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SPECIES LEVEL DIFFERENCES IN THE ECOLOGY OF TWO NEOTROPICAL TADPOLE SPECIES: RESPONSES TO NONLETHAL PREDATORS AND THE ROLES OF COMPETITION AND RESOURCE USE

Zacharia Costa
Virginia Commonwealth University

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SPECIES LEVEL DIFFERENCES IN THE ECOLOGY OF TWO NEOTROPICAL TADPOLE SPECIES: RESPONSES TO NONLETHAL PREDATORS AND THE ROLES OF COMPETITION AND RESOURCE USE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE AT VIRGINIA COMMONWEALTH UNIVERSITY

by

ZACHARIA J. COSTA
Bachelor of Science, University of California, Davis 2007

Director
DR. JAMES VONESH DEPARTMENT OF BIOLOGY

Virginia Commonwealth University
Richmond, Virginia
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ABSTRACT

SPECIES LEVEL DIFFERENCES IN THE ECOLOGY OF TWO NEOTROPICAL TADPOLE SPECIES: RESPONSES TO NONLETHAL PREDATORS AND THE ROLES OF COMPETITION AND RESOURCE USE

By Zacharia J. Costa, M.S.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
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Virginia Commonwealth University, 2011
Major Director: Dr. James Vonesh
Assistant Professor, Department of Biology

Closely related species at the same trophic level are often considered to be ecologically equivalent. However, it is clear that individuals species can have unique functional roles that drive community and ecosystem processes. In this study we examine the growth responses of two Neotropical hylid tadpole species, Agalychnis callidryas and Dendropsophus ebraccatus, to intraspecific and interspecific competition. We also look at density-dependent effects of each on phytoplankton, periphyton and zooplankton, as well as their responses to a caged dragonfly predator through ontogeny.
Intraspecific competition affected both species similarly, and their effects on resources were qualitatively similar but quantitatively different. Predators affected resource levels and interspecific competition. Predator effects on tadpole size varied in both magnitude and direction through ontogeny for both species. This study shows that closely related species at the same trophic level can have different ecological roles and that tadpoles are more functionally unique than previously thought.
Chapter 1: Species specific effects of two Neotropical hylid tadpoles on aquatic resources and other primary consumers

Introduction

Natural communities are often too complex to study on a species-by-species basis. Therefore, ecologists often simplify food webs by grouping species together based on shared characteristics such as phylogeny and/or trophic status. Species within such groups are assumed to be functionally redundant, or to have similar effects on population, community or ecological processes (Chalcraft & Resetarits 2003a,b, Harris 1995, Lawton & Brown 1993). In fact, functional redundancy often serves as a null hypothesis when looking at the consequences of biodiversity for ecosystem functioning and is the main assumption underlying neutral theory (Loreau 2004, Holoyoak & Loreay 2006, Hubbell 2001). It is increasingly clear however, that even closely related species at the same trophic level can vary greatly in their functional roles. This is often attributed to a suite of traits that influence consumers’ interactions with their resources, such as habitat use, metabolic requirements, and/or foraging modes, often making it difficult to make generalizations about species and their functional roles based on individual traits (Beckerman et al. 2010, Fox et al. 2009, Resetarits & Chalcraft 2007, Schmitz & Suttle 2001). Thus, it is likely that investigations into qualitative and quantitative differences in species’ interactions will give insight into their specific impacts on communities,

The functional roles of aquatic herbivores often involve various direct and indirect interactions with primary producers. For example, besides direct consumptive effects, herbivores can both regulate and facilitate their resources indirectly through their interactions with other factors that control primary productivity such as nutrient cycling (Vanni 2002). Thus, aquatic herbivores can have dramatic effects on communities by simultaneously exerting consumptive top-down effects and bottom-up effects (e.g. facilitation of primary producers) on food web structure and dynamics, making it difficult to predict their functional roles a priori (Flecker et al. 2002, Knoll et al. 2009, Kupferberg 1997, Power 1990). In the tropics, the drivers of food web dynamics in freshwater systems remain unclear (Danger et al. 2009, Lazzaro et al. 2003, Menezes et al. 2010). However, in a few cases single species have found to be functionally unique (Flecker 1992, 1996, Taylor et al. 2006, Vanni et al. 2002), suggesting the need to evaluate the assumption that species are functionally redundant in these systems if we want to understand the role of these herbivores in food web dynamics.

Larval anurans are some of the least understood consumers in regards to their functional roles, especially in the tropics where they are more diverse and exhibit more trophic variation than in temperate systems (Wells 2007). Traditionally, diverse groups of herbivorous tadpoles have been lumped together as redundant primary consumers, although their effects on aquatic ecosystems can be highly species-specific and involve
direct and indirect impacts on primary producer and invertebrate communities (Connelly et al. 2008, Flecker et al. 1999, McDiarmid & Altig 1999, While et al. 2006). In order to assess the role of these important consumers in a tropical lentic food web, we manipulated the identity and density of two larval Neotropical hylid frog species. *Agalychnis callidryas* is a mid-water suspension feeder thought to primarily feed on phytoplankton, and *Dendropsophus ebraccatus* is a smaller benthic grazer that feeds on filamentous algae and periphyton (Duellman & Treub 1986, McDiarmid & Altig 1999, Wassersug & Rosenberg 1979, Wells 2007). Because of their different resource and habitat use, we expected *a priori* that these species would have different density dependent direct and indirect effects on a simplified food web. Specifically, we expected *D. ebraccatus* to have strong density-dependent consumptive effects on periphyton and *A. callidryas* would not. Since phytoplankton and periphyton compete for nutrients (Confer 1972, Hansson 1988, 1990), by reducing periphyton levels these tadpoles could indirectly facilitate phytoplankton. We also expected *A. callidryas* to have a density dependent impact on phytoplankton levels, which would indirectly benefit periphyton and would indirectly decrease zooplankton through exploitative competition. Thus, we investigated how species identity and two different intraspecific densities affected tadpole size and relative growth rates, their resource levels, and zooplankton.
Methods

Experiment 1

This study was conducted at the Smithsonian Tropical Research Institute in Gamboa, Panama (9°7′17″ N, 79°42′11″W) between 18 June and 10 July 2010. *Agalychnis callidryas* and *Dendropsophus ebraccatus* are Neotropical treefrogs that commonly co-occur in lowland ponds throughout their ranges from southern Mexico to Panama. At the study site they are locally abundant, and eggs, tadpoles and adults commonly co-occur throughout the rainy season (May-December) in Gamboa area ponds (J.C. Touchon & J.R. Vonesh unpubl. data).

To examine the effects of intraspecific competition on the growth and survival of *A. callidryas* and *D. ebraccatus* and the effects of these species on resources and other aquatic primary consumers I conducted an experiment using five different treatments. These were a control with no tadpoles and each species crossed with two densities, twenty five and fifty tadpoles per tank. Each treatment was replicated five times. Initial tadpole (0.125 and 0.0625 tadpole/L) densities were within the natural range that were observed during field estimates across five breeding ponds in the Gamboa area (J.C. Touchon & J.R. Vonesh unpubl. data). Treatments were randomly assigned to 400L mesocosms (0.75 x 0.8 m) with screened drain holes at 0.75 m height, arranged in five blocks of five tanks each in an open field. Tanks were filled with a mix of filtered aged rain and tap water, 75-85g of dried leaves confined to a mesh bag and 1.5 g of Sera
Micron® powdered algae on 6 June. Each tank received 100 ml of standardized pond inoculate on 8 June and again on 17 June. Pond inoculate was collected from an artificial pond adjacent to secondary forest, by repeatedly sweeping a plankton net through the water column and filtering it through a 1 mm mesh filter to exclude large invertebrates but to allow communities of phytoplankton, periphyton and zooplankton to establish. Tanks were covered with fine nylon mesh secured with rubber to prevent the colonization by non-experimental organisms.

