Responses of Xishuangbanna's frog species to their environment:

habitat selection in adults

and phenotypic plasticity in tadpoles

By

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Dedication

This dissertation is dedicated to the memory of Josh Posner.
His vision and leadership got a big project off the ground.
His kindness and friendship made it a success.
He will be missed.

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Introduction

Amphibians are the most threatened vertebrate group and the main driver of amphibian population declines is habitat loss (Alford & Richards 1999; Collins & Storfer 2003; Gardner, Barlow & Peres 2007; Sodhi *et al.* 2008; Sodhi *et al.* 2009). Habitat loss can take many forms, and implies that an aspect of the habitat that a species needs for survival is missing in a given location. Thus habitat loss can encompass subtle changes to an ecosystem like the addition or loss of a species, to full scale ecosystem removal. In order to determine that a population is declining due to habitat loss requires knowing the habitat needs of a species. Unfortunately, hotspots of amphibian diversity generally contain poorly studied species, and are located in areas with high rates of land cover change (Sodhi *et al.* 2004; Gardner *et al.* 2007a; Rowley *et al.* 2010). This makes it a challenge to determine whether a landscape modification in a given area constitutes habitat loss for a species. Therefore, it is valuable to understand how species use and respond to natural and recently modified components of their environment in order to make informed predictions about their future persistence.

Xishuangbanna prefecture in southern Yunnan province, China, is part of the Indo-Burma biodiversity hotspot (Myers *et al.* 2000), and is a frog diversity hotspot within China (Xie *et al.* 2007). The frog fauna in Xishuangbanna is more similar to Southeast Asia than East Asia, and natural history and ecological information about the species is limited. Over the past thirty-five years, Xishuangbanna has converted over two-thirds of its native rainforests into plantations of exotic rubber trees (Li *et al.* 2007). This expansion of rubber agriculture has been accompanied by a 10-fold increase in household income and great improvements in living standards in many areas (Liu *et al.* 2006), yet the impact of this land conversion on biodiversity has yet to be quantified . One of the main obstacles preventing an assessment of rubber plantations on frog populations is the lack of information regarding frog species habitat needs.

The first chapter of this dissertation coauthored with Xiaodong Yang and Jin Chen from the Xishuangbanna Tropical Botanical Garden (XTBG), fills the void by examining the terrestrial and aquatic habitats frogs need to complete their full life cycle. Previous studies have investigated whether frog species use other types of tropical plantations as habitat, and generally conclude that some disturbance-tolerant species are consistently found in plantations (Gardner *et al.* 2007b; Wanger *et al.* 2009; Gibson *et al.* 2011). However, in these studies only the terrestrial habitat needs of juveniles and adults are considered, and aquatic breeding habitats are ignored (but see Parris & Lindenmayer 2004). Our work

demonstrates that both aquatic and terrestrial habitats should be considered since both are required for population persistence. We show that disturbance-tolerant species are present in rubber plantation terrestrial habitat, but no species use aquatic sites in rubber plantations for breeding. Therefore, rubber plantations represent a complete loss of habitat for disturbance-intolerant species, and a loss of breeding habitat for all species.

In addition to the well-documented changes to Xishuangbanna's terrestrial landscape, changes in the aquatic habitats have also been happening but are much more poorly documented. Native fish populations in rivers and streams have been severely reduced (J. Behm, *pers. obs.*), likely through overfishing and introductions of exotic species. By far the most common fish species encountered during formal and informal surveys was tilapia (*Oreochromis aureus*).   Tilapia are native to Africa and were introduced to Xishuangbanna about 30 years ago for food (Zhuangfang Yi, XTBG, *pers. comm*.) and have established in the Luosuo River, a tributary to the Mekong River. In addition, it is common for farmers in villages to raise tilapia in manmade fish ponds. The drastic expansion of this novel species was the inspiration for focusing on tadpole ecology in aquatic sites for my second and third chapters.

During breeding site surveys associated with Chapter 1, the three most common tadpole species we encountered were *Fejervarya limnocharis, Microhyla fissipes,* and *Polypedates leucomystax.* Ecologically, the three species are quite different. *F. limnocharis* is dark-colored and stays on the bottoms of pools. Its diet mainly comprises algae it scrapes off of leaves and rocks, and detritus. *M. fissipes* is the smallest of the three species and is a filter feeder. It is light-colored and mainly stays near the water surface in pools, constantly filtering particles out of the water. *P. leucomystax* is the largest of the three species and gray-colored. Unlike the other two species, it swims throughout the entire water column, but its mouthparts and diet are similar to *F. limnocharis*.

Despite their ecological differences, these three species generally co-occur in the same ephemeral pools, meaning they are exposed to the same suite of predators and competitors. In surveys, all three tadpole species frequently co-occurred in pools containing larval dragonflies (odonates) which are tadpole predators, whereas only *P. leucomystax* tadpoles were found in pools containing tilapia. Many tadpole species exhibit phenotypic plasticity in traits following exposure to predators and competitors, yet plastic responses in these three tadpole species, or any species from Xishuangbanna have never been studied. Phenotypic plasticity is often suggested as a mechanism by which species can adjust to changing environmental conditions (Agrawal 2001; Chevin & Lande 2011). Therefore, the goals

of chapters 2 and 3 were to understand the role phenotypic plasticity plays in helping these species adjust to their natural and recently introduced predators.

First, in a separate study not included in this dissertation, we conducted an experiment to quantify the strengths of intraspecifc competition within species and interspecific competition among pairs of species (Edmonds *et al. In Prep*). *M. fissipes* experienced the highest intraspecific competition strength, and *F. limnocharis* experienced the strongest interspecific competition in the form of reduced survival due to interactions with *P. leucomystax* (Edmonds *et al. In Prep*). I then conducted preliminary predation trials to quantify predation rates on the three tadpole species by tilapia and odonate predators. Odonates had the highest predation rates on *F. limnocharis* while tilapia had the highest predation rates on *M. fissipes.* Based on the results from these experiments about how each species experience predation and competition, I could make predictions for chapters 2 and 3.

The goal of chapter 2 was simply to quantify the patterns of phenotypic plasticity exhibited by each species in response to predation and competition. I exposed tadpoles to 3 levels of predation: no predator, tilapia, or odonate, crossed by 3 levels of competition: low competition, interspecific competition and intraspecific competition. I expected that *F. limnocharis* should have strong responses to interspecific competition and odonates, *M. fissipes* should have strong responses to intraspecific competition and tilapia, and *P. leucomystax* should have responses to intraspecific competition and minimal responses to predators. Overall, the three tadpoles matched my predictions in response to competition. In response to predation, however, *M. fissipes* had strong responses to both odonates and tilapia, whereas *P. leucomystax* also responded to odonates. The majority of previous work on phenotypic plasticity in tadpoles in response to predation and competition has been conducted in temperate systems (Relyea 2002; McCoy 2007; Teplitsky & Laurila 2007; Van Buskirk *et al.* 2011). In these systems, there is conformity across tadpole species in their responses to predation and competition according to their shared environment (Relyea 2002). My study differs, because although all three species share a common environment, their responses to predation and competition are species-specific.

Although a species may have a plastic response to a predator or competitor, it does not mean the response provides a fitness advantage. For example, when *Hyla chrysoscelis* tadpoles are exposed to odonates, tail depth increases and overall weight decreases. When these tadpoles are tested with an odonate, increased tail depth is correlated with increased survival, whereas smaller body size is correlated with decreased survival (McCollum & VanBuskirk 1996). In chapter 3, I examined which of

the trait changes I documented in chapter 2 were correlated with increased survival with a predator. In addition, because all three tadpole species exhibited strong responses to competition, I asked whether exposure to competition altered survival with a predator. I exposed tadpoles to no predators, odonates or tilapia while experiencing inter- or intraspecific competition. I then tested these tadpoles with predator- and competitor-induced phenotypes in predation trials with the same predator and the same competitive context in which they were raised. Previous exposure to odonates translated into increased survival with odonates, whereas previous exposure to tilapia translated into decreased survival with tilapia. In addition, the competitive context changed the outcome of these results. Since tilapia are invasive species, it is possible that tadpoles with previous exposure to tilapia were responding to a general fish cue, but the phenotype they expressed evolved in response to a native fish predator with a different hunting style than tilapia. Thus they are maladapted to responding to tilapia.

In conclusion, the results from all three chapters have several implications. First, in terms of general ecology, it is important to consider the ecological context of a system when designing studies. In chapter 1 if we had only considered terrestrial habitat, or in chapters 2 and 3 if I had only considered intraspecific competition, the conclusions would have been very different. Second, in terms of conservation, the changes in the landscape in Xishuangbanna are not advantageous for the frog species there. Frogs do not use rubber plantations for breeding habitat and tadpoles may be maladapted to responding to invasive tilapia. The most pressing species in need of conservation are the disturbance-intolerant species that are restricted to rainforests. Most of these species are endemic to Southeast Asia, thus losing them would mean losing a substantial amount of phylogenetic diversity.   At present, there does not appear to be a conservation concern for disturbance-tolerant species; however, the influx of wealth into Xishuangbanna has been accompanied by an increase in development. Most of this development is concentrated in the lowland valleys where it is easiest to build (Figure 1). These areas are also where disturbance-tolerant frog species breed.   If too many lowland, fish-free breeding sites are eliminated, then disturbance-tolerant species populations could decline.
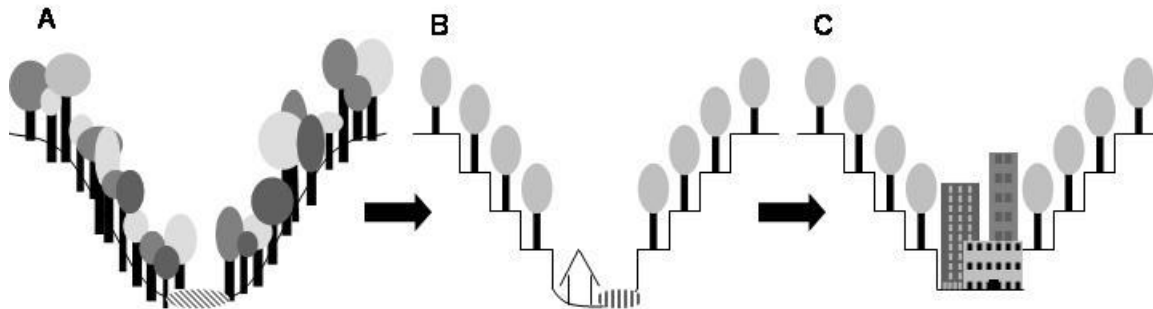
Figure 1: Diagram depicting transformation of Xishuangbanna's landscape in the lowlands. A) In the past, hillsides were covered by primary or secondary forest. Ephemeral pools could easily accumulate on valley bottom (diagonally shaded oval). B) In present day Xishuangbanna, forested sloping hillsides have been converted into terraced rubber plantations, and valley bottoms are covered with villages and agriculture. Disturbance-tolerant frog species use ephemeral pools that accumulate in valley bottoms (vertically shaded oval). C) In the future, demand for rubber in China and abroad will likely maintain rubber plantations on hillsides. Local economic development in Xishuangbanna will promote further development of valley bottoms in some localities increasing impervious surface area and eliminating breeding sites for ephemeral pool breeding frogs. Rubber plantation terraces may be remaining area where ephemeral pools accumulate in these areas.

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Gilman, R. T. and J. E. Behm. 2011. Hybridization, species collapse, and species reemergence after disturbance to premating mechanisms of reproductive isolation. Evolution. 65: 2592-2605.

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Behm, J.E., B. E. Knaebe, V. W. K. Fu, and D. A. Edmonds. *In Press. Polypedates leucomystax* (Java Whipping Frog) communal nesting. Herpetological Review

Fu, W. K. V., and J. E. Behm. *In Press.* Field guide to the frogs of the Xishuangbanna Tropical Botanic Garden.

Edmonds, D. A. and J.E. Behm *In Press. Raorchestes menglaensis* (Mengla small treefrog) coloration. Herpetological Review.

Edmonds, D. A. and J.E. Behm *In Press. Raorchestes menglaensis* (Mengla small treefrog) geographic distribution update. Herpetological Review.

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Chapter 1

Surveys incorporating breeding and post-metamorphic habitat use reveal rubber plantations are poor habitat for frogs

Jocelyn E. Behm, Xiaodong Yang, and Jin Chen

Abstract

Habitat loss is a major threat to tropical biodiversity, yet it can be difficult to identify for species with complex life histories. If all habitat needs of a species are not considered, there is a risk of overestimating the conservation value of altered habitat. Here we investigate whether the conversion of tropical rainforest into rubber plantations constitutes habitat loss for a community of frog species in Xishuangbanna, China. We conducted surveys to quantify habitat use for breeding and post-metamorphic life history activities in rainforest, rubber plantation and other human impacted sites. Post-metamorphic habitat surveys indicated the frog community comprises disturbance-tolerant generalist species that were present in all three areas and disturbance-intolerant species that were confined to the rainforest. Breeding surveys showed no species bred in rubber plantation sites. Canopy cover was the major driver of breeding site selection with disturbance-tolerant species in general preferring low canopy and disturbance intolerant-species preferring high canopy sites. We demonstrate that the absence of species breeding in the high canopy rubber plantation sites is due to a habitat mismatch: species that use the rubber plantation as post-metamorphic habitat breed in low canopy sites and species that breed in high canopy sites never go in the rubber plantation. We corroborate these results with a series of three experiments which demonstrate that rubber plantation aquatic sites are suitable for tadpole growth and survival. Rubber plantations represent complete habitat loss for disturbance-intolerant species, and an absence of breeding habitat for disturbance-tolerant species. Rubber plantations alone cannot support frog populations. Our findings indicate that considering habitat use for disparate life history activities provides a more complete assessment of the conservation value of altered habitat for biodiversity.

Introduction

Habitat loss is a major threat to tropical biodiversity (Gibson *et al.* 2011), yet identifying that habitat has been lost can be difficult. This is because deforestation is a complex process where forest is not wholly and immediately eliminated. Rather, driven largely by the conversion of forest into agriculture, continuous forest landscapes are converted into networks of remnant forest patches isolated by a matrix of agriculture, roads and development. Initially, the matrix was viewed as a barrier to dispersal- a space species passed through temporarily between ideal habitat patches (Gascon *et al.* 1999). However, as deforestation has escalated, forest patch sizes are smaller and more isolated by an extensive matrix (Li *et al.* 2007; Ribeiro *et al.* 2009) and the matrix-as-barrier model is less applicable. Species persistence is not simply a function of successfully passing through the matrix but rather directly using it as habitat for sustaining life history activities. Therefore, there is a strong need to quantify how species use matrix as habitat to determine if converting forest to agriculture constitutes habitat loss (Gardner, Barlow & Peres 2007).

Southeast Asia has one of the highest tropical deforestation rates and plantation agriculture is a predominant matrix feature in the Southeast Asian tropics (Sodhi *et al.* 2004). Because plantations often provide a well-needed livelihood source for local residents, there have been high hopes that plantations would also support high levels of biodiversity (Schroth *et al.* 2004). Plantations are structurally and biologically simplified versions of natural forests and in some cases harbor significant numbers of species, yet there is a lack of consensus on the conservation value of plantations for biodiversity (Barlow *et al.* 2007).

Standard methods for investigating the impact of plantations on native species involve comparing the species composition between plantations and primary forests. While these methods are useful, they may be limited in scope for species with complex life histories, like amphibians, if they do not consider the multiple habitat types required for species to complete their life cycle. Overall, studies show a decrease in abundance, richness or composition of amphibian species in plantations compared to primary forest, and the pattern seems to be stronger for more intensively managed plantation types, although there are exceptions (Gardner, Barlow & Peres 2007).

We argue that these studies may over-estimate the value of plantations for tropical amphibians for three reasons. First, an appreciable number of studies surveyed leaf-litter amphibians in the neotropics

(Gardner *et al.* 2007b). This group is in part composed of species from the endemic Leptodactylidae, Brachycephalidae, and Craugastoridae families that are often characterized by terrestrial development, forgoing the need for aquatic habitats for reproduction. While plantations may be suitable for terrestrial development species, other sympatric species that do require aquatic habitats for breeding are more vulnerable to disturbance (Becker *et al.* 2007). Therefore these results may not be applicable to leaf-litter amphibians in other regions.

Second, most studies conduct visual encounter surveys of amphibian fauna. These methods are useful for obtaining rapid inventory data on species that are present; however, they only survey individuals in the post-metamorphic phase of their life cycle and do not consider breeding habitat (e. g. Wanger *et al.* 2010). We have a limited understanding of the quality of breeding habitat that plantations provide (Parris & Lindenmayer 2004), thus there is a strong need to understand whether plantations can provide adequate breeding habitat for amphibians.

Finally, while many studies are conducted in a community context, the individual species are treated as independent units. In fact, closely related species have similar ecological requirements and can have similar responses to disturbance (Helmus *et al.* 2010). If this is the case, there may be a phylogenetically non-random assemblage of species that is absent from plantations which results in a greater loss of evolutionary diversity that if species were lost at random.

Here, we present an investigation into a poorly studied amphibian group from tropical Asia in a region experiencing rapid conversion of rainforests into rubber plantations. The ultimate goal of our study was to determine whether the conversion of rainforests into rubber plantations constitutes habitat loss for the local amphibian species. We reached this goal by 1) conducting surveys of species' breeding and post-metamorphic habitat use; 2) identifying environmental variables related to habitat selection; 3) quantifying the quality of breeding habitat provided by rubber plantations. We interpret our results in a phylogenetic context to provide a deeper insight into how this community is being affected by rubber plantations. Finally, by integrating breeding and post-metamorphic habitat use we conclude that the conversion of rainforests into rubber plantations does constitute habitat loss and threatens the native species in the area.

Methods

Study System

We performed our study in the tropical Xishuangbanna Autonomous Prefecture in southern Yunnan Province, China. Xishuangbanna is part of the Southeast Asian Indo-Burma biodiversity hotspot (Myers *et al.* 2000) and is a frog diversity hotspot within China (Xie *et al.* 2007) with over 35 species from six families. Bordering Laos and Burma, Xishuangbanna prefecture is 19,700 km^2, with elevations ranging from 550-1980 m. It has a typical seasonal tropical monsoon climate with the rainy season ranging from May to October (Cao *et al.* 2006) which is the breeding season for the ephemeral pool-breeding frog species we studied. Over the past 30 years, nearly two-thirds of the native lowland rainforests in Xishuangbanna have been converted to rubber plantations (Li *et al.* 2007), leaving a landscape of small, isolated lowland rainforest patches separated by agriculture, human development and rubber plantations. The impact of this land conversion on native biodiversity has yet to be quantified. Our in-depth surveys and experiments took place during the rainy seasons of 2008, 2009 and 2010 at the 900 ha Xishuangbanna Tropical Botanic Garden (XTBG) (Longitude: 101°15' 9.7"E, Latitude: 21°55' 44.63"N). XTBG has one of the few remaining remnant patches of lowland rainforest located adjacent to a 195 ha working rubber plantation.

Methods Overview

Our goal was to understand habitat use for the ephemeral pool-breeding species from Xishuangbanna to infer whether rubber plantations constitute habitat loss for this species assemblage. Therefore we conducted surveys to quantify breeding and post-metamorphic habitat use within the 3 major land cover types in Xishuangbanna: rainforest, rubber plantation and impacted sites. Impacted is a general category for land-cover types that were not rubber plantation or rainforest and had some level of human impact, ranging from grassy fields to dirt roads. In 2008, we conducted preliminary surveys in each land cover type to identify aquatic sites and learn the frog calls. In 2009, we established 2.8 km, 3.1 km, and 3.8 km routes for intensive surveys in rubber plantation, rainforest and impacted areas respectively. Survey routes consisted of aquatic sites connected by intervening stretches of terrestrial habitat and were used for both breeding and post-metamorphic surveys.

During our surveys we discovered that no frogs were breeding in the aquatic sites in the rubber plantation. Most rubber plantations in Xishuangbanna are placed on hillsides. Unlike the rainforests they replaced, rubber plantations have terraces which provide locations for water to collect. In addition, rubber farmers dig small fertilizer pits between trees along the terraces for twice annual fertilizing. Rain water collects in the pits and may provide additional breeding sites. Initial investigations of the pits

revealed they had few aquatic invertebrates and potentially could not support tadpoles. Therefore, we conducted three experiments to determine if the quality of the rubber plantation fertilizer pits is high enough to support frog breeding.

Breeding Survey

Our breeding surveys consisted of visiting aquatic sites along our survey routes at night to record calling activity and then revisiting the same sites the following day to record environmental site variables and oviposition activity. Our nighttime breeding survey methods were based on the North American Amphibian Monitoring Program protocol (Weir & Mossman 2005). The majority of our breeding sites were small, temporary rainwater pools; however, we also surveyed a few larger and more permanent man-made pools. We surveyed each route once per week for 11 weeks in the rainforest and impacted areas and 9 weeks in the rubber plantation areas during the 2009 breeding season. At each breeding site, we listened for 5 minutes and recorded the total number of species calling. We then listened for an additional 2 minutes, and if no new species were heard the survey was over. If we did record new species, we kept adding 2 minute segments until no new species were heard. Species calling abundance was recorded as 0, 1, 2, or 3 in accordance with the North American Amphibian Monitoring Program Protocol (Weir & Mossman 2005).

For our day surveys, we returned to the sites surveyed the previous night and recorded the total number of egg masses per species. Once egg masses were identified, we conducted 5, 1-meter dipnet sweeps to quantify the density of tadpoles, invertebrates and fish. Tadpoles were identified to species and fish and invertebrates were identified to the lowest taxonomic level possible. We also recorded the following abiotic data for each site: surface area of water (length x width of pool), mean depth (mean of 5 depth measurements), percent vegetation cover in water, pool substrate (rocks, gravel, cobble, silt, mud, leaf litter), and percent canopy cover.

Analyses of the breeding survey data were conducted on calling and oviposition data separately, with evidence of oviposition defined as tadpoles and/or eggs at a site. To determine which environmental variables influenced breeding site choice, we conducted separate partial correspondence analyses on the presence of calling and oviposition at breeding sites. Partial correspondence analysis performs an ordination of the site data based on species composition which is then constrained by the environmental variables. The significant effect of each environmental variable is considered independently of the other variables. The invertebrate taxa were split into 3 categories prior to analyses:

predatory invertebrates (based on firsthand knowledge of tadpole predation or from the literature; hereafter referred to as predators), non-predatory invertebrates (hereafter referred to as invertebrates) and snails. When snails were present at a site their densities were orders of magnitude higher than other non-predatory invertebrates and thus obscured the signal of other invertebrates, so they were placed in their own category.   The variables surface area, mean depth, vegetation, canopy cover, fish density, predator density, invertebrate density, and snail density were log transformed and standardized to have mean 0 and variance 1 prior to analyses. Since the pool substrate classes were not mutually exclusive (i.e. a pool could have leaf litter, silt and cobble), we conducted a PCA on all 5 pool substrate variables to derive a composite pool substrate variable. The first 2 PCA axes represented 41% and 25% of the variation, respectively, and summarized different aspects of the pool bottom with silt loading strongly and positively to PC1 and mud and leaf litter loading negatively to PC2. Therefore, we used both PC1 and PC2 in analyses. We also included tadpole density as an environmental variable in the analysis for calling. Note this includes the density of all tadpoles, not just conspecifics at a site.

We used linear multilevel models (Gelman & Hill 2008) to calculate regression coefficients for each species for the environmental variables that were statistically significantly associated with calling and oviposition. In our models, the environmental variables were treated as fixed effects and species were treated as random effects.

Post-metamorphic Survey

Post-metamorphic surveys involved visually searching the intervening habitat between breeding sites for frogs and were conducted at the same time as our breeding surveys. Searches were started once we were far enough away from the breeding site as to not include individuals who were actively engaged in breeding. While it is possible that we counted individuals who were migrating to breeding sites in our surveys, they were not actively engaged in breeding activity (e.g. calling) at the time of our encounter.

