration at the end of the experiment. (André et al. 2003) reported that N is mostly released in excretion while P in the form of fecal pellets. Thus, I expected higher amount of P in the water column at the end of the experiment (hypothesis H2). However, patterns of these results comply with the findings of (Liess and Haglund 2007) who reported increase in

dissolved N in water column with time in a snail fecal pellet experiment. P typically sinks to the bottom, increasing its availability for basal resources (Liess and Haglund 2007), and it is possible that, in this experiment, P were immediately used by microorganisms. However, this assumption still needs to be tested experimentally.

In line with hypothesis H3, tadpole identity (i.e., mouthpart), tadpole density and leaf litter toughness influenced litter decomposition. Tadpoles can influence litter decomposition either directly by consuming leaves or indirectly by stimulating decomposer microbial activities through nutrient recycling (Rugenski et al. 2012). One limitation of the experiment was the inability to control leaf chemistry, although we must recognize the complexity of such an experiment. Leaf chemistry and toughness strongly influence litter decomposition (see (Bakker et al. 2011) for a review), and it is possible that decomposition in this study could be the result of interaction between leaf chemistry and toughness, not the sole effect of toughness per se. I believe, however, that at least, it is possible to infer the importance of leaf toughness on litter decomposition in tadpoles. Leaf toughness is known to negatively affect litter decomposition (Cornelissen et al. 1999) and could represent the most important factor predicting decomposition (Pan et al. 2015).

Tadpoles (at least those with generalized mouthpart) were thought to be not effective at consuming leaf litter because their jaw structure and teeth are not strong enough to fragment whole leaves (Iwai et al. 2009). However, previous studies have suggested that tadpoles can feed on relatively hard materials (Ramamonjisoa et al. 2016b; Taylor et al. 1995), meaning tadpoles can still facilitate leaf breakdown, possibly by opening frays in the leaf surface, allowing them to gradually feed on the inner part of the leaves (Iwai et al. 2009). Whether litter represents an important resource for tadpoles is unclear because I limited the food available to the subjects in enclosures. Leaf litter may only represent a secondary resource for tadpoles in nature because of its low nutritional value and in Chapter 3, I did not find enough evidence to support the assumption that tadpoles would majorly feed on leaf litter. Rather, they might receive energy from associated bacteria than from the litter itself (Altig et al. 2007; Ramamonjisoa et al. 2016a).

My results contrast with previous studies that found either no effect or little contribution of tadpoles to litter decomposition without the presence of shredder macroinvertebrates (Connelly et al. 2011; Iwai et al. 2009; Rugenski et al. 2012). But, this could be primarily because of differences in experimental design. First, I manipulated tadpoles at different densities, ranging from low to high while previous studies used one

density treatment. Second, I used leaf litter of different toughness. Had I limited the design to hard leaves and low tadpole density, I would have concluded an absence of effects.

BQ and MM had differential effects on litter decomposition. Soft leaves decomposed at higher rate than hard ones (e.g., (Cornelissen and Thompson 1997). There were no effects of tadpoles on litter decomposition at low density, irrespective of litter type. However, for soft leaves, BQ influenced litter decomposition at medium and high densities. In contrast, MM influenced litter decomposition only at high density. Why such a difference in effects? Although I did not conduct any experiment to disentangle these mechanisms, difference in species feeding behavior could be one explanation. BQ is grazer and can exert direct physical activities on leaf litter through consumption. Conversely, MM is constrained by its mouthpart (funnel-mouthed) and is limited to feeding on floating particles, thus MM may not directly affect decomposition. It may, however, enhance microbial activities through its higher excretion rate (Fig. 1–2). I did not measure leaf biofilm nor leaf respiration rate but previous studies suggested that even minor increases in nutrients in nitrogen ($<33 \mu\text{g NO}_3\text{-N. l}^{-1}$) (Ferreira et al. 2006) and in dissolved phosphate (0.1 mg.l^{-1}) (Suberkropp 1998) can significantly influence microbial dynamics and consequently enhance litter decomposition.

I expected higher decomposition rate in the mix-species treatment (hypothesis H3) following predictions of common biodiversity-ecosystem functioning experiments (Tilman et al. 2014), but I found no additive effects (i.e., the observed effects in mix treatments did not equal the combined effect of monospecific treatments) of tadpole diversity on litter decomposition, either at low or at high density. This is probably due to the absence of species complementary effects on litter decomposition. MM could have enhanced microbial activities through its excretion rate, but BQ might suppress such effect by grazing on the leaf surfaces, thereby removing decomposer microbes from the leaves, thus conversely decelerating decomposition.

Conclusions and implications

Tadpoles are highly diverse and can represent the largest biomass in streams. This study suggests that ecomorphologically dissimilar tadpoles can differentially impact the ecosystem.

Tadpoles have been thought to play minor roles or indirect effects on leaf litter decomposition in streams. This study reveals that tadpoles can directly affect such a process, but this ability depends on tadpole identity, density and leaf litter trait. In lotic environments

with poor fish fauna, tadpoles can represent the largest consumer groups (Strauß et al. 2010) and potentially play critical roles in these ecosystems. The absence of complementary effects on litter decomposition suggests that species richness does not necessarily translate into enhanced ecosystem function. It does not mean, however, that diversity has minor weight as we found that species differentially recycled nutrient and affected litter decomposition through different pathways. As reported in previous studies, in highly diverse communities, species abundance (biomass) could play more important role than diversity in delivering ecosystem services (Winfrey et al. 2015). Had I used more species may have resulted in higher decomposition rate, but doing so may not be ecologically relevant because the other functional groups (e.g., sand-eaters, suctorial tadpoles) do not occur in pools, thus may have no direct effects on litter decomposition.

Changes in species diversity of functional traits can affect ecosystem function by altering nutrient recycling rates, and more diverse communities may create biogeochemical ‘hotspots’ that regulate nutrient flow and fuel productivity (Vanni, 2002; McIntyre et al., 2008). These results suggest that a reduction in tadpole abundance can dramatically affect ecosystem functioning. It may be fruitful to further explore the relative contribution of each member in species rich communities and understand which ones can deliver a significant part of services. This information would definitely help guide conservation strategies in prioritizing species that are mostly critical to the ecosystem.

4.5. Figures

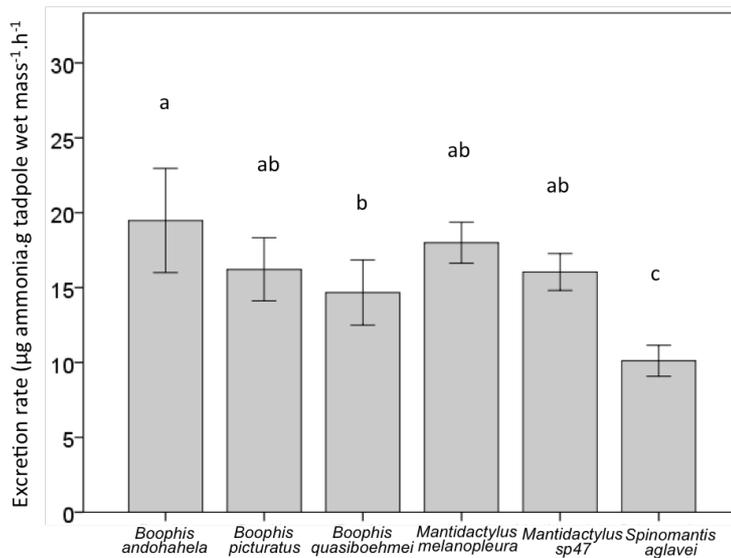


Figure 1. Excretion rate in ammonia in tadpoles that differ in mouthparts: *Boophis andohahela* (suctorial mouthpart), *Boophis quasiboehmei* (generalized mouthpart), *Boophis picturatus* (sand-eater), *Mantidactylus melanopleura* (funnel mouthed), *M. sp47* (fossorial), *Spinomantis aglavei* (Spinomantis generalized). Values are mean \pm SD. N = 4-6. Different letters mean significant differences.

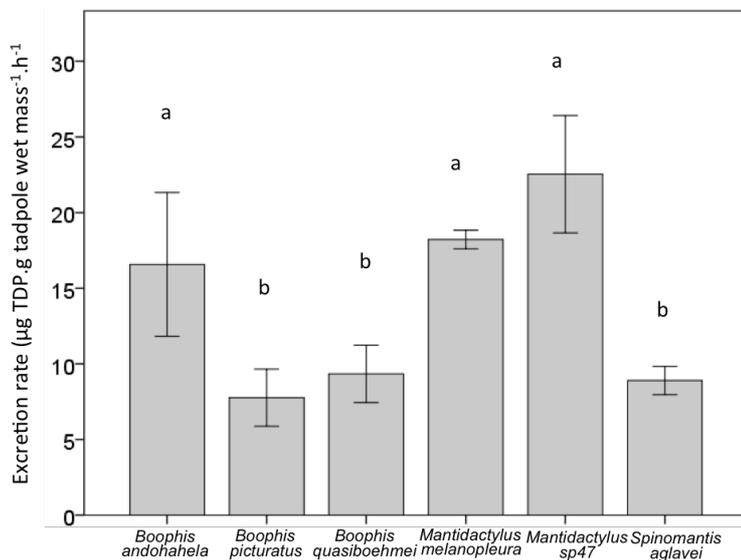


Figure 2. Excretion rate in total dissolved phosphorus in tadpoles that differ in mouthparts *Boophis andohahela* (suctorial mouthpart), *Boophis quasiboehmei* (generalized mouthpart), *Boophis picturatus* (sand-eater), *Mantidactylus melanopleura* (funnel mouthed), *M. sp47* (fossorial), *Spinomantis aglavei* (Spinomantis generalized). Values are mean \pm SD. N = 4-6. Different letters mean significant differences.