On 11 June I collected 12 egg clutches of D. ebraccatus from Ocelot Pond and 16 clutches of A. callidryas from vegetation surrounding Experimental Pond. Eggs were kept in an ambient temperature laboratory and misted often to prevent desiccation. Eggs from both species were the same age but D. ebraccatus eggs hatch earlier because they are smaller and develop more rapidly (Touchon & Warkentin 2010), so they were three days post-hatching when A. callidryas eggs hatched on 16 June. Tadpoles were dorsally photographed in a white tray with a Nikon D40x digital camera and added to mesocosms on 18 June. All tadpoles were dip-netted again on 9 July and re-photographed. All photographs included a ruler for calibration in order to use ImageJ (http://rsbweb.nih.gov/ij/) to obtain measurements of tadpole length.

To measure the effects of tadpoles on their resources and other potential competitors, phytoplankton and zooplankton were sampled on 8 July. I used a 1 L integrated tube sampler to collect four samples from the water column of each tank. To measure water column chlorophyll a to indicate phytoplankton levels, 100 ml were sub-
sampled and put on ice before vacuum filtered on Whatman GF/A glass filters. Filters were extracted in ethanol for 24 hours under refrigeration in the dark. Samples were then read in Aquafluor fluorimeter following standard protocol to estimate μg of chlorophyll a per unit volume (Welschmeyer 1994).

Periphyton samples were collected by hanging a piece of flagging tape (625 x 29 mm) in the center of the tank at the water level (Austin et al. 1981). Periphyton was sampled on 10 July. The flagging tape was scraped onto a filter with a razor blade, as were two additional 70 x 70mm squares on the north and south walls of the tanks, approximately 30 cm from the top. These solutions were vacuum filtered on pre-weighed Whatman GF/A glass filters. Filters were then put in a drying oven at 30° C for 48 hours and weighed on a digital balance and then converted to μg of periphyton per cm² (Aloi 1990).

Zooplankton samples were obtained from water from the tanks that had previously been collected with the tube sampler. Four liters were filtered on a 20 μm Nitex® filter and rinsed with tap water to form a more concentrated solution (~15ml). Four to six drops of Lugol’s solution were added as a preservative and to stain the zooplankton (Stoecker et al. 1994). Zooplankton were then quantified in the lab at 30X magnification with a stereo microscope, and individuals were classified into morphospecies.
Experiment 2

In 2011 a follow up experiment was performed to investigate the effects of *A. callidryas* on the size structure of the phytoplankton community and their indirect effects on periphyton and zooplankton. Following the procedure from 2010, ten mesocosms were filled on 7 July with a mix of filtered aged rain and tap water, 75-85g of dried leaves confined to a mesh bag and 1.5 g of Sera Micron®. Each tank received 100 ml of standardized pond inoculate on 7 July and again on 16 July swept from Experimental Pond. On 17 July five tanks were randomly assigned fifty tadpoles each. Tadpoles were hatched from 8 clutches collected from Experimental Pond at ages 4-6 days. Experimental tadpoles were introduced at seven days post-oviposition and were randomly distributed. To separate the direct consumptive and possible indirect effects of tadpoles (e.g. due to nutrient cycling) on periphyton, tadpole exclosures (9 cm diameter plastic pots) open at the surface with mesh sides were suspended in the middle of the mesocosms at the surface with pieces of tape (approx. 3.8 x 12 cm) attached to two cards on the sides of both the outside and inside of the exclosures. On 5 August tape samples from each side of the exclosure were dried and extracted in ethanol to measure chlorophyll a with fluourometry. To examine the effect of tadpoles on the size structure of the phytoplankton community three additional 200 ml samples from each tank were taken on 4 August and analyzed with fluourometry as in 2010, after being filtered through 20 μm, 5 μm and 1 μm Nitex to look at the relative abundances of these different plankton size classes. Before vacuum filtering, in addition to the regular (non filtered) samples. Zooplankton samples
were also collected on this date by filtering 2 L through a 20 μm filter and then quantified to morphospecies. Two tadpole tanks and one control tank were colonized by other organisms and were excluded from analyses.

Analysis

All statistical analyses were conducted on tank means using R version 2.11.0 (R Developmental Core Team 2010). We first analyzed univariate treatment effects using ANOVA. To test for species and density effect compared to controls, we performed additional univariate tests as a priori planned contrasts. For the factorial treatments with tadpoles (i.e., excluding controls) we also performed a two-way ANOVA to examine the interaction between species identity and density. Due to size differences between the species, if species effects were significant we also performed an ANCOVA to determine if tadpole effects on their resources and zooplankton were driven by differences in biomass rather than species identity per se. Data for tadpole growth and primary production were analyzed with mixed linear models treating block as a random effect. Periphyton biomass and phytoplankton chlorophyll a measurements were log transformed to meet assumptions of homogeneity of variance. Zooplankton count data were analyzed using a generalized linear mixed model approach to account for both block effects and non-normal error distributions. To account for non-normality in the error distribution Poisson error structure was assumed (Pinheiro & Bates 2000). Zooplankton data from 2011 had substantial overdispersion so a quasi-Poisson error distribution was used. All
pair wise comparisons were tested using Tukey’s test of honestly significant differences (HSD).

For data from 2011 we first analyzed univariate treatment effects on the four different phytoplankton measures using ANOVA. To examine possible differences between direct and indirect effects of tadpoles on periphyton (outside and inside the exclosures respectively) we used a two-way ANOVA to examine possible interactions between treatments and the side of the exclosure periphyton was grown. A mixed linear model used tank as a random factor to avoid nested sampling errors. Primary production values were log transformed to homogenize variance.
Results

Tadpole Growth

Initially, *A. callidryas* tadpoles were 12.78 ± 0.34 mm (mean ± SD) and *D. ebraccatus* tadpoles were 6.92 ± 0.18 in total length (TL). There was no difference in initial size within species across treatments (*A. callidryas*: $F_{1, 4} = 0.169$, $P = 0.70$; *D. ebraccatus*: $F_{1, 4} = 5.61$, $P = 0.08$). Species identity ($F_{1, 13} = 73.54$, $P < 0.001$) and density ($F_{1, 13} = 37.03$, $P < 0.001$; Fig. 1A) both had significant influences on final tadpole TL. Low density *A. callidryas* tadpoles (34.41 ± 0.50) were 18.6% larger than high density tadpoles (29.00 ± 2.11) ($F_{1, 4} = 31.05$, $P = 0.005$). Tadpoles of *D. ebraccatus* from low density treatments (26.92 ± 2.61) were 21.4% larger than high density tadpoles (22.17 ± 1.78) ($F_{1, 4} = 11.33$, $P = 0.028$). Comparing the two species, at low densities *A. callidryas* tadpoles were 27.8% larger than *D. ebraccatus*, and at high densities they were 30.8% larger than *D. ebraccatus*. Thus, the effects of increasing conspecific density were similar for both species and there was no species by density interaction ($F_{1, 12} = 0.145$, $P = 0.71$).