Rubber Plantation Breeding Habitat Quality

Laboratory experiment

We conducted a laboratory experiment to test whether water from the rubber plantation fertilizer pits is suitable for tadpole growth and development using three common species: *Fejervarya limnocharis*, *Polypedes leucomystax,* and *Rhacophorus rhodopus.* All three species are listed as least concern according to IUCN. *F. limnocharis* adults were recorded in the rubber plantation during post-

metamorphic habitat surveys, and were recorded breeding in disturbed sites. Neither *P. leucomystax* nor *R. rhodopus* adults were observed in the rubber plantation, but *P. leucomystax* did breed in disturbed sites. *F. limnocharis* has a larval period of 4-7 weeks and the other two species have larval periods of 6-8 weeks (J. Behm *unpublished data)*. We collected early-stage (stages 25-30) (Gosner 1960) tadpoles from ten pools. Tadpoles were randomly assigned to two treatments: "rubber" water which was collected directly from the fertilizer pits and filtered through ¼ mm mesh before use in the experiment, and rain water which was collected from a large catchment. We recorded initial weights by haphazardly selecting three individuals of the same species and weighing them together because they were too light to weigh individually. The group of three was then transferred to a 2.5 L bucket containing either rubber water or rain water. Due to the availability of tadpoles, *F. limnocharis* treatments were replicated ten times while *P. leucomystax* and *R. rhodopus* treatments were replicated three times each. Throughout the course of the experiment, tadpoles in both treatments were fed an *ad libitum* diet of spirulina-based fish food, and water was changed every three days. At the end of 14 days, we calculated survival for each bucket, and tadpoles were weighed individually and identified to their Gosner developmental stage. While 14 days does not encompass the entire larval period, weight declines in the later stages of larval development during tail absorption and oral transformation. We chose 14 days because it covers a period of rapid growth before weight declines, and other work has demonstrated experimental responses to growth and survival in 14 days for temperate species with a much longer larval period (~3 months) (Skelly, Freidenburg & Kiesecker 2002).

We used relative growth per day, survival and final Gosner developmental stage as response variables in statistical analyses. Analyses were conducted on rearing container means within each treatment, and growth per day was log transformed, survival was arcsine square root transformed, and Gosner stage was square root transformed to meet the assumption of normality of variances prior to analysis. We used MANOVA with growth per day, final Gosner stage and survival as the multivariate response variable, and treatment and species as the independent variables.

Field Experiment

We conducted a field transplant experiment to determine whether the rubber plantation fertilizer pits are suitable for tadpole growth and development. Specifically, we predicted that the pits would be food limited for tadpoles based on their apparent low productivity.   Therefore, we raised tadpoles under two treatments:"food added" and "no food added". We used *F. limnocharis* tadpoles only for this

experiment due to tadpole availability. We identified 12 fertilizer pits in XTBG's rubber plantation with the potential to remain full of water for the duration of the experiment. Pits were on average 50 L (+/- 11.78 L) in volume (0.6 m x 0.4 m x 0.2 m). Invertebrates were scarce, but the ones that were present were removed from each pit before the pit was covered with 1 mm mesh to permit rain water to enter, retain tadpoles in the event of flooding, and prevent predators or frogs from entering the pits. We haphazardly selected nine early-stage *F. limnocharis* tadpoles and weighed them as a group, then added the group to a pit. Pits were randomly assigned to a treatment for a total of six replicates per treatment. The food-added pits received a food addition of spirulina-based fish food for two consecutive days followed by one day without a food addition. The no food added pits received no food additions. Water in the pits was closely monitored, and if the water evaporated to less than half full, we refilled it using water from neighboring pits that were not in the experiment. After 14 days, we calculated survival for each pit, and we weighed each tadpole individually and identified them to Gosner developmental stage.

We used log transformed growth per day, arcsine square root transformed survival and square root transformed stage as the response variables in statistical analyses. We calculated a fluctuation index for each pit as the total amount of water added to each pit over the course of the experiment. We used MANOVA to analyze these data again with growth per day, final Gosner stage and survival as the multivariate response variable, and treatment, pit fluctuation index and pit volume as the independent variables.

Pits in other habitats

Rubber plantation fertilizer pits are similar in volume to many of the ephemeral pools where we observed breeding; however, they are narrower and deeper than most commonly used pools in other habitats. To determine whether frogs have an aversion to breeding in pools this shape, we dug similar-sized pits at one rainforest and two impacted sites. At each site we dug 10 pits spaced 3-4 m apart. After each large rainfall, we monitored these pits and recorded which species used these pits for oviposition. Because these pits were freshly dug, no pit contained its own vegetation; however, pits were surrounded by ambient vegetation, and we expected that species that oviposited in ephemeral pools in the area should also oviposit in these pits.

Results

Post-metamorphic Surveys

During our post-metamorphic surveys we encountered 22 species across the three land-cover areas (Fig. 1a). Rainforest had the most species (n=18) followed by impacted (n=15), and rubber plantation (n=11). Ten species were shared by all three areas. There were 7 species unique to rainforest, 2 species unique to impacted and no species unique to rubber plantation sites. All 11 species found in rubber plantation were also found in impacted sites. Comparisons of species composition among areas yielded strong phylogenetic patterns. Twenty of the 22 species we found were from 3 families: the Microhylidae, Ranidae and Rhacophoridae. Microhylids were the most tolerant of disturbance with species abundant in both impacted and rubber plantation sites (Fig. 1a). Ranids were somewhat tolerant of disturbance with three out of seven species in both impacted and rubber plantation areas (Fig. 1a). Rhacophorids were the least tolerant with no species present in rubber plantation sites (Fig. 1a).

Breeding Surveys

In our breeding surveys we heard 19 species with 12 species calling at rainforest, 13 species calling at impacted, and no species calling at rubber plantation sites (Fig. 1b). The rainforest and impacted sites shared 6 species, 6 species were unique to the rainforest and 7 species were unique to disturbed sites.

We recorded oviposition for 11 species with 5 species ovipositing in rainforest sites, 8 species ovipositing in impacted sites and 0 species ovipositing in rubber plantation sites (Fig. 1c). The rainforest and impacted areas shared 2 species, and there were 3 species unique to rainforest and 6 species unique to impacted sites. The phylogenetic patterns for breeding were similar to the patterns exhibited in the post-metamorphic surveys. Species that were unique to rainforest sites were mostly Rhacophorids and species that were unique to impacted sites were mostly Microhylids; however, there were Microhylids, Rhacophorids and Ranids present in both areas. In fact, in both areas the three most common tadpoles were a Microhylid, a Ranid and a Rhacophorid, yet the identity of the tadpoles differed at the rainforest versus impacted sites (Fig. 1c).

In our daytime breeding site surveys, we encountered 4 fish taxa, 9 predatory invertebrate taxa and 10 non-predatory invertebrate taxa (Appendix Table A1). The partial correspondence analysis showed that depth, canopy cover and invertebrate density significantly influenced the presence of calling at breeding sites (Table 1). The correspondence analysis plot for calling (Fig. 2a, 2b) shows that both canopy cover and PC1 affect species composition at sites in the same direction and were positively associated with the majority of rainforest sites. Predators had an equal but opposite effect on species

composition compared to canopy cover and PC1, and was positively associated with the majority of the impacted sites.   Invertebrates and tadpoles affected species composition in the same direction which was opposite to depth, fish and snails.

PC1, canopy cover, fish and snails significantly influenced the presence of oviposition at breeding sites (Table 1). The correspondence analysis plot (Fig. 2c, 2d) again shows canopy and PC1 influence oviposition in the same direction, and were positively associated with the majority of rainforest sites. Fish and snails influence oviposition in the direction perpendicular to canopy and PC1.

Because the partial correspondence analysis considers the impact of each environmental variable on species composition independently, two variables that affect species composition in the same direction (e.g. fish and snails Fig. 2B) are not necessarily correlated. Therefore in order to understand the correlation structure of the variables, we calculated correlation coefficients for all pair-wise combinations of environmental variables (Appendix Table A2). Based on the results of the correspondence analysis and the correlation coefficients, general patterns emerge in terms of how environmental variables influence species composition at breeding sites. In general sites are split into two main groups: sites with fish and sites without fish. Sites with fish have low breeding activity are correlated with low predator abundance. Sites without fish have higher breeding activity and are further split into two groups: high and low canopy sites. High canopy sites are positively correlated with PC1 and negatively correlated with predators.

We used a multi-level linear model to calculate regression coefficients for each species for the significant environmental variables that affected calling and oviposition (Table 2).   Regression coefficients for canopy cover were the largest for both calling and oviposition. Species that bred only in the rainforest on average had positive regression coefficients for canopy cover while species that bred in disturbed sites had negative coefficients for canopy cover.

Habitat mismatch

A major result from the breeding surveys is that frogs are not breeding in rubber plantation sites, and canopy cover is the strongest environmental factor influencing breeding site selection at sites where breeding was recorded. The post-metamorphic surveys revealed that there are frogs that use rubber plantation as post-metamorphic habitat, yet they leave the rubber plantation to breed. To understand the relationship between canopy cover and post-metamorphic habitat use, we calculated the mean canopy cover across all breeding sites that each species used for calling or oviposition (Fig. 3). When

plotted with respect to post-metamorphic habitat use, it becomes apparent that the species that use the rubber plantation as post-metamorphic habitat prefer to breed in much lower canopy cover sites and will not breed in the rubber plantation. The species that breed in high canopy cover sites within the range of canopy cover in the rubber plantation use the rainforest as post-metamorphic and breeding habitat. They never leave the rainforest and go in the rubber plantation, so they will never breed in the rubber plantation.

Rubber Plantation Breeding Habitat Quality

Laboratory Experiment

There was a significant effect of treatment ($F_{1,26}$=6.35, P<0.01), species ($F_{2,26}$=21.72, P<0.01) and no significant treatment by species interaction ($F_{2,26}$=1.31, P=0.27) for the lab experiment. The significant treatment effect for growth per day was due to *Fejervarya limnocharis* tadpoles raised in rubber water growing significantly more than tadpoles grown in rainwater ($F_{1,26}$= 12.30, P<0.01)(Fig. 4a). The significant treatment effect for developmental stage was due to *Polypedates leucomystax* tadpoles raised in rubber water having a higher final stage than those in rain water ($F_{1,26}$= 4.82, P<0.04, Fig. 4b). All three species had equally high survival in rubber water and rain water (Fig. 4c). Significant species effects for growth ($F_{2,26}$= 89.22, P<0.01) and stage ($F_{2,26}$= 90.08, P<0.01) were due to *P. leucomystax* tadpoles having significantly higher growth and lower development stage than the other two species.

Field Experiment

There was no effect of treatment ($F_{1,8}$=0.06, P=0.98), pit volume ($F_{1,8}$=0.58, P=0.65) or pit fluctuation ($F_{1,8}$=1.07, P=0.43) on *F. limnocharis* growth, development or survival. Tadpoles displayed equally high growth ($F_{1,8}$=0.13, p=0.73, Fig. 4a), development ($F_{1,8}$= 0.08, P=0.78, Fig. 4b), and survival ($F_{1,8}$= 0.21, P=0.66, Fig. 4c) in the food added and no food added treatments. *F. limnocharis* tadpoles grown in the lab and field had comparable growth rates, and development rates, while tadpoles grown in the lab had slightly higher survival (Fig. 4).

Pits in other habitats

We recorded oviposition repeatedly throughout the breeding season in both areas where we dug pits similar in shape to rubber plantation fertilizer pits. In the rainforest, we recorded oviposition by

Limnonectes bannaensis and *R. rhodopus.* In the impacted sites, we recorded oviposition by *Microhyla fissipes, M. butleri, M. heymonsi, Micryletta inornata, F. limnocharis,* and *P. leucomystax.*

Discussion

We argued that surveys quantifying both breeding and post-metamorphic habitat use were necessary for determining the impact of rubber plantations on the native frog fauna in Xishuangbanna, China. We demonstrated that the conversion of rainforests into rubber plantation represents a complete loss of habitat for disturbance-intolerant species. Rubber plantations do provide post-metamorphic habitat for disturbance-tolerant species, but are not used for breeding by any species. By quantifying both types of habitat use, we acquired a more complete picture of the value of rubber plantations for supporting frog diversity in Xishuangbanna.

Post metamorphic habitat selection

In terms of post-metamorphic habitat, rubber plantation was inferior to the other two land cover types. Rubber plantation had the lowest species richness, and no species were unique to the rubber plantation. The species missing from rubber plantation were phylogenetically non-random. No species from the Rhacophorid family were found in the rubber plantation, rather the species found were three disturbance-tolerant Ranids and all seven Microhylids. Work in cacao plantations in Sulawesi suggested that amphibian richness is influenced by microclimates provided by plantation features such as logs and leaf litter (Wanger *et al.* 2009). We hypothesize that these factors are likely contributing to the diversity patterns we observed as well. Rubber plantations lack understory vegetation and are likely more dry than the other two land cover types (Guardiola-Claramonte *et al.* 2008). The disturbance-tolerant Microhylids are leaf litter frogs that are more resistant to desiccation than other species and can likely better tolerate conditions in the rubber plantation. The three species from the *Rhacophorus* genus were among the most sensitive species we encountered. These are gliding treefrogs with webbing between their front and rear toes which allows them to glide between branches in the forest canopy. Therefore, structural and ecological components of the rubber plantation may also be unsuitable for these species. This is consistent with work from Madagascar which showed arboreal species were highly sensitive to habitat modification (Vallan 2000).

The conversion of rainforest into rubber plantation clearly represents a loss in post-metamorphic habitat for these disturbance-intolerant species. For disturbance-tolerant species, it is not yet clear whether rubber plantations add high quality post-metamorphic habitat. All disturbance-

tolerant species that were found in rubber plantation were also found at impacted sites, and most were also found in rainforest, indicating that the absence of rubber plantations would not eliminate post-metamorphic habitat. It is possible that rubber plantations could be a sink habitat if, for example, food resources or pesticide levels caused low fitness for individuals in the rubber plantation. Alternatively, rubber plantations may be a high quality habitat for some species. The invasive earthworm, *Pontoscoles corthrurus,* is present in high abundance in rubber plantations, and we observed *Fejervarya limnocharis* juveniles eating earthworms (J. Behm *pers obs)*. Quantifying the quality of post metamorphic habitat provided by rubber plantations should be an area for future research.

Our survey was likely a best-case scenario for frog diversity in rubber plantations in Xishuangbanna as XTBG's rubber plantation is relatively old (ca. 30y) compared to most of the plantations, is not as intensively managed, and is adjacent to a relatively large remnant rainforest patch which likely acts as a source of frog species. Given that Rhacophorids avoided this plantation may indicate that rubber plantations are dispersal barrier for Rhacophorids and other disturbance-intolerant species. Confirming the extent of genetic isolation for these populations is a necessary avenue for future work.

Breeding Habitat Selection

Rubber plantation was also inferior to the other two land-cover types with respect to breeding as all species avoided breeding in rubber plantation sites. While there were species from all three major families breeding in both areas, the species assemblages in rainforest and impacted sites more or less mimicked the assemblages for post-metamorphic habitat with disturbance-tolerant Microhylids breeding mostly in impacted sites and disturbance-intolerant Rhacophorids breeding in rainforest sites.

The main environmental drivers of breeding site selection were presence of fish and canopy cover. While fish was not a significant explanatory variable in the calling analyses, it is likely that the significant effect of invertebrates was the result of frogs avoiding calling in pools with fish rather than directly choosing to call at pools with invertebrates because pools without fish have invertebrates as invertebrates are fish food. For the most part, avoiding breeding in pools with fish was a uniform trait across species. Fish are egg and tadpole predators, and it is common for frogs to avoid breeding in pools with fish (e. g. Resetarits & Wilbur 1989). Of the four fish taxa we encountered, Gambusia and tilapia, are notorious invasive species that can easily establish in poor quality habitats and negatively impact native species (Canonico *et al.* 2005; Stanback 2010). Fish-free aquatic sites will need to be maintained in Xishuangbanna to insure adequate frog breeding sites are available.

In contrast to their more or less uniform avoidance of fish, frog species were bimodal in responses to canopy cover with some species breeding in high canopy sites and others breeding in low canopy sites. For the most part, impacted sites had low canopy, although there were a few exceptions and all rainforest sites had high canopy. Canopy cover is a driver of breeding site selection in other systems (Skelly, Freidenburg & Kiesecker 2002) and either directly or indirectly influences a host of variables such as temperature, food resources, and invertebrate community. In our system, this bimodal distribution possibly evolved due to some species to taking advantages of gaps caused by tree falls or elephant activities. Breeding in higher temperature, low canopy sites comes with the advantage of faster tadpole development, but the risk of lower tadpole survival (Hawley 2010).

The bimodal distribution in species responses to canopy cover is also in part responsible for the habitat mismatch that resulted in no species breeding in the rubber plantation. Species that prefer to breed in high canopy sites, like what the rubber plantation offers, never go in the rubber plantation and only use rainforest for both breeding and post-metamorphic habitat. The disturbance-tolerant species that do use the rubber plantation as post-metamorphic habitat breed in low canopy sites, and as a result, no species bred in rubber plantation. Adult migration between post-metamorphic and breeding habitat is well-documented in pond-breeding species, but has been more difficult to study in ephemeral pool breeding species (Semlitsch 2008). Our work suggests that some disturbance-tolerant species are migrating between habitats during the breeding season.

It is also possible that species are avoiding breeding in the rubber plantation because it is detrimental to tadpole health, and our initial observations of aquatic sites in rubber plantations indicated that this might be the case. Our lab and field experiments demonstrated the opposite: tadpoles grown in rubber water and rubber plantation sites grew and developed well. In some cases, tadpole growth and development in rubber water exceeded that in rain water. Despite our efforts to filter the rubber water, some small particulates may have entered the rubber water lab treatments, providing an extra resource that enhanced growth and development. Nevertheless, rubber plantation sites are sufficient for tadpoles. Since *F. limnocharis,* the species we used in both experiments, did not breed in the rubber plantation indicates that there are likely other sites on the landscape (e.g. low canopy sites) that confer higher fitness to their offspring (Skelly, Freidenburg & Kiesecker 2002).

There are two disturbance-tolerant species, *Microhyla heymonsi* and *Occidozyga martensii* that used the rubber plantation as post-metamorphic habitat and also bred in both high and low canopy sites. Based on these preferences, we would expect that these are two good candidate species for

breeding in the rubber plantation. Ephemeral pool-breeding species are generally good at finding and colonizing new aquatic sites and we observed *O. martensii* and *F. limnocharis* adults swimming in rubber plantation aquatic sites on several occasions, indicating that finding the sites is not an issue. The fact that we did not record *O. martensii* and *M. heymonsi* breeding in the rubber plantation indicates that there may be an additional environmental variable that frogs are avoiding that we did not measure.

Conclusions

By considering the habitat needs for major life history activities, we are able to conclude that rubber plantations alone cannot sustain frog populations. Our result underscores the importance of considering the suite of habitat needs a species requires before making major conclusions about the value of altered habitat for biodiversity.

In their current state, upland rubber plantations adjacent to lowland impacted areas may provide sufficient habitat resources for the entire life cycle for some species. Like many areas, development in Xishuangbanna is non-random with respect to topography, and lowland areas generally are developed first (Silva, Simoes & Simonetti 2007). The continuation of this trend will further eliminate breeding sites for disturbance-tolerant species.

It may be possible to modify rubber plantations to make them more conducive to frog diversity. One of the most disturbance tolerant Rhacophorids, *Polypedates leucomystax,* is commonly found in urban sites (Bickford *et al.* 2010), and one individual lived in a hole in a bathroom of an XTBG office building for several months (J. Behm *pers. obs.)*. Despite their obvious tolerance to human disturbance, no *P. leucomystax* were found in the rubber plantation during post-metamorphic surveys. Perhaps increasing the amount of understory vegetation would provide necessary microclimates that would protect against desiccation for slightly more sensitive species. During our breeding surveys, we found tadpoles of many species in garbage piles, construction sites, and puddles in the middle of roads. Again despite this obvious tolerance to disturbance, no species bred in the rubber plantation. At this point our only suggestion for modifying rubber plantations to make them more attractive for frog breeding would be to reduce canopy cover.

In conclusion, we agree with (Gibson *et al.* 2011) in that there is no substitute for primary forests for biodiversity. The conversion of rainforests to rubber plantations eliminated habitat for disturbance-intolerant species. In order to conserve this unique assemblage of disturbance-intolerant species, remnant forest patches must be preserved.

Tables

Table 1: P-values for the impact of environmental variables on the presence of calling and oviposition from partial canonical correspondence analysis. Values in bold are statistically significant at the $p<0.05$ level.

Variable	Calling	Oviposition
Surface Area	0.113	0.102
Depth	0.001	0.106
Vegetation	0.666	0.898
PC1	0.120	0.005
PC2	0.078	0.087
Canopy Cover	0.000	0.001
Fish	0.201	0.011
Predators	0.158	0.832
Invertebrates	0.010	0.582
Snails	0.221	0.044
Tadpoles	0.049	-

Table 2: Multilevel model regression coefficients for each species with respect to environmental variables that significantly impact calling and oviposition. Breeding habitat was determined by as follows: for species that only called, calling area indicated breeding habitat. If species oviposited, oviposition area indicated breeding habitat (e.g. POLE called in both rainforest and impacted sites, but only oviposited in impacted sites, so it was categorized as "breeds in impacted").