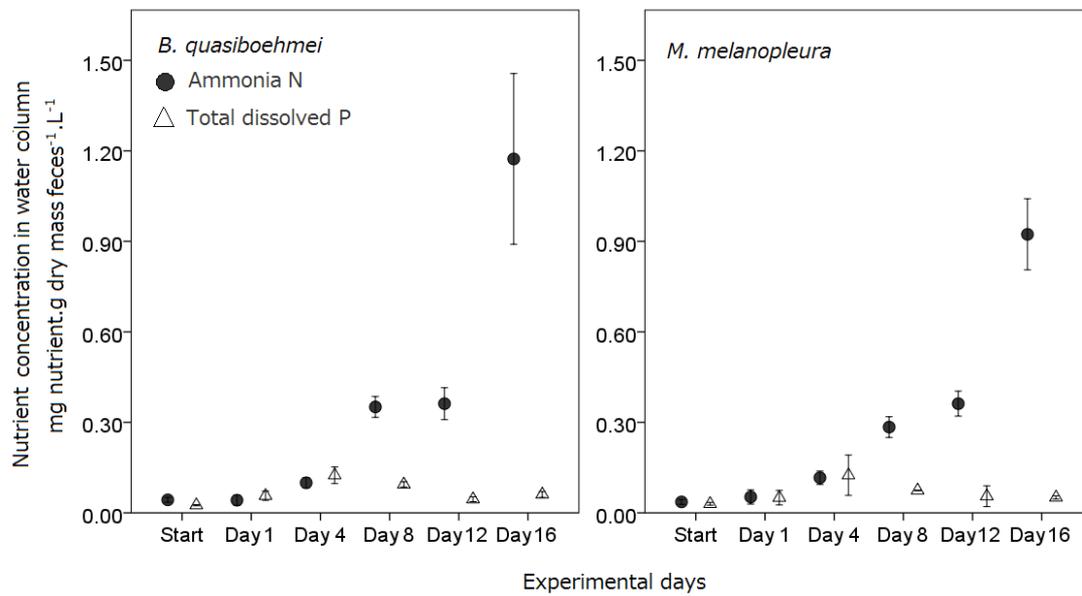


Figure 3. Mean concentration of nutrients (ammonia and total dissolved phosphorus) released in the water column from fecal pellets of *B. quasiboehmei* (generalized mouthpart) and *M. melanopleura* (funnel-mouthed). Pellets were incubated in stream water and sampling was done every 4 days. Values are mean \pm SD. N = 2

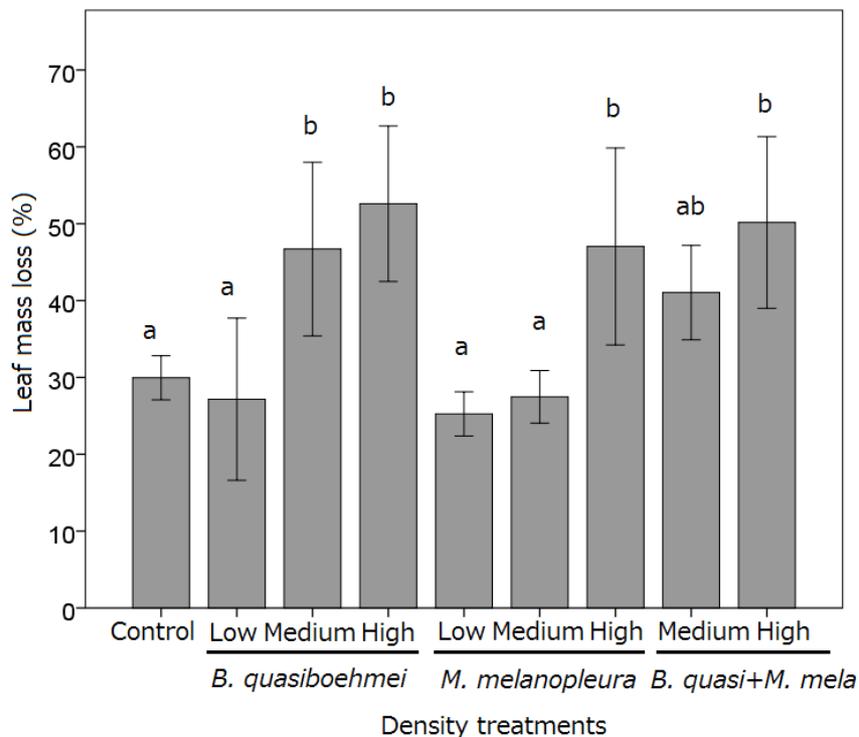


Figure 4a. Effect of tadpoles of *B. quasiboehmei* (generalized mouthpart) and *M. melanopleura* (generalized mouthpart) when alone or together on leaf mass loss of soft leaves. The control had only leaves but no tadpoles. Values are mean \pm SD. N = 5

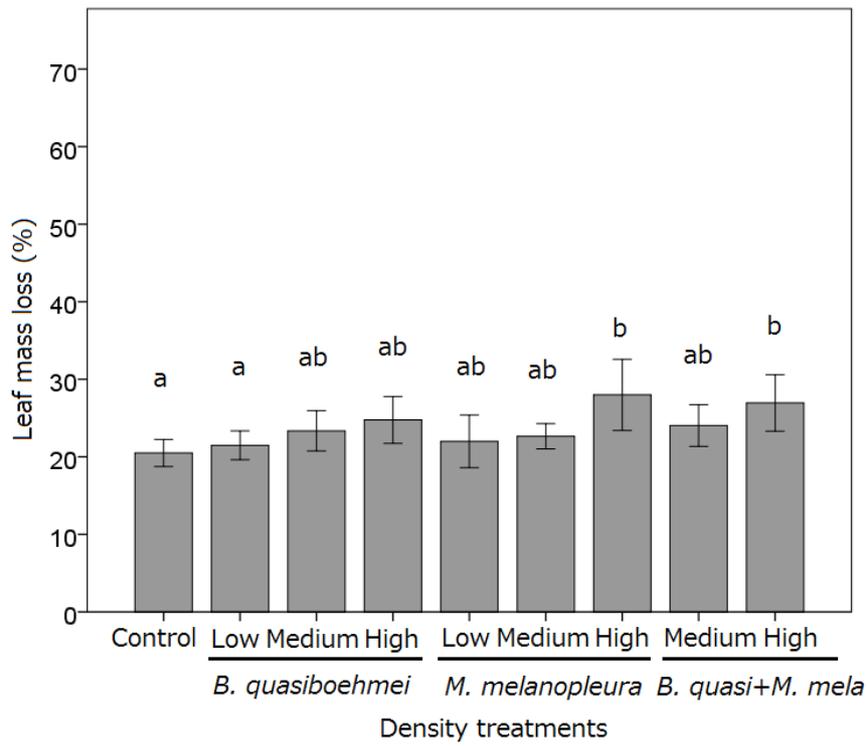


Figure 4b. Effect of tadpoles of *B. quasiboehmei* and *M. melanopleura* when alone or together on leaf mass loss of hard leaves. The control had only leaves but no tadpoles. Values are mean \pm SD. N = 5

4.6. Tables

Table 1. Results of mixed-effect models analyzing the effects of tadpole density, leaf toughness and species treatment (alone or mix species) and their interactive effects on litter decomposition. P-values were obtained by likelihood ratio-test.

	<i>Chi-square</i>	d.f.	P
Tadpole density (dens)	20.515	2	<0.001
Leaf toughness (tough)	61.896	1	<0.001
Treatment (treat)	4.143	2	0.125
Dens x Tough	17.741	2	<0.001
Dens x Treat	8.688	3	0.033
Tough x Treat	9.774	2	0.007
Dens x Tough x Treat	5.712	3	0.126

Chapter 5: Importance of phenotypic plasticity in alleviating strength of competition in tadpoles

One tenet in ecology is that ecologically similar species should strongly compete for the same resources and cannot persist indefinitely in the same system. It has been observed, however, that many species with little niche difference can persist in nature with no evidence of exclusion. Many hypotheses have been proposed, among these are life-history trade-offs (McPeck 2004), species-specific nutritional niche (Behmer and Joern 2008), storage effects (Angert et al. 2009) and phenotypic plasticity (Turcotte and Levine 2016). Particularly, when dispersal is limited, phenotypic plasticity, which is the ability of an organism to modify its phenotypes (behavior, morphology) in different environments, may allow species to stably coexist in their environment. I will present two studies to test the hypotheses that (1) generalist species can shift to the less preferred resources under strong competition (2) inferior competitors are able to invest in phenotype allowing them to alleviate the pressure from superior competitors. The first study investigates the ability of tadpoles to shift in their food preference i.e., move to the less preferred resource, in the presence of competitors. The second study examines how the strength of interaction between species can vary with the level of food resources and how tadpoles can modify their phenotype under asymmetric competition.

Part 1: Food properties and competitor density influence feeding behavior in a generalist omnivorous tadpole.

Abstract

I tested the food preference of an omnivorous pond-dwelling tadpole when exposed to animal and high protein algal materials at different toughness and concentrations (low and high concentrations, X and 2X amount of food per volume), used as a proxy indicator of energy gain. I subsequently tested how tadpole feeding behaviour and food choice change with competitor density. Food type, toughness and concentration influenced food choice; no interactions between these factors were observed though. The tadpoles did not feed randomly and preferred the algal materials when these were softer than or as tough as the animal materials. The tadpoles discriminated foods of different concentrations and readily fed on the high concentration food materials independently of resource toughness. The level of competition led to behavioral diversification in foraging decisions. Indeed, the presence of one competitor did not change individual feeding behaviour and food preference. In a group, however, feeding activities and grazing on less-preferred foods increased. Food toughness, energy gain per bite and the presence of competitors influenced feeding behaviour in the tadpoles. The ability of tadpoles to shift towards less preferred foods is suggested to alleviate the negative effect of competition on individual fitness.