Results for relative tadpole growth rates parallel those for TL. Species identity and density affected growth (species: $F_{1, 13} = 108.87$, $P < 0.001$; density: $F_{1, 13} = 34.54$, $P < 0.001$; Fig. 1B), with no interaction between the two ($F_{1, 12} = 0.40$, $P = 0.54$). Low density *A. callidryas* (0.43 ± 0.01) grew 21.9% faster than high density treatment tadpoles (0.35 ± 0.05) ($F_{1, 4} = 13.79$, $P = 0.021$). *Dendropsophus ebraccatus* tadpoles in
low density treatments (0.60 ± 0.04) grew 19.3% faster than high density tadpoles (0.50 ± 0.03) (F₁,₄ = 19.67, P = 0.011). Within density treatments, at low density treatments D. ebraccatus grew 38% faster than A. callidryas. In high density treatments D. ebraccatus grew 40.9% faster than A. callidryas. Overall, D. ebraccatus (0.55 ± 0.06) grew 39.3% faster than A. callidryas (0.39 ± 0.05).

Total tank tadpole biomass was only affected by species identity (F₁,₁₂ = 5.42, P = 0.038; density: F₁,₁₂ = 2.54, P = 0.14; interaction: F₁,₁₂ = 0.58, P = 0.81; Fig. 1C), with D. ebraccatus (5.29 ± 1.26g) having 19.2% less biomass than A. callidryas (6.55 ± 1.20g).

**Primary Producers**

Water column chlorophyll a levels were strongly affected by treatment (F₄,₁₆ = 8.12, P < 0.001; Fig. 2A). When pooled across densities there was a strong species effect as A. callidryas and D. ebraccatus each significantly increased chlorophyll a by 197% and 84.3% relative to controls, respectively (F₂,₁₈ = 8.36, P = 0.003). When pooled across tadpole species, high density treatments had 39% more chlorophyll a compared to low density treatments and 180% more than controls (F₂,₁₈ = 5.94, P = 0.011). Looking at the main and interactive effects of species type and tadpole density within treatments with tadpoles, there was a species (F₁,₁₂ = 7.50, P = 0.018) and species-by-density interaction (F₁,₁₂ = 11.08, P = 0.006) while density itself was not significant (F₁,₁₂ = 1.86, P = 0.20). This was due to the fact that high density A. callidryas’ treatments increased chlorophyll a by 120% compared to low densities (F₁,₄ = 9.38, P = 0.038), but D. ebraccatus density treatments were not different from one another (F₁,₄ = 1.38, P = 0.30).
Similarly, species identity ($F_{1,12} = 6.73, P = 0.017$) and its interaction with biomass were both significant ($F_{1,12} = 10.34, P = 0.007$) while biomass itself was not ($F_{1,12} = 0.77, P = 0.40$). This is because there were biomass effects within *A. callidryas* treatments ($F_{1,4} = 11.65, P = 0.03$), but not for *D. ebraccatus* ($F_{1,4} = 1.9, P = 0.24$).

In the 2011 follow-up experiment with *A. callidryas*, total water column chlorophyll a was also affected by treatment ($F_{1,5} = 16.95, P = 0.009, R^2 = 0.73$; Fig. 4A), as *A. callidryas* increased it by 123% compared to controls. All measured size classes responded similarly to tadpole presence as the $< 20 \mu m$ ($F_{1,5} = 13.36, P = 0.015, R^2 = 0.67$), $< 5 \mu m$ ($F_{1,5} = 41.49, P = 0.001, R^2 = 0.87$) and $< 1 \mu m$ ($F_{1,5} = 30.97, P = 0.003, R^2 = 0.83$) size classes increased by 124%, 104% and 118%, respectively.

In 2010, periphyton dry mass was also affected by treatment ($F_{4,16} = 6.94, P = 0.002$). When pooled across densities there was a significant species effect as both species reduced periphyton similarly as compared to controls ($F_{2,18} = 15.06, P < 0.001$; Fig. 2B). Pooled across species there was also a density effect as controls had 591% more periphyton than high density treatments and 625% more than low density treatments ($F_{2,18} = 10.16, P = 0.001$). Within tadpole treatments only species identity was relevant as *A. callidryas* reduced periphyton by 67.7% more than *D. ebraccatus* (species: $F_{1,12} = 4.95, P = 0.046$; density: $F_{1,12} = 0.12, P = 0.73$; interaction: $F_{1,12} = 0.04, P = 0.85$), and this was not due to a biomass effect due to the larger size of *A. callidryas* ($F_{1,12} = 0.18, P = 0.68$).
In 2011, periphyton chlorophyll a levels were affected by tadpoles ($F_{1,5} = 14.99, P = 0.01$), side of exclosure ($F_{1,5} = 32.78, P = 0.002$), and their interaction ($F_{1,5} = 35.26, P = 0.002$; Fig. 4B). Post-hoc pair wise comparisons showed tadpoles decreased periphyton on the outside of exclosures by 91.2% compared to the inside of hose tanks ($P < 0.001$). In addition, tadpole decreased periphyton by 89.3% compared to the outside ($P < 0.001$) and 88% compared to the inside ($P < 0.001$) of control tanks.

**Zooplankton**

The total number of zooplankton varied among treatments ($F_{4,16} = 6.34, P = 0.003$). Specifically, across density species effects were significant ($F_{2,18} = 12.41, P < 0.001$) because *A. callidryas* (138.53 ± 71.67 per L) reduced zooplankton 54% compared to controls (303.2 ± 109.76) and by 61.2% compared to *D. ebraccatus* (363.33 ± 117.34; Fig. 3F), but pooled across species, density had no effect ($F_{2,18} = 0.46, P = 0.64$). Within tadpole treatments only species effects were significant, as *A. callidryas* reduced total zooplankton levels (Table 1). Copepod densities were affected by treatment ($F_{4,16} = 6.20, P = 0.001$). Pooled across species there was no density effect ($F_{2,18} = 0.22, P = 0.81$), but across densities there was a significant species effect ($F_{2,18} = 13.02, P < 0.001$). Specifically, *A. callidryas* (1.93 ± 1.34) reduced copepods 87% relative to controls (15.85 ± 7.27) and by 91% compared to *D. ebraccatus* (21.50 ± 14.56; Fig. 3A). When looking at only tadpole treatments and possible interactions between species identity and density, only species effects were significant (Table 1). There was no interaction between species identity and biomass, but both were significant (species: $F_{1,12} = 31.80, P < 0.001$;
biomass: $F_{1,12} = 10.26$, $P = 0.008$; interaction: $F_{1,12} = 1.18$, $P = 0.30$). When pooled by species, biomass was marginally significant for *D. ebraccatus* ($F_{1,4} = 6.80$, $P = 0.06$) as copepods increased with increasing tadpole biomass, but this was not the case with *A. callidryas* ($F_{1,4} = 0.04$, $P = 0.85$). Nauplii, early copepod instars, were also affected by treatment ($F_{4,16} = 11.48$, $P < 0.001$) and when pooled across densities, by species ($F_{2,18} = 19.63$, $P < 0.001$). They were reduced by *A. callidryas* (52.10 ± 38.15) 75.8% relative to controls (215.50 ± 117.30) and by 81% compared to *D. ebraccatus* (273.95 ± 98.54; Fig. 2B), but density was not significant ($F_{2,18} = 0.34$, $P = 0.72$). Within tadpole treatments, there was a significant species effect and a marginal species by density interaction ($F_{1,12} = 4.33$, $P = 0.06$) in which low density *A. callidryas* (29.10 ± 8.59) reduced nauplii compared to high density treatments (75.10 ± 43.35) ($F_{1,4} = 10.08$, $P = 0.03$) but overall, density had no effect (Table 1), and neither did biomass ($F_{1,12} = 0.03$, $P = 0.87$). There were no predictors of cladoceran density (treatment: $F_{4,16} = 2.05$, $P = 0.14$; species: $F_{2,18} = 1.36$, $P = 0.28$; density: $F_{2,18} = 2.39$, $P = 0.12$; Fig. 3C). For rotifers, univariate tests comparing treatment effects were not significant ($F_{4,16} = 1.82$, $P = 0.17$), but pooled across densities *A. callidryas* (53.85 ± 70.00) increased rotifers 140% compared to *D. ebraccatus* (22.45 ± 25.06) and 161% compared to controls (20.65 ± 25.18) ($F_{2,18} = 3.80$, $P = 0.04$; Fig. 3D), and this was not due to tadpole biomass ($F_{1,12} = 0.05$, $P = 0.82$). There were no other predictors of rotifer abundances (Table 1). None of the treatments affected ostracod abundances (treatment: $F_{4,16} = 0.47$, $P = 0.76$; density: $F_{2,18} = 0.99$, $P = 0.39$; species: $F_{2,18} = 0.12$, $P = 0.89$; Fig. 3E).
In 2011, no zooplankton groups responded to the presence of *A. callidryas* tadpoles: copepods ($F_{1,5} = 3.25, P = 0.13$), nauplii ($F_{1,5} = 0.20, P = 0.68$), ostracods ($F_{1,5} = 0.66, P = 0.45$), and total zooplankton ($F_{1,5} = 0.21, P = 0.67$; Fig. 4C), although cladocerans ($F_{1,5} = 4.93, P = 0.08$) and rotifers ($F_{1,5} = 5.11, P = 0.07$) were marginal. Post-hoc pair wise comparisons however, showed tadpoles decreased rotifers (44.33 ± 27.27) $(P = 0.03)$ compared to controls (108.5 ± 45.75) and increased cladocerans (40.67 ± 30.72) $(P < 0.05)$ compared to controls (8.13 ± 10.28).
Discussion