Species	Calling			Oviposition			
	Depth	Canopy	Invertebrate	PC1	Canopy	Fish	Snail
Breeds in Rainforest							
Limnonectes bannaensis	0.09	0.84	-0.03	0.11	2.42	0.03	-0.20
Rhacophorus rhodopus	0.21	3.66	0.16	0.12	1.47	-0.08	-0.05
Rhacophorus kio	0.13	0.29	0.07	0.42	0.44	-0.29	0.11
Rhacophorus maximus	0.06	0.83	0.12	-	-	-	-
Leptolalax ventripunctatus	0.05	3.98	-0.09	-	-	-	-
Breeds in Impacted							
Micryletta inornata	0.13	0.08	0.05	-	-	-	-
Kaloula pulchra	-0.20	-0.24	0.30	-0.09	-0.13	-0.45	0.15
Microhyla pulchra	0.39	-1.89	0.05	0.28	-1.72	-0.39	0.17
Microhyla fissipes	-0.18	-1.32	-0.09	-0.87	-0.71	-0.03	-0.08
Fejervarya limnocharis	-0.30	-1.26	-0.05	-0.17	-1.24	-0.19	-0.29
Hoplobatrachus rugulosus	-0.07	-0.59	0.08	0.09	-0.85	-0.40	0.10
Chiromantus doriae	0.01	-2.31	-0.23	-	-	-	-
Polypedates leucomystax	-0.43	-0.80	0.08	-0.11	-0.61	3.44	-0.24
Breeds in Both							
Microhyla butleri	-0.01	-1.95	-0.01	0.31	0.02	-0.61	0.28
Microhyla heymonsi	-0.01	0.28	-0.06	0.12	-0.41	0.52	-0.51
Occidozyga martensii	-0.32	-0.93	0.14	-0.59	0.53	2.96	0.22
Hylarana nigrovitata	0.45	0.78	-0.40	-	-	-	-
Kurixalus odontotarsus	0.13	0.08	0.05	-	-	-	-

Figures
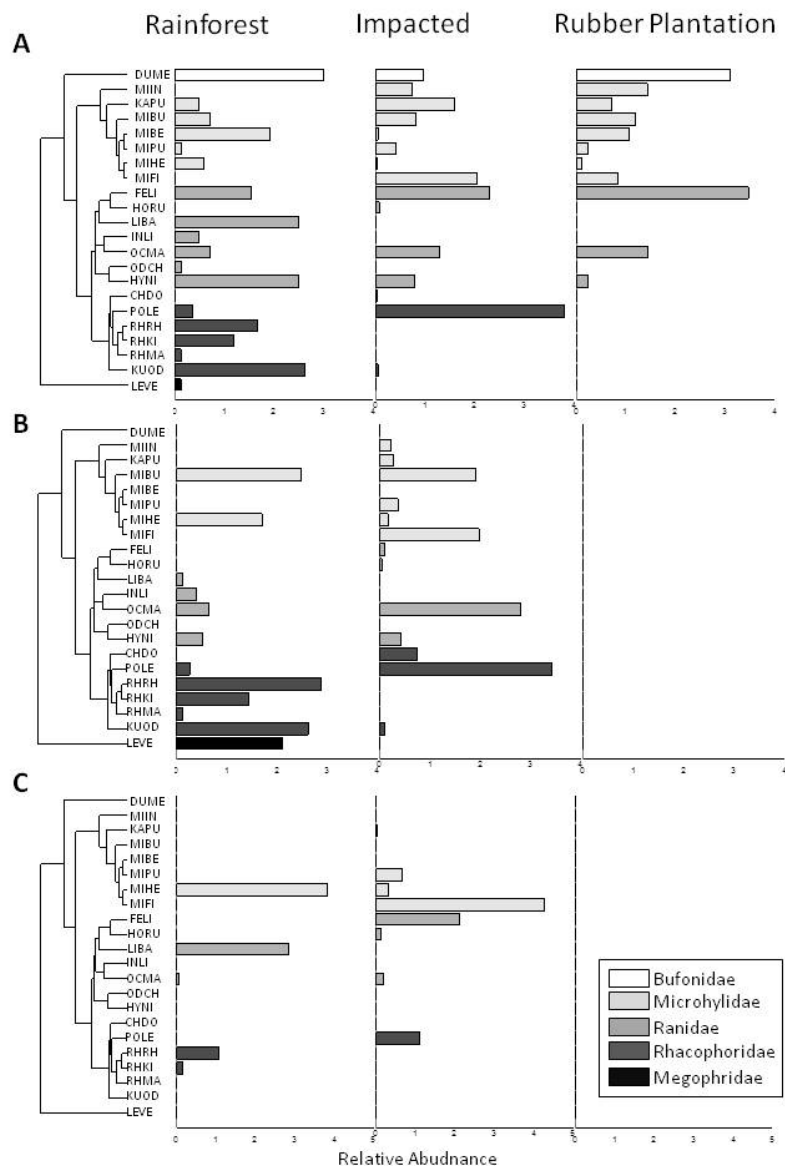
Figure 1: Standardized relative abundances of the 22 species we encountered in A) post-metamorphic surveys, B) calling surveys and C) oviposition surveys in rainforest, impacted and rubber plantation sites. Color of bars corresponds to family species belongs to. Phylogeny was built using genetic data available at phylota.net. Species codes are: DUME: *Duttaphrynus melanostictus*, MIIN: *Micryletta inornata,* KAPU: *Kaloula pulchra*, MIBU: *Microhyla butleri,* MIBE: *Microhyla berdmorei,* MIPU: *Microhyla pulchra*, MIHE: *Microhyla heymonsii*, MIFI: *Microhyla fissipies*, FELI: *Fejervarya limnocharis*, HORU: *Hoplobatrachus rugulosus*, LIBA: *Limnonectes bannaensis*, INLI: *Ingerana liui,* OCMA: *Occidozyga martensii*, ODCH:

Odorrana chloronota, HYNI: *Hylarana nigrovittata,* CHDO: *Chiromantis doriae,* POLE: *Polypedates leucomystax,* RHRH: *Rhacophorus rhodopus,* RHKI: *Rhacophorus kio,* RHMA: *Rhacophorus maximus,* KUOD: *Kurixalus odontotarsus,* LEVE: Leptolalax ventripunctatus.
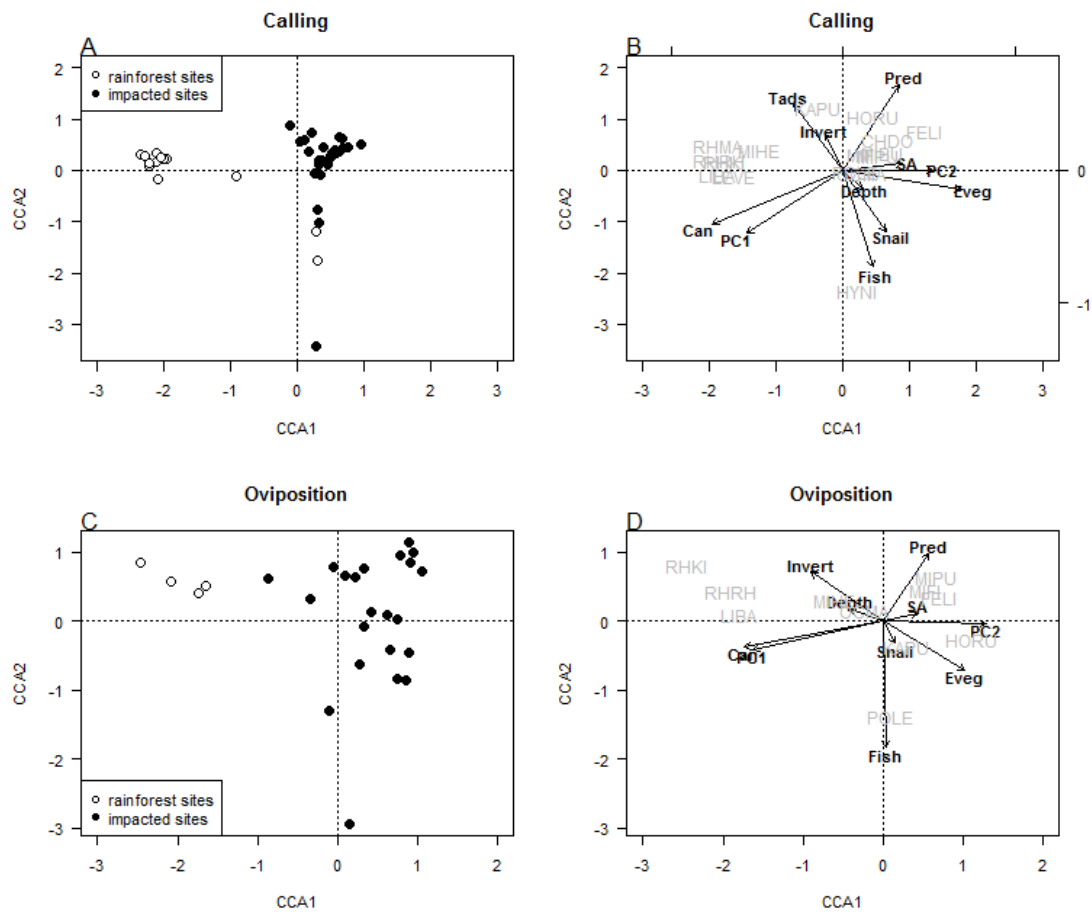
Figure 2: Plots from correspondence analysis from breeding surveys showing distribution of A) calling survey sites and B) 11 environmental variables and 18 species from calling surveys, and C) oviposition survey sites and D) 10 environmental variables and 12 species from oviposition surveys. Species codes are the same as in Figure 1.
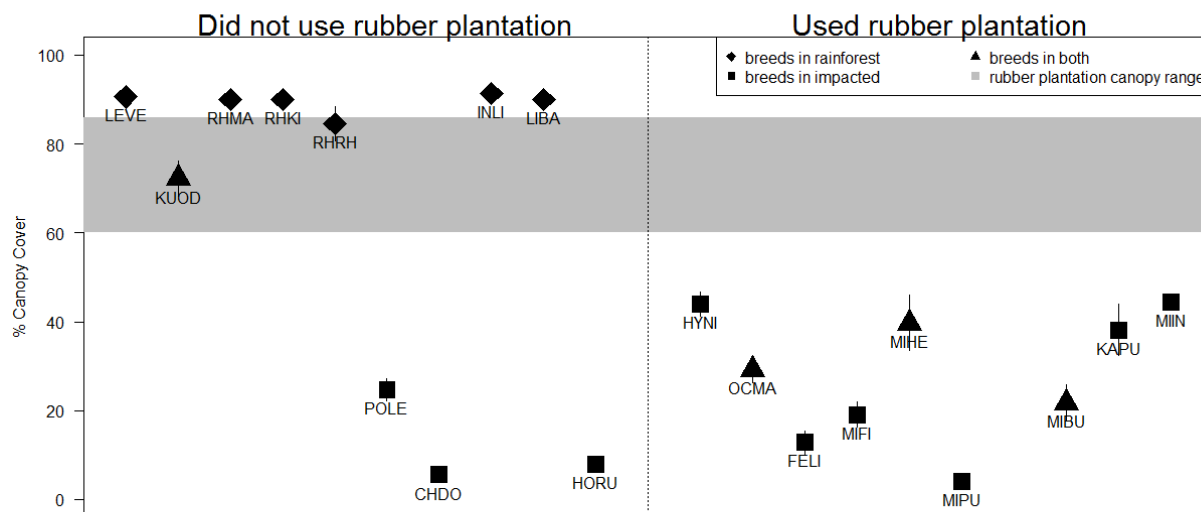
Figure 3: Symbols indicate the mean percent canopy cover (+/- SE) of breeding sites used by each species. The symbol shapes indicate where the species were recorded breeding. Species in the left panel were never found using rubber plantation for post-metamorphic habitat. Species in the right panel used the rubber plantation as post-metamorphic habitat. The gray band indicates the range of percent canopy cover recorded in the rubber plantation. Species codes under each symbol are the same as in Figure 1.
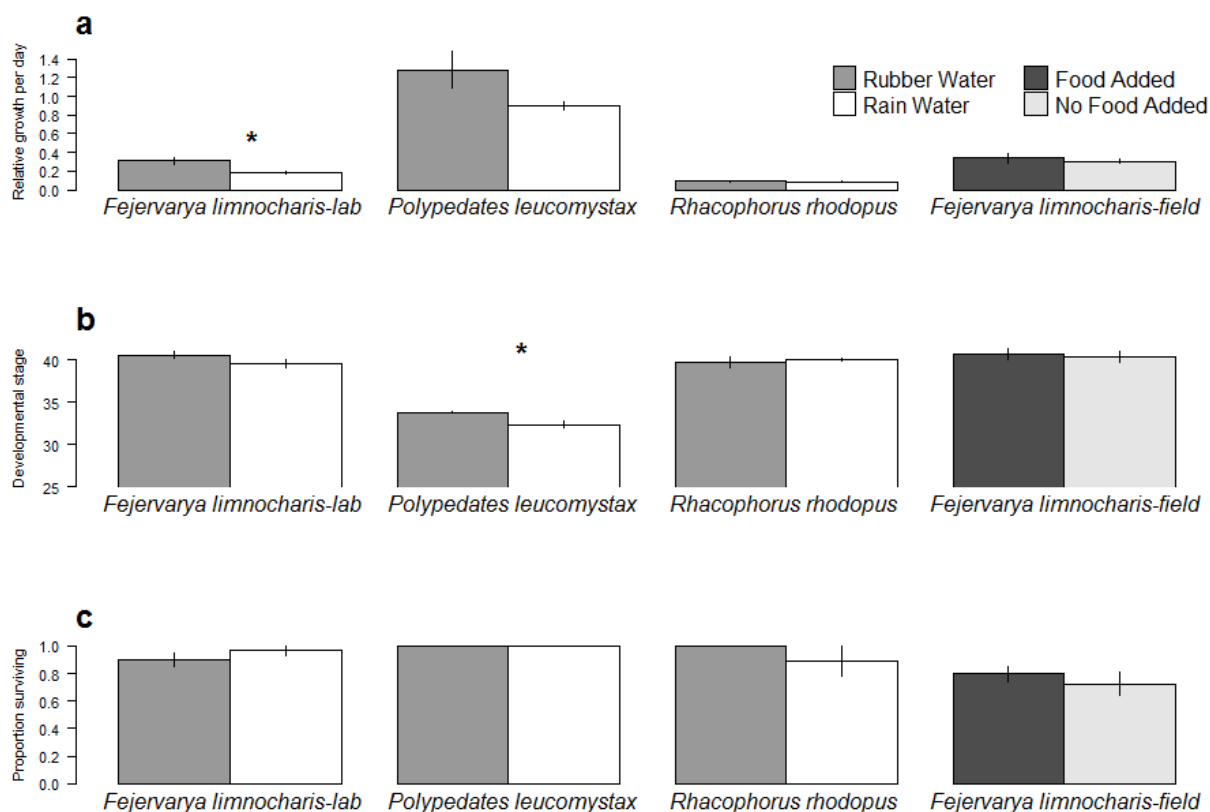
Figure 4: Results from laboratory (rubber water vs. rain water) and field experiments (food added vs. no food added) using tadpoles. A) relative growth per day; b)final developmental stage; c) proportion surviving. Bars indicate means +/- SE. Asterisks indicate significant differences between treatments within a species according to a Tukey post hoc test that accounts for multiple comparisons.

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Appendix A

Table A1: List of fish, predatory invertebrates, and non-predatory invertebrates identified in breeding site surveys.

Fish	Predatory Invertebrates	Non Predatory Invertebrates
Tilapia spp	Epiprocta larvae	Gastropod (snail)
Gambusia spp	Zygoptera larvae	Caridea (shrimp)
Carassius spp	Notonectidae	Brachyura (crab)
Small darter-like *Percidae*	Belastomatidae	Culicidae larvae
	Aquatic Araneae	Chrionomid larvae
	Dytiscid larvae	Hirudinea
	Dytiscid adults	Oligochaeta
	Gerridae adults	Coleopteran larvae
	Ranatra adults	Ephemeropteran larvae
		Trichopteran larvae

Table A2: Correlation Table of environmental variables below diagonal is calling, above diagonal is oviposition. Values of 0.45 and above are in bold.

	SA	Depth	Veg	PC1	PC2	Can	Fish	Pred	Invert	Snail
SA	-	0.73	0.18	0.00	0.31	-0.41	-0.10	0.38	0.30	0.12
Depth	0.73	-	-0.17	0.18	0.02	-0.16	0.02	0.27	0.43	0.03
Veg	0.15	-0.03	-	-0.32	0.57	-0.20	0.32	0.00	-0.21	0.14
PC1	-0.10	0.11	-0.39	-	-0.43	0.46	0.23	-0.28	0.12	0.30
PC2	0.25	0.09	0.44	-0.12	-	-0.42	0.16	0.16	-0.01	0.00
Can	-0.56	-0.26	-0.25	0.48	-0.24	-	0.21	-0.48	0.08	0.02
Fish	-0.07	0.18	0.36	0.26	0.11	0.25	-	-0.36	-0.10	0.40
Pred	0.47	0.28	-0.03	-0.40	0.02	-0.67	-0.46	-	0.38	-0.17
Invert	0.25	0.31	-0.27	-0.01	-0.01	-0.08	-0.23	0.36	-	-0.02
Snail	0.13	0.09	0.09	0.30	-0.07	0.03	0.35	-0.23	-0.06	-

Tads   -0.09   0.05   -0.41   -0.10   -0.26   -0.15   -0.42   0.45   0.41   -0.20

Chapter 2

Individual natural history rather than shared environmental context explains predator- and competitor- induced phenotypic plasticity for three tropical tadpole species

Jocelyn E. Behm

Abstract

Evolution of phenotypic plasticity is driven by environmental heterogeneity, as different phenotypes are adaptive under different environmental conditions. However, complex natural environments can generate forces that favor different phenotypes simultaneously, thereby leading to tradeoffs. Tadpoles are often characterized as having tradeoffs in plastic traits for fish versus insect predators, and in plastic traits for predation versus competition. These tradeoffs are largely based on work from temperate, semi-permanent pool systems that have relatively simple environments containing either fish or insect predators, and either predators or competitors. I investigated whether these tradeoffs apply to three tadpole species from a complex tropical ephemeral pool system in which predators and competitors almost always co-occur, and in which presence of both fish and insect predators may be frequent. I exposed each tadpole species to three predator treatments (no predator, odonate larva, fish) crossed with three competition treatments (low competition, intraspecific competition, intraspecific competition). Overall, the three species responded to both types of predators and to competition; however, the responses of each species were distinct even when they shared the same environmental conditions. In addition, the most frequent environment encountered by all three species in nature – odonate predators and interspecific competition – was the environment with fewest differences between traits in predation and competition treatments for all species. Thus, the tradeoffs predicted by temperate systems were not consistently encountered in my system. These results underscore the importance of examining phenotypic plasticity in the context of the ecological community in which species reside.

Introduction

       Phenotypic plasticity, the ability of one genotype to produce different phenotypes according to environmental context, evolves in response to environmental heterogeneity (Moran 1992; Sultan & Spencer 2002; Ernande & Dieckmann 2004). Ideally, plastic traits are well-matched to a specific environment resulting in increased fitness relative to individuals lacking plasticity (Lively 1986; McCollum & VanBuskirk 1996). Since optimal values of the same trait differ in different environments, tradeoffs can arise between environments (Gomulkiewicz & Kirkpatrick 1992). If environmental heterogeneity is arranged into discrete, non-overlapping and predictable environments, phenotypic plasticity can be highly advantageous, because the costs of expressing the wrong phenotype in the wrong environment are rarely realized. Conversely, if environments are less-predictable or more overlapping, phenotypic plasticity may be less advantageous (Gomulkiewicz & Kirkpatrick 1992). In situations where current cues are poor predictors of future environmental conditions, phenotypes may have less environmental specificity to reduce the associated costs of expressing the wrong phenotype in the wrong environments (Zhivotovsky, Feldman & Bergman 1996). Alternatively, trait plasticity may be too costly and absent altogether (Dayton & Fitzgerald 2011).

       Many species of larval anurans (tadpoles) have high plasticity in traits and are an excellent system for the study of phenotypic responses in alternate environments. Substantial research attention has focused on the plasticity of tadpole traits in response to the threat of predation (McCollum & VanBuskirk 1996; Van Buskirk 2002; Relyea 2003; Teplitsky, Plenet & Joly 2005). Tadpoles modify their phenotypes in response to karimones, chemical cues exuded by a predator that has consumed a conspecific tadpole (Schoeppner & Relyea 2005). Predators can alter tadpole life-history traits by increasing or retarding growth and development rates (Relyea 2004; Teplitsky, Plenet & Joly 2005; Capellan & Nicieza 2007; McCoy 2007; Gonzalez, Touchon & Vonesh 2011). The general morphological response to predators is increased tail size and decreased body size (Relyea & Hoverman 2003; Teplitsky, Plenet & Joly 2005); however, many tadpole species have specific responses to specific predators related to how the predator attacks (Relyea 2004; Teplitsky, Plenet & Joly 2004; McCoy 2007). In response to sit-and-wait, grasping predators like larval odonates (dragonflies), tadpoles generally grow deeper tail fins to divert attacks towards the tail rather than their more valuable head (Van Buskirk *et al.* 2003; Johnson, Burt & DeWitt 2008). In response to predators that pursue their prey, like fish, tadpoles develop thicker tail muscles to help them swim better to escape predation (Teplitsky, Plenet & Joly 2004; Teplitsky, Plenet & Joly 2005). Thus, fish-induced and insect-induced tail morphologies are in

opposing directions, indicating a trade-off in investing in tail fin depth versus tail muscle thickness (Teplitsky, Plenet & Joly 2004; Benard 2006; Touchon & Warkentin 2008).

Tadpoles also have plastic responses to competition (Relyea 2000; Relyea 2002; Relyea & Hoverman 2003; Relyea 2004; Teplitsky & Laurila 2007). Competition affects life-history traits in a consistent manner across species by reducing growth and development (Gurevitch, Morrison & Hedges 2000; Relyea 2002). Morphological responses to competition are less well-studied, but there is evidence for a consistent pattern of increased body size and decreased tail size across species (Relyea 2002; Van Buskirk 2009). This may depend on the type of competition. Most studies focus only on intraspecific competition, and phenotypic responses to interspecific competition have rarely been investigated (but see Relyea 2002). At present it is not known whether tadpoles respond plastically to inter- and intraspecific competition as unique forces that would warrant unique traits and potentially result in tradeoffs. Alternatively inter- and intraspecific competition may represent two forces along a continuum warranting similar phenotypic responses.

Given that the general morphological responses for predation and competition are in opposing directions, there is also an inherent tradeoff between responding to predation and competition. It has been confirmed experimentally for several species that predation and intraspecific competition induce traits in opposing directions (Relyea 2002; Relyea & Hoverman 2003; Resetarits, Rieger & Binckley 2004; Relyea & Auld 2005; Teplitsky & Laurila 2007), and tadpoles with predator-induced traits are poor competitors (Van Buskirk, McCollum & Werner 1997; Van Buskirk & Relyea 1998; Relyea 2002). In these studies, when tadpoles are exposed to competition and predation simultaneously, the results are mixed. In some cases, traits in the mixed predation-competition treatment are similar to the corresponding competition-only treatment (Relyea 2002). In other cases, traits in the mixed predation-competition treatment are similar to the corresponding predation treatment or are intermediate between the two (Relyea 2002; Resetarits, Rieger & Binckley 2004; Teplitsky & Laurila 2007).

The two established trade-offs between fish and insect predators, and predation and competition are likely maintained through the arrangement of environmental heterogeneity in temperate, semi-permanent pool systems (Wellborn, Skelly & Werner 1996; Relyea 2002) where most work on phenotypic plasticity in tadpoles has been conducted. In these systems, environments are generally predictable and non-overlapping; therefore, costs associated with these tradeoffs between responses to different threats are likely rarely experienced. Because temperate pools where tadpoles occur are often semi-permanent, fish are not ubiquitous. When fish are present, they eat insects and

tadpoles, leaving fish as the only predator and competitor density low. When fish are absent, if insect predators have colonized the pool competitor density is low (Wellborn, Skelly & Werner 1996). Thus tadpoles rarely encounter competitors and predators simultaneously and rarely encounter fish and insect predators simultaneously.

The environment faced by tadpoles in tropical communities is often more complex, with different predators, and predators and competitors, occurring in the same breeding sites (Gonzalez, Touchon & Vonesh 2011). I examined predator- and competitor-induced plasticity in ephemeral rain water pools in tropical southern China. In a survey of pools in this region, high densities of tadpoles were found in pools with high densities of invertebrate predators (Chapter 1), indicating that tadpoles likely experience predation and competition simultaneously. For the most part, frogs avoid breeding in more permanent pools containing fish; however, fish in this system may be more mobile than in temperate systems. Predatory snakehead fish from the genus *Channa* are endemic to the region; they can breathe air and disperse across land from permanent to temporary pools (J. Behm *pers. obs*.). Furthermore, during heavy monsoon rains permanent water bodies (rivers, streams, manmade pools) can become temporarily connected to ephemeral water bodies allowing fish to invade the ephemeral pools in which frogs breed. In addition, the long duration of the breeding season (6-8 months for some species), insures a constant stream of interspecific competitors colonizing pools. Given that the environmental heterogeneity in this system differs substantially from temperate systems, I asked whether the expected tradeoffs from temperate systems were occurred in my system.

I experimentally exposed three tadpole species that commonly co-occur in the same ephemeral pools, *Fejervarya limnocharis, Microhyla fissipes* and *Polypedates leucomystax,* to competition and the threat of predation separately and simultaneously. Phenotypic responses to predation and competition have never been quantified for these species or other species from this system. My aims for each species were to: 1) determine if fish and odonate predators caused phenotypic responses in opposite directions; 2) determine if inter- and intraspecific competition caused phenotypic responses in opposite directions; and 3) determine if the four pair-wise combinations of predation and competition caused phenotypic responses in opposite directions, and if so, determine whether tadpoles exposed simultaneously to predation and competition expressed traits that matched the corresponding competition-only or predation-only treatment.

I anticipated that different species may show different responses and different tradeoffs to predation and competition. Although the three species share the same environment, my previous

research showed that they experience different relative strengths of predation and competition. Therefore, my experiments allow me to assess whether the different relative strengths of predation and competition cause different patterns of phenotypic plasticity among species.

Methods

Study System

My study was conducted at the Xishuangbanna Tropical Botanic Garden (XTBG) in Xishuangbanna prefecture, southern Yunnan Province, China. Xishuangbanna has a typical tropical monsoon climate with the rainy season running from May to October. Xishuangbanna is a frog diversity hotspot within China (Xie *et al.* 2007), and about half of the 36 native species breed in ephemeral rain water pools during the rainy season. I focused on three species, *Fejervarya limnocharis, Microhyla fissipes,* and *Polypedates leucomystax* (hereafter referred to FELI, MIFI and POLE, respectively) which frequently co-occur in the same pools as tadpoles. FELI and POLE have roughly equal development times ranging from 5 to 7 weeks, while MIFI develops faster on the order of 3-5 weeks. FELI dark-colored bodies are longitudinally compressed with eyes on the tops of their heads and mainly stay on the bottom of pools. Their mouths have scraping mouthparts and their diets largely comprise algae they scrape from rocks and detritus gleaned from the bottom of the pools. POLE tadpoles' gray-colored bodies are box-like and the tadpoles swim throughout the entire water column. Their mouthparts and thus diets are similar to FELI. MIFI tadpoles' bodies are round and clear to white. They are filter-feeders that constantly filter particles from the water and mainly remain near the surface of the water. All three species are explosive breeders in response to rainfall, with clutch sizes on the order of 200-500 eggs.

In previous breeding site surveys, FELI, MIFI and POLE were the most common tadpoles in breeding sites (Chapter 1). The majority of sites where we found one focal species, we also found another focal species indicating that all three tadpole species likely experience both intra- and interspecific competition in nature (Table A1). In addition, all three species occurred with odonates, while POLE was the only species found with fish, specifically tilapia (*Oreochromis aureus*) (Table A1).