5.1.1. Introduction

Optimal theory predicts that an organism will maximize its fitness by maximizing its net energy intake per unit time, and will usually choose the resource that yields the most calories for the effort it takes to consume it (Pyke et al. 1977). From herbivores and omnivores perspectives, food protein content has frequently been suggested to govern food choice (Mattson 1980; Sørensen et al. 2008). However, studies have suggested that more than protein content, energy gain (e.g. (Le Gall and Behmer 2014)), toughness (e.g. (Coley 1983)) and the social influence (e.g. (Giraldeau et al. 2002)) can strongly affect food selection. The trade-offs made when deciding among these factors have received less attention.

Anuran larvae are model organisms to study the feeding behaviour of omnivores. Tadpoles are still some of the least understood in terms of their feeding behavioural ecology, although they can represent the major biomass in freshwater environments (Altig et al. 2007). Information on feeding behaviours is central to understanding the ecological roles of tadpoles

because feeding behaviour is often linked to functional roles (Altig et al. 2007). Tadpoles were previously thought to be indiscriminate feeders, adjusting their feeding rates to food availability (Test and McCann, 1976; Wagner, 1986). When offered limited choices, however, tadpoles are known to feed selectively (Taylor et al., 1995). Many tadpoles with general morphology are omnivores (Whiles and Altig 2010), feeding on both animal and plant materials, but their food preference remains unclear. A long-held hypothesis holds that tadpoles mainly feed on plant materials because of the frequent abundance of visible, algal-based items (green material) in their long guts and the successful rearing of tadpoles on plant-based materials (Altig et al., 2007). Observations of opportunistic oophagy, carnivory or necrophagy and reports of substantial amounts of animal materials found in the gut, however, have suggested a potential affinity for animal materials (Petranka and Kennedy, 1999; Altig et al., 2007; Schiesari et al., 2009).

Previous studies suggested that both the protein content and toughness of a food resource could be important factors in food discrimination in tadpoles. Under limited food choice, tadpoles choose animal-based over plant-based materials, and preferred energy-rich and softer materials (Petranka and Kennedy 1999; Richter-Boix et al. 2007; Taylor et al. 1995). These patterns should not be surprising for at least two reasons: (1) proteins are often limited in the plant materials commonly consumed by tadpoles (Bowen et al. 1995; Kupferberg 1997b); and (2) animal materials are excellent sources of the protein necessary for growth (Crump 1990), are generally softer (e.g., decaying animal materials), and thus, should be readily selected upon availability. In particular, toughness (the ability to resist mechanical abrasion and penetration; *sensu* Watson and Norton 1985) can strongly influence resource edibility and feeding niche in omnivores (Deraison et al. 2014). Indeed, increasing food toughness reduces food ingestion rate and assimilation, and thus energy gain, with adverse consequences on development and fitness (Simpson et al. 2004; Clissold et al. 2009). In contrast to terrestrial herbivores that cut and shear plant materials, tadpole feeding is generally accomplished by anchoring the keratinized mouth to a substrate and raking materials off of it (de Sousa et al. 2014; Venesky et al. 2010). As such, strength limitation of their mouthparts might result in the avoidance of hard materials by tadpoles.

Although food properties should drive food preference in the absence of constraints, competition typically influences food choice in nature (Galef and Giraldeau 2001; Giraldeau et al. 2002). Foraging models predict that under low competition, individuals should feed on the preferred resource but should consider moving to lower quality patches when competition

increases. Shift in food preference in the presence of competitor has been documented in mammals (Kausrud et al. 2006), arthropods (Sherratt and Harvey 1993), fishes (Schindler et al. 1997) and birds (Weale et al. 2000) but has relatively received less research attention in amphibians. (Griffiths and Foster 1998) and (Altig and Christensen 1981) revealed that tadpoles increase their activity levels in groups, but whether or not higher foraging results into diversification in foraging decisions remains elusive. (Eterovick 2000) found that single and aggregative tadpoles of *Bufo rhinella* may exhibit the same diet but in a group, individuals tend to consume a higher amount of food with increasing preference for low-ranked foods. Using stable isotopes, (Arribas et al. 2015) found that tadpoles can shift their diet in the presence of competitors. In sum, these studies suggest that density-dependent feeding behaviour could be pervasive in tadpole feeding strategies, yet the underlying mechanism remains unclear.

In this study, I investigated the food choice of an omnivorous tadpole when offered the choice between animal and high protein algal materials at different toughness and concentration levels, which was used as a proxy indicator of energy gain per bite. I subsequently analysed how tadpole feeding behaviour and food choice change as a function of competitor density. I predicted that tadpoles prefer animal materials, softer and high concentration food materials. I predicted that foraging activities and preference for low-ranked foods increase with increasing competitor density following predictions of optimal foraging models.

5.1.2. Materials and methods

Forest Green Tree Frogs, *Rhacophorus arboreus*, are relatively large frogs found in Honshu, Japan, and the island of Sado, off the eastern coast of Honshu. The species occurs from sea level to mountainous regions at altitudes 2000 m (Wilkinson 2003). After mating, the female deposits eggs in a foam nest (300–800 eggs) on vegetation near standing water (Uchiyama et al. 2002). The tadpoles have generalized morphology (Wilkinson 2003), and the labial tooth row formula is 4-5/3. An earlier experiment indicated that tadpoles of this species are omnivores and can develop well on algal and animal diets (Iwai and Kagaya 2005).

On June 16th 2014, I collected four egg masses from a pond located in Shiga Prefecture, Japan (35.147368°N, 135.87881°E; datum WGS84). Upon hatching (after 2–3 d), I kept the tadpoles in 3-L aquaria filled with 2 L of Gosner 1960). The mean (\pm 1 SD) body

mass of the tadpoles at this stage was 0.291 ± 0.061 g ($n = 20$). The tadpoles were not fed for 24 h before the start of each experiment. I did not measure the water temperature of the test aquaria, but the room in which the experiments were conducted was maintained at 25° C with a natural photoperiod regime.

Experiment 1: Food preference in tadpoles when offered foods of different concentration and toughness

I first tested for the influence of food concentration and toughness on tadpoles' preference for algal and animal materials. I used food materials of nearly the same protein content: green-blue algae (Sun Nutrition Kabushikugaisha, Japan; protein 5.57%, lipid 5.7%, C/N 5.525 : 6.085%; CN Corder MT 700, Yanaco) and dry sludge worm (Itomimizu Kamihata Fish Industry Group, Japan; protein 5.52%, lipid 5.12%, C/N 5.541 : 6.095%; CN Corder MT 700, Yanaco). Food nutritional information is reported as indicated on the food packages. The general experimental design follows Taylor et al. (1995). I manipulated food concentrations (13 and 23 amount of food per unit volume) and food toughness (low and high) by mixing 2.5 g or 5 g of the respective food in 100 mL of either 0.5% or 1% agar solutions. I moulded the food solutions into bricks of 2.5 mL each. The toughness of each food brick was 0.23 ± 0.04 N and 1.01 ± 0.08 N ($n = 25$; measured with a tension gauge, Ouba Instrument, Ltd.), for the 0.5% and 1% agar solutions, respectively. In 1-L containers filled with 0.5 L of aged tap water, I introduced groups of eight tadpoles with two food bricks of animal materials of each concentration and two food bricks of algal materials of each concentration. The experimental design used three different treatments of food toughness: in treatment A, the four food bricks were of the same toughness (agar concentration 0.5%); in treatment B, the algal materials were tougher than the animal ones (agar concentration 1% vs. 0.5%) and in treatment C, the animal materials were tougher (agar concentration 1% vs. 0.5%). Each treatment was replicated 11 times, making a total of 33 experimental units. Controls consisted of containers with food bricks but with no tadpoles and were replicated six times. The controls were used to estimate the amount of food that was potentially dissolved in water. I left the tadpoles to feed for 48 h, and the foods remaining after that time were dried at 55° C for 36 h. The percentage of removed food was estimated by dividing the dry weight of remaining food brick in question by the mean dry weight of food bricks from the associated control (Taylor et al. 1995).

Experiment 2: Density dependence of feeding behaviour and food choice in tadpoles

In this experiment, I tested how the feeding activities of individual tadpoles changed with increasing competitor density. I observed the food choice and feeding duration of individual tadpoles under three different group sizes (one, two, or five individuals per container; hereafter, Density 1, Density 2, and Density 5). (Punzo 1992) applied similar densities when testing social facilitation in tadpoles.

As in Experiment 1, the replicates consisted of 1-L containers filled with 0.5 L of aged tap water and four food bricks of animal and algal materials of different concentrations (1X and 2X) but of the same toughness (0.5% agar solution). I introduced the respective number of tadpoles into each container, allowed them to acclimate for a period of 5 min, and then recorded tadpole feeding behaviour for 15 min with a digital camera at 24 frames/s. The foods were tagged with small pins to differentiate between high and low concentration foods. Within each group, individual tadpoles were identified by stopping the recording at the onset of each filming period. From each recording, a single focal tadpole was randomly selected. Feeding was defined as when the tail is raised up and undulated to keep the mouth next to the food (Altig and Christensen 1981). In contrast to the first experiment, I used feeding duration as an indicator of food preference because the manner by which I manipulated the food materials did not allow us to measure individual food intake, especially at Density 1. Each density treatment was replicated 32 times.

Data Analysis

In Experiment 1, I analysed the effect of food properties on tadpole preference using conventional linear models, as this is more appropriate for my percentage data (Crawley 2013). I combined the results from the three treatments and performed a linear mixed-effects model with the package lme4 (Bates et al. 2012) using the function lmer. My response variable, the proportion of removed food, was arcsine square root transformed before analysis. The predictors were food type (animal or algal material), food concentration (high and low), and food toughness (high and low). I had two random effects with container (11 replicates per treatment) nested within treatments (the three combinations of food block toughness).