This experiment shows that these two tadpole species can have dramatically different roles within these lentic communities. Both species reduced periphyton and increased phytoplankton. For A. callidryas their effect on phytoplankton was density dependent, as higher densities had a stronger facilitative effect. This strong bottom-up effect on these food webs was contrasted by the fact that they also decreased the abundances of several zooplankton taxa, while D. ebraccatus did not. Thus, the assumption that closely related species at the same trophic level have similar functional roles within communities are not warranted with these tropical tadpole species.

Similar to previous studies, we show that an increased number of conspecifics reduced both the final sizes and growth rates of the two focal species, supporting the idea that intraspecific competition plays an important role in structuring communities (Gurevitch et al. 2000, Morin 1999). Species identity and density were both important determinants of final length and growth rates, as A. callidryas tadpoles were larger and grew slower than D. ebraccatus, and tadpoles of both species from high density treatments were smaller and grew slower than those from low density treatments. The growth rates and sizes of both species were affected similarly by the number of conspecifics, so there was no interaction between these two factors. This is surprising given the initial differences in size between the two species, and that smaller tadpoles are thought to tolerate competitive effects better than larger tadpoles, and larger tadpoles
have a greater per capita effect on resources (Werner 1994). The lack of pair-wise
differences in biomass across all tadpole treatments supports the idea that the final total
tank masses of both species, at both densities, was limited by conspecifics due to possible
resource limitations, and that the per unit biomass competitive effects of tadpoles were
similar across across species.

Both tadpole species had strong effects on primary producers. All tadpoles
reduced periphyton dry mass compared to controls, but *A. callidryas*, the putative filter
feeder, consumed 67.7% more periphyton than *D. ebraccatus*. This was a species
specific effect that was not attributable to size or biomass, suggesting *A. callidryas*
consumes more periphyton than previously thought (McDiarmid & Altig 1999,
Wassersug & Rosenberg 1979) and is a more efficient grazer per unit biomass and per
individual than *D. ebraccatus*. The lack of density dependent effects on periphyton by
both species shows they were limited by this resource, as both densities nearly grazed it
completely. Phytoplankton biomass was also not affected by density across treatments,
but both species increased its levels relative to controls. Across *D. ebraccatus* treatments
total phytoplankton was increased compared to controls, and this effect was stronger in
low density treatments. *Agalychnis callidryas* increased phytoplankton compared to
controls, and this effect was strongest in high density treatments. Given that *A. callidryas*
has behavioral and anatomical features that strongly suggest filter feeding (McDiarmid &
Altig 1999, Wassersug & Rosenberg 1979), and they graze periphyton, there are several
possible mechanisms that may explain this counterintuitive result.
Consumer-mediated nutrient cycling can provide substantial amounts of nutrients required for phytoplankton and periphyton growth (Knoll et al. 2009, Vanni & Layne 1997, Vanni 2002). Thus, it is possible *A. callidryas*’ indirect facilitation of phytoplankton due to their nutrient excretion could overcome their consumptive effects. This is supported by the fact that their facilitation of phytoplankton was density dependent, as more individuals excrete more nutrients. Final tadpole tank biomass was not a significant predictor of phytoplankton abundance however, but species and the interaction between the two were. This was due to the fact that lower biomass *D. ebraccatus* treatments actually increased chlorophyll a more than higher biomass treatments, while the opposite was true for *A. callidryas*. It is then likely that not just the amount of nutrients, but possible differences between these two species in their body nutrient composition, excretion rates and ratios (stoichiometry) can all contribute to their indirect effects on primary producers (Elser & Urabe 1999, Vanni et al. 2002). Although we did not address any of those questions, in 2011 we attempted to separate the consumptive effects of tadpoles on periphyton from nutrient-mediated indirect effects using tadpole exclosures. The indirect effects of tadpoles did tend to benefit periphyton on the inside of exclosures as they had the highest periphyton chlorophyll a levels, but this was not statistically significant.

Filter feeders can also have dramatic effects on phytoplankton communities due to size selective feeding. By altering phytoplankton size composition, filter feeders can indirectly alter plankton biomass, primary productivity and photosynthetic efficiency
(Watson et al. 2003). Some filter feeding fish can actually enhance total phytoplankton levels by consuming large phytoplankton, which reduces competition between large and small size classes (Byers & Vinyard 1990, Drenner et al. 1996, Lazzaro et al. 1992). This is because nanoplanckton (<20 μm) are often too small to be effectively filtered, have higher growth rates (Smith & Kalff 1983) and have higher primary productivity per unit of algal biomass relative to larger phytoplankton (Schlesinger et al. 1981). As A. callidryas increased total phytoplankton and did not affect any of the measured size classes (<1 μm, <5 μm, <20 μm) differently, this suggests they increased total phytoplankton levels by increasing nanoplanckton < 1 μm.