In a previous experiment, we quantified the relative strengths of intra- and interspecific competition for all pair-wise combinations of these three tadpole species. We found that while all three species experience both intra- and interspecific competition, MIFI experiences the strongest intraspecific competition and FELI experiences the strongest interspecific competition in the form of reduced survival when paired with POLE (Figure A1). In preliminary predation trial experiments where I

tested each species separately with an odonate or tilapia predator, I found that odonates had the highest predation rate on FELI, and tilapia had the highest predation rate on MIFI (Table A2).

Experimental design

I used a 3 x 3 factorial experimental design to test the effects of predation and competition alone and in combination on phenotypes for each tadpole species. For each tadpole species I had three levels of competition: low competition (20 tadpoles of focal species), intraspecific competition (60 tadpoles of the focal species) and interspecific competition (20 tadpoles of the focal species plus 20 tadpoles of each of the other two species). The three competition treatments were crossed with one of three predation treatments: no predator, odonate predator, tilapia predator. Each treatment was replicated five times within five temporal blocks per focal tadpole species.

My experiment was conducted in an outdoor laboratory that was completely enclosed by shade cloth that blocked 60% of the direct solar radiation. I collected fresh egg masses for each tadpole species from ephemeral pools at XTBG and separated them by species into hatching basins in the laboratory. Once tadpoles reached Gosner stage 25 (Gosner 1960) (about 5 days post hatching), they were haphazardly assigned to experimental treatments. A subset of 20 individuals per species was retained at the beginning of each block for measuring initial weight.

Each experimental treatment was maintained in a 30L plastic basin. Basins were filled with rain water, tadpole food and 5g of leaf litter two days prior to the addition of tadpoles. Food consisted of 5g of chopped frozen earthworms, 2g of dried algae and 0.5g of ground spirulina. Following the addition of tadpoles, each basin was covered with 0.5mm mesh. I began the predator treatments the day following the addition of tadpoles to the basins. Odonate and tilapia predators were placed individually in 0.5mm mesh bags that were anchored to the top of the basin and suspended in the water. Basins assigned to an odonate treatment received 4 odonates (4 bags), basins assigned to a tilapia treatment received one tilapia plus three empty bags, and basins in the no predator treatment received 4 empty bags. Every three days we rotated predators from the treatment basins with predators from my stock colony to insure that treatment basins did not have the same individual predators for the duration of the experiment. On rotation days, I also rotated empty bags within same-predator treatments. Odonates in the stock colonies that were not actively being used in an experiment were maintained on a diet of tadpoles and insect larvae, while tilapia were maintained on a diet of tadpoles and fish food. I fed the predators by adding tadpoles of the focal species to their bags and then placed the bags in the

treatment basins immediately following feeding. I added enough tadpoles such that 300mg of tadpoles was consumed in total by predators in a treatment after each feeding (i.e., 75mg per odonate, 300mg per tilapia). This design allows the focal tadpoles in the experimental basin to experience the cues associated with predation, without directly being eaten themselves. The feeder tadpoles were from the same clutches of tadpoles that the focal tadpoles in the experiments hatched from. Focal tadpoles were in their treatments for 14 days starting from the day the predators were first introduced. The experiments needed to be terminated at 14 days to avoid *M. fissipes* tadpoles metamorphosing. On the eighth day of the experiment, I changed 50% of the water in each treatment basin and added a second addition of food identical to the original addition. The odonates used in the experiment were two larval morphospecies from the family Libellulidae collected from ephemeral pools and basins filled with water we placed around the XTBG campus. Neither morphospecies was abundant enough to use alone in the experiment; however, both had similar hunting styles and elicited similar plastic responses in tadpoles. The tilapia were juveniles (ca. 50mm) provided by a local farmer.

The five temporal blocks were started on June 18, June 20, July 18, August 8, and August 22, 2010. Onset of an experimental block was contingent on availability of eggs which depended on rainfall. At the conclusion of the experimental period, 10 tadpoles per species were collected from each basin, anesthetized with MS-222, weighed, staged and photographed. The response traits we measured were three life history traits: final developmental stage, growth rate (final weight – initial weight), survival, and seven morphological traits: body depth, body length, tail depth, fin depth, tail area, tail muscle depth, and tail muscle area.

Statistical Analyses

I conducted analyses on the 10 response traits I measured (hereafter referred to as 'individual' traits) plus one composite variable that summarized the life-history and morphological variables, and one composite variable that summarized tail morphology. My overall analysis strategy was to first conduct ANOVAs on individual and composite traits to determine if there was an effect of competition, predation and their interaction on traits. I then followed ANOVAs with planned contrasts to determine whether specific treatments differed significantly. We used the results from the contrasts to determine the pattern of trait expression for the predation, competition and mixed predation-competition treatments. Prior to analyses, stage was square-root transformed, growth was logarithm transformed and survival was arcsine-square-root transformed to meet the variance assumptions for ANOVA. I

regressed each morphological dimension on total body length and retained the residuals for use in subsequent analyses in order to remove the effect of size on each variable.   Then each variable was logarithm transformed and individually standardized to have a mean zero and variance one for each species. All analyses were conducted separately for each tadpole species using the R statistical program (version 2.13.0, http://www.r-project.org/).

Redundancy analyses

I used redundancy analysis to analyze simultaneously the variables of stage, growth and the seven morphological dimensions. Redundancy analysis is similar to principal component analysis except it is performed on only that component of the variance in the response variables that is explained by the independent (environmental) variables of interest. In this case we used predation and competition treatments as the independent variables constraining the data. All of the response variables were standardized to have a mean zero and variance 1 separately for each species, and I included all three species in the same analysis. I used scores from the first two RDA axes in subsequent analyses.

Because I have an *a priori* expectation that there should be tradeoffs in tail depth versus tail muscle in response to odonate versus fish exposure, I analyzed a second composite variable encompassing only tail morphology. I used redundancy analysis again to explain variation in the 5 tail morphological variables we measured, constraining the analysis by using predation and competition treatments as independent variables. I included all three tadpole species in the same redundancy analysis. I used scores from the first two RDA axes in subsequent analyses.

Models and contrasts

I used separate ANOVA models to analyze the three life-history variables, (stage, growth and survival), and the first two axes of each redundancy analysis for each tadpole species. I used MANOVA to analyze morphology, with all seven morphological variables as the multivariate response variable. For both ANOVA and MANOVA models, I used predation, competition, their interaction and block as independent variables.

My planned contrasts corresponded to the three aims outlined in the introduction of this manuscript. The first set of contrasts analyzed how traits responded to predation treatments. I contrasted the no predator treatments to predator treatments in general, and the three pair-wise contrasts of each predator treatment type (no predator vs. odonate, no predator vs. tilapia, odonate vs.

tilapia). My second set of contrasts was analogous to the first, only substituting competition treatments for predator treatments (low competition vs. high competition, low vs. interspecific, low vs. intraspecific, inter- vs intraspecific). My final set of contrasts involved two steps and compared responses to predation and competition treatments. I calculated the four pair-wise contrasts of the odonate and tilapia treatments at low competition to the interspecific and intraspecific competition treatments without predation. When trait values in the pair of predation and competition treatments differed, I then compared traits in the mixed predation-competition treatment to the corresponding predation-low competition and competition- no predation treatment.

Results

I first present the results from my redundancy analyses on overall phenotype and tail morphology. I then describe how each species' traits responded to predation treatments, competition treatments and simultaneous exposure to predation and competition.

Redundancy Analyses

To assess overall phenotype, I performed redundancy analysis on growth, stage and the seven morphological variables. Stage and growth were positively associated with the first axis (hereafter referred to as RDA1). The second axis (RDA2) was associated with morphological traits; body depth, tail depth and body length were positively associated with RDA2 and the remaining four tail traits were negatively associated with RDA2 (Figure 1). The associations of morphological variables on RDA 2 suggest a tradeoff between body and tail morphology such that as body size increases, tail size decreases. The orthogonal nature of the two axes suggests that tadpole life-history traits may be decoupled from morphology in response to competition and predation risk. RDA1 explained 44.3% and RDA2 explained 8.9% of the constrained variation. I calculated the difference between the two most extreme points on each axis as a coarse metric of the overall degree of plasticity for each species. POLE was the most plastic along RDA 1 (1.12) followed by MIFI (0.92) and FELI (0.80). MIFI was the most plastic along RDA 2 (2.02) followed by FELI (0.88) and POLE (0.69).

To assess tail phenotype independent of body phenotype, I performed a separate redundancy analysis on the five tail morphological variables. All five tail variables were positively associated with the first axis (RDATail1), indicating this axis summarizes overall tail size (Figure 2). The dimensions associated with tail muscle, muscle depth and muscle area were positively associated with the second

axis (RDATail2) while tail depth and fin depth were negatively correlated to RDATail2. The associations of variables to RDATail2 suggest a tradeoff between the amount of tail muscle and the overall size of the tail fin. RDATail1 explained 10.9% and RDATail2 explained 4.6% of the constrained variation.

FELI

Predation

Odonate treatments caused increased growth and decreased tail muscle area, while tilapia caused increased tail size. Specifically, there were significant effects of predation on growth, muscle area, RDA2, and RDATail2, with all of these variables showing significant differences in odonate and tilapia treatments (Table 1A). Tadpoles in odonate treatments grew larger than tadpoles in tilapia treatments (Table B1, Figure B2). Odonates caused reduced muscle area compared to tadpoles grown with tilapia and in the absence of predators (Table C1, Figure C1). Tadpoles in tilapia treatments had lower RDA2 scores than odonate or no predator treatments (Table 1A, Figure 1) indicating relatively larger tails and smaller heads. Tadpoles in odonate treatments had lower RDATail2 scores than tilapia and no predator treatments (Table 1A, Figure 2) indicating smaller tail muscle.

Competition

Competition caused reduced growth and development, and reduced tail size. Specifically, there were significant effects of competition on growth, development, muscle depth, muscle area, tail area, RDA1, RDATail1, and RDATail2, with only the muscle dimensions and RDATail2 showing a difference between inter- and intraspecific competition (Table 1A). Inter- and intraspecific competition had equal effects on reducing growth and development (Table 1A, Figures B1 and B2). This pattern was echoed in the RDA1 scores (Figure 1). For muscle depth, there was as a significant competition X predation interaction where tadpoles in the interspecific competition treatment had low muscle depth in the absence of predators and high muscle depth in the presence of predators (Table C1). This was the only significant competition X predation interaction in the entire study. Interspecific competition treatments had lower muscle depth and muscle area than the other two competition treatments (Table 1A, Figure C1). Tadpoles in low competition treatments had larger tail area than the other two competition treatments (Table 1A, Figure C1). Tadpoles in low competition treatments had more positive RDATail1 scores than tadpoles in either of the high competition treatments, indicating larger overall tail size,

while tadpoles in the interspecific treatment had lower RDATail2 scores than tadpoles in the intraspecific treatment indicating larger tail depth and smaller tail muscle (Figure 2).

Mixed Predation and Competition

When FELI tadpoles were simultaneously exposed to competition and predation, their growth rates and developmental stages matched those of the corresponding competition-only treatment for all treatment combinations for growth, developmental stage and RDA1 (Table 1A). With respect to morphology, there were far fewer instances of significant differences between competition and predation treatments. Of the treatments that had significant differences, none went in the direction of competition; they were either in the direction of the predator or intermediate between the two treatments (Table 1A).

MIFI

Predation

Overall, predators caused reduced growth and development, reduced body size, and increased tail size. Specifically, there were significant effects of predation on growth, developmental stage, RDA1, body depth, body length, tail depth, max fin depth, RDA2, and RDATail1, with stage, body depth, tail depth and RDA2 showing significant differences between traits in odonate and tilapia treatments (Table 1B). Growth and development were reduced in the presence of predators with odonates causing a larger reduction in stage than tilapia or no predators (Table 1B, Figure B1 and B2). Body depth was lower in tilapia treatments relative to odonate and no predator treatments (Table 1B, Figure C2). Tail depth was higher in odonate treatments relative to tilapia and no predator treatments, while fin depth was higher in both predator treatments relative to no predator treatments (Table 1B, Figure C2). Tadpoles in tilapia treatments had the lowest RDA2 scores, indicating larger tails and smaller heads, followed by odonate treatments and no predator treatments (Figure 1). Finally, tadpoles in no predator treatments had the smallest RDATail1 scores compared to both predator treatments indicating smaller tail size in the absence of predators (Figure 2).

Competition

Competition caused reduced growth and development, reduced tail muscle, and increased tail fin depth. Specifically, there were significant effects of competition on growth, stage, fin depth, muscle

area, RDA1, RDA2, and RDATail2, with significant differences between inter- and intraspecific competition for growth, stage, RDA1 and fin depth (Table 1B). For growth, stage, and RDA1, there was no difference between low competition and interspecific competition, while tadpoles in intraspecific competition treatments had reduced values for all three variables (Table 1B). Low competition treatment tadpoles had lower RDA2 scores than tadpoles in both high competition treatments indicating larger relative tail size (Figure 1). Tadpoles in interspecific competition treatments had the largest fin depth compared to low competition and intraspecific competition treatments (Figure C2). Tadpoles in low competition treatments had larger muscle area than the intraspecific competition treatment (Figure C2) and higher RDATail2 scores than the inter- and intraspecific competition treatments indicating larger tail muscle dimensions (Figure 2).

Mixed Predation and Competition

For growth and development variables, there were only two treatments with significant differences; the odonate-interspecific treatment was intermediate for stage, and the tilapia-intraspecific treatment was in the direction of competition for RDA1 (Table 1B). With respect to morphological variables, when there were significant differences between predation-only and competition-only treatments the mixed predation-competition treatment never went in the direction of competition (Table 1B). In most cases mixed-predation competition treatments traits were intermediate between their corresponding predation-only and competition-only treatments. When traits did go in the direction of the predator, it was when tilapia was the predator. This was true for the tilapia-intraspecific treatments for body depth, body length, fin depth, and RDA2, and also the tilapia-interspecific treatment for RDA2 (Table 1B).

POLE

Predation

Overall, predators caused reduced development and increased tail size. Specifically, there was a significant effect of predation on stage, tail depth, fin depth and RDATail1, with tail depth only showing a significant difference between tilapia and odonate treatments (Table 1C). Both predators reduced developmental stage equally relative to the no predator treatment (Table 1C, Figure B1). Odonates caused increased tail depth relative to tilapia and no predator treatments, while both predators caused increased max fin depth relative to no predator treatments (Figure C3). Finally, tadpoles in both

predator treatments had higher RDATail1 scores than tadpoles in the no predator treatment indicating predators caused increased tail size (Figure 2).

Competition

Competition caused reduced growth and development, reduced body size, reduced tail muscle and reduced tail size. Specifically, there was a significant effect of competition for growth, stage, RDA1, body depth, muscle depth, tail depth, fin depth, tail area, and RDATail1, and for all of these variables there were significant differences between inter- and intraspecific competition values (Table 1C). Tadpoles in intraspecific treatments exhibited lowest growth, development and RDA1 scores, followed by interspecific treatments and low competition treatments. Tadpoles in intraspecific competition treatments had lower body depth, muscle depth, tail depth, fin depth and tail area than tadpoles in low competition and interspecific competition treatments (Figure C3). Finally, tadpoles in intraspecific treatments had lower RDATail1 scores than tadpoles in low competition and interspecific treatments, indicating lower overall tail size (Figure 2).

Mixed Predation and Competition

For all treatment combinations for growth, developmental stage and RDA1, the mixed predation-competition treatments matched the corresponding competition-only treatment in all cases (Table 1C). With respect to morphology, when there were significant differences between competition-only and predation-only treatments, there was only one instance of the mixed competition-predation treatment matching the predation-only treatment (Table 1C). This was for the odonate-interspecific treatment for max fin depth.   There were six instances for the mixed predation-competition treatment matching the competition-only treatment and all six instances involved intraspecific competition treatments (Table 1C). The remaining five significant contrasts were intermediate between the predation-only and competition-only treatments.

Discussion

I exposed tadpoles from three tropical frog species that co-occur in the same habitat to combinations of predation and competition to determine whether their traits showed similar or dissimilar patterns of phenotypic plasticity. Overall, the three species responded differently to the different treatments, with

their responses being explained by their individual life histories and relative susceptibilities to each threat.

Comparisons with previous studies on tadpole plasticity

Predation

Previous studies have demonstrated mixed effects of predator exposure on life-history traits (Teplitsky, Plenet & Joly 2005; McCoy 2007; Gonzalez, Touchon & Vonesh 2011), and consistent effects of predation on morphological traits with many tadpole species exhibiting predator-specific morphologies (Relyea 2004). In my study I also demonstrated mixed effects of predator exposure on life-history traits: odonates increased FELI growth, both predators reduced all MIFI life-history traits, and both predators reduced development stage for POLE. Overall, when my three tadpole species were exposed to predators, each species exhibited the general predator-induced phenotype of increased tail size (Relyea & Hoverman 2003; Teplitsky, Plenet & Joly 2005), but MIFI was the only species to clearly express different morphological traits for each predator- increased tail depth for odonates, and increased tail size and decreased body depth for tilapia. POLE also expressed increased tail depth when exposed to odonates, but had no specific trait responses for tilapia. Increased tail depth in POLE and MIFI with odonates may be an adaptive trait because in other species increased tail depth gives odonates a target to attack which diverts their strikes from the body (Van Buskirk *et al.* 2003; Johnson, Burt & DeWitt 2008). FELI showed decreased tail muscle area when exposed to odonates. Other studies report decreased tail muscle when tadpoles are exposed to insects and increased tail muscle when tadpoles are exposed to fish. FELI showed no change in tail muscle in response to tilapia, but was the only species to have increased growth in response to a predator, specifically odonates. Increased growth may be an adaptive strategy to outgrow the upper size limit of odonate predators (Capellan & Nicieza 2007). It is possible that increased investment in growth came at the cost of decreased investment in tail muscle. However, at this point the adaptive significance of any of these trait changes is unknown.

Other studies have demonstrated strong phenotypic differences in response to fish and insect predators, in some cases with the same traits responding in opposite directions relative to the no predator treatment (e. g., Benard 2006; Touchon & Warkentin 2008). In my study, all three species showed statistically significant differences in at least one trait when exposed to odonates versus tilapia. However, no species exhibited the pattern of odonates and tilapia inducing the same trait in opposite

directions; in all but one case of significantly different odonate and tilapia-induced traits, one of the predator treatments was statistically identical to the no predator treatment. The lone exception is *M. fissipes* treatments along RDA 2 which form a gradient of tilapia treatments having the most extreme values followed by odonates then no predator controls. In addition, when odonate and tilapia treatments do affect the same trait, it is in the same direction.

Tilapia is not a native species, and my focal tadpole species do not share a long evolutionary history with them. Therefore, selective pressure from tilapia is likely not the evolutionary force that shaped the phenotypic responses exhibited by my tadpole species. It is likely that the response tadpoles exhibited to tilapia is the same response they would have to their native fish predators. If that is the case, odonates and native fish may have similar hunting styles (sit-and-wait), which warrant similar morphological responses. Because native fishes are now rare in this system, having been removed by fishing and replaced by tilapia, the ability to understand the evolutionary forces driving evolution of phenotypic plasticity to fish predators has been eliminated.

Competition

Similar to other work, competition had a strong effect on life-history traits for all three species (Relyea 2002). I expected the intraspecific competition treatments to have the largest effect on life-history traits, followed by interspecific competition and then the low competition treatments, but this was only the case for POLE. For FELI, inter- and intraspecific competition had equal effects on life history traits. In a previous experiment, FELI experienced strong interspecific competition from POLE (Edmonds *et al. In Prep*), and I suspect that this same mechanism is responsible for the patterns I observed in the current study. For MIFI the effect of interspecific competition was equal to the effect of low competition on life history traits. In the same previous experiment, MIFI experienced high interspecific competition from FELI (Edmonds *et al. In Prep*). Because the previous experiment only considered pairwise species combinations, MIFI may have been released from interspecific competition with FELI because POLE was present in my study.

The general competition-induced morphology described in other studies is large body and small tail (Relyea 2002), but I did not observe this pattern. Competition affected the most morphological variables in POLE*,* resulting in decreased trait size for both body and tail traits. FELI and MIFI did exhibit reduced tail size following competition, but this was not accompanied by an increase in body size. Other researchers have speculated on the adaptive significance of morphological trait changes in response to

competition. I suspect that in my system given that competition resulted in only reduced morphological traits, they are likely not adaptive changes.

Few studies have specifically addressed whether tadpoles have unique responses to inter- versus intraspecific competition. *Rana sylvatica* tadpoles can distinguish between reduced food density due to low food availability versus increased conspecific density (Relyea 2002). In addition, *Rana temporaria* tadpoles can adjust morphological responses according to conspecific density (Van Buskirk *et al.* 2011). Therefore, I predicted that tadpoles would respond different to interspecific versus intraspecific competition. I observed statistical differences in traits when exposed to inter- versus intraspecific competition in all three tadpole species. POLE had six examples of this for morphological traits and all three life-history traits; however, in all nine cases, the pattern was intraspecific competition causing the smallest trait relative to interspecific competition and low competition. Thus, it is not clear if POLE distinguish inter- and intraspecific competition as unique forces or differing levels of the same force along a continuum. On the other hand, it is clear that both FELI and MIFI respond to inter- and intraspecific competition as unique forces. FELI expressed the lowest muscle depth in interspecific treatments relative to intraspecific and low competition treatments. Similarly, MIFI expressed largest fin depth in interspecific treatments relative to intraspecific and low competition treatments. Given that the most extreme trait is expressed in the interspecific treatment, it indicates that these two species do not perceive the two types of competition as a continuum, with respect to these traits. This is even more noteworthy given that interspecific competition had equal effects on life history traits as intraspecific competition for FELI and low competition for MIFI. To my knowledge this is the first demonstration that tadpole species have species-specific responses to interspecific competition.

Predation and competition

Previous studies have demonstrated that plastic traits can respond to predation and competition in opposite directions (Relyea 2002; Relyea & Hoverman 2003; Resetarits, Rieger & Binckley 2004; Relyea & Auld 2005; Teplitsky & Laurila 2007). While my study supports this, my results were not consistent across traits or across species. With respect to life-history traits, FELI and POLE showed the same pattern of all predation and competition combinations being statistically different, with the mixed predation-competition treatments expressing traits that match the corresponding competition-only treatment. These results highlight the strong effect of competition on life-history traits for these two species. MIFI did not show this pattern; only two out of the twelve combinations showed significant

differences between competition and predation treatments. MIFI showed the strongest effect of predation on life-history traits, and therefore the effects of predation and competition were relatively equal on these traits.

In contrast, the relative effects of predation and competition on morphological traits were more varied. FELI had the fewest significant differences between competition and predation for morphological variables, and when traits were different, they never matched the competition-only treatment. MIFI had a higher number of significant differences than FELI, and like FELI no mixed predation-competition treatments matched the competition-only treatment. In addition, when mixed predation-competition treatments matched predator-only treatments, it was when tilapia was the predator. On the other hand, POLE had mixed predation-competition treatments that matched the competition-only treatment, but this was only for intraspecific competition treatments.

The most striking result from these analyses is that in the most common environment that species are found according to surveys, with odonate predators and interspecific competitors (Table A1), none of the tadpole species has a substantial tradeoff between predation and competition for morphological traits. For FELI, muscle depth is the only trait significantly different between interspecific and odonate treatments and the mixed competition-predation value is intermediate between the two. Muscle depth does not influence survival in odonate attacks, and its adaptive significance for interspecific competition is not known. MIFI had the fewest significant differences between competition and predation for the odonate-interspecific treatment. POLE did have a significant difference for fin depth which in other species is correlated with surviving odonate attacks. This likely explains why the trait value in the odonate-interspecific treatment matched the odonate treatment.

Conformity between species responses and ecology

While my results do not conform nicely to predictions made from other systems, they are in accord with each tadpole species' ecology and natural history. FELI and odonates are mainly distributed on the bottoms of pools along the sediment, which likely causes high encounter rates between the two species. In my preliminary predation trials, odonates had the highest predation rate on FELI (Table A2). In the current study, FELI had more responses to odonates than to tilapia. In addition, FELI experiences high interspecific competition (Edmonds *et al. In Prep*), and this was reflected in life-history traits responding equally to inter- and intraspecific competition, and morphological variables having unique responses to interspecific competition.