In Experiment 2, I used general linearized mixed model fitted by maximum likelihood assuming a Laplace approximation to the likelihood function (package lme4) using the function glmer. I fitted the data to a negative binomial distribution (following a comparison

between a Poisson, a binomial, and a negative binomial distribution using Akaike Information Criterion). The response variable was the duration that an individual tadpole feeds on a specific food type (measured in seconds). Our predictors were tadpole density (Densities 1, 2, and 5), food type (algal or animal materials), and food concentration (1X and 2X). I set container (32 replicates per treatment) as a random effect. For the analyses of both experiments, 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`. I considered a fixed effect as significant when the difference between the likelihood of two models (with and without the effect in question) was significant. To test for food preference, I ran pairwise differences with the function `lsmeans` (Lenth et al. 2015) with Tukey adjustment after bootstrapping method using the package `pbkrtest` (Halekoh and Højsgaard 2014). In Experiment 1, post hoc tests were conducted across treatments. In Experiment 2, I first analyzed how foraging activities changed with the number of competitors, and subsequently compared food preference in the individual tadpole at Densities 1, 2, and 5. All statistical analyses were performed with R (v3.2.3, R Core Development Core Team) with a significance level held at 0.05.

5.1.3. Results

Experiment 1: Tadpole food choices between foods of different concentration and toughness

The tadpoles preferred algal to animal materials when the foods were similar in toughness or the animal materials were tougher (Fig. 1). Food type, concentration and toughness influenced food preference. The only significant interaction effect was observed between “food type” and “food toughness” (Table 1). This interaction was likely because food preference was reversed when the algal materials were tougher than the animal ones (Fig. 1). The other two-way interactions: “food concentration” by “food toughness” and “food concentration” by “food type” were not significant. This means that “food toughness” and “food type” have little effect on tadpoles’ preference for high concentration foods (Fig. 1). The ability of tadpoles to discriminate food concentration diminishes when food toughness increases though (Fig. 1). The three-way interaction “food concentration” by “food toughness” by “food type” was not significant, meaning that although the tadpoles reversed their food choice when the animal materials were tougher, they still preferred the high concentration to the low concentration foods (Table 1).

Experiment 2: Density-dependent feeding behaviour and food choice

Tadpole feeding duration did not change in the presence of one competitor ($Z = -1.30$, $P = 0.39$) but significantly increased in groups (Density 1 vs. Density 5, $Z = 4.24$, $P < 0.001$; Density 2 vs. Density 5, $Z = 2.99$, $P = 0.007$). Competitor density, food type and food concentration significantly influenced feeding activity in the tadpoles (Table 2). In line with the first experiment, there was no interactive effect of food type and concentration on tadpole food choice (Table 2). The number of competitors influenced food preference; the effect is marginally significant though ($\chi^2_2 = 5.44$, $P = 0.06$). When alone or in pairs, the tadpoles preferentially fed on the high concentration algal material. In groups, however, the tadpoles still exhibited a preference for the high concentration algal material but increased their feeding on the less-preferred food items, here including animal materials (Fig. 2).

5.1.4. Discussion

In line with previous studies (Taylor et al. 1995; Kupferberg 1997), the tadpoles fed selectively. Although aggressive individuals pushing others into adjacent food patches may cause a portion of the difference, the fact that all food bricks were fed upon indicates that samplings were made but feeding was not random, otherwise the amount of food removed from the food bricks would not have been different. The results indicate that food type, food concentration and competitor density strongly influence food choice in *Rhacophorus arboreus* tadpoles. Food choices were clear, in general the tadpoles preferred algal materials, high concentration and soft foods. Food preference was reversed when the algal materials were tougher, and consequently feeding on the animal materials increased. In the second experiment, we observed that feeding activities in the tadpoles did not change under low competition but almost doubled in the presence of a group of competitors. The less preferred food items in the absence of competitor became more preferred when competitors are present, following predictions of simple foraging models (Pyke et al. 1977)

As for many omnivores, feeding on both plant and animal foods may ensure that tadpoles meet the necessary nutrients they cannot acquire from one food alone. When the foods were of the same toughness, the tadpoles preferred the algal to animal materials. It is not clear whether food choice was driven by calorie gain, nutrient content or palatability in our experiment but a previous study suggested that even as a small component of the diet, animal materials can greatly contribute to growth in freshwater omnivores (Evans-White et al. 2003). (Schiesari et al. 2009) revealed that carnivory could be ubiquitous in many pond

dwelling tadpoles; still, compared to filamentous algae and other plant materials, animal materials represent a relatively smaller proportion of the diet. It is not known whether in nature food choice is mediated by preference or food availability but in a parallel experiment, I found that animal materials become toxic when they occupy the most part of tadpoles' diet (Ramamonjisoa, personal observation). This may explain why animal materials were consumed at a relatively lower rate compared to algal materials when the two resources were of the same toughness in our experiment. Further experimental works are needed to test this assumption though.

Food toughness strongly influenced food selection. Recent studies suggested that biomechanical traits (e.g., bite strength) predict feeding niche and food choice in herbivores (Deraison et al. 2014; Ibanez et al. 2013). Indeed, increased toughness reduces the rate of nutrient supply by reducing food ingestion and assimilation (Clissold et al. 2009) with direct consequences on development. In contrast to terrestrial omnivores that use shearing and tearing to break down and process food resources, tadpoles generally feed via filter-feeding or scraping, collecting and vacuuming the surface of substrates (Alford 1999). Feeding occurs by anchoring the oral disc onto a substrate and raking material from it (Venesky et al. 2010). A tadpole's keratinized labial teeth may not be strong enough to penetrate tough materials; thus, strength limitations result in the avoidance of hard resources by tadpoles. This may explain why the tadpoles could not clearly differentiate food concentrations at higher food toughness. Similarly, limitation in bite strength may justify the reverse in food choice when the algal materials were tougher than the animal ones. This situation may be common in nature because plants usually show the greatest variation of toughness and most animal tissues are relatively soft and decompose more quickly. So far, resource protein content has often been suggested to be the prime factor of food choice in tadpoles. However, a previous laboratory work (Ramamonjisoa et al., personal observation) established that, like in some herbivores, resource toughness could exert a larger effect than food protein content on food choice. One striking results was the ability of tadpoles to distinguish foods of different concentrations. Indeed, tadpoles readily fed on high concentrations food materials, 2X amounts of food per volume. That is, the tadpoles removed the same food volume but gained twice the actual nutrients per bite. This result complies with the predictions of foraging models (Pyke et al. 1977). The ability to discriminate foods of different concentrations may not be pervasive among tadpoles though. For example, although the larvae of *Lithobates sphenoccephalus* and *Anaxyrus woodhousii* have the same labial structure, only *Anaxyrus*

woodhousii tadpoles could distinguish materials with different food concentration (Taylor et al. 1995). The ability of tadpoles to discriminate foods of different concentrations is intriguing but taste and cue intensity emanating from the foods were probably involved.

The second key finding is consistent with the prediction that a common arousal mechanism modulates feeding duration (Altig and Christensen 1981; Ziv et al. 1991). Interestingly, feeding duration only increased in groups; the presence of one conspecific did not trigger a competitive behavior that should have increased feeding activities in the individual tadpole (Altig and Christensen 1981). This pattern is not in line with the common observations reported in some other taxa such as rodents (Harlow 1932), molluscs (Ziv et al. 1991) and birds (Plowright and Redmond 1996) where the mere presence of one conspecific induces higher activity level among individuals. The size of my experimental unit, which could be relatively large at a tadpole scale, may be one of the reasons. The exact cues responsible for the arousal are still unclear, but tadpoles are assumed to be sensitive to flapping tails (Michimae et al. 2005), and the stimuli from a larger group could be more detectable than that from one individual.

Within the short behavioural observations, the tadpoles were able to discriminate foods on a small spatio-temporal scale and exhibited a preference for high concentration algal food, in line with the first experiment. I observed that the tadpoles generally browsed briefly on each food brick before foraging longer on a typical food, so physical contact may be required to assess food quality. The ability of tadpoles to change their diet in the presence of competitors has been documented (Arribas et al. 2015; Caut et al. 2012), and such ability has been suggested to help tadpoles adapt to changing environments. The pattern of food choice was density-dependent. Food preference did not change at low competitor density but did in group i.e., at higher intensity of competition (Fig. 2), following the pattern observed in some fish and mammal taxa (Kausrud et al. 2006; Schindler et al. 1997; Weale et al. 2000). The tadpoles remained selective, but the presence of a group increased the preference for low-ranked food following the predictions of foraging models. (Eterovick 2000) reported similar results where single and aggregated *Rhinella crucifer* tadpoles exhibited similar diets, but aggregated tadpoles incorporated more of the less-preferred food in their diet. Our experiment could not clearly answer whether the increasing preference for the less-preferred food was driven by competition (aggressive tadpoles may displace others onto adjacent food bricks), by social facilitation (Sontag et al. 2006) or by individual specialization (Bolnick et al. 2011). In any case, the presence of conspecifics induced higher activity, allowed the

tadpoles to explore more food choices and opened the opportunity to feed on “not priority” foods, ultimately allowing them to optimize individual fitness in competitive environments. Broadly, the ability of tadpoles to shift their food preference allow them circumvent the negative effects of competition and ultimately coexist with competitors (Arribas et al. 2015).

In conclusion, palatability (animal versus plant materials) resource profitability, competitor density and, importantly, resource toughness are important factors of resource discrimination in tadpoles. One point worth considering is would tadpoles maintain the same pattern of food choice if they were presented the same foods along ontogeny, given that tadpole nutritional needs may vary with body size and the state of the environment? Future studies will be needed to assess the relationship between bite force and food choice. Another venue is to gain a better understanding of the functional significance of a sole resource diet or switching between plant and prey and how, in turn, this regulation affects tadpole fitness.