Another mechanism by which these consumers could benefit their resources is mediated by competition between the resources. Phytoplankton and periphyton compete for nutrients (Confer 1972, Hansson 1988, 1990), so it is possible that by reducing periphyton levels these tadpoles could potentially facilitate phytoplankton, as has been proposed previously (Bronmark et al. 1991, Leibold and Wilbur 1992). This may be true however, within A. callidryas treatments there was no density effect on periphyton, but strong density dependent facilitation of phytoplankton. Similarly, pair wise comparisons show D. ebraccatus’ reduction of periphyton was not density driven, but its effect on phytoplankton was; low density treatments increased phytoplankton more than high density treatments. It is possible that we missed density effects by sampling after periphyton has already been completely grazed, but regardless, the evidence suggests other mechanisms than competition between the two resources are at work.
Aquatic consumers can also indirectly affect phytoplankton through their interactions with zooplankton. Changes in the abundance and/or species composition of zooplankton communities can alter their consumption and/or excretion rates and ratios, which can have strong impacts on phytoplankton communities (Brett & Goldman 1996, Elser & Urabe 1999, Vanni & Findlay 1990, Vanni & Layne 1997). It is possible this mechanism also influenced *A. callidryas*’ effect on phytoplankton as they had strong effects on the zooplankton community while *D. ebraccatus* did not. Specifically, the abundances of *Mesocyclops* cyclopoid copepods, the largest sized zooplankton (0.6-1.1mm) in our mesocosms, were strongly reduced by *A. callidryas* compared to all other treatments, and this was independent of biomass and density. These copepods are omnivorous (Hopp et al. 1997, Kumar and Rao 1999) and can have strong impacts on plankton communities (Blumenshine & Hambright 2003, Chang & Hanazato 2005a, Nagata & Hanazato 2006). Therefore, by decreasing their abundances, tadpoles could indirectly interact with phytoplankton and other zooplankton taxa.

Nauplii, the younger instars of *Mesocyclops*, followed a similar pattern as *A. callidryas* reduced their abundances compared to other treatments. Within *A. callidryas* treatments there was a marginal density effect because low density treatments had fewer nauplii than high density treatments. Other tadpole species can feed on zooplankton (While et al. 2010), yet examination of *A. callidryas*’ stomach contents and feces have failed to show evidence that they do (Warkentin pers. comm.), so the mechanisms behind these reductions are unclear. Previous studies have shown tadpoles can reduce
zooplankton biomass (Leibold & Wilbur 1992) and cladoceran densities (Mokany 2007), but the mechanisms were also unknown. In 2010 there were no predictors of cladoceran density, but pair wise comparisons showed low densities of *A. callidryas* reduced them more than all other treatments. In contrast, rotifers were increased by *A. callidryas* compared to other treatments, regardless of density. These results suggest *A. callidryas*’ impact on some zooplankton taxa are not density dependent. In 2011 there were no significant treatment effects of *A. callidryas* on any of the zooplankton, while they had a similar impact on phytoplankton and periphyton, suggesting that their interactions with zooplankton are not driven by their effects on primary producers. However, it is possible that we lacked the statistical resolution to detect such effects since the sample size was reduced because other anurans invaded the mesocosms.

*Agalychnis callidryas*’ effects on zooplankton may also be explained by a trophic cascade due to their reduction of predatory *Mesocyclops* copepods. If this were true we would expect a subsequent increase in their zooplankton prey items, which are cladocerans and rotifers (Chang & Hanazato 2003, 2005a,b, Nagata & Hanazato 2006). This did not happen with cladocerans in 2010 but this may be the case with rotifers as there was a marginal species effect (*P* = 0.056) as *A. callidryas* increased rotifers compared to other treatments. In 2011 there were no treatment effects of *A. callidryas* on any zooplankton, but pair-wise comparisons showed they increased cladocerans and reduced rotifers, suggesting other factors influence zooplankton dynamics. Previous work has shown zooplankton community responses to changes in the abundances of
Mesocyclops can be highly species-specific and likely involve various indirect effects within the plankton community (Chang & Hanazato 2005a, Nagata & Hanazato 2006).

Our results show that tadpole effects on aquatic food webs can be highly species specific and are not driven by biomass or trophic status per se. Besides the importance of their direct consumptive effects, the roles of these tadpoles within aquatic food webs are also driven by various indirect effects that most likely involve simultaneous processes that include their interactions with other consumers, nutrient cycling and competition among primary producers. Specifically, by both regulating and facilitating different primary producers and consumers (zooplankton) simultaneously, the indirect effects of tadpoles on aquatic food webs may be more important and complex than previously thought. Understanding the functional roles of these aquatic consumers will require further work that addresses the specific mechanisms that drive the relative importance of both their bottom-up and top-down influences on aquatic food webs.
Chapter 2: Nonlethal Effects of Predators Change Interactions Between Two Neotropical Hylid Tadpoles Through Ontogeny

Introduction

Predators can affect the growth rates and traits of their prey (trait-mediated interactions; TMIs), independent of their influence on prey mortality, which can have strong effects on prey populations (Preisser et al. 2005). In order to lower predation risk prey must make a trade-off between the acquisition and metabolic use of resources and the expression of defensive phenotypes (Johansson et al. 2001, Steiner 2007, Werner & Anholt 1996, Van Buskirk & Schmidt 2000). By altering prey traits such as behavior, morphology, and physiology, predators can indirectly affect how their prey interact with other species (Peacor & Werner 2001, Werner & Peacor 2003). Such trait-mediated indirect interactions (TMIIs; Abrams 1995) can have important consequences for population dynamics and the structure and functioning of ecological communities (Bolker et al. 2003, Miner et al. 2005, Schmitz et al. 2008, Werner & Peacor 2003).

Theoretical and empirical work has shown that the magnitude and even the direction of TMIs and TMIIs are context dependent, shifting with changes in prey resource use, resource responses to those changes, and their effects on intra- and interspecific competition (Peacor 2002, Peacor & Werner 2004, Relyea 2000, Werner &
Anholt 1996). For example, under conditions of high resources/low competition, prey growth rates can be negatively affected by the presence of predators due to reduced foraging and energy income. Further, because net herbivory is low and resources are primarily self-limiting, reduced prey foraging will only have a weak influence on resource levels. However, as competition increases and resources become limited by herbivory (e.g. prey are getting larger), predator induced reductions in prey foraging can have a strong positive effect on resources, via a trait-mediated trophic cascade (Beckerman et al. 1997). In fact, this can indirectly increase resource availability to the extent that it compensates for reduced prey foraging, resulting in a net positive effect of predators on prey growth (Bolnick & Preisser 2005, Peacor 2002, Peacor & Werner 2004). Because the trait-mediated indirect interactions of predators with resource levels can affect their trait-mediated interactions with prey, and that these effects can change over time, it is necessary to look at resource dynamics when examining how predators influence prey growth.

Predators can also decrease the impacts of competition on prey growth (Gureveitch et al. 2000), and can alter and even reverse competitive interactions among prey (Peacor & Werner 2000, 2001, Relyea 2000). This is attributed to predator-induced changes in prey behavior and morphology that affects their acquisition and use of resources (Reylea 2000, 2002a, Werner & Anholt 1996), thus indirectly affecting competitors. However, in the majority of these studies resource levels are not measured through time, instead the growth rates of focal organisms are used as an indirect
measurement of resource levels (e.g. Peacor 2002, Peacor & Werner 2004). This method is problematic because it is difficult to distinguish between trait-mediated interactions driven by prey interactions with resources vs. other factors such as costs of plasticity (Callahan et al. 2008, Relyea 2002b), or other indirect effects being propagated through the food web (Wootton 1994). For example predators can indirectly increase resources due to nutrient excretion and egestion that is independent of their effects on prey phenotypes (Vanni 2002). In this case, predators could reduce competitive interactions among consumers because their facilitation of resources, which could be independent of effects on prey traits. Because generating predator cues usually involves feeding caged predators prey that are external to the focal system, this represents a potentially large nutrient subsidy to the system that is seldom addressed in the experimental studies of the non-lethal effects of aquatic predators. Therefore, besides monitoring resources levels over time, it is also necessary to examine nutrient mediated effects of predators on resources without the presence of prey in order to address possible mechanisms explaining predator effects on prey, their competitors and their resources.