MIFI is the smallest of the three tadpoles and the most ecologically different. Because it is small, it likely has a difficult time outgrowing the upper size limits of predators. It is a filter feeder that stays near the surface of pools constantly filtering particles out of the water, which makes it especially vulnerable to predation by fish. This was confirmed in my preliminary predation trials. In addition, it is likely also susceptible to predation by odonates on vegetation, although my predation trials did not specifically address this. In the current study, MIFI had the strongest response to predation in general and tilapia specifically, showing responses to both predators in life-history and morphological traits. In addition, MIFI also experiences high intraspecific competition and this was consistent with the responses in life history traits.

POLE was the largest of the three tadpole species, the most resistant to predation in predation trials and experienced relatively low interspecific competition. POLE are active tadpoles that spend time on the substrate and in the water column, which should expose them to both odonate and tilapia predators. In addition, they were the only species to breed in pools containing tilapia. Thus, I predicted that they would have minimal responses to predators and only to tilapia. This was partially true; POLE did have minimal responses to predators in most traits, but a strong response to odonates in tail depth. As predicted, POLE also had a strong response to intraspecific competition, which was evident in both life history and morphological variables.

Importance of ecological context

While the three focal species share the same larval environment, they experience the environment very differently, which explains their distinct responses to the same threats. Therefore, in my system, each species' response can only be explained in the context of how it experiences the environment.

By using three tadpole species from the same pools, I was able to demonstrate that there is no environment-specific response shared by all species. In contrast, many tadpole plasticity studies focus on one tadpole species. While this may be reasonable in the face of logistical constraints, it limits the scope of conclusions that can be drawn from the studies. Using my study as an example, if I had only investigated MIFI, I would have drawn very different conclusions regarding the importance of predators on morphological and life-history plasticity than if I had only investigated POLE. Therefore, I encourage studies that simultaneously address multiple species, and multiple environmental drivers, to fully explore phenotypic plasticity and potential tradeoffs under naturally complex ecological conditions.

Tables

Table 1: Planned contrasts for each response variable and treatment combination for A) *Fejervarya limnocharis* (FELI), B) *Microhyla fissipes* (MIFI) and C) *Polypedates leucomystax* (POLE). Cells with letters or asterisk (*) indicate the contrast or main effect was significant at α=0.05 level, respectively. For contrasts within the predation and competition treatments, the letter indicates the treatment with the larger trait value: P=predation, N=No Predator, O=Odonate, T=Tilapia, H=high competition, L=low competition, R=interspecific competition, A=Intraspecific Competition. For the contrasts between predation and competition treatments, the letter indicates the direction of the trait in the mixed predation-competition treatment: P=predation, C=competition, and I= intermediate between the two.

A) FELI

	Life History				Morphology								
	Growth	Stage	RDA 1	RDA 2	Body Depth	Body Length	Muscle Depth	Tail Depth	Fin Depth	Muscle Area	Tail Area	RDA Tail 1	RDA Tail 2
Predation	*			*						*			*
Predator vs No Predator	-	-	-	-	-	-	-	-	-	-	-	-	-
No Predator vs. Odonate	-	-	-	-	-	-	-	-	-	N	-	-	N
No Predator vs. Tilapia	-	-	-	N	-	-	-	-	-	-	-	-	
Odonate vs. Tilapia	O	-	-	O	-	-	-	-	-	T	-	-	T
Competition	*	*	*				*			*	*	*	*
High Competition vs Low Competition	L	L	L	-	-	-	-	-	-	-	L	L	-
Low Competition vs Interspecific	L	L	L	-	-	-	L	-	-	L	L	L	-
Low Competition vs Intraspecific	L	L	L	-	-	-	-	-	L	-	L	L	-
Interspecific vs Intraspecific	-	-	-	-	-	-	A	-	-	A	-	-	A
Predation x Competition							*					*	*
Mixed Predation and Competition Treaments													
Odonate - Interspecific	C	C	C	-	-	-	I	-	-	-	-	-	-
Odonate - Intraspecific	C	C	C	-	-	-	-	-	-	P	-	-	P
Tilapia - Interspecific	C	C	C	-	-	-	P	-	-	-	I	P	-
Tilapia - Intraspecific	C	C	C	-	-	-	-	-	-	-	-	-	I

B) MIFI

	Life History				Morphology								
	Growth	Stage	RDA1	RDA2	Body Depth	Body Length	Muscle Depth	Tail Depth	Fin Depth	Muscle Area	Tail Area	RDA Tail 1	RDA Tail 2
Predation	*	*	*	*	*	*		*	*			*	
Predator vs No Predator	N	N	N	N	N	N	-	-	P	-	-	N	-
No Predator vs. Odonate	N	N	N	O	-	-	-	O	O	-	-	N	-
No Predator vs. Tilapia	N	-	-	N	N	N	-	-	T	-	-	N	-
Odonate vs. Tilapia	-	T	-	O	O	-	-	O	-	-	-	-	-
Competition	*	*	*	*					*	*			*
High Competition vs Low Competition	L	L	L	L	-	-	-	-	-	L	-	-	-
Low Competition vs Interspecific	-	-	-	-	-	-	-	-	R	-	-	-	L
Low Competition vs Intraspecific	L	L	L	L	-	-	-	-	-	L	-	-	L
Interspecific vs Intraspecific	R	R	R	-	-	-	-	-	R	-	-	R	-
Predation x Competition Mixed Predation and Competition Treaments													
Odonate - Interspecific	-	I	-	I	-	I	-	-	-	-	-	-	I
Odonate - Intraspecific	-	-	-	I	I	I	-	-	-	-	-	I	-
Tilapia - Interspecific	-	-	-	P	I	I	-	-	-	I	-	-	I
Tilapia - Intraspecific	-	-	C	P	P	P	-	-	P	I	-	-	I

C) POLE

	Life History				Morphology								
	Growth	Stage	RDA1	RDA2	Body Depth	Body Length	Muscle Depth	Tail Depth	Fin Depth	Muscle Area	Tail Area	RDA Tail 1	RDA Tail 2
Predation		*						*	*			*	
Predator vs No Predator	-	N	-	-	-	-	-	P	-	-	-	-	-
No Predator vs. Odonate	-	N	-	-	-	-	-	O	O	-	-	O	-
No Predator vs. Tilapia	-	N	-	-	-	-	-	-	T	-	-	-	-
Odonate vs. Tilapia	-	-	-	-	-	-	-	O	-	-	-	-	-
Competition	*	*	*		*		*	*	*		*	*	
High Competition vs Low Competition	L	L	L	-	-	-	L	-	-	-	L	-	-
Low Competition vs Interspecific	L	L	L	-	-	-	-	-	-	-	-	-	-
Low Competition vs Intraspecific	L	L	L	-	L	-	L	L	L	-	L	L	-
Interspecific vs Intraspecific	R	R	R	-	R	-	R	R	R	-	R	R	-
Predation x Competition													
Mixed Predation and Competition Treaments													
Odonate - Interspecific	C	C	C	-	-	-	-	-	P	-	-	I	-
Odonate - Intraspecific	C	C	C	-	-	-	C	I	I	-	C	C	-
Tilapia - Interspecific	C	C	C	-	-	-	-	-	I	-	-	-	-
Tilapia - Intraspecific	C	C	C	-	-	-	-	C	C	-	I	C	-

Figures

Figure 1: Redundancy analysis results to assess tadpole phenotype. An RDA was performed on developmental stage, growth rate and seven morphological response traits and was constrained by the competition and predation treatments. The first three panels show the mean RDA1 and RDA2 scores for each treatment and species. The fourth panel shows how the 9 variables used in the analyses were associated with the two axes; stage and growth have a positive association with RDA1, body dimensions and tail depth have a positive association with RDA2 and the remaining tail variables are negatively associated with RDA2. Variable abbreviations for morphological traits are: bodyd = body depth; bodyl= body length; taild = tail depth; taila= tail area; maxfin = fin depth; muscd= tail muscle depth; musca= tail muscle area.
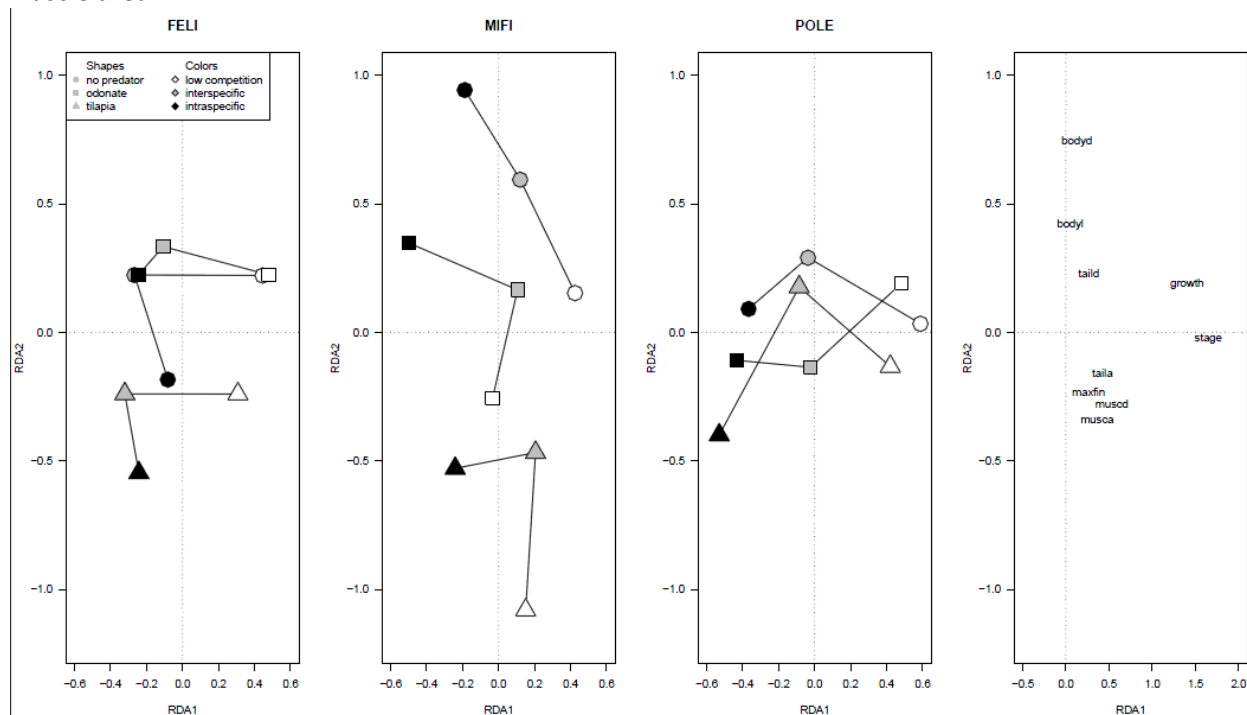
Figure 2: Redundancy analysis results to assess tail phenotype. An RDA was performed on the 5 variables describing tail morphology. The first three panels show the mean axis scores for each treatment and species, and the fourth shows how the 5 tail traits are associated with the two RDA axes; all variables correlate negatively with RDA1, tail muscle variables correlate negatively with RDA2 and tail depth variables correlate positively with RDA2. Morphological variable name abbreviations are the same as in Figure 1.
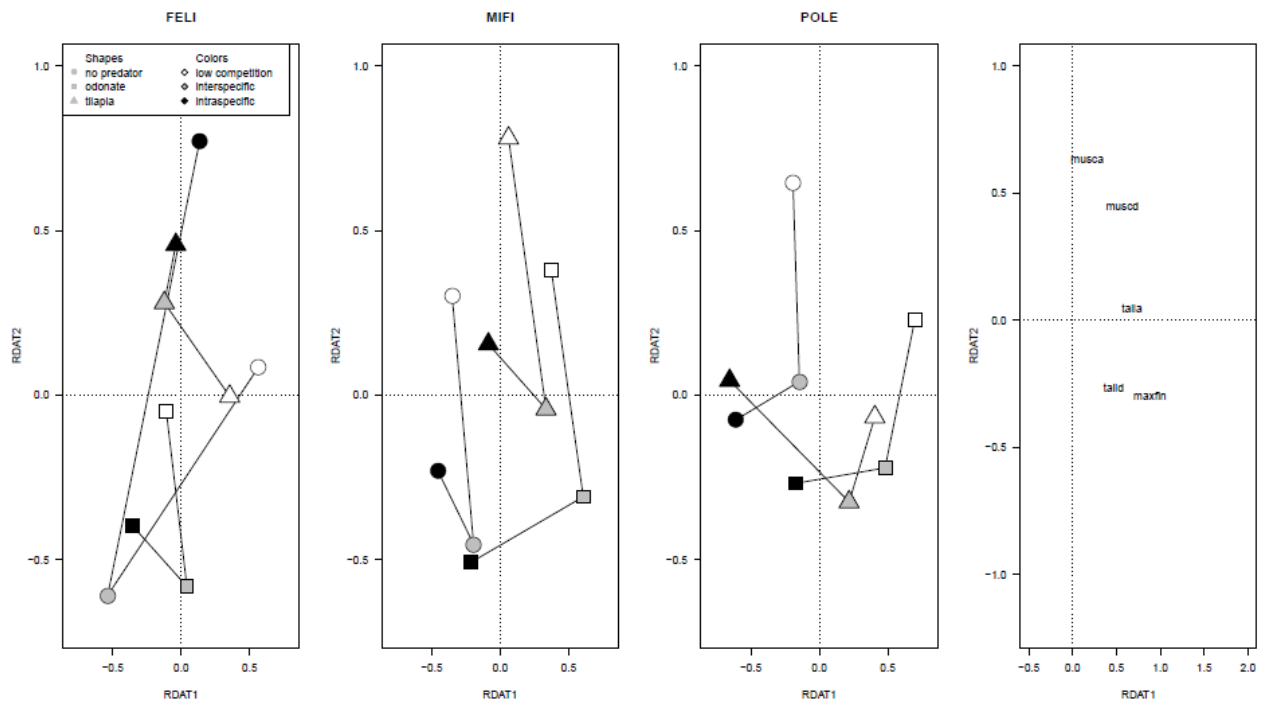
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Appendix A- Summary of breeding site surveys, predation trials and competition experiments

Table A1: Rows summarize breeding site surveys for each tadpole species and show the proportion of sites containing each of the focal tadpole species, mean species richness across all breeding sites, proportion of sites with invertebrate predators, odonate predators, fish, and tilapia.

	Prop FELI	Prop MIFI	Prop POLE	Tadpole Spp Richness	Invert Predators	Odonates	Fish	Tilapia
FELI	1.00*	0.91	0.70	4.76	0.97	0.48	0.00	0.00
MIFI	0.59	1.00	0.69	4.49	0.94	0.37	0.04	0.04
POLE	0.40	0.60*	1.00*	4.14	0.72*	0.34	0.22*	0.12*

*indicates value is significantly different from values in that column at the $\alpha=0.05$ level after adjusting for multiple comparisons.

Table A2: Results from predation trials for each tadpole species where naïve tadpoles that had not previously been exposed to predators were exposed separately to tilapia and odonates and the proportion surviving at the end of 180 minutes and 24 hours respectively was recorded.

	Odonate	Tilapia
FELI	0.39*	0.31
MIFI	0.54	0.16*
POLE	0.72	0.35

*indicates value for that tadpole species is significantly different from values of other species in that column at the $\alpha=0.05$ level after adjusting for multiple comparisons

Figure A1: Reproduced from Edmonds et al. (*In Prep*). Summary of significant competition strengths from experiment testing competition among all pairwise tadpole species combinations with respect to four life history response variables (weight, length, developmental stage and survival). Higher values indicate a higher effect of competition on that variable. Arrows point from the species causing the effect to the species receiving the effect. MIFI experiences strongest intraspecific competition (values are highest and most response variables affected), while FELI experiences the strongest interspecific competition from POLE in the form of significantly reduced survival.

weight: 1.25
length: 1.15
stage: 1.27

weight: 0.57
length: 0.76
stage: 1.81

weight:1.07
length: 0.72

MIFI → FELI

weight:0.93

survival: 3.43

weight: 0.64
length:0.63
stage: 0.99

length: 0.49

POLE

length: 0.55
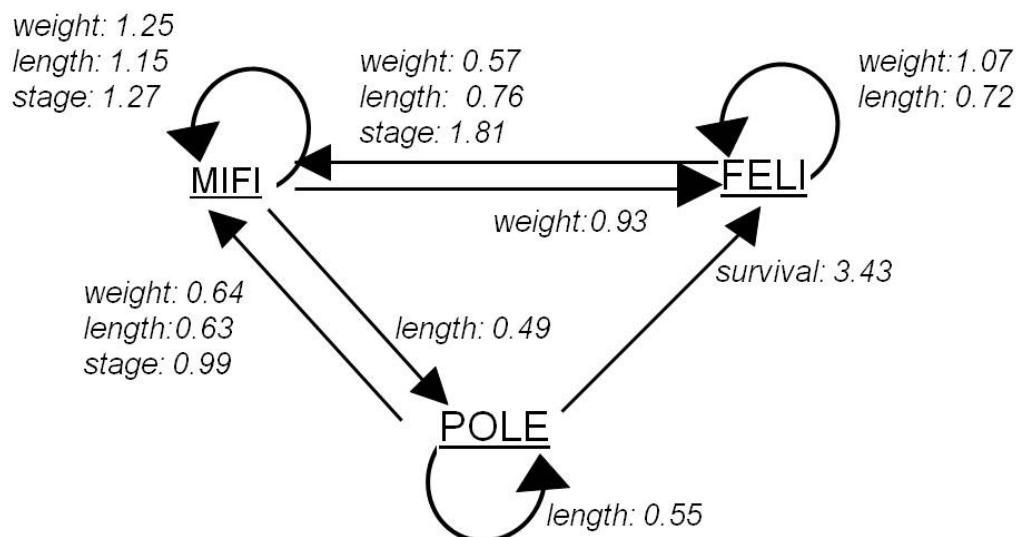
Appendix B- Life history variables: Growth, developmental stage and survival

Table B1: Results from separate ANOVAs on non-morphological response variables for each tadpole species. Statistically significant P values are in bold.

	FELI			MIFI			POLE		
Stage	SS	F	P	SS	F	P	SS	F	P
Competition	12.64	37.85	<0.001	6.02	10.50	<0.001	13.45	45.63	<0.001
Predation	0.58	1.75	0.19	4.34	7.57	<0.01	1.21	4.09	<0.05
Block	7.24	10.84	<0.001	2.71	2.36	0.07	7.99	13.55	<0.001
Competition x Predation	0.17	0.26	0.90	1.21	1.05	0.40	0.65	1.11	0.37
Growth									
Competition	6.01	21.89	<0.001	3.07	12.70	<0.001	15.51	52.06	<0.001
Predation	1.35	4.90	<0.05	2.30	9.53	<0.001	0.35	1.19	0.32
Block	19.21	34.97	<0.001	24.16	49.95	<0.001	14.86	24.93	<0.001
Competition x Predation	0.10	0.18	0.95	0.54	1.13	0.36	0.09	0.15	0.96
Survival									
Competition	1.24	0.76	0.48	0.87	0.42	0.66	1.18	0.86	0.43
Predation	0.11	0.06	0.94	0.68	0.33	0.72	3.72	2.72	0.08
Block	14.55	4.45	<0.001	6.31	1.51	0.22	16.84	6.16	<0.001
Competition x Predation	1.95	0.60	0.67	2.74	0.66	0.63	0.38	0.14	0.97

Figure B1: Standardized develomental stage for three tadpole species.
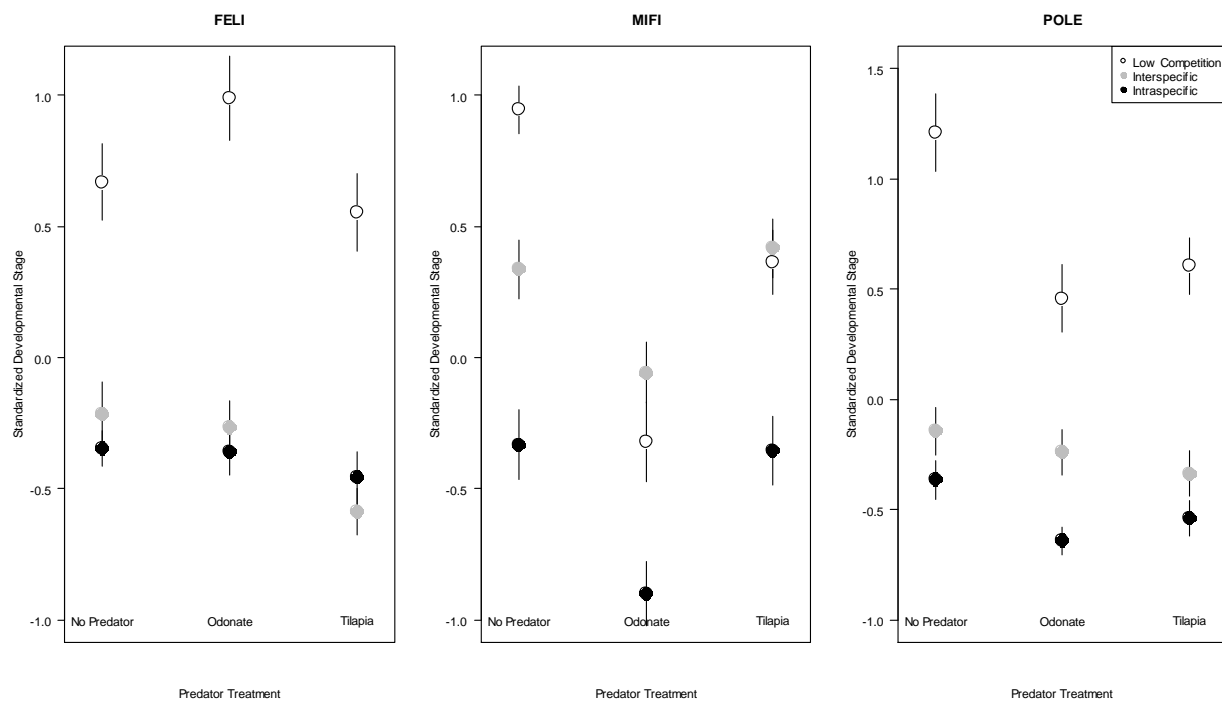
Figure B2: Standardized growth rate for the three tadpole species.
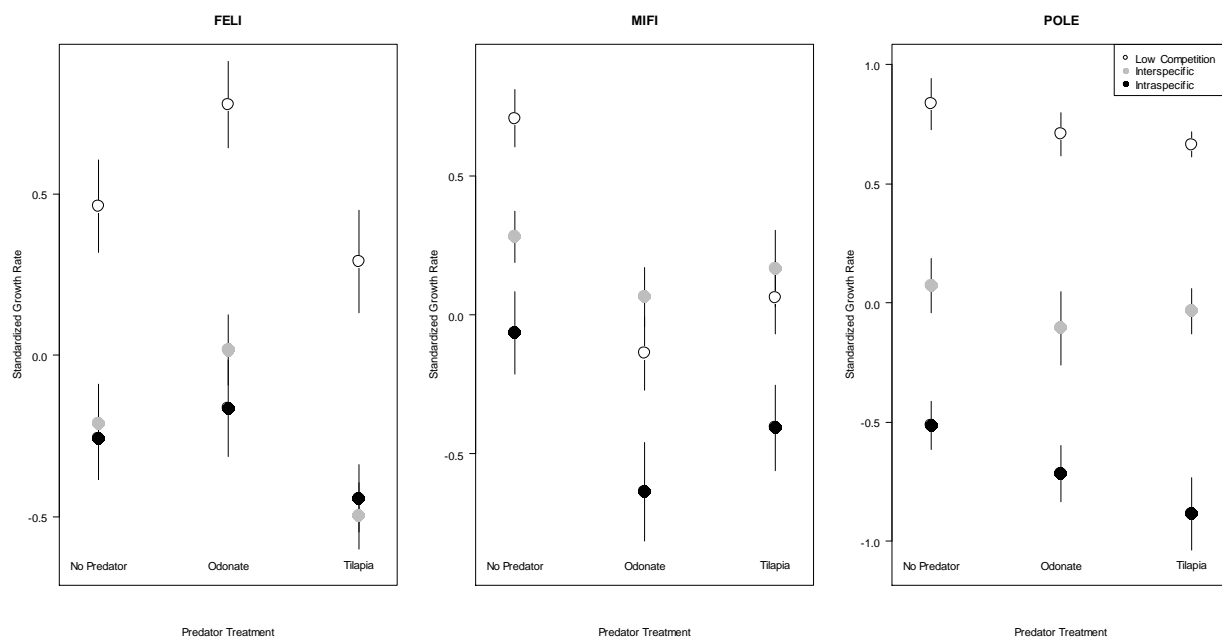
Figure B3: Survival for the three tadpole species.