5.1.5. Figures

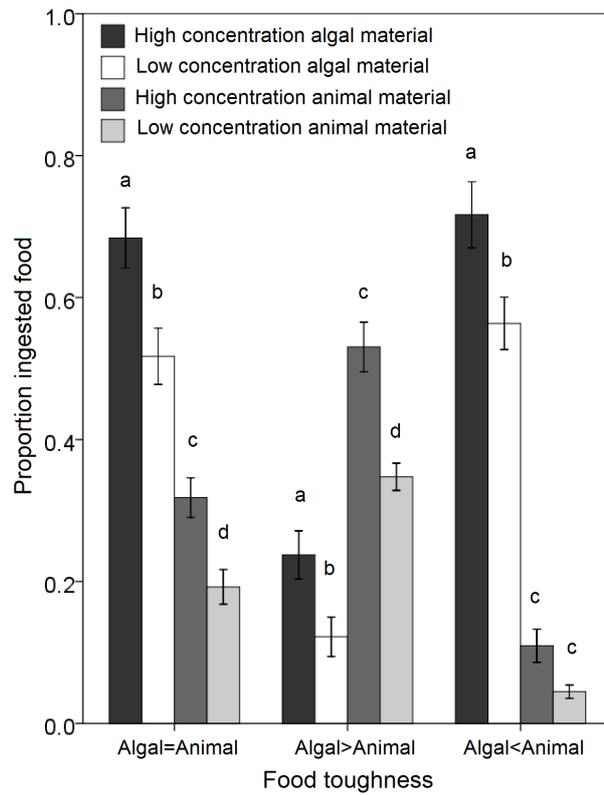


Figure 1. Tadpole food preference when presented with animal and algal materials of the same protein content. Resource toughness (same or two times tougher) and food concentrations (Low and High, X and 2X amounts of food per volume) were manipulated over three treatments. Different letters indicate significant differences (post-hoc tests with Tukey adjustment, $\alpha = 0.05$) in each combination of food toughness. Values are the mean \pm 1 SD. N = 11.

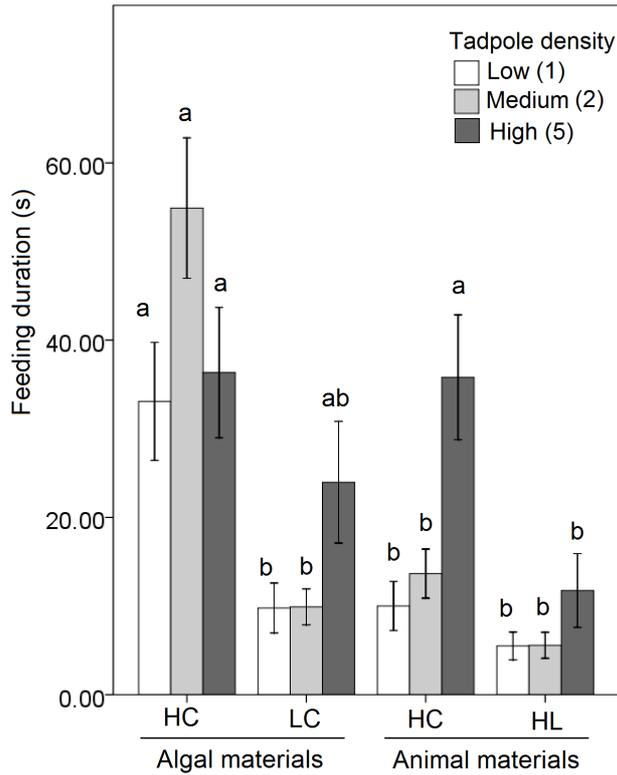


Figure 2. Changes in individual tadpole feeding activities with increasing competitor density. The food materials were high protein algal materials and animal materials of the same toughness, using two levels of food concentration, namely High Concentration (McGoey and Stinchcombe) and Low Concentration (LC) (2X or X amount of food per volume). Different letters indicate significant differences (Adjusted post-hoc Tukey following a negative binomial GLMM, $\alpha = 0.05$). Data were analyzed with GLMM followed by post-hoc adjusted Tukey. The values are the mean \pm 1 SE. N = 32 per density treatment.

5.1.6. Tables

Table 1. Interactive effects of food type (animal or algal materials), toughness (0.5% or 1% agar) and concentration (1x and 2x amounts of food per unit volume) on food choice in tadpoles of *Rhacophorus arboreus*. P-values were obtained by likelihood ratio tests following a linear mixed-effects model (statistically significant values indicated in bold).

Predictor	χ^2	df	<i>P</i>
Food concentration (Concentration)	11.09	1	< 0.001
Food toughness (Toughness)	104.41	1	< 0.001
Food type	96.89	1	< 0.001
Food type × Toughness	18.76	1	< 0.001
Food type × Concentration	0.43	1	0.51
Concentration × Toughness	0.79	1	0.37
Food type × Concentration × Toughness	0.21	1	0.64

Table 2. Interactive effects of competitor density (one, two or five tadpoles in a container), food type (animal or algal materials) and food concentration (1x or 2x of food amount per unit volume) on the feeding preferences of *Rhacophorus arboreus* tadpoles. P-values were obtained by likelihood ratio tests following a generalized linear mixed-effects model with negative binomial distribution.

Predictor	χ^2	df	<i>P</i>
Food type	29.42	1	< 0.001
Tadpole density (Density)	11.72	2	0.002
Food concentration (Concentration)	47.55	1	< 0.001
Food type × Density	3.42	2	0.18
Food type × Concentration	0.68	1	0.41
Density × Concentration	2.26	2	0.32
Density × Food type × Concentration	5.44	2	0.06

Part 2: Hierarchical competitive ability and phenotypic investments in prey: inferior competitors compete and defend

Abstract

It is commonly assumed that prey must make growth-defense trade-offs when in the presence of predators under resource-limited conditions. Thus, it is predicted that prey will allocate limited resources either to competitive phenotypes to grow or to defensive phenotypes to defend, not both. Evidence from plants, however, has suggested that under strong selection prey might evolve phenotypes that allow them to simultaneously compete and defend. In a controlled laboratory experiment, I investigated phenotypic investment in asymmetrically competing tadpoles of *Rhacophorus schlegelli* (RS) and *Pelophylax nigromaculatus* (PN) in the presence and absence of predatory dragonfly larvae. I predicted that, because of differences in resource acquisition abilities, tadpoles would invest in competitive and defensive phenotypes differently, depending on their relative competitive ability in the presence of predators. RS was the superior competitor and depressed the growth of PN whether predators were present or absent. As expected, the inferior competitor (PN) responded to competition by elongating its gut in predator-free environments. In contrast, the superior competitor (RS) did not. In the presence of predators, both tadpoles invested in larger tails, a common defensive trait, while the presence of competitors did not influence tail size. When reared alone in the presence of predators, PN grew faster and tended to have a longer gut. In contrast to theoretical predictions, however, in the presence of both predators and competitors the inferior competitor (PN) still maintained the competitive (long gut) and defensive (large tail) phenotypes despite being depressed in growth. Optimal digestion theory suggests that gut elongation should enhance assimilation efficiency and energy gain. In this view, when a competitive phenotype also serves to benefit prey in predator environments, prey may be able to both compete and defend, ultimately allowing inferior competitor to persist in their environment under strong competition.

5.2.1. Introduction

Competition and predation often act as selective pressures that drive prey adaptation (Gurevitch et al. 2000). Animals and plants respond to competition and predation with adaptive plastic phenotypes, which increases fitness in the presence but not in the absence of a threat (Schlichting and Pigliucci 1998). But because of the often conflicting demands of

competition and predation on prey phenotypes, their independent and simultaneous impacts can strongly differ.

Under competition, organisms generally reduce investment in structures that are costly to maintain, investing instead in phenotypes that enhance competitive ability and growth. For example, competition-induced plastic changes in plants often involve increased root density and stem length, leading to better nutrient and light acquisition (Dudley and Schmitt 1996). In animals, responses to competition for food involve increased foraging activity (Anholt and Werner 1995), aggressive behavior (King 1973), and elongation of the alimentary tract in order to improve nutrient assimilation (Relyea and Auld 2004; Sibly 1981). Conversely, in the presence of predators prey phenotypes that increase survival are favored, but they generally come at the cost of slower growth and development. In plants, responses to herbivory involve the production of defense chemicals (Karban and Baldwin 2007) at the cost of reduced root length and density, making plants less able to compete for nutrients and water (Armitage and Fourqurean 2006; Karban and Strauss 1993; Ohgushi 1997) but see (Whittaker 2003). Animal prey species typically reduce foraging activity (Lima and Dill 1990) and/or build defense structures, at the cost of diverting energy from growth in order to maintain these structures (Tollrian and Harvell 1999). Recent evidence shows that one way in which prey can compensate for investment in defense structures is through the shortening of the gut (Kehr and Gómez 2009; Venesky et al. 2013), reducing resource assimilation efficiency and thus reducing growth (Relyea and Auld, 2004).

Two types of models have typically been used to predict the interactive effects of competition and predation on prey phenotypes (Teplitsky and Laurila 2007). The simple allocation model predicts low investment in defense under strong competition, when limited available resources should be invested in growth (Harvell 1990), but prioritization of defense under weak competition, when increased resource availability would allow such investment (Relyea 2004). The second, the growth-defense model (Herms and Mattson 1992), predicts the converse: that prey should invest less in defense and prioritize growth in high resource environments (e.g., (van Velzen and Etienne 2015)), but should increase investment in defense when there is little opportunity for growth and they will have to spend more time remaining in risky environments (e.g., (Parejko and Dodson 1991; Pauwels et al. 2010).