Predicting how predators will alter prey traits and the consequences of these trait shifts for communities is further complicated by the fact that prey responses to predation risk can vary between species and even through ontogeny within a species (Benard 2004, Hettyey et al. 2010, Reylea 2003). Predator effects on prey phenotype can change through ontogeny because prey often exhibit responses to predators that reflects their relative predation risk. For example, larger individuals often respond less or not at all to
the presence of gape-limited predators (Peacor & Werner 2000, Riessen & Trevett-Smith 2009, Werner & Anholt 1996). Thus, it is necessary to examine prey responses to predators through ontogeny, as their trait-mediated interactions with predators can change, making it likely the resulting indirect effects of these changes on other taxa will change through prey ontogeny as well.

Research on the community consequences of the non-lethal effects of predators has focused on the interactions between anuran larvae and their predators in temperate ponds (McDiarmid & Altig 1999, Wilbur 1997). In many of these studies resources are often not measured or measured only at the end of the study. Most tadpoles are assumed to be generalist periphyton grazers, although recent work suggests their may be substantial trophic variation between tadpole species (Altig et al. 2007, Wells 2007, Whiles et al. 2010). This is despite the fact that competition is thought to be relatively common within tadpole assemblages (Gurevitch et al. 2000), yet little is known about how they actually compete (Alford 1999). This is especially true in tropical lentic systems where many tropical anuran species have prolonged breeding periods, increasing the opportunity for cohorts of different species and different ages to interact. The effects of predators on such interactions are also relatively unexplored, in fact, predator induced trait-mediated effects in tropical anurans been investigated only recently (Warkentin 2000, Touchon & Warkentin 2008, Vonesh & Warkentin 2006, Warkentin & Caldwell 2009), despite the fact that predation is thought to be more important structuring tropical than temperate communities (Azevedo-Ramos et al. 1999, Hero et al. 2001, Paine 1969).
In this experiment we examine the independent and combined effects of interspecific competition and a caged dragonfly predator on the resources and growth of tadpoles of two commonly co-occurring Neotropical hylid frogs, the red-eyed treefrog (*Agalychnis callidryas*) and the hourglass tree frog (*Dendropsophus ebraccatus*) over their larval period. It has been shown that *A. callidryas* increases phytoplankton and decreases some zooplankton taxa, and both species consume periphyton (Costa, MS thesis, Chapter 1.). Thus, it is likely both species compete for periphyton, but they interact differently with basal resources and other consumers.

In this experiment we address: (1) The independent and combined effects of predators and tadpoles on periphyton and phytoplankton levels over time. (2) The effects of predators on the growth rates of tadpoles through ontogeny. (3) The importance of interspecific competition for both species. We also examine if (4) the presence of predators affects competitive interactions between the tadpoles through time and (5) The independent and combined effects of predators and tadpoles on other consumers (zooplankton). And finally, (6) can resource and zooplankton dynamics provide insight into the effects of predators and competitors on tadpole growth.
Methods

*Dendropsophus ebraccatus* and *A. callidryas* commonly co-occur in lowland ponds throughout their ranges from southern Mexico to Panama. At the study site they are locally abundant and eggs, tadpoles and adults commonly co-occur throughout the rainy season (May-December). The larvae of the Amazon darner dragonfly (*Anax amazili*) are also found in the breeding ponds shared by the two frog species near Gamboa (J.C. Touchon & J.R. Vonesh unpublished data). Gonzalez et al. (2011) used lethal dragonfly predators in combination with a substitutive design to examine predation’s effect on competition between these two species. They found that predators dramatically reduced the growth rates of both species and erased the competitive asymmetry that favored *A. callidryas* in the absence of predators, despite the fact that *A. callidryas* was more vulnerable to predation. However, this experiment was performed over a short-time period (8 days), food was supplemented (i.e., limited internal resource dynamics), and because they used a substitutive design, it was not possible to separate the absolute strength of inter- and intraspecific competition between the two species.

**Experimental design:** This study was conducted at the Smithsonian Tropical Research Institute in Gamboa, Panama (9°7’17” N, 79°42’11”W) between 30 September and 28 October 2010. To examine the interaction between predator presence and competition on the growth and survival of *A. callidryas* and *D. ebraccatus* through ontogeny, I conducted
a completely randomized 2 x 4 factorial design in which the presence or absence of a
caged *A. amazili* was crossed with four levels of tadpole species composition (25 *A.
callidryas*, 25 *D. ebraccatus*, 25 *A. callidryas* and 25 *D. ebraccatus* or no tadpoles).
Each treatment was replicated five times and the experiment ran for 28 days. I used an
additive design to look at the absolute strength of interspecific competition. I also
measured periphyton, phytoplankton and zooplankton periodically throughout the
experiment. Treatments were randomly assigned to 400L mesocosms (0.75 m diameter x
0.8 m high, with screened drain holes at 0.75 m height) arranged in five blocks of eight
tanks each. Tanks were filled with a mixture of filtered aged rain and tap water on 9
September. On 10 September 15 g of rabbit food (primarily alfalfa) pellets, fifteen large
*Inga* tree leaves (~250 cm²), and 100ml aliquots of concentrated pond inoculate were
added to each tank. A second 100 ml pond inoculation was added on 19 September.
Pond inoculate was collected from an artificial pond by repeatedly sweeping a plankton
net through the water column and then filtering it through a 1 mm mesh filter to exclude
large invertebrates but allowing experimental communities of phytoplankton, periphyton
and zooplankton to establish. Tanks were securely covered with fine nylon mesh to
prevent colonization by non-experimental organisms. Aquatic communities were allowed
to establish for twenty-one days after first inoculation before the start of the experiment.
Four additional tanks were stocked with 115 tadpoles of each species to provide prey for
caged predators. These tanks were filled on 29 September and given 6.0 g of rabbit food
and 3.0 g of Sera Micron® powdered algar. Additional resources of 3.0 g of rabbit food and 1.5g of sera-micron were added to these tanks on 4 October.

On 22 September, I collected twelve one-day old *A. callidryas* clutches from Experimental Pond to use as focal tadpoles. On 23 September thirteen newly laid *D. ebraccatus* clutches were collected from Ocelot Pond and twelve clutches were collected from Experimental Pond. All eggs were kept in an ambient temperature laboratory and misted frequently to prevent desiccation. Most clutches hatched on 26 September, and some *A. callidryas* unhatched clutches were manually stimulated to induce hatching (Warkentin 2000). For *D. ebraccatus*, focal tadpoles captured from Ocelot Pond but feeder tadpoles included a combination of tadpoles from both ponds. *Agalychnis callidryas* feeder tadpoles were hatched on 26 September from some individuals collected from Experimental Pond on 22 Sept, in addition to 13 clutches ages 0-2 days collected from Ocelot Pond on 22 September. Thus, focal and feeder tadpoles came from egg clutches of different ages, but they were all hatched on the same day and were introduced into mesocosms on 1 October after they were haphazardly sampled from their respective groups and assigned to treatments randomly.

*Anax amazili* were collected from Quarry Pond and placed in mesh cages (40 cm deep x 10 cm diameter) constructed from mesh window screen (1.2 mm mesh diameter). Cages were suspended at the top of the water column on the south side of each tank. Predators were introduced on 1 October and fed six tadpoles every three days. Predators in single species tadpole treatments were only fed tadpoles of that species. The mixed
tadpole and no tadpole-predator treatments were fed three individuals of each tadpole species. The number of feeder tadpoles stayed fixed through the experiment, but they grew in a similar manner to focal tadpoles so feeder tadpole biomass increased over time. Predators that did not eat all tadpoles during each three day period were replaced. Feeding stopped on 21 October, and all feeder tadpoles were eaten by 25 October and no predators were replaced. For each round of feeding an equal number of tadpoles were haphazardly chosen from each feeder tank and haphazardly assigned to predator treatments.