FELI

MIFI

POLE

Low Competition
Interspecific
Intraspecific

Proportion Surviving

Predator Treatment

Appendix C- Morphological variables

Table C1: Results from MANOVA on morphological variables. Statistically significant *P* values are in bold.

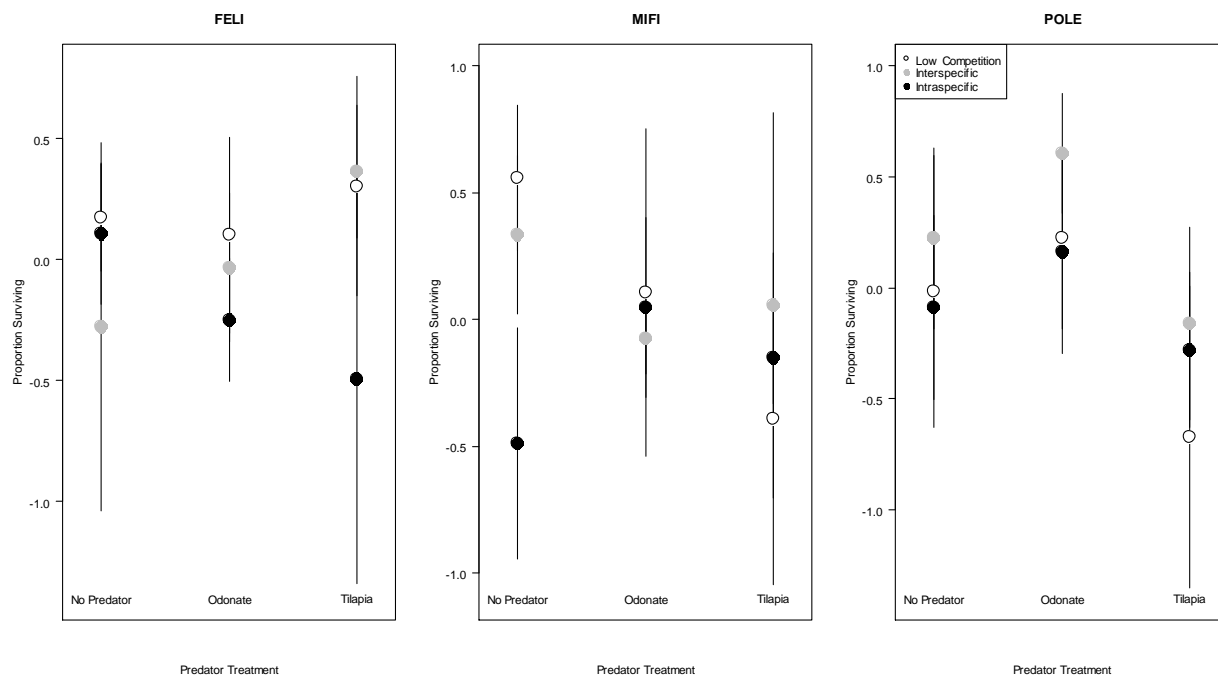|		FELI			MIFI			POLE		
Overall		Pillai's Trace	*F*	*P*	Pillai's Trace	*F*	*P*	Pillai's Trace	*F*	*P*
	Competition	0.83	2.74	<0.01	0.71	2.14	<0.05	0.55	1.44	0.16
	Predation	0.65	1.88	0.05	0.91	3.21	<0.01	0.94	3.41	<0.001
	Block	1.68	3.01	<0.001	1.66	2.93	<0.001	1.38	2.19	<0.01
Competition x Predation		0.85	1.12	0.33	0.37	0.43	0.99	0.73	0.93	0.57
Body Depth		SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
	Competition	0.33	0.61	0.55	1.49	2.87	0.07	1.14	3.73	<0.05
	Predation	1.66	3.07	0.06	5.18	9.96	<0.001	0.71	2.33	0.11
	Block	2.36	2.19	0.09	1.75	1.68	0.18	1.55	2.53	0.06
Competition x Predation		0.41	0.38	0.82	0.45	0.44	0.78	0.87	1.42	0.25
Body Length										
	Competition	0.18	0.27	0.77	1.80	2.98	0.06	0.32	1.00	0.38
	Predation	0.33	0.49	0.62	2.46	4.08	<0.05	0.15	0.46	0.64
	Block	5.07	3.81	<0.05	1.46	1.21	0.33	1.61	2.49	0.06
Competition x Predation		0.76	0.57	0.69	0.18	0.15	0.96	1.05	1.61	0.20
Tail Depth										
	Competition	1.75	3.25	0.05	0.60	1.42	0.26	2.63	7.56	<0.01
	Predation	0.48	0.88	0.42	2.68	6.40	<0.01	2.44	7.02	<0.01
	Block	2.57	2.39	0.07	4.48	5.36	<0.01	0.95	1.36	0.27
Competition x Predation		0.82	0.76	0.56	1.04	1.24	0.31	0.48	0.68	0.61
Tail Area										
	Competition	1.81	4.99	<0.05	1.64	3.25	0.05	1.72	3.98	<0.05
	Predation	0.15	0.40	0.67	0.71	1.40	0.26	1.09	2.54	0.09
	Block	2.86	3.93	<0.05	7.85	7.77	<0.001	0.27	0.32	0.86
Competition x Predation		1.76	2.43	0.07	0.39	0.39	0.82	0.12	0.14	0.97
Muscle Depth										
	Competition	1.35	3.30	<0.05	2.11	2.92	0.07	4.25	7.36	<0.01
	Predation	1.04	2.54	0.10	1.51	2.08	0.14	0.60	1.05	0.36
	Block	1.73	2.11	0.10	10.71	7.39	<0.001	2.29	1.98	0.12
Competition x Predation		2.28	2.79	<0.05	1.01	0.70	0.60	0.08	0.07	0.99
Max Fin Depth										
	Competition	0.87	2.95	0.07	2.18	4.97	<0.05	2.36	6.19	<0.01
	Predation	0.06	0.19	0.83	3.19	7.27	<0.01	3.44	9.02	<0.001
	Block	4.49	7.63	<0.001	4.20	4.80	<0.01	0.81	1.07	0.39
Competition x Predation		1.43	2.43	0.07	0.05	0.05	0.99	1.13	1.48	0.23
Muscle Area										
	Competition	1.47	3.70	<0.05	2.09	3.34	<0.05	0.80	1.81	0.18
	Predation	2.27	5.73	<0.01	2.01	3.22	0.05	0.13	0.29	0.75
	Block	1.91	2.40	0.07	4.55	3.64	<0.05	0.97	1.11	0.37
Competition x Predation		1.78	2.25	0.09	0.29	0.23	0.92	0.29	0.33	0.86

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Figure C1: FELI morphological variables with significant effects of predation and/or competition: muscle depth, muscle area and tail area.
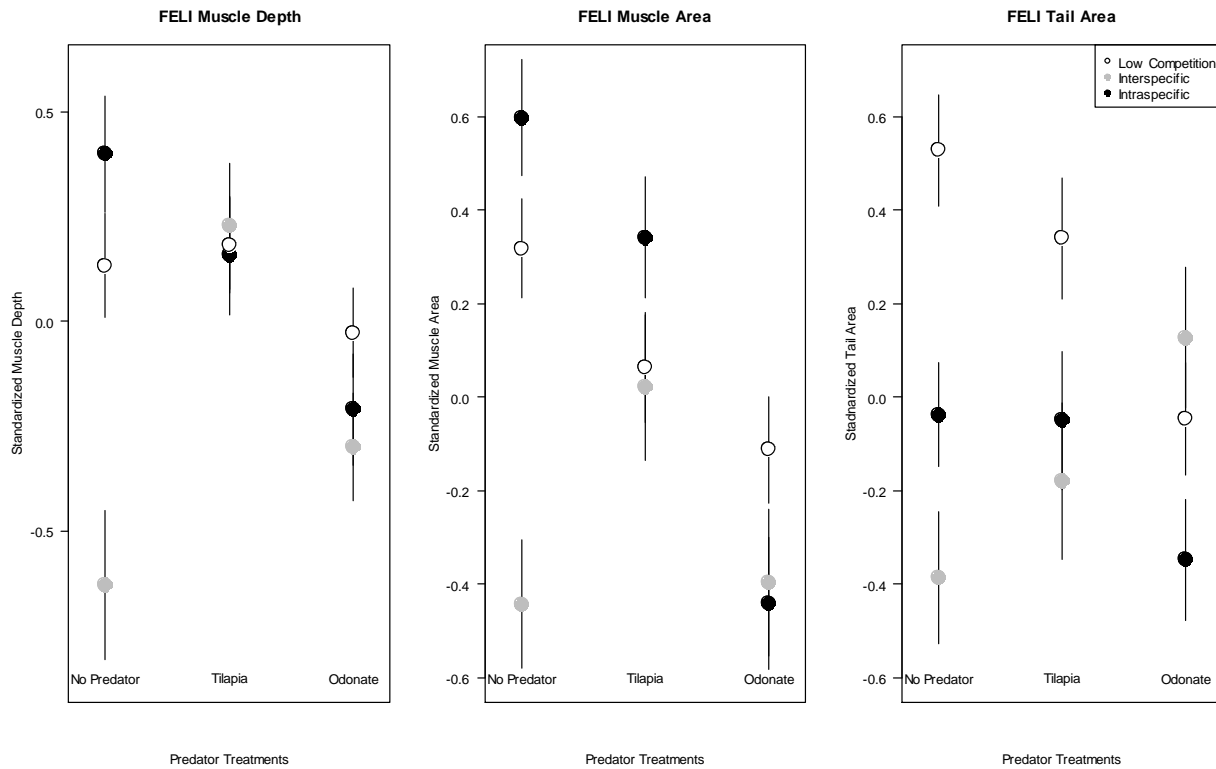
Figure C2: MIFI morphological variables with significant effects of predation and/or competition: body depth, body length, tail depth, fin depth, and muscle area
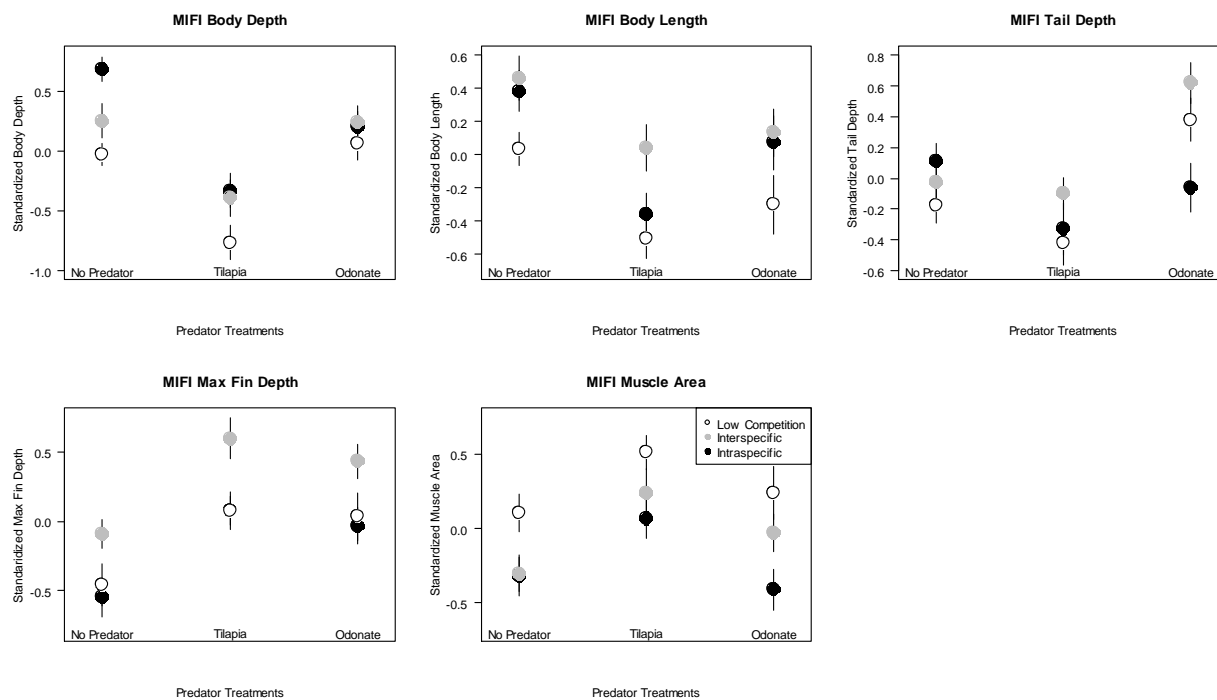
Figure C3: POLE morphological variables with significant effects of predation and/ or competition: body depth, tail depth, tail area, muscle depth and fin depth.
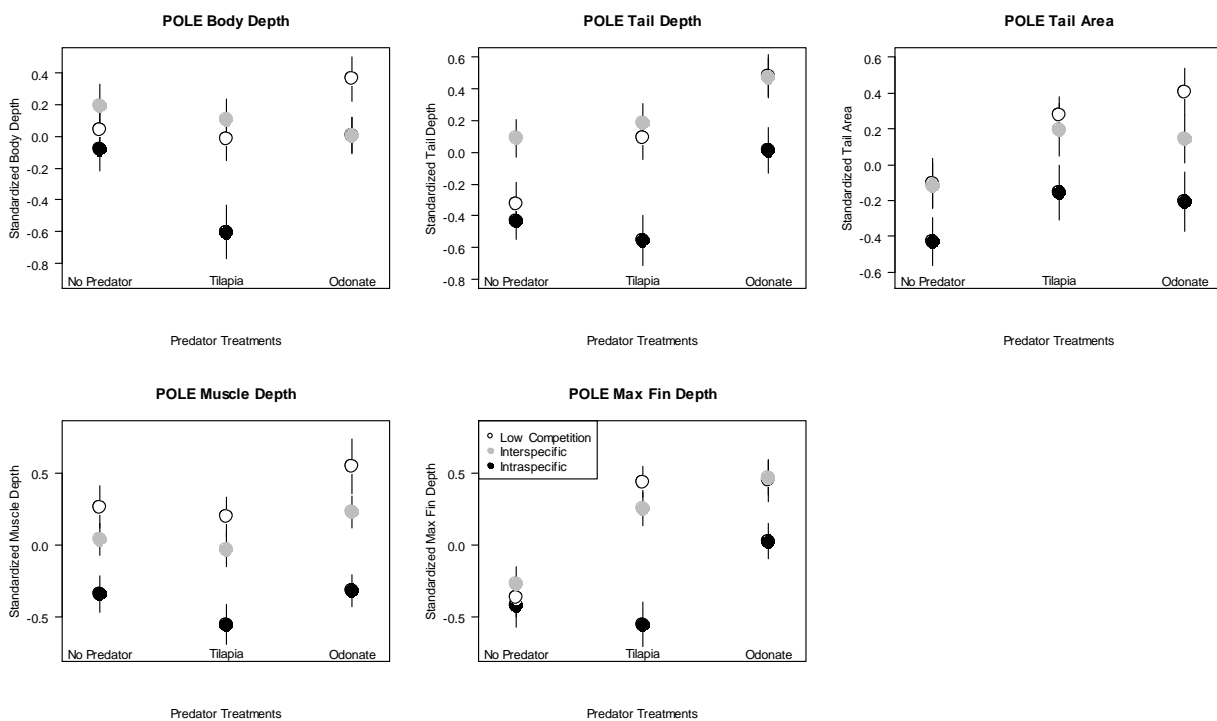
Chapter 3

Competitive context alters the outcome of predation in tadpoles with predator-induced phenotypic plasticity

Jocelyn E. Behm

Abstract

Many species have adaptive, predator-induced phenotypes which provide enhanced survival during predator encounters. If plastic traits responsible for enhanced survival also respond to other environmental forces, like competition, the adaptive nature of the predator-induced phenotype may be reduced. I tested whether inter- or intraspecific competition affected survival rates of three tadpole species (*Fejervarya limnocharis, Microhyla fissipes,* and *Polypedates leucomystax*) with tilapia (fish) or larval odonate-induced phenotypes relative to non-induced tadpoles. Previous exposure to odonates led to increased survival for all tadpole species, yet it was dependent on competition in all cases. *F. limnocharis* experienced increased survival in the interspecific competition treatment only, while the other two species experienced increased survival in the intraspecific competition treatment only.   In contrast, previous exposure to tilapia led to decreased survival for all three species relative to non-induced tadpoles, and this effect was only expressed in intraspecific treatments.   The differing effect between predators may be due to odonates being native and tilapia being exotic. The phenotype tadpoles express in response to tilapia may be one expressed in response to native fish predators with hunting styles different from tilapia. Thus, this phenotype may be maladaptive for surviving with tilapia predators. Finally, while all three species exhibited changes in morphological and life-history traits in response to predation and competition, their responses were unique, and different traits were correlated with tadpole survival for each species. These results highlight the importance of considering competitive context when examining predator-prey interactions.

Introduction

Phenotypic plasticity, the ability for one genotype to produce different phenotypes according to environmental context, is widespread across many taxa (Agrawal 2001; Pigliucci 2001; West-Eberhard 2003; Fordyce 2006). A substantial portion of phenotypic plasticity studies in ecology focus on the environmental context of predation, because many species exhibit plasticity in traits following exposure to a predator. In general, individuals with the predator-induced phenotype have higher survival rates during predator encounters relative to individuals without the induced phenotype (Lively 1986; McCollum & VanBuskirk 1996). If induced phenotypes do result in higher fitness when tested with a predator, they are adaptive (Auld, Agrawal & Relyea 2010).

Predator-induced phenotypes are complex and may be the product of multiple traits changing together. For example, *Hyla chrysoscelis* tadpoles exposed to larval odonate predators are smaller and have relatively deeper tails than those not exposed to predators, and tadpoles with predator-induced phenotypes have higher survival during predator encounters (McCollum & VanBuskirk 1996). Both growth rate and tail depth are plastic traits that change following exposure to a predator; however, smaller body size is correlated with lower survival when exposed to odonate predators (McCollum & VanBuskirk 1996). Thus, tail depth appears to be a trait that significantly contributes to the adaptive nature of the induced phenotype, while body size does not. Understanding the contributions of individual traits is necessary to understand the adaptive nature of an induced phenotype.

This is especially true under natural conditions, because predation is not the only force that induces phenotypic change. Although less intensively studied, competition also induces plastic responses in many species. Plastic traits in response to competition can also be adaptive in that they provide a competitive advantage in a competitive environment (Van Buskirk, McCollum & Werner 1997; Van Buskirk & Relyea 1998; Relyea 2002). Competition can induce changes in the same traits that respond to predation, yet may interact with predation to alter the trait value (Resetarits, Rieger & Binckley 2004; Van Buskirk *et al.* 2011). For species that encounter predation and competition simultaneously, competition may severely affect the adaptive nature of predation-induced phenotypes. The potential interactions between plastic responses to predation and competition have received little research attention.

Here, I address how competitive context affects the adaptive nature of predator-induced phenotypes in larval amphibians. Larval amphibians exhibit high plasticity in morphological and life-history traits in response to competition and predation. In general, predators induce relatively

consistent morphological changes of small body size and large tail size across tadpole species (Relyea & Hoverman 2003; Teplitsky, Plenet & Joly 2005). Within this framework, tadpoles alter specific body and tail traits in response to specific predators and these predator-specific trait changes can be adaptive (McCollum & VanBuskirk 1996; Van Buskirk *et al.* 2003; Benard 2006; Johnson, Burt & DeWitt 2008). Similarly, tadpoles also exhibit adaptive morphological changes in response to competition, specifically, increased body size and decreased tail size. This morphological change is thought to increase digestive efficiency (Relyea & Auld 2004; Relyea & Auld 2005), and tadpoles with competitor-induced phenotypes are better competitors than those without the phenotype (Relyea 2002; Relyea & Auld 2005). Given that the general morphological responses for predation and competition are in opposing directions, there is a tradeoff between responding to predation and competition. It has been confirmed experimentally for several species that predation and intraspecific competition induce traits in opposing directions (Relyea 2002; Relyea & Hoverman 2003; Resetarits, Rieger & Binckley 2004; Relyea & Auld 2005; Teplitsky & Laurila 2007), and tadpoles with predator-induced traits are poor competitors (Van Buskirk, McCollum & Werner 1997; Van Buskirk & Relyea 1998; Relyea 2002).   The converse situation, whether competitor-induced tadpoles have low survival with predators has not been tested.

    While competition has the potential to change plastic trait responses to predation due to competition, it also can change the context of predation. If an individual is experiencing strong intraspecific competition, it likely also experiences predation in a group of conspecifics. Conversely, if an individual is experiencing strong interspecific competition, it likely also experiences predation in a group of heterospecifics. An individual's chance of survival is based on its phenotype relative to the phenotypes of the rest of the individuals in the group. Thus, I would expect that the adaptive benefits of plastic phenotypes in response to predation will depend on the presence of competitors, and whether these competitors are con- or heterospecific.

    I tested how previous exposure to a predator affected survival with that predator in different competitive contexts. I contrasted responses of three tadpole species that commonly co-occur in the same ephemeral pools in tropical southwest China. All three species compete, are eaten by the same predators and demonstrate plastic responses in morphological and life history traits in response to predation and competition (Chapter 2). The adaptive significance of the predator-induced phenotypes has not been investigated for any of these species. I exposed all three species to combinations of intra- or interspecific competition and predation, and then tested them with a free-roaming predator in the

same competitive context in which they were raised to determine 1) if these species have adaptive responses to predators, 2) if so, which traits are responsible for the adaptive phenotypes.

Methods

Study Species

My study was conducted at the Xishuangbanna Tropical Botanic Garden (XTBG) in Xishuangbanna prefecture, southern Yunnan Province, China.   For my experiments, I used three species, *Fejervarya limnocharis, Microhyla fissipes,* and *Polypedates leucomystax* (hereafter referred to as FELI, MIFI and POLE, respectively) which frequently co-occur in the same ephemeral pools. During breeding site surveys, I found that invertebrate predators are present in 97%, 94% and 72% of the pools surveyed containing FELI, MIOR, and POLE respectively, and the most common invertebrate predator I found was larval Libellulidae (odonates). In addition, FELI and MIFI completely avoided breeding in pools containing tilapia, whereas 12% of the pools where POLE bred also contained tilapia. Tilapia, which are native to Africa, were introduced to Xishuangbanna within the past 30 years (Zhuangfang Yi, XTBG, *pers. comm.*) primarily for food, and have established in rivers and permanent pools. In the few instances where POLE tadpoles and tilapia co-occurred, it was in permanent pools where tilapia were present prior to oviposition.

Rearing Treatments

Tadpoles of all three species were collected as eggs in the field and relocated to an outdoor laboratory for hatching. Once tadpoles reached Gosner stage 25 (Gosner 1960)(about 5 days post hatching) they were haphazardly assigned to rearing treatments. Rearing treatments were conducted in a 2 x 3 factorial design for each tadpole species with two competition treatments (intraspecific, interspecific) crossed with three predation treatments (no predator, odonate, tilapia). The intraspecific competition treatment consisted of 60 individuals of focal species, and the interspecific competition treatment contained 20 individuals of focal species plus 20 individuals each of the other two species; this design ensured consistent initial density across all treatments. Predators were contained in mesh bags suspended in treatment basin water and fed tadpoles of the focal species from the same clutch that focal species were hatched from. This is a typical predator-induced phenotypic plasticity experimental design and allows focal tadpoles to experience cues of predation without being eaten themselves.

After 14 days, predators were removed from experimental treatments and a subset of 10 individuals per species per basin was removed for measuring. I anesthetized tadpoles in MS-222 and weighed, staged and photographed the right side of each tadpole. Tadpoles were then returned to their rearing basins for recovery from anesthetic for a minimum of 20h prior to the predation trials.

A detailed description of the experimental rearing conditions is provided in the methods of Chapter 2.