These two models both assume that prey can invest in either a competitive or a defensive phenotype, but not in both (Lind et al. 2013). Evidence from plants, however, has suggested that prey do not always make such a trade-off. This may occur in the presence of

strong selection by competition and weaker selection by predation (Uriarte et al. 2002), or when a single phenotype is beneficial under both competition and predation (Siemens et al. 2002). Evidence of such a situation is still scarce in animals, but a recent study reports that prey facing increasing predation risk can prioritize investment in growth and in morphological defenses even when resource availability is reduced (Costa and Kishida 2015), suggesting that prey may also compete and defend.

In this study I examine the phenotypic investment of asymmetrically competing prey species in both the presence and absence of predators. Because of disproportionality in resource acquisition, I expected superior and inferior competitors to invest differently in competitive and defense phenotypes. From a prey perspective, the presence of interspecific competitors is potentially more challenging than the presence of conspecifics because of potential interspecific differences in competitive ability and resistance to predators (Cipollini 2004). Thus, maladaptive phenotypic investment in the presence of interspecific competitors may increase selection against the less defended or the less competitive prey. This situation might induce phenotypic investment in prey to not follow theoretical models. Asymmetric competition is pervasive in nature (Schoener 1983), and is common among anuran larvae (Morin and Johnson 1988; Parris and Semlitsch 1998; Smith et al. 2004; Werner 1992), which are often model systems in studies of phenotypic plasticity. Tadpoles typically respond to competition by reducing their tail size (Relyea 2004) and elongating the gut (Relyea and Auld 2004). Conversely, in the presence of predators, tadpoles grow larger tails and shorten their gut, likely to compensate for building this structural defense (Kehr and Gómez 2009; Relyea and Auld 2004). Here, I used gut length and tail fin size as proxy estimates of investment in competitive and defensive phenotypes, respectively.

Following the predictions of theoretical models, I expected 1) that in predator-free environments, inferior competitors would invest less in costly structures (i.e., smaller tail fin size) and more in phenotypes that foster growth (i.e., longer gut) in response to lower resource gain; 2) that in asymmetric competition in the presence of predators, inferior competitors would develop either a long gut to compete or a large tail to defend, but not both, and that which phenotype developed would depend on the relative effects of predation and competition on growth; and 3) that due to higher resource gain, superior competitors would not develop an elongated gut and would maintain a larger tail in the presence of competitors and predators.

5.2.2. Materials and methods

Rhacophorus schlegelli (hereafter RS) and *Pelophylax nigromaculatus* (hereafter PN) are two coexisting anuran species occurring on the main island of Japan, breeding in small ponds and paddy fields. The two species differ in breeding mode: *Pelophylax nigromaculatus* produces more offspring (500-600 eggs per clutch) than *Rhacophorus schlegelli* (200-300 eggs per clutch) (Uchiyama et al. 2002). Under limited resources, RS and PN are known to compete asymmetrically (Ramamonjisoa & Natuhara, unpublished data) and represent a good system for this experiment. I collected four and two egg masses of *Rhacophorus schlegelli* and *Pelophylax nigromaculatus*, respectively. Eggs were collected in early June 2013 from two rice paddy fields in Toyota city, Japan. Eggs were hatched in the laboratory and tadpoles were kept in plastic containers at 15 individuals.L⁻¹. Tadpoles were fed rabbit chow every other day until the start of the experiment.

To test my predictions, I conducted a laboratory factorial design in which the presence or absence of a caged predator dragonfly larva (*Orthetrum* sp) was crossed with the absence or presence of competitors (8 RS, 8 PN, 4 RS + 4 PN). *Orthetrum* sp. larvae are important tadpole predators in paddy fields in Japan. Densities were manipulated to fall within the range of their density in nature (Nakanishi et al. 2009). For each species, tadpoles from different egg masses were pooled and then randomly allocated to the containers. This allowed us to homogenize the composition of each experimental unit while introducing genetic variability. Tadpoles were at Gosner 26-27 (Gosner, 1960) at the start of the experiment and initial body masses did not differ at the start of the experiment (t-test, n=15, $t_{28}=1.488$, p=0.148).

I conducted the experiment in rectangular plastic tubs (15 x 24 x 12 cm) filled with 2.5 L of aged tap water, following the same experimental design as Smith, Dingfelder & Vaala (2004) in studying asymmetric competition in tadpoles. Each treatment was replicated six times. I used pellets (described below) to simulate clumped resources and to stimulate species interaction (Kiesecker et al. 2001). Under such conditions, PN and RS tadpoles compete asymmetrically and the strength of interspecific interaction typically increases with decreasing resource levels (Ramamonjisoa and Natuhara, unpublished data). This laboratory setting allowed us to control for the amount of resources and the environmental conditions the tadpoles are exposed to, facilitating the inference of causal factors of observed effects.

The food materials consisted of a mixture of rabbit chow (Marukan, Ltd protein: 15%, lipid=5%) and commercial algae (Sun Nutrition, protein = 57%, lipid = 7%) in equal weights. Food protein content (~36%) is within the range of dietary protein content that pond dwelling tadpoles meet in the field (Schiesari, Werner & Kling, 2009). Foods were pulverized into fine powder and bound into a 0.5% (in mass) agar solution. The solution was poured into petridishes (85 mm x 15 mm) and allowed to harden at 7°C. To create pellets, I bored in the agar-bound food material using a 6 mm diameter cylinder (pellet dimension 6 mm x 15 mm). As applied in previous studies (e.g., Bennett, Pereira & Murray, 2013), I provided food weighing about 4-5% of the total mass of the tadpoles per aquarium every day to simulate environments with moderate food restriction.

Dragonfly larvae were collected from an experimental paddy field inside Nagoya University campus. Each aquarium contained one small cylinder covered with a net that was either empty or held a dragonfly larva. This method allowed simulating the threat of predation while avoiding actual predation, prevented tadpoles from feeding on predator's egestion but allowed predator waterborne chemical cues to diffuse in the water. Predators who died during the course of the experiment were immediately replaced with living ones. I fed predators with two tadpoles every other day. Predators in single-species tadpole treatments were only fed tadpoles of that species. The mixed tadpole-predator treatments were fed individuals of each tadpole species (Costa and Vonesh 2013). I provided approximately the same prey biomass to reduce variation among treatments. To maintain the chemical cues in containers, half of the water volume was siphoned and replaced every 3-4 days; feces were removed with a pipette. Containers were randomly reorganized in the experimental room every time water was changed. There were no food leftovers in the containers prior to feeding. The experiment was conducted in a room at 24 °C with natural daylight regime. The experiment was terminated after 35 days when the tadpoles were at Gosner 34-36.

At the end of the experiment, the tadpoles were removed from their containers, blotted dry and weighed. Feeding ended 30 hours before the end of the experiment, which allowed tadpoles to "clear" their gut. As applied in previous studies (e.g., (Bennett et al. 2013).), mean individual species growth rates were computed by the differences in body mass (final body mass – initial body mass) over the number of experimental days (35 days) and the number of tadpoles of the respective species in each container. Mortalities occurred in the

first days of the experiments (until day 3), during which dead individuals were immediately replaced by new alive ones.

Morphological responses

At the end of the experiment, I randomly sampled a subset of three tadpoles of each species from each container and took digital images of the tadpoles. I measured body length, tail length, body height, tail height, tail depth, muscle depth and tail fin area following (Warkentin 1999) (on some occasions, morphometrics were conducted on only two individuals). When needed, a piece of glass was placed under the tail to avoid distortion during measurement. Morphometric measures were performed with the software ImageJ (<http://imagej.nih.gov/ij/>). Upon completion of morphological measurements, the tadpoles (not necessarily the ones used for morphometrics) were euthanized by an overdose of MS-222, weighed to the nearest 0.1mg and dissected or frozen (-20°C) for later measurement. I uncoiled the entire gut system without stretching and measured length to the nearest 0.1mm with a digital caliper. Measurements were repeated two times and averaged for one individual tadpole.

Data analyses

I evaluated the effects of “competition” and “predation” (factors) on tadpole “growth rates”, “gut length”, and “tail fin area” (response variables) with general linear models (GLM). Upon significance, I conducted post-hoc TukeyHSD tests to compare tadpole growth rates among treatments. Partial etas squared are reported as estimate of effect sizes. All statistical analyses were conducted on tank means (N = 6) for all response variables. Data were log-transformed when necessary to improve assumptions of normality and homogeneity of variances.

Gut length was standardized using the Scaled Mass Index, a robust technique to correct metric size to body mass (Peig & Green, 2009). Tail fin area was corrected following (Berner 2011): I first entered a subset of the metric traits (body length, tail length, body height, tail height and muscle depth), log-transformed when necessary to increase linearity, into a Principal Component Analysis to estimate a latent size PC1. I then size-corrected the tail fin area (square-rooted) using that PC1 as covariate in a GLM. This method has been suggested to be more reliable than common techniques based on residual analysis (Berner 2011). PC1 scores were averaged and centered prior to analysis (Gabriela et al. 2016). I used Sigmaplot

(ver. 13, SYSTAT Software Inc.) and SPSS (ver. 17, IBM) to perform the analyses and SPSS (ver. 17, IBM) to make the figures.

5.2.3. Results

Growth rates

Competition and predation influenced tadpole growth rates but competition exerted a larger effect (Table 1). The two species competed asymmetrically: RS was the superior competitor whether in the presence or absence of predator (Fig. 1). Compared to when reared alone, in mixed rearing, RS grew faster at the expenses of PN (Table 2). Intraspecific competition was more detrimental to RS than interspecific competition while the opposite pattern was observed in PN. The presence of predators did not reverse the outcome of interaction: RS still grew faster at the detriment of PN (in comparison to when reared alone, Table 2). PN grew faster when reared alone in the presence of predators but RS did not (Table 2). Competition and predation significantly interacted on PN growth: PN decreased growth under competition but grew faster in the presence of predators.