Focal tadpoles were dorsally photographed in a white tray with a Nikon D40x digital camera and added to mesocosms on 1 October. Approximately twenty tadpoles were dip-netted from each tank on 13 October and photographed (twenty of each species in mix-species treatments), and all tadpoles were dip-netted again on 28 October and re-photographed. All photographs included a ruler for calibration to use ImageJ (http://rsbweb.nih.gov/ij/) to obtain measurements of tadpole length.

To measure the effects of tadpoles and predators on resources and other potential competitors, I sampled phytoplankton and zooplankton from each tank on day 0 (before tadpole additions), 12, 20 and 27 using a 1 L integrated tube sampler. Three samples were collected from the water column of each tank and a 100 ml subsample was taken and put on ice and then vacuum filtered on Whatman GF/A glass filters. Filters were extracted in 95% ethanol for 24 hours under refrigeration in the dark. Samples were then
read in Aquafluor fluorimeter following standard protocol to estimate μg of chlorophyll a per unit volume (Welschmeyer 1994).

Zooplankton samples were obtained by filtering 2 L on a 20 μm Nitex® filter which was rinsed with 15 ml of tap water. Four to six drops of Lygol’s solution were added to stain the soft tissue and silhouette. Zooplankton were counted in the lab at 30X magnification with a stereo microscope.

Periphyton was sampled on 13 October and 28 October. Before the tanks were filled with water, 38 cm² pieces of tape were stuck to the north and south walls of the tank at both 70 and 45 cm from the bottom (Austin et al. 1981). On 13 October tape was removed with forceps, dried, and placed into 30 ml of ethanol to extract chlorophyll a as above. On 28 October two 115 cm² sections of tank wall were scraped from both the north and south sides of the tanks (60 cm from bottom) and vacuum filtered on Whatman GF/A glass filters to estimate μg of chlorophyll a per mm² (Aloi 1990).

Analysis: All statistical analyses were conducted on tank means using R version 2.11.0 (R Development Core Team 2010). For phytoplankton, periphyton and for tadpole total length (TL) and growth rates, values were log transformed to homogenize variances, and analyzed using repeated measure linear mixed effects models (LMM) to examine their responses to interactions among predator presence, tadpole species composition, and time, with repeated measures on the same tank treated as the random factor. Model selection in all analyses was based on minimizing Akaike information criterion (AIC; Burnham & Anderson 2002) but retaining significant (p < 0.05) explanatory variables.
For each model, if necessary, we also chose specific variance-covariance structures, and heterogeneity of variances at different time points, based on minimizing Akaike information criterion. For TL analysis, each species was considered separately and initial measurements were excluded because there were no differences between treatments for each species, and there was low variance. Because *A. callidryas* is a larger species than *D. ebraccatus*, we analyzed relative growth rates as log (final or mid TL/ mid or initial TL) to facilitate comparisons of species responses to competitors and predators. Total tank biomass was calculated using length-mass regressions (Vonesh & Costa unpub.) for each species from the number of tadpoles remaining at the end of the experiment. Values were log transformed and analyzed with a mixed linear model examining the interaction between tadpole composition and predator presence treating block as a random factor. Percent mortality of each species from each tank was calculated using the number of remaining tadpoles and was analyzed with generalized linear model (GLM) with a binomial error distribution. For zooplankton data we used generalized linear mixed models (GLMM) to examine the interactions among time, predator presence and tadpole composition. We minimized overdispersion in the count data by using either a Poisson or Gamma distribution (Pinheiro & Bates 2000), and also specifying the variance-covariance structure and treating repeated measures of tanks as a random factor. Post-hoc comparisons of treatments were conducted using Tukey’s HSD.
Results

Phytoplankton chlorophyll a levels were affected by time (F_{1,118} = 8046, P < 0.001) and tadpole composition (F_{3,35} = 5.09, P = 0.005; Figure 1B). Phytoplankton levels decreased in all treatments by day 12 and then increased through the end of the experiment. The main effect of predators was marginal (F_{1,35} = 3.78, P = 0.06), but there was a significant predator by time interaction (F_{1,118} = 7.67, P = 0.007) in which predators tended to increase chlorophyll a levels over time (Figure 1A). Averaged across the entire experiment phytoplankton levels were the same in the mixed and *A. callidryas* treatments (35.45 μg/L⁻¹), which represented an increase of 21.3% and 28.3% as compared to the *D. ebraccatus* and no tadpole treatments, respectively.

Significant predictors of periphyton chlorophyll a levels included time (F_{1,39} = 555, P < 0.001), predators (F_{1,35} = 13.47, P < 0.001; Figure 2A) and tadpole composition (F_{3,35} = 3.68, P = 0.02; Figure 2B), no interactions were significant. Midway through the experiment periphyton levels were highest in the no tadpole and *D. ebraccatus* treatments, and across all treatments predators doubled periphyton compared to no predator treatments. By the end of the experiment overall periphyton levels were greatly reduced, but predators increased periphyton by 85.1%, compared to no predator treatments. Tadpoles decreased periphyton at both time points and this effect was more pronounced at the end of the experiment.
Initially, *A. callidryas* tadpoles were 15.69 ± 0.31 mm (mean ± SD) in total length (TL), and there were marginal differences across treatments (*F*<sub>3,16</sub> = 3.02, *P* = 0.061). This was because by chance when tadpoles were assigned to tanks there were marginal differences between the two no predator treatments in which tadpoles from the single species treatment (15.89 ± 0.06) were larger than those from the mixed treatment (15.42 ± 0.23; Tukey’s HSD; *P* = 0.07). Total length of *A. callidryas* was affected by time, predators, and the interactions between predators and predators and competitors (Table 1, Figure 3A). Predators reduced tadpole TL by 21.9% and 6.4% at the midpoint and end of the experiment respectively. In the absence of predators *D. ebraccatus* initially reduced *A. callidryas*’ size by 2.8%, but in the presence of predators *D. ebraccatus* actually increased their growth by 12.9%. At the end of the experiment in the absence of predators there was little difference between competitor treatments (0.6% reduction in TL by *D. ebraccatus*) while in the predator treatments, *D. ebraccatus* increased *A. callidryas*’ size by 7.1%. Growth rates of *A. callidryas* were influenced by time and the interaction between predators and time (Figure 4A), in which predators initially decreased growth rates by 32.3%, but by the end of the experiment, growth rates were increased by 128% in the presence of predators (Table 1).

Initial sizes of *D. ebraccatus* tadpoles were 7.74 ± 0.15 mm TL, and there were no differences in size across treatments (*F*<sub>3,16</sub> = 1.98, *P* = 0.157). Total length of *D. ebraccatus* was affected by time, and the interaction between predators and time and competitors and time (Table 2, Figure 3B). Initially predators had no affect on length,
but at the end of the experiment predators increased length by 13.3% compared to no predator treatments. Similarly, initially, *A. callidryas* initially had no affect on *D. ebraccatus* length, but by the end of the experiment they had reduced tadpole size by 13%. Time and competitors were significant predictors of *D. ebraccatus* growth rates, as *A. callidryas* initially had no affect, but in the second half of the experiment they reduced *D. ebraccatus* growth by 44.4%. Predators also had a marginal effect on *D. ebraccatus* growth rates: midway through the experiment there were no differences, but in the second half of the experiment predators increased growth rates by 49.8% (Figure 4B; Table 2). There were no interactions among these factors.