Predation Trials

Tadpoles in all rearing treatments were subjected to predation trials; tadpoles from tilapia treatments were tested with tilapia, tadpoles from odonate treatments were tested with odonates, and tadpoles from no predator treatments were tested with both tilapia and odonates separately. Because tilapia and odonates differ in their predation rates, predation trials were conducted differently for each predator. Predation trials for tilapia were conducted in 35L basins and predation trials for odonates were conducted in 1.5L basins. Basins were filled with rain water a minimum of 20h prior to the start of the predation trials. For tilapia trials involving tadpoles reared in intraspecific treatments, I used 12 tadpoles of one species reared together in the same rearing basin. For tilapia trials involving tadpoles reared in interspecific treatments, I used 4 tadpoles per species that were reared together in the same basin. I allowed tadpoles to acclimate to the predation trial basin for about 1h prior to the addition of the tilapia. Following tilapia addition, I recorded the number of surviving tadpoles every 10min for 90min and then every 15min for an additional 90min.

For the odonate trials involving tadpoles reared in intraspecific treatments, I used 6 tadpoles of one species reared together in the same rearing basin. For odonate trials involving tadpoles reared in interspecific treatments, I used 2 individuals per species that were also reared together in the same rearing basin. I allowed tadpoles to acclimate to the predation trial basin for about 1h prior to the addition of the odonate. Following odonate addition, I recorded the number of surviving tadpoles every hour for 11h and then once more at 24h following odonate addition.

The predators used in predation trials were the same individuals used in the rearing treatments, thus, all individuals had previous experience consuming tadpoles prior to the predation trials. In addition, all predators were starved for the 24h prior to the start of the predation trials. Tilapia used in predation and rearing treatments were juveniles (ca. 50mm, mean mass= 3.89g, 1.1-8.1g) provided by a local farmer. I used two larval odonate morphospecies from the Libellulid family, because neither

species was abundant enough to use alone. I refer to the two morphospecies as species A and species B. While species A was the superior predator, both species induced plastic responses in tadpoles (Chapter 2).   Prior to the start of all predation trials, predator weights and predator species in the case of odonates, were recorded and used as covariates in statistical analyses.

Each set of rearing treatments was replicated using 5 temporal blocks. The five temporal blocks were started on June 18, June 20, July 18, August 8, and August 22, 2010. Since each temporal block was not run concurrently, tadpoles in predation trials where always from the same block. My goal was to have 3 tilapia and/ or 3 odonate predation trials from each rearing treatment basin; however, this was dependent on tadpole survival during the rearing portion. Given limited survival in rearing treatments in some blocks, I could not use all rearing treatments in every predation trial; details of sample sizes are given in Table A1.

Statistical Analyses

My ultimate goal was to determine whether tadpole survival during predation trials could be attributed to previous predator exposure and/or competitive context (intra- vs interspecific), or whether other morphological and life history variables better explained survival. The first step in my analyses was to determine how rearing treatments affected morphological and life history traits. Based on the responses of these traits and the correlations among them, I selected a subset of uncorrelated variables that best described trait variation for use in subsequent analyses of survival in predation trials.   I then conducted analyses on tadpole survival in predation trials using statistical models containing only rearing treatment variables, only trait variables, and then combined rearing treatment and trait variables into the same model; the last analyses were designed to assess the potential ability of tadpole trait values in explaining survival in the different treatments. In the event that different variables were responsible for survival early versus later in the predation trials, I analyzed data at my first time point for each trial type, and then a second later time point that corresponded to approximately 50% of tadpoles being eaten; I selected the second point, because at 50% survival, the statistical power of the tests should be high. All analyses were conducted separately for each tadpole species using the R statistical program (version 2.13.0, http://www.r-project.org/).

Responses to rearing treatments

I used three life-history variables (final mass, final developmental stage, final body length) and seven morphological variables (body depth, body length, tail depth, fin depth, tail area, tail muscle depth, tail muscle area) as the response variables to rearing treatments. All morphological variables and total length were measured from photographs using ImageJ software (version 1.45h, rsbweb.nih.gov/ij/), while final mass and developmental stage were recorded while tadpoles were anesthetized. Prior to analyses, mass, length, and all morphological variables were logarithm transformed, and developmental stage was square-root transformed. I conducted separate regressions for each tadpole species of each morphological variable on total body length, and retained the residuals from the regressions for length-independent metrics of the morphological traits. Then, all life-history and morphological traits were standardized to have a mean equal to zero and variance equal to one to facilitate comparison among tadpole species.

I used redundancy analysis (RDA, (Legendre & Legendre 1998) to obtain a qualitative description of how the seven morphological variables responded to the rearing treatments. All seven standardized morphological variables were used in the analysis with the constraining matrix of predator and competition rearing treatments. Since the morphological variables were standardized within each species, I conducted the redundancy analysis on data from all three species together.

I calculated all pair-wise correlations of variables in order to facilitate selection of uncorrelated variables for analyses of survival in predation trials.

To understand how morphological and life history variables were affected by the rearing treatments, I used MANOVA. I used the three standardized life-history variables, seven standardized morphological variables, and scores from RDA1 and RDA2 as my multivariate response variables, and predator, competition and predator by competition interaction as my predictor variables. I performed separate MANOVAs for each tadpole species and for each rearing predator treatment. For example, for the MANOVAs for tilapia-reared tadpoles, I included only the tilapia and no predator replicates that were used in subsequent predation trials. This is because different no predator treatments were used in the odonate and tilapia predation trials (Table A1), and because morphological variables were used as predictor variables explaining tadpole survival in predation trials in subsequent analyses.

Analyzing survival in predation trials

I analyzed survival for each tadpole species with each predator at the first time point of the predation trial and the point where approximately 50% of the tadpoles had been eaten. To account for

the differences in the starting number of tadpoles in the inter- versus intraspecific competition trials, I analyzed the number of tadpoles surviving using a general linear mixed model fit with a binomial distribution. My 'treatment' model analyzed the effect of rearing treatments on tadpole survival. I used predator treatment, competition treatment, their interaction, predator mass and, in the case of odonate trials, predator species as fixed effects, and block as a random effect. I conducted the analyses for the treatment model and subsequent models with predator mass and predator species as random effects nested within block, but the results were qualitatively the same as having them as fixed effects; therefore, to use the simplest models with the fewest assumptions about the distribution of predator mass and species, I opted to code them as fixed effects. I followed each treatment model with planned contrasts to determine whether there was a difference in survival for predator-exposed and unexposed tadpoles within a competition treatment category.

Based on the responses of the morphological and life-history traits to the rearing treatments, I identified one life-history and three morphological variables that best summarized the trait data and analyzed them in my 'trait' model. The trait model was similar in structure to the treatment model, treating traits as fixed effects, along with predator species and predator mass, and including a random block effect.

I put all variables from the treatment and trait models into a single 'full' model and then used backwards selection to find the 'reduced' model that retained only significant variables from the full model. All analyses were conducted using R, and general linear mixed model analyses were done using 'lmer' in the lme4 library.

Results

Selecting morphological and life history variables for predation trial analyses

In the redundancy analysis on morphological variables, the first axis (RDA1) explained 12.3% of the variation and the second axis (RDA2) explained 7.8% of the variation. Because a redundancy analysis only explains the variation limited by the constraining matrix, the percentage of variation explained is typically less than in principle components analysis. In comparison, a principal components analyses on the same morphological variables results in similar associations between variables and axes and PC1 explaining 44.6% and PC2 explaining 17.7% of the variation. Six out of the 7 morphological variables were negatively associated with RDA1, indicating this axis represents overall size or magnitude of the trait. Body and tail morphology were split along RDA2 with body traits having positive scores and

tail traits having negative scores (Figure 1). The morphologies of all three tadpole species were affected by the rearing treatments. MIFI showed a strong influence of both predation and competition on morphology relative to the other species, POLE was influenced primarily by competition, and FELI responded to tilapia treatments differently from the rest (Figure 1).

I used MANOVA to determine the influence of the predator and competition rearing treatments on the life-history and morphological variables. The univariate tests from each MANOVA are reported in Table 1. The three tadpole species differed in their responses to the treatments. For FELI, there was no difference between inter- and intraspecific competition on life-history variables, whereas MIFI and POLE both experienced higher growth and development in interspecific treatments relative to intraspecific treatments (Table 1). Thus, the negative effects of interspecific competition on life-history traits were less than intraspecific competition for MIFI and POLE. Interspecific competition caused decreased muscle size in FELI, but increased tail size in MIFI and increased tail and body size POLE (Table 1).

For predation, being raised with tilapia had no effect on MIFI life-history variables, but caused reduced mass in FELI and a reduction in all three life-history variables in POLE, relative to no predator treatments (Table 1A). Tilapia also caused reduced body and tail size in FELI and POLE relative to the no predator treatments, while MIFI experienced decreased body depth but increased fin depth and muscle size (Table 1A). Odonates had no effect on FELI life-history variables, yet caused reductions in life-history variables for MIFI and POLE relative to no predator treatments (Table 1B). Odonates caused reduced body length and increased fin depth for all three species (Table 1B). For MIFI and POLE, odonates also increased tail size, while odonates decreased muscle size in FELI (Table 1B).

Based on the RDA results (Figure 1), MANOVA results (Table 1) and the correlations among traits (Table B1), I chose mass, body depth, fin depth and muscle depth as the four variables to use in my trait model. All three life-history variables were correlated (Table B1), responded similarly to rearing treatments (Table 1), and mass was the least correlated with morphological traits. Body depth and fin depth were the most extreme values along RDA2 (Figure 1). Body depth was correlated with body traits and fin depth was correlated with tail traits (but not tail muscle traits) (Table B1); thus, they represent the two extremes of body and tail morphology. In previous work, I demonstrated a trade-off between tail fin and tail muscle morphology in response to competition and predation (Chapter 2). Therefore, I also included muscle depth and it was not strongly correlated with the other three traits I chose. Figure 2 shows the influence of rearing treatments on my four chosen trait variables.

Tadpole survival in tilapia predation trials

Although the tilapia trials lasted for 180 minutes, all three species reached the 50% survival mark by 30 minutes, thus I analyzed survival at 10min and 30min.

Survival of FELI tadpoles with tilapia was influenced by both previous exposure to tilapia and competitive context. For the trials involving interspecific treatments, tadpoles with prior exposure to tilapia survived better than those that had no exposure (Table 2A,B, Figure 3). This was significant for the 10min (P <0.01), and a trend for the 30min time point (P<0.1). For the intraspecific treatment this pattern was reversed for both 10min (P<0.01) and 30min (P<0.01); tadpoles with previous exposure to tilapia had lower survival than those with no exposure (Table 2A,B, Figure 3). This pattern may have been in part influenced by tail muscle depth, which also had a significant predation X competition interaction (Table 1A). Survival with tilapia was also correlated with increased mass and decreased fin depth (Table 2A,B). Because the tilapia rearing treatment caused a reduction in mass (Table 1A), previous exposure to tilapia may have indirectly resulted in reduced survival. Neither competition nor predation rearing treatments affected fin depth (Table 1A).

Previous exposure to tilapia resulted in decreased survival for MIFI tadpoles relative to tadpoles with no previous exposure (Table 2C,D, Figure 3). This pattern was a trend at 10min (P<0.1) and statistically significant at 30min (P<0.05) for tadpoles in the intraspecific competition treatments. Exposure to tilapia resulted in decreased body depth (Table 1A), and decreased body depth was correlated to increased survival. Therefore, tilapia-induced changes in body depth predict the oppose effect of being reared with tilapia on survival from that which was observed. After 30min, increased body mass was also correlated to survival (Table 2D).

There was an overall negative effect of previous exposure to tilapia on POLE survival relative to tadpoles with no previous exposure at both 10 and 30 minutes (Table 2E,F, Figure 3). Tilapia exposure resulted in decreased mass relative to unexposed tadpoles (Table 1A), and increased mass was significantly correlated with increased survival (Table 2E,F). Therefore, the negative effect of predation on tadpole survival may have been due in part to weight. This was supported by the results comparing the trait and reduced models; while there was a significant effect of mass on survival in the trait model, this effect was removed by inclusion of the treatment effects in the reduced model (Table 2E,F). In addition, competitive context affected tadpole survival. Tadpoles in the interspecific competition treatments had lower survival relative to their counterparts in the intraspecific competition treatments. This effect was independent of mass, as interspecific competition resulted in increased mass (Table 1A).

Tadpole survival in odonate predation trials

All three tadpole species reached the 50% survival level at 24h; therefore, I present results for odonate trials after 1h and 24h.

For the 1h time point, only treatment effects were significant for FELI survival. There was a significant predation X competition interaction, where tadpoles raised with odonates had significantly higher survival relative to unexposed tadpoles for the interspecific competition treatments only ($P<0.05$, Table 2G, Figure 3). This pattern was a non-significant trend at 24h ($P< 0.1$). The significant predation X competition interaction at 1h was likely not due to any life-history or morphological variables, given that no traits were significant predictors in the trait model (Table 2G). At 24h, tadpole survival was only correlated with increased fin depth (Table 2H). While fin depth was positively correlated with the odonate and interspecific rearing treatments (Table 1B), no rearing treatments were significant in the treatment model (Table 2H). Thus, the effect of fin depth on tadpole survival was independent of rearing treatment.

While there was no effect of previous exposure to predators after 1h (Table 2I), MIFI tadpoles exposed to odonates survived better than unexposed tadpoles in intraspecific competition treatments after 24h ($P<0.05$, Table 2J, Figure 3). At both 1h and 24h, tadpole survival was correlated with increased mass and body depth (Table 2I, J). Exposure to odonates resulted in decreased mass and decreased body depth (Table 1B); thus, previous predator exposure may have indirectly caused reduced survival.

At both 1h and 24h, POLE tadpoles exposed to predators survived better than tadpoles with no previous exposure in intraspecific treatments (1h: $P<0.001$, 24h: $P<0.01$; Table 2K, L; Figure 3). At 1h, the significant effect of predator exposure was independent of life-history and morphological variables, as no variables were significant in the trait model (Table 2K).   Tadpole survival at 24h was also correlated with increased mass (Table 2L).

Discussion

I exposed three tadpole species to two different predators and then tested the tadpoles in predation trials with those predators to determine if previous experience with the predator translated into increased survival. In addition, predator treatments were crossed with intra- and interspecific competition treatments in which focal tadpoles were both raised and tested with only intraspecific competitors and with individuals of the other two species. My goal was to determine how phenotypic

plasticity in species traits affected their survival from predation when tadpoles are faced with developmental trade-offs between antipredator defenses and competition. Furthermore, by measuring tadpole traits in the different rearing treatments, I identified at least some of the plastic phenotypic responses to predation and competition, and used these to try to explain the treatment effects of rearing on tadpole survival when confronted by predators.

Previous exposure to predators

My expectation was that exposure to predators would induce traits that make tadpoles less susceptible to predation by those predators. Contrary to expectation, exposing tadpoles to predators had negative, positive and no effects on tadpole survival relative to tadpoles with no previous exposure. The relative success of tadpoles with predator-induced phenotypes was in part due to the predator species. All three tadpole species experienced enhanced survival when previously exposed to odonates during at least one time period, whereas FELI was the only species with increased survival when previously exposed to tilapia. In contrast, all three species experienced decreased survival when previously exposed to tilapia during at least one time period, and no species experienced a direct negative effect of previous exposure to odonates. This striking contrast may be due to the evolutionary history the tadpoles share with these predators because tilapia are exotic and odonates are native.

The libellulid odonates species I used are native to the Southeast Asian tropics, thus my three tadpole species also native to this area likely have coevolved with these odonates somewhat. This may explain why the odonate-induced phenotype had higher survival than the non-induced phenotype. Different traits of the odonate-induced phenotype appear to be responsible for each species' success at different time points. At 1h, survival for FELI and POLE depended solely on rearing treatments and was not related to the traits I measured. At 24h, however, increased fin depth affected FELI survival, and mass affected POLE survival in addition to previous exposure to odonates.   Thus, it is possible that behavioral aspects of the phenotype that were expressed due to previous exposure to the predator were responsible for their survival early on, then morphological aspects became more important. FELI and POLE have similar trophic ecologies in that both species consume detritus from the bottom of pools. This likely puts them in contact with odonates, therefore both tadpole species would benefit from behavioral modifications that reduce their exposure to odonates.   In contrast, *M. fissipes* is a filter feeder which spends most of its time near the surface of the water filtering particles. They likely only encounter odonates that are on vegetation or in shallow pools; therefore, investing in traits which

possibly make them more difficult for odonates to handle may be a better strategy. Survival for MIFI at 1h and 24h was due to increased mass and body depth only. While it seems contradictory that these traits were associated with increased survival because exposure to odonates resulted in reduced mass and reduced body depth, these effects were small. There was a significant competition x predation interaction for body depth such that only intraspecific-no predator treatments had greater body depth than the odonate treatments. In addition, the effect of odonates on mass was small relative to the effect of competition on mass.

In contrast to native odonates, tilapia were introduced to Xishuangbanna only within the past 30 years. There is evidence for (Kiesecker & Blaustein 1997) and against (Sosa, Ryan & Schlaepfer 2009; Gomez-Mestre & Diaz-Paniagua 2011) the ability of tadpoles to evolve defenses in response to introduced predators. Populations of red-legged frogs (*Rana aurora*) exposed to introduced bullfrog (*Rana catesbeiana)* predators for 60 years evolved behavioral defenses which facilitated enhanced survival relative to naïve populations (Kiesecker & Blaustein 1997). However, lowland leopard frogs (*Rana yavapaiensis)* exposed to green sunfish (*Lepomis cyanellus)* for a comparable time period did exhibit phenotypic changes in response to fish exposure, but previous exposure did not correlate to increased survival (Sosa, Ryan & Schlaepfer 2009). In my system, all three species had responses in morphological variables, and FELI and POLE had responses in life history variables due to tilapia rearing treatments. In fact, tilapia caused reduced body depth in MIFI and reduced body depth was correlated with increased survival, yet there was still an overall negative effect of previous exposure to tilapia on tadpole survival.

While there are many examples of tadpoles with predator-induced phenotypes having no difference in survival relative to non-induced individuals (Benard & Fordyce 2003; Alvarez & Nicieza 2006; Benard 2006), there are scant examples of predator-induced tadpoles having reduced survival relative to non-induced tadpoles. Conversely, there are many examples of induced phenotypes incurring a cost in the wrong environment (Auld, Agrawal & Relyea 2010). Although my three focal tadpoles do not share an evolutionary history with tilapia, they did evolve with fish. In general all three species in my study area breed in ephemeral rainwater pools and avoid breeding in pools that contained fish. However, it is possible that during floods ephemeral pools could join pools containing fish and fish could become isolated in a pool. In addition, fish from the genus *Channa* are native to Southeast Asia and can breathe air and disperse over land from permanent pools to ephemeral pools. Thus, my three focal tadpole species have likely evolved plasticity in traits in response to fish cues, and I suspect that

the tilapia-induced phenotypes my tadpoles expressed were generalized responses to fish cues. The reason the tilapia-induced phenotype caused reduced survival, could be due to differences in hunting style between tilapia and the native fish species. Tilapia are generalists and quite plastic in their feeding behaviors. They are not exclusively benthic like many of the native fish such as *Channa* that tadpoles encounter. Tilapia remain in the water column and pursue prey in the water column (http://www.fishbase.org/summary/Oreochromis-aureus.html). MIFI and POLE are in the water column and likely encounter tilapia the most and these species had consistent reductions in survival when previously exposed to tilapia. Thus it is possible that their predator-induced phenotype is in response to a benthic fish. Regardless, non-native fish introductions are a significant driver of amphibian population declines (Collins & Storfer 2003), and my data suggest that my focal species may be maladapted to respond to introduced tilapia.

Competitive context

In my study competitive context referred to both the rearing environment and the predation trial environment, and impacted the outcome of predation trials for all three species for both predators. Overall, there were significant effects of competitive context on survival for FELI and POLE in tilapia trials. The significant effects of competitive context on survival could be due to two mechanisms. First, because the survival of tadpoles in the interspecific competition treatments were conducted with all three species present, a difference in predation rate between a predator-induced intraspecific versus interspecific trial could be due to the relative capturability of species in the trial. For example, if one species' predator-induced phenotype made it very difficult to capture, the predation rates on the other two species could increase. In effect, the predation trials in the interspecific competition treatment were choice tests in which predators could select different prey species to pursue. This hypothesis was not consistent with my data; predation rates for predator-induced and non-induced tadpoles were identical between interspecific and intraspecific treatments in odonate trials. In tilapia trials, there were differences in predation rates between the inter- and intraspecific treatments for FELI and POLE; however, even though FELI's predation rate decreased in the predator-induced treatment, POLE's remained unchanged.

Alternatively, significant effects of competitive context could be due to differences in traits induced by inter- versus intraspecific competition in the rearing treatments. My data support this hypothesis. For FELI there was a significant predation x competition interaction where tilapia-induced

tadpoles had higher survival in interspecific competition treatments and lower survival in intraspecific competition treatments. This pattern may in part be driven by muscle depth as there was a significant predation x competition interaction on muscle depth from the rearing treatments. In the absence of predators, tadpoles had reduced muscle depth in interspecific competition treatments and greater muscle depth in intraspecific competition treatments, and in my trait model muscle depth was positively correlated with survival. For POLE, tadpoles in the interspecific tilapia predation trials had lower survival than tadpoles in the intraspecific predation trials at both 10 and 30 minutes. This may have been due to increased fin depth in interspecific competition treatments relative to intraspecific competition treatments, because increased fin depth was correlated with decreased survival in my trait model.

When there was a difference in survival between the predator-induced and non-induced phenotypes, seven times out of nine it was for intraspecific competition treatments, and the two instances of differences in survival for interspecific treatments were for FELI. This underscores the importance of considering ecological context for phenotypic plasticity studies. If my experiments were conducted on single species at raised high density, I would have concluded that phenotypic plasticity in response to the presence of predators was more effective at deterring predation than what I found. Situations involving high densities of conspecifics and high densities of heterospecifics both occur in nature, and therefore it is important to consider both in experiments. In my system the three species are found with heterospecifics at the majority of the sites I sampled, therefore interspecific competition is common and the most frequent situation in nature corresponds to my interspecific competition treatments.

The two instances where survival in the predator-induced phenotype exceeded the non-induced phenotype for interspecific competition treatments were for FELI. FELI experiences strong interspecific competition from POLE (Edmonds *et al. In Prep*), and there are equal effects of inter- and intraspecific competition on life-history traits. However, inter- and intraspecific competition have different effects on morphological traits, and this can change according to the predation context. According to my results, changes in morphological traits, and possibly other aspects of the predator-induced phenotype, are advantageous in an interspecific context. In both tilapia and odonate treatments, FELI predator-induced tadpoles had higher survival than non-induced tadpoles in only the interspecific treatments.

My data show that FELI achieves a survival advantage when with heterospecifics, while POLE is at a survival disadvantage with heterospecifics. These patterns are reflected in the species' distributions

at breeding sites. In ephemeral pools where I found FELI, 91% also had MIFI and 70% had POLE. This is in contrast to pools were I found POLE: 40% had FELI and 60% had MIFI (Chapter 1).

Conclusion

      In conclusion, my work demonstrates that the outcome of predator-prey interactions changed according to competitive context because competition altered predator-induced phenotypes. Although my work considered how competition altered the effectiveness of predator-induced plasticity, tadpoles are highly plastic and respond to other non-biotic factors. Temperature (Touchon & Warkentin 2011), pesticides (Teplitsky *et al.* 2005), and UV radiation (Alton *et al.* 2012) all alter the phenotypic responses of tadpoles to predators.   Given that amphibians are living in increasingly altered environments (Sodhi *et al.* 2008) with novel stressors and novel predators (Collins & Storfer 2003), it may be worthwhile investigating how these stressors interact to affect tadpole survival.

Tables

Table 1: Univariate components of MANOVA explaining the influence of predation and competition rearing treatments on life history and morphological variables for the three tadpole species, *Fejervarya limnocharis* (FELI), *Microhyla fissipes* (MIFI), and *Polypedates leucomystax* (POLE).   Competition refers to interspecific treatments and predator refers to tilapia for A) and odonates for B). Symbols in table indicate direction of effect size relative to intraspecific treatment for competition or no predator treatment. Rows highlighted in gray are variables used in analyses on survival in predation trials.   Note: these analyses are based on the tadpoles that were used in predation trials, therefore because the tilapia trials had one fewer no predator-interspecific treatment, the results for competition are slightly different for A) and B).