Morphology

PN and RS exhibited larger tails in the presence of predators (Fig. 2, Table 2). In contrast to expectations, investment in tail defense was not affected by the presence of competitors (Table 2). The inferior competitor PN elongated its gut in the presence of competitors and predators (Fig. 2, Table 2). Gut length in the superior RS did not change in any treatments.

5.2.4. Discussion

I investigated how the relative competitive abilities of two tadpole species affected competitor and predator induced phenotypes. PN and RS tadpoles competed asymmetrically in which RS was the superior competitor in both the presence and absence of predators. This may be due to the fact that the relative impacts of competition on tadpole growth exceeded the positive impacts of predation. Such a pattern has been previously reported in other systems in which predation can unidirectionally benefit one competitor (Chase et al. 2002). I used food pellets and this may have increased the strength of interaction between the two species by simulating clumped resources (Kiesecker et al. 2001).

Both tadpole species grew larger tails in the presence of dragonfly larvae predators, consistent with previous reports (e.g., (Van Buskirk 2000; Van Buskirk et al. 2003). A larger

tail functions as a lure and is thought to increase survival in predator environments (Van Buskirk et al. 2003). Typically the expression of an inducible defense, such as larger tails in tadpoles, is thought to result in a growth/predation risk trade-off, in which vulnerability to predators decreases at the cost of decreased growth and development (Tollrian and Harvell 1999). However, none of the tadpoles in this experiment exhibited reduced growth when reared alone in the presence of dragonfly larvae. To the contrary, PN grew even faster in the presence of predators. Growth acceleration in response to predators is not unusual, and has been seen in many taxa, including tadpoles with induced morphological defenses (Bennett et al. 2013; Costa and Kishida 2015; Relyea 2002). Adopting a high growth strategy is thought to be adaptive: faster growth constitutes a quicker means of reaching a size refuge against predators, and a quicker path to metamorphosis and escape from the hostile environment (Urban 2007). In tadpoles, vulnerability to predators typically decreases with increasing body size (Brodie Jr and Formanowicz Jr 1983; Costa and Kishida 2015; Urban 2007), and in my system, larger tadpoles are less susceptible to predation from dragonflies (Ramamonjisoa, unpublished data). Furthermore, gape-limited newts are also top predators in this system, suggesting that larger, faster growing tadpoles will have increased fitness in the presence of these predators.

I predicted smaller tails in the inferior competitor species (PN) because of lower resource gain (e.g., (Relyea 2004; Teplitsky and Laurila 2007), but instead I found that PN elongated its gut while also enlarging its tail. A prediction of the optimal digestion theory is that an organism can modify gut length in response to the quantity and the quality of its diet (Sibly, 1981) as a longer gut increases food assimilation efficiency and energy gain (Sibly 1981), and is considered an adaptive competitive trait to foster growth in tadpoles (Relyea and Auld 2004). Although a previous experiment indicated RS can elongate its gut on a low quality diet (N. Ramamonjisoa, unpublished data), only PN did in this experiment, likely as a consequence of it being the inferior competitor.

The fact that the inferior competitor PN simultaneously grew a competitive phenotype (long gut) and a defensive one (large tail) in the presence of predators goes against the predictions of two classic phenotypic investment models (simple allocation models and growth-defense models; see Introduction). Typically, these models predict a trade-off between competitive and defense traits; that is, I expected tadpoles to invest in either a larger tail for predator defense or a longer gut for competition, but not both (Relyea and Auld 2004). So why did I find the opposite pattern, in which the inferior competitor invested in both traits,

even with decreasing resources? A similar pattern has recently been reported, in which tadpoles facing predation from gape-limited salamanders can prioritize investment in both growth and morphological defenses even when resource availability is reduced (Costa and Kishida 2015). Although this study was not designed to identify the mechanisms underlying such patterns, several recent studies have shown that prey in the presence of a predator can alter various physiological traits which can reduce metabolic rates, increase assimilation efficiency, and allow for the investment of energy in defense structures (McPeck 2004; Thaler et al. 2014; Thaler et al. 2012). Assuming that the digestive tract is the key organ mediating digestion efficiency and nutrient assimilation in animals (Clissold et al. 2010; Sibly 1981), elongating the gut might not be an unusual prey response to predators (but see (Steiner 2007)).

Investing in a competitive phenotype while maintaining defense is hypothetically adaptive. For example, Teplitsky & Laurila (2007) has suggested that investing in morphological defense is an important compensation for the commonly induced behavioral risk-taking that prey experience at low resource levels (Lima and Dill 1990). Because I did not conduct behavioral observations, it is not known whether foraging activity increased in the inferior competitor, but optimal digestion theory (Sibly 1981) predicts that a longer gut may help tadpoles increase fitness by increasing energy intake to accommodate the nutritional demands in the presence of both competitors and predators. A simple scenario may further illustrate why such a simultaneous investment occurred: lowering investment in competitive phenotypes can be costly and may ultimately exclude the least competitive prey from the local system (Holt et al. 1994). On the other hand, reducing defense investment in the presence of a better-defended superior competitor would not be adaptive because selection may intensify against the less defended prey species (Cipollini 2004). Thus, selection for plasticity in both defensive and competitive traits may occur simultaneously, resulting in tadpoles exhibiting larger tails and longer guts with no apparent trade-off between the two traits.

Although predation and competition typically have opposite effects on prey phenotypes (Relyea and Auld 2004), the fact that the inferior competitor simultaneously grew a longer gut to compete and a larger tail to defend does not indicate that trade-offs did not exist. Instead, it is possible that trade-offs were not apparent because I looked at a limited set of morphological traits. Clearly, an integrative analysis of physiological, behavioral and morphological traits would provide a more thorough understanding of the phenotypic trade-

offs in these tadpoles, although I acknowledge the difficulty of conducting such a study. Furthermore, another drawback of my experiment is the simplified laboratory conditions. Tadpoles typically live in dynamic and complex environments, so the phenotypic responses reported in this simplified scenario might differ from what tadpoles would exhibit in nature. For instance, the tadpoles in this study are typical to small ponds and ephemeral water bodies (paddy fields) in which desiccation could potentially influence prey strategies and phenotypic investments (Richter-Boix et al. 2011).

In conclusion, these prey species differentially invested in gut length, with the inferior competitor (PN) increasing its gut length in response to interspecific competition. Furthermore, this species also increased its gut length in the presence of predators while also maintaining a large tail for defense. This may indicate that when a competitive phenotype (here, the gut) also functions to benefit prey in predator-environments (e.g., increased growth), prey might evolve the ability to simultaneously compete *and* defend. Investment in defense typically induces costs, which arise from diverting energy needed for growth into defense structures. In this experiment, predation did not impair growth, and this apparent lack of defense cost may partially explain the current results (e.g., Siemens *et al.*, 2002). While the ability to simultaneously express competitive and defense phenotypes was first thought to be specific to plants (Jones et al. 2006; Siemens et al. 2002), these results indicate that this ability is present in animals too. The costs associated with these investments remain unclear and present interesting avenues for future research.

5.2.5. Figures

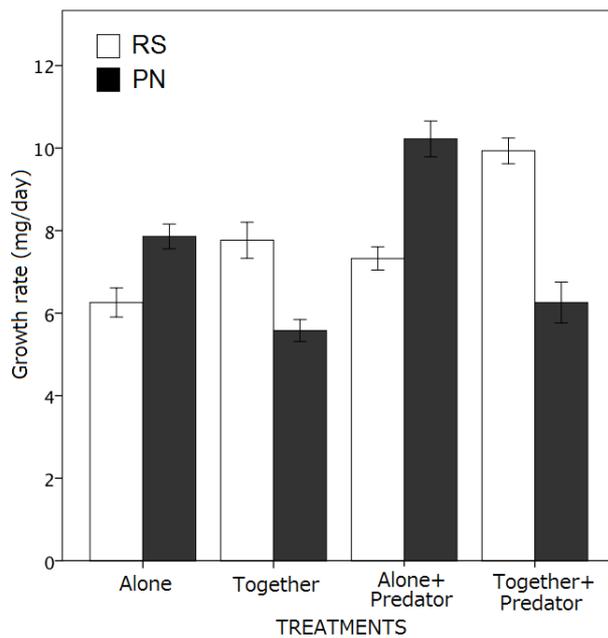


Figure 1. Growth rates of the tadpoles of *Rhacophorus schlegelli* (RS) and *Pelophylax nigromaculatus* (PN) when reared alone or together in the presence and absence of non-lethal dragonfly larvae. Data are mean \pm SE.