A) Tilapia

	FELI			MIFI			POLE		
	Competition (Inter)	Predator (Tilapia)	Competition x Predator	Competition (Inter)	Predator (Tilapia)	Competition x Predator	Competition (Inter)	Predator (Tilapia)	Competition x Predator
Mass		-		+			+	-	
Length				+			+	-	
Stage				+			+	-	
Body Depth		-			-		+	-	
Body Length			+						+
Fin Depth				+	+		+		+
Tail Depth		-			-		+		
Tail Area				+			+	-	
Muscle Depth	-	-	+		+		+		
Muscle Area	-		+		+				
RDA1		+		-				-	-
RDA2	+	-		-				-	

B) Odonate

	FELI Competition (Inter)	FELI Predator (Odonate)	FELI Competition x Predator	MIFI Competition (Inter)	MIFI Predator (Odonate)	MIFI Competition x Predator	POLE Competition (Inter)	POLE Predator (Odonate)	POLE Competition x Predator
Mass				+	-		+		
Length				+	-		+	-	
Stage				+	-		+	-	
Body Depth	-		+	-	-	+			-
Body Length		-		-			-		
Fin Depth	+	+	+	+	+		+	+	
Tail Depth			+	-	+	+	+	+	
Tail Area	-		+	+	+		+	+	
Muscle Depth	-	-	+	-		+	+		
Muscle Area	-	-	+	-		+	+		
RDA1	+	+	-	-	-	-	-	-	
RDA2				-	-		+	-	

Table 2: Results from a generalized linear mixed model fit with a binomial distribution to explain tadpole survival in predation trials. The response variables in all models was the inverse logit transformed proportion of tadpoles surviving. Treatment models included as predictors only the rearing treatment categorical variable of predator (no predator and odonate or tilapia predator), competition (inter- and intraspecific), and their interaction. Trait models included mass, body depth, fin depth, and muscle depth as predictors. Full models include all variables from treatment and trait models. Reduced models were obtained through backwards selection and include only significant predictors. All models also included predator mass and predator morphospecies (for odonate trials) as predictors (these were included in reduced models only if significant. Chi-squared values (ChiSq) were obtained through likelihood ratio chi-squared tests. The ES column provides the sign of the effect size of continuous variables.

A) FELI Tilapia 10

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	1.30	0.25				0.30		0.58	0.01		0.97
Competition	1.93	0.16				0.02		0.90	0.74		0.39
Predator x Competition	12.12	0.001				13.27		<0.001	15.21		<0.001
Mass			13.39	+	0.001	25.98	+	<0.001	30.35	+	<0.001
Body Depth			0.21	-	0.65	1.7	-	0.19			
Fin Depth			3.09	-	0.08	3.78	-	0.06	6.34	-	<0.05
Muscle Depth			6.71	+	0.01	0.03	-	0.85			
Predator Mass	11.99	0.001	4.93		0.03	13.27		<0.01	10.81	-	<0.01

B) FELI Tilapia 30

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	3.43	0.07				0.76		0.38	0.15		0.70
Competition	1.68	0.20				0.11		0.74	0.48		0.49
Predator x Competition	7.86	0.001				13.29		<0.001	13.00		<0.001
Mass			11.33	+	<0.001	24.51	+	<0.001	25.46	+	<0.001
Body Depth			0.09	+	0.76	1.44	-	0.23			
Fin Depth			3.17	-	0.08	4.96	-	<0.05	5.26	-	<0.05
Muscle Depth			4.69	+	<0.05	0.01	-	0.98			
Predator Mass	10.85	0.001	7.09		<0.01	8.28		<0.01	10.38	-	<0.01

C) MIFI Tilapia 10

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	3.68	0.06				5.88		<0.05	10.81	-	<0.01
Competition	2.26	0.13				0.34		0.56			
Predator x Competition	0.04	0.85				0.05		0.83			
Mass			9.35	+	0.002	2.80	+	0.09			
Body Depth			2.74	-	0.10	7.21	-	<0.01	5.93	-	<0.05
Fin Depth			1.71	-	0.19	0.29	-	0.59			
Muscle Depth			0.23	-	0.63	0.90	+	0.34			
Predator Mass	13.12	0.001	14.94		0.001	5.28		<0.05	6.52	-	<0.05

D) MIFI Tilapia 30

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	8.02	0.005				9.51		<0.01	18.37	-	<0.001
Competition	0.52	0.47				1.54		0.21			
Predator x Competition	0.09	0.76				0.46		0.50			
Mass			9.26	+	<0.01	3.83	+	<0.05	6.68	+	<0.01
Body Depth			0.82	-	0.37	6.15	-	<0.05	9.36	-	<0.01
Fin Depth			1.14	-	0.28	0.02	+	0.88			
Muscle Depth			0.18	-	0.67	0.93	+	0.33			
Predator Mass	6.51	0.01	10.82		<0.01	2.48		0.11			

E) POLE Tilapia 10

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	10.54	0.01				1.35		0.25			
Competition	0.02	0.90				8.64		<0.01	11.45	-	<0.001
Predator * Competition	0.22	0.64				1.19		0.27			
Mass			16.66	+	<0.001	23.47	+	<0.001	36.58	-	<0.001
Body Depth			0.13	+	0.72	0.22	+	0.64			
Fin Depth			2.61	-	0.11	0.89	-	0.34			
Muscle Depth			0.84	+	0.36	0.78	+	0.38			
Predator Mass	6.02	0.05	1.96		0.16	6.28		<0.05	12.38	-	<0.001

F) POLE Tilapia 30

Treatment	Trait	Full	Reduced

	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	5.10	0.02				0.33		0.56			
Competition	0.76	0.38				10.11		<0.01	11.65		<0.001
Predator x Competition	0.31	0.58				1.46		0.22			
Mass			28.11	+	<0.001	35.31	+	<0.001	45.97	+	<0.001
Body Depth			0.93	-	0.33	0.15	-	0.70			
Fin Depth			4.27	-	<0.05	2.51	-	0.11			
Muscle Depth			4.29	+	<0.05	4.45	+	<0.05			
Predator Mass	5.96	0.01	3.28	-	0.07	8.73	-	<0.01	13.39	-	<0.001

G) FELI Odonate 1h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	0.01	0.91				0.05		0.82	0.42		0.52
Competition	0.05	0.82				0.45		0.50	0.42		0.52
Predator x Competition	4.00	<0.05				5.40		<0.05	5.40		<0.05
Mass			0.002	-	0.96	0.68	+	0.41			
Body Depth			0.26	-	0.61	0.79	-	0.37			
Fin Depth			1.17	+	0.29	2.36	+	0.12			
Muscle Depth			0.01	+	0.93	2.45	-	0.12			
Predator Mass	5.85	0.02	2.87		0.09	6.17		<0.05	4.19	-	<0.05
Predator Species	5.84	0.06	7.68		<0.05	5.52		0.06			

H) FELI Odonate 24h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	1.17	0.28				0.05		0.82			
Competition	0.22	0.64				2.64		0.10			
Predator * Competition	2.16	0.14				2.04		0.15			
Mass			0.41	+	0.52	1.54	+	0.21			
Body Depth			0.39	+	0.53	0.35	+	0.55			
Max Fin			8.06	+	<0.01	11.00	+	<0.001	9.41	+	<0.01
Muscle Depth			1.76	-	0.18	4.76	-	<0.05			
Predator Mass	4.96	0.03	4.35		<0.05	7.55		<0.01			

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator Species	23.59	<0.001	30.56		<0.001	23.44		<0.001	32.09		<0.001

I) MIFI Odonate 1h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	3.46	0.06				0.10		0.76			
Competition	0.79	0.37				0.02		0.88			
Predator x Competition	1.83	0.18				0.42		0.51			
Mass			5.84	+	<0.05	2.88	+	0.09	7.08	+	<0.01
Body Depth			5.71	+	<0.05	4.38	+	<0.05	6.64	+	<0.01
Fin Depth			0.49	+	0.48	0.67	+	0.41			
Muscle Depth			0.03	-	0.86	0.24	-	0.63			
Predator Mass	3.78	0.06	5.65		<0.05	5.58		<0.05	5.63	-	<0.05
Predator Species	21.80	<0.001	25.15		<0.001	21.95		<0.001	28.68		<0.001

J) MIFI Odonate 24h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	4.25	0.04				1.37		0.24			
Competition	0.51	0.47				0.03		0.87			
Predator x Competition	0.17	0.68				0.07		0.79			
Mass			17.11	+	<0.001	17.00	+	<0.001	17.03	+	<0.001
Body Depth			3.88	+	<0.05	3.24	+	0.07	8.80	+	<0.01
Fin Depth			0.42	-	0.52	1.55	-	0.21			
Muscle Depth			0.05	-	0.82	0.05	+	0.82			
Predator Mass	10.01	0.002	12.96		<0.001	10.09		<0.01	13.50	-	<0.001
Predator Species	30.65	<0.001	46.51		<0.001	30.35		<0.001	45.93		<0.001

K) POLE Odonate 1h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	5.07	0.02				5.49		<0.05	5.45	+	<0.05
Competition	0.02	0.88				0.78		0.38			
Predator * Competition	0.15	0.69				0.08		0.78			
Mass			1.15	+	0.28	1.24	+	0.27			

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Body Depth			0.06	+	0.80	0.19	-	0.66			
Fin Depth			1.46	+	0.23	1.35	-	0.25			
Muscle Depth			0.11	-	0.74	1.05	+	0.30			
Predator Mass	9.11	0.003	6.91		<0.01	8.07		<0.01	9.54	-	<0.01
Predator Species	25.85	<0.001	20.74		<0.001	13.40		<0.01	27.58		<0.001

L) POLE Odonate 24h

	Treatment		Trait			Full			Reduced		
	ChiSq	P	ChiSq	ES	P	ChiSq	ES	P	ChiSq	ES	P
Predator	5.78	0.02				6.92		<0.01	7.80	+	<0.001
Competition	2.28	0.13				0.03		0.87			
Predator x Competition	1.69	0.19				0.19		0.66			
Mass			7.86	+	<0.01	7.73	+	<0.01	7.14	+	<0.01
Body Depth			4.09	+	<0.05	1.91	+	0.17			
Fin Depth			0.86	+	0.35	1.44	-	0.23			
Muscle Depth			0.97	-	0.33	0.01	-	0.95			
Predator Mass	6.51	0.01	6.89		<0.01	7.61		<0.01	7.82	-	<0.01
Predator Species	33.19	<0.001	34.09		<0.001	22.10		<0.001	35.06		<0.001

Figures

**Figure 1:** Mean rearing treatment scores for each tadpole species from a redundancy analysis on morphological variables. The panel on the right shows how the morphological variables correlate to RDA1 and RDA2. Codes for morphological variables are: bodyd= body depth; bodyl= body length; taild= tail depth; muscd = muscle depth; musca = muscle area; taila = tail area; maxfin= maximum fin depth.
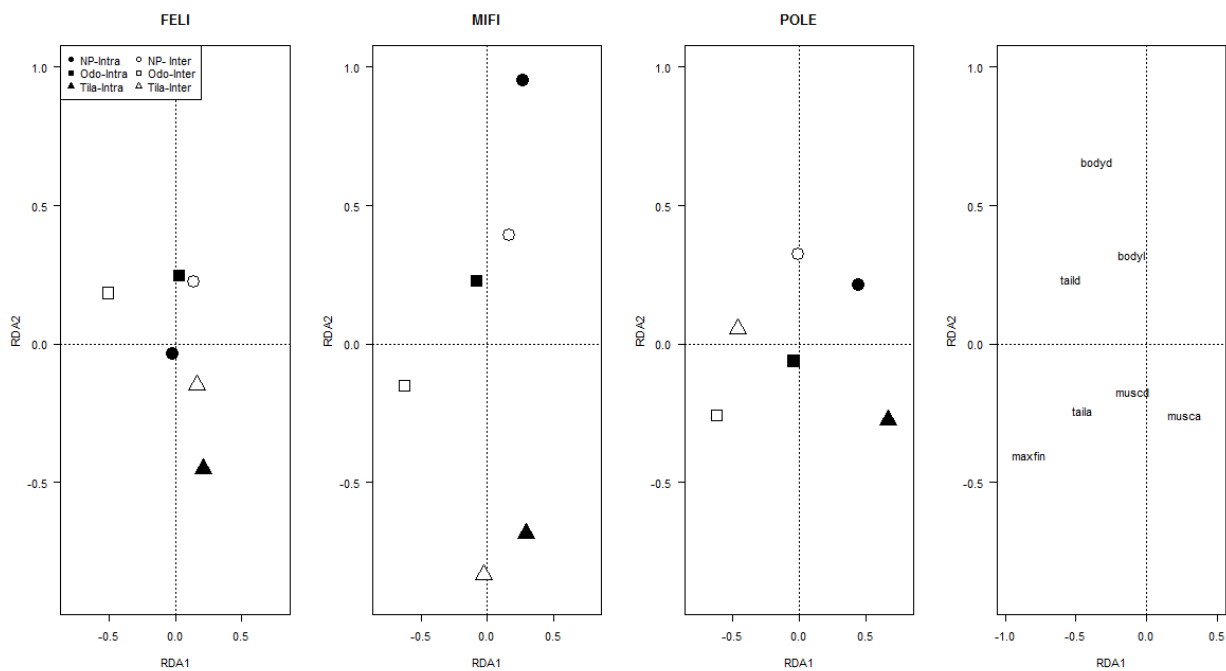
Figure 2: Influence of competition and predator rearing treatments on the life-history and morphological variables used as predictor variables in analyses of tadpole survival in predation trials for the three tadpole species, FELI, MIFI and POLE. Predator treatments were no predator (NP), odonate (Odo) and tilapia (Tila). Competition treatments were intraspecific (Intra), and interspecific (Inter). Mass in this figure is untransformed and the three morphological variables shown here are residuals following a regression on total body length. Error bars in figure are one standard error of the mean and are shown for a qualitative description of the data.
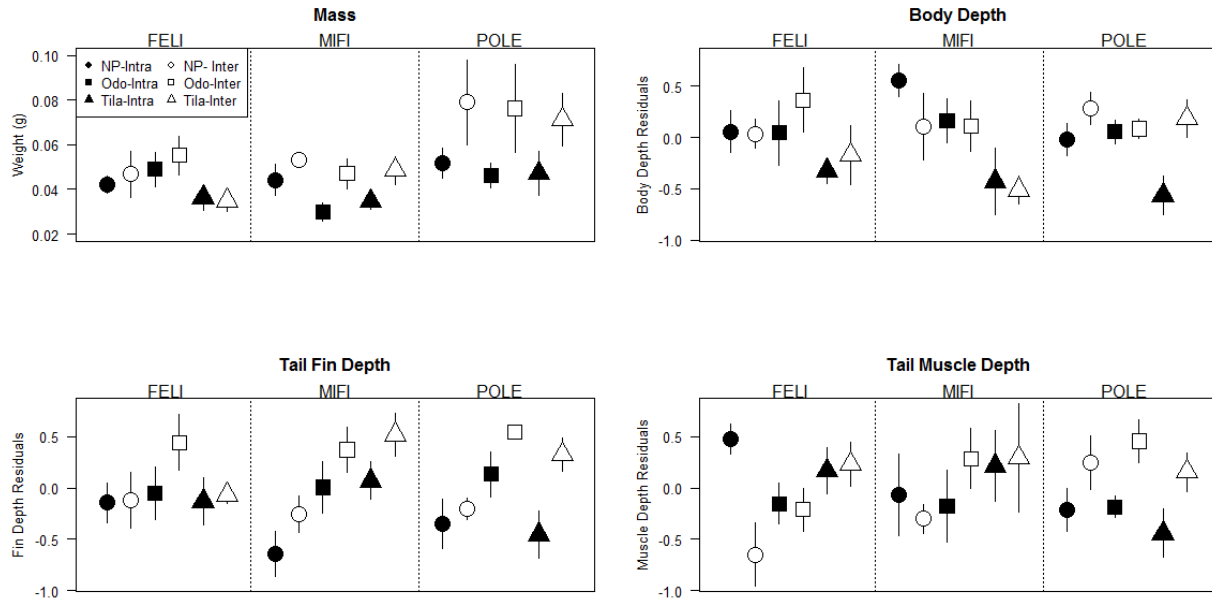
Figure 3: Inverse logit proportion of tadpoles surviving following exposure to free roaming odonate predator for 1 hour and 24 hours, and tilapia predator for 10 minutes and 30 minutes. Symbols indicate rearing treatment (codes are the same as figure 2).   Plotted values are residuals after removing variation explained by predator mass and predator species (for odonates), and morphological and life-history variables of the tadpoles. Thus, the panels present the effects of rearing treatments on tadpole survival. Asterisks indicate survival in the predator vs. no-predator rearing treatments are significantly different in the contrasts containing the same competition treatment; for example, for Tilapia at 10 minutes, in the presence of interspecific competition there was a statistically significant positive effect of being raised with Tilapia on survival for FELI. Error bars are one standard error of the mean and are shown only for a qualitative description of the data.
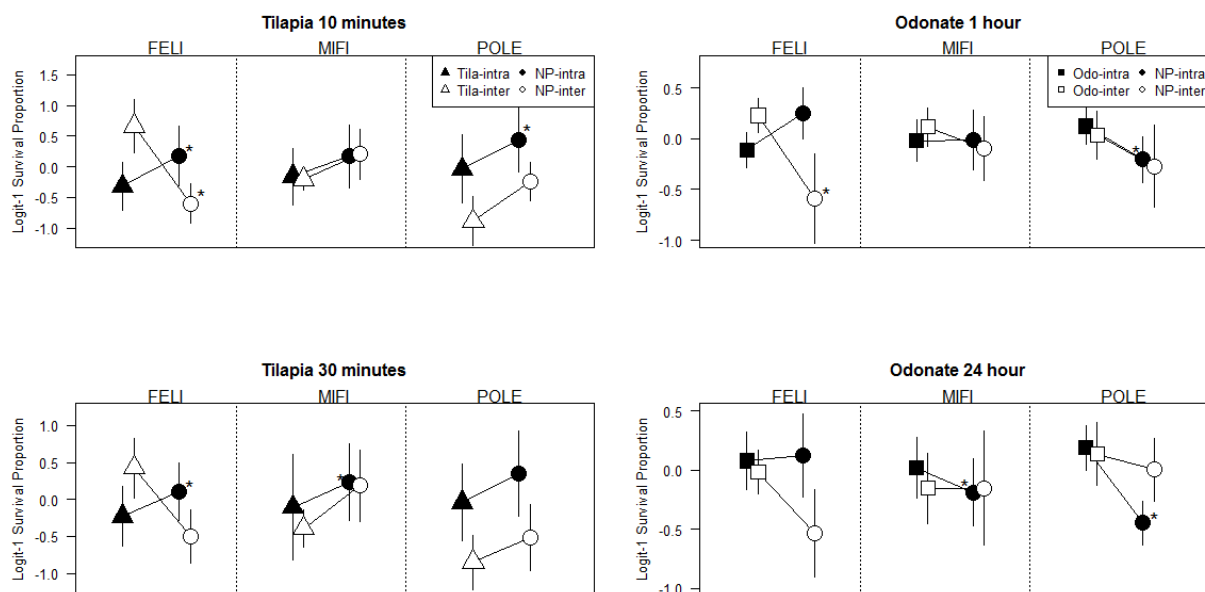
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Appendix A- Sample sizes of predation trials

Table A1: Sample sizes from each rearing treatment during each temporal block for tilapia and odonate predation trials. Rearing treatment codes are: NP = No Predator; Tila = Tilapia; Odo = Odonate; Inter = Interspecific; Intra = Intraspecific. For odonate trials I used two morphospecies, which I refer to as Species A and Species B. Species B was more abundant and thus used more often than Species A.

A) Tilapia Trials

	FELI				MIFI				POLE			
	NP-Intra	NP-Inter	Tila-Intra	Tila-Inter	NP-Intra	NP-Inter	Tila-Intra	Tila-Inter	NP-Intra	NP-Inter	Tila-Intra	Tila-Inter
June 18	3	3	3	2	2	3	2	2	3	3	4	2
June 20	3	1	3	3	2	1	2	3	2	1	4	3
July 18	2	0	1	1	2	0	2	1	2	0	2	1
August 8	1	2	2	3	0	2	2	3	2	2	2	3
August 22	2	3	2	2	2	3	2	2	3	3	2	2

B) Odonate Trials

	FELI				MIFI				POLE			
	NP-Intra	NP-Inter	Odo-Intra	Odo-Inter	NP-Intra	NP-Inter	Odo-Intra	Odo-Inter	NP-Intra	NP-Inter	Odo-Intra	Odo-Inter
June 18												
Species A	0	1	3	4	2	1	3	4	2	1	2	4
Species B	1	1	1	1	0	1	1	1	1	1	4	1
June 20												
Species A	0	1	2	2	2	1	0	2	3	1	0	2
Species B	2	1	2	3	1	1	2	2	0	1	4	3
July 18												
Species A	0	0	1	2	1	0	0	2	0	0	1	2
Species B	3	1	5	1	1	1	6	1	3	1	5	1
August 8												
Species A	0	1	1	1	2	1	0	1	0	1	0	1
Species B	2	2	2	1	0	1	6	1	3	2	3	1
August 22												
Species A	0	1	0	0	1	1	1	0	1	1	0	0
Species B	4	1	3	4	2	1	1	4	0	1	4	4

Appendix B- correlations of life history and morphological traits

Table B1: Correlations of life history and morphological traits for the three tadpole species. Correlations were based on odonate predation trials in order to include the maximum amount of data.

A) FELI

	Length	Stage	Body Depth	Body Length	Tail Depth	Tail Area	Fin Depth	Muscle Area	Muscle Depth	RDA1	RDA2
Mass	0.97	0.92	0.13	-0.33	0.15	0.10	0.06	0.25	0.18	-0.08	-0.14
Length	-	0.93	0.03	-0.36	0.09	-0.05	-0.03	0.20	0.14	0.04	-0.14
Stage		-	-0.03	-0.48	0.26	-0.09	-0.16	0.30	0.21	0.10	-0.14
Body Depth			-	0.48	-0.02	0.34	0.32	0.20	0.00	-0.46	0.57
Body Length				-	-0.39	0.15	0.28	-0.10	-0.14	-0.23	0.46
Tail Depth					-	0.38	0.26	0.35	0.48	-0.5	-0.29
Tail Area						-	0.87	0.41	0.46	-0.89	-0.48
Fin Depth							-	0.09	0.31	-0.94	-0.38
Muscle Area								-	0.72	-0.18	-0.38
Muscle Depth									-	-0.37	-0.58
RDA1										-	0.25

B) MIFI

	Length	Stage	Body Depth	Body Length	Tail Depth	Tail Area	Fin Depth	Muscle Area	Muscle Depth	RDA1	RDA2
Mass	0.96	0.89	-0.01	-0.03	-0.05	0.06	0.13	0.07	0.19	-0.08	-0.11
Length	-	0.93	-0.13	-0.07	-0.06	0.00	0.17	0.04	0.16	-0.04	-0.16
Stage		-	-0.26	-0.05	-0.14	0.05	0.24	0.13	0.18	-0.04	-0.27
Body Depth			-	0.54	-0.19	-0.40	-0.40	-0.46	-0.16	0.12	0.83
Body Length				-	-0.50	-0.62	-0.49	-0.23	0.02	0.47	0.70
Tail Depth					-	0.80	0.53	0.52	0.50	-0.85	-0.52
Tail Area						-	0.65	0.65	0.46	-0.84	-0.75
Fin Depth							-	0.43	0.38	-0.84	-0.75
Muscle Area								-	0.86	-0.47	-0.72
Muscle Depth									-	-0.48	-0.48
RDA1										-	0.59

C) POLE

	Length	Stage	Body Depth	Body Length	Tail Depth	Tail Area	Fin Depth	Muscle Area	Muscle Depth	RDA1	RDA2
Mass	0.99	0.96	-0.16	0.25	0.18	0.20	0.20	0.13	0.33	-0.21	-0.21
Length	-	0.96	-0.25	0.22	0.09	0.10	0.12	0.03	0.25	-0.11	-0.17
Stage		-	-0.20	0.27	0.05	0.08	0.05	0.08	0.22	-0.05	-0.11
Body Depth			-	0.40	0.05	0.22	0.09	0.26	0.36	-0.26	0.32
Body Length				-	-0.19	-0.16	-0.19	-0.11	0.23	0.07	0.54
Tail Depth					-	0.71	0.80	0.50	0.54	-0.89	-0.62
Tail Area						-	0.74	0.87	0.52	-0.82	-.76
Fin Depth							-	0.53	0.64	-0.95	-0.78
Muscle Area								-	0.45	-0.58	-0.67
Muscle Depth									-	-0.71	-0.41
RDA1										-	0.68