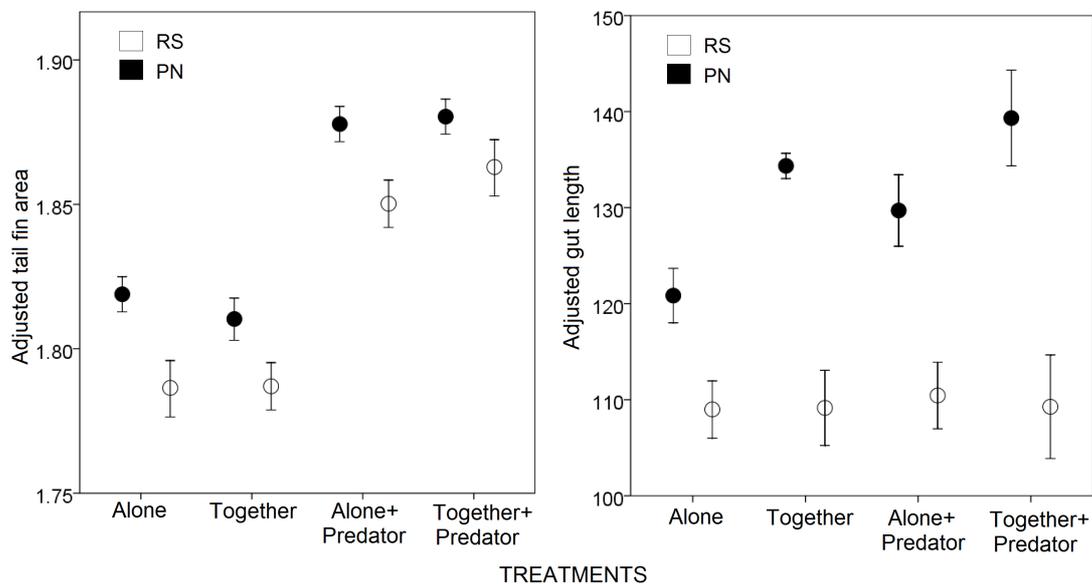


Figure 2. Morphological responses of the tadpoles of *Rhacophorus schlegelli* (superior competitor, RS) and *Pelophylax nigromaculatus* (inferior competitor, PN) when reared alone, together in the presence and absence of non-lethal dragonfly larvae. Tail fin areas and gut length were size-corrected prior to analysis. Tail fin areas were square-rooted and log-transformed. Data are mean \pm SE

5.2.6. Tables

Table 1. Results of general linear models summarizing the interactive effects of predation and competition on tadpole growth rate and morphological traits in the superior (*Rhacophorus schlegelli*, RS) and the inferior competitor (*Pelophylax nigromaculatus*, PN). Morphological traits were size-corrected before analysis (see Materials and methods). P-values in bold indicate significant effects at alpha = 0.05, N = 6. “Eta” represents partial eta squared.

		Competition			Predation			Comp. x Pred.		
		<i>F</i> (1, 20)	<i>P</i>	<i>Eta</i>	<i>F</i> (1,20)	<i>P</i>	<i>Eta</i>	<i>F</i> (1,20)	<i>P</i>	<i>Eta</i>
	Growth rate	34.56	<0.001	0.64	21.31	<0.001	0.51	2.46	0.132	0.11
RS	Tail fin area*	1.232	0.281	0.06	91.65	<0.001	0.82	1.97	0.177	0.09
	Gut length	0.01	0.902	0.01	0.03	0.847	0.01	0.03	0.87	0.01
	Growth rate	65.76	<0.001	0.77	15.61	<0.001	0.43	4.79	0.04	0.20
PN	Tail fin area*	0.04	0.846	0.01	49.3	<0.001	0.72	0.69	0.417	0.03
	Gut length	12.49	0.002	0.38	5.78	0.026	0.22	0.87	0.36	0.04

* *F* (1, 19)

Table 2. Tukey post-hoc tests following general linear models testing the effects of predation and competition on tadpole growth rate, tail fin area and gut length in the superior (*Rhacophorus schlegelli* RS) and the inferior competitor (*Pelophylax nigromaculatus* PN). *P*-values in bold indicate significant effects at alpha = 0.05, N = 6. GR=growth rate, TFA= tail fin area; GL=gut length.

Treatments	PN (inferior competitor)			RS (superior competitor)		
	GR	TFA	GL	GR	TFA	GL
Alone vs. together	0.002	0.996	0.023	0.029	0.998	0.998
Alone vs. alone+pred	0.001	<0.001	0.117	0.17	<0.001	0.994
Alone vs. together+pred	0.037	<0.001	0.002	<0.001	<0.001	0.999
Alone+pred vs. together	<0.001	0.002	0.854	0.808	<0.001	0.996
Alone+pred vs. together+pred	<0.001	0.975	0.286	<0.001	0.257	0.997
Together vs. together+pred	0.606	<0.001	0.73	0.001	<0.001	0.999

Chapter 6: Thesis summary and conclusions

This study investigated the mechanism of coexistence in species rich lotic tadpole community in Madagascar and in generalist pond dwelling tadpole species in Japan.

I demonstrated that tropical streams are heterogeneous enough at the microhabitat level to harbor different assemblages (Hypothesis 1). Habitat partitioning was a first axe that likely mediated tadpole co-occurrence in streams. In line with the predictions of environmental filter hypothesis, the tadpoles differentially responded to environmental variation. By inducing changes in habitat structures, disturbance affected species composition, which consequently might affect species interactions and species coexistence (Hypothesis 1). Forest specialist species were replaced by species adapted to open habitats outside forest, and such a complementary effect could be one mechanism that allowed abundances and species richness to be maintained between habitats despite changes in species composition. Tadpole assemblages were strongly structured by the environment.

Trophic analyses provided a better understanding of the mechanism of tadpole species co-occurrence in Madagascar streams. There was little variation in trophic levels among tadpoles (as inferred by their N isotopic values). Much trophic variation was observed in carbon isotope values, suggesting differences in food resources consumed by the tadpoles (Hypothesis 2). Although niche partitioning was not evident in some species from isotope analysis, examination of gut content revealed that species differed in their food resources. This study highlights the importance of combining these two methods, though time and resource consuming, in analyzing the trophic ecology of consumers. Subtle differences in food resource partitioning may not have been detected, had we limited analysis to stable isotope or gut analysis alone. Future studies should not dissociate these methods, as it has typically been done in previous tadpole studies.

Surprisingly, not only phenotypically divergent species but also species that are similar in mouthpart differed in their food resources. But, as it could be expected, phenotypically similar species shared more commonality in their food resources than more dissimilar ones. It was also observed that species may feed on similar food items (e.g., sediment and algal materials) but ingested these resources at different proportions; indeed, exploiting resources at different proportions can allow sympatric species coexist without strict partitioning (Behmer and Joern 2008; Simpson et al. 2004).

One remaining question is whether these species compete in their environment. Indeed, having manipulated species density in a side project, I did not find evidence of resource limitation in at least two species, but this was probably because resources were abundant during the experiment (unpublished data). A previous study revealed, however, that competition is important in these tadpoles, especially during the dry season when resources are limited (Strauß et al. 2016). In this respect, interspecific competition allows phenotypic divergence to be maintained among species.

In analyzing the relationship between tadpole morphology and functional role, I found that tadpoles, though many occupy similar trophic level, are not functionally redundant in nutrient recycling and in litter decomposition, two central ecosystem processes in streams (Hypothesis 3). I observed that tadpole abundance exerted a larger effect than species richness. It is possible that species complementarity effects were not evident in these tadpoles on the ecosystem processes I measured. But, because these species exhibit resource partitioning, it is very likely that they play differential roles on the ecosystem. Practically, resource partitioning can help understand and predict how species loss would affect ecosystem functioning. If one species disappear, so too is the ability of other groups to exploit the resources the local extinct species was adapted to use. Amphibian decline can dramatically impact ecosystem functioning as documented in some parts of the tropics (Whiles et al. 2006), and understanding their functional role is crucial to predict any trophic cascade that may result from their loss.

The last chapter provided evidence that phenotypically similar tadpoles can compete asymmetrically. Such a form of competition has the potential to exclude worst competitors (Holt et al. 1994). However, phenotypic plasticity allowed inferior competitor to compete and defend in the presence of superior competitors and predators (Hypothesis 4). It is not clear from this experiment whether, in the long term, these investments would be adaptive and what would be the trade-offs associated with these investments. Phenotypic plasticity can at least alleviate the strength of interaction between species and may help understand why generalists with little niche partitioning can coexist in nature.

Collectively, this study indicates that resource partitioning allows multiple tadpoles to coexist in lotic environments in Madagascar. Although mechanisms of species coexistence of tropical species have been the subject of debate (neutral vs. deterministic processes), this study provides solid evidence that coexistence in this system is strongly niche-based. Further, this study suggests that not only niche partitioning but also phenotypic plasticity may refrain

the exclusion of inferior competitors from local system. Ecologists have only begun to appreciate the importance of phenotypic plasticity in maintaining biodiversity, (Turcotte and Levine 2016), it would be interesting to investigate to what extent phenotypic plasticity can compensate for differences in competitive abilities in ecologically similar species.

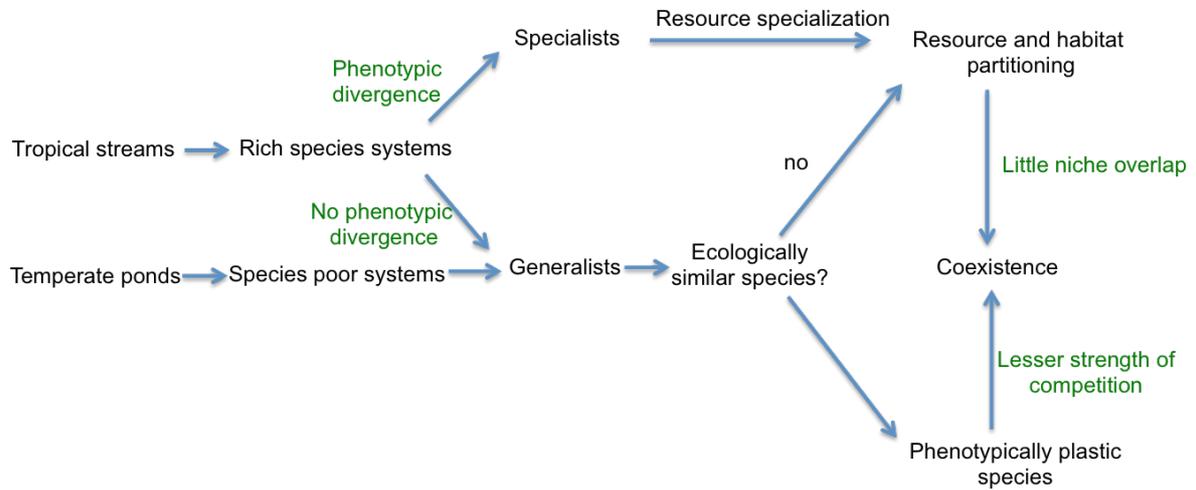


Figure 1. Simplified scenarios of mechanisms of species coexistence covered in this study.

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