The biomass of each species was not affected by treatment (*A. callidryas*: $F_{3,12} = 1.81, P = 0.20$; *D. ebraccatus*: $F_{3,12} = 1.42, P = 0.29$). Within species, *A. callidryas* biomass was marginally decreased by predators, but not competitors or their interaction (Table 1). The biomass of *D. ebraccatus* was not affected by predators, competitors or their interaction (Table 2). Total tank biomass was affected by tadpole composition ($F_{2,20} = 24.76, P < 0.001$), but not predators ($F_{1,20} = 0.05, P = 0.83$) or their interaction ($F_{2,20} = 1.14, P = 0.34$). This was because *D. ebraccatus* ($3.26 \pm 2.15$g) had much lower biomass than *A. callidryas* ($8.77 \pm 2.66$g), regardless of treatment. Overall, tadpole mortality was low for *D. ebraccatus* ($0.058 \pm 0.055$) and *A. callidryas* ($0.02 \pm 0.038$), but there were no differences in mortality rates between the species ($\chi^2 = 0.38, P = 0.54$). Mortality was not affected by treatment ($\chi^2 = 0.56, P = 0.99$), predator presence ($\chi^2 < 0.01, P = 0.95$), or competitors ($\chi^2 = 0.59, P = 0.9$).
The total number of zooplankton was affected by tadpole composition and time (Table 4, Figure 9A). Total zooplankton numbers decreased over time and averaged across all time points, tadpole treatments decreased total zooplankton by 17.9% compared to no tadpole treatments. Copepod abundances were affected by predators and time (Figure 9B). Initially copepods were slightly higher in predator treatments, and this trend continued over time. Averaged across the entire experiment, predators increased copepods by 88.1% compared to no tadpole treatments. Nauplii (early copepod instars) did not respond to any experimental treatments but they gradually decreased through the experiment. Similarly, ostracods were also only affected by time, but they gradually increased. Rotifers were only affected by tadpole composition (Figure 9C). Averaged over the entire experiment tadpoles decreased rotifer abundances by 38.5% compared to no tadpole treatments. No treatments predicted cladoceran abundances except for the predator by tadpole composition interaction. Overall predators increased cladocerans compared to no predator treatments, but this increase depended on the tadpole treatment.
Discussion

In order to elucidate possible mechanism explaining tadpole growth responses to predators and competitors, we first looked at the independent and combined effects of tadpole composition and predators on periphyton and phytoplankton levels. We found that the chlorophyll a levels of phytoplankton initially decreased, and then increased toward the end of the experiment, and more so in the *A. callidryas* and mixed tadpole treatments. Phytoplankton has previously been shown to respond positively to the presence of *A. callidryas*, but the mechanisms for this remain unknown (Costa, MS thesis, Chapter 1). Although the main effect of predators on phytoplankton was marginal, there was a significant predator by time interaction in which predators increased phytoplankton as the experiment progressed. Periphyton chlorophyll a decreased in all treatments over time, but there was a main effect of predators that increased periphyton abundances. All tadpole treatments reduced periphyton and this effect was more pronounced at the end of the experiment, as periphyton reached relatively low levels (< 2.5 μg/cm²), suggesting that all tadpole treatments were limited by this resource, regardless of predators. For both periphyton and phytoplankton there were no tadpole by predator interactions, suggesting that the responses of these resources to the presence of predators was not dependent on the presence of tadpoles. Thus the main positive effect of predators on both resources was not occurring solely due to a trait-mediated trophic cascade. Instead, is likely that nutrient cycling due to the excretion and egestion of the
dragonflies also contributed to the growth of both resources (Schmitz et al. 2010, Vanni 2002).

The fact that predators tended to facilitate both basal resources may explain how their trait-mediated effects on tadpole growth changed over time. In this experiment, *A. callidryas*’ TL and growth rates were initially reduced by dragonflies but this affect was ameliorated over time, as shown by the significant predator by time effects on total length and growth rates. In the second half of the experiment, predators actually increased growth rates by 128% compared to no predator treatments, which almost completely compensated for the initial decrease in growth. As predators increased phytoplankton over time, and increased periphyton as well, it is possible these tadpoles benefited from the presence of predators later in the experiment due to an increase in their resources as *Agalychnis callidryas* filter feeds phytoplankton and grazes periphyton (Costa, MS thesis, Chapter 1, McDiarmid & Altig 1999, Wassersug & Rosenberg 1979). It is unknown to what extent *A. callidryas* depends on both of these resources but it is likely that predators increased tadpole growth later in ontogeny because they increased phytoplankton over time, and they reduced competition for periphyton, which became more important as it became a limiting resource toward the end of the experiment.

Similarly, the size of *D. ebraccatus* was also affected by the interaction between predators and time. Midway through the experiment predators had no affect on their length or growth rates, but in the second half predators increased their growth rates and their final size. This result is surprising as this species has been shown to reduce their
growth in response to dragonflies (Gonzalez et al. 2001), and that these tadpoles respond morphologically to dragonflies and other aquatic invertebrate predators by altering the size and color of their tails (Costa unpublished data, Touchon & Warkentin 2008). So it is possible that similar to A. callidryas, these tadpoles’ responses to these predators were changing through ontogeny due to predator facilitation of their resources. The tadpoles of D. ebraccatus do not filter feed, but they do consume periphyton (Costa, MS thesis, Chapter 1, McDiarmid & Altig 1999). As periphyton appeared to be a limiting resource in all tadpole treatments by the end of the experiment it is likely predators were able to increase tadpole size and although marginally, their growth rates as well, due to their facilitation of periphyton.

For both tadpole species it is likely that predator effects on their growth changed over time due to increased competition for resources (Bolnick & Preisser 2005, Relyea 2004). Although we were not able to distinguish between the two most plausible mechanisms, it is clear that predators can indirectly facilitate resources both through nutrient cycling (Vanni 2002) and through induced changes in the foraging of their prey (Beckerman et al. 1997, Peacor & Werner 2001). Given the nonlinear nature between resource growth rates and total resource levels, a reduction in consumer foraging and/or an increase in limiting nutrients can potentially cause a proportionately larger increase in resource levels than one might expect. In this case the indirect negative effect of predators on prey growth will be less than the indirect positive effect due to the increase in total resource levels. This positive effect of predators on resource levels can then
compensate for reduced foraging by tadpoles, to the extent that predators can have a net positive effect on the growth rates of their prey (Peacor 2002). These results suggest that the trait-mediated effects of predators on tadpole growth rates can change through ontogeny and are strongly dependent on resource dynamics (Peacor & Werner 2004, Werner & Anholt 1996).

Alternatively, for both species it is likely that tadpoles are altering their responses to predators to reflect their relative predation risks. For *A. callidryas* tadpoles it has been shown that they reach a size refuge from predation as they get larger, so larger tadpoles do not change their growth in response to predators (Vonesh et al. unpublished data). The tadpoles of *D. ebraccatus* also approach a size refuge to most predators at they near metamorphosis (Costa unpublished data), so like other tadpole species, it is likely their trait-mediated interactions with predators also change through ontogeny (Hettyey et al. 2010, Relyea 2003, Werner & Anholt 1996). It is interesting to note that Gonzalez et al. (2011) found that these same predators strongly reduced the growth of both of these tadpole species over 8 days, despite the fact that dragonflies consumed > 60% of both species. However, they also found that *A. callidryas* tadpoles were more vulnerable to predation, so perhaps in our experiment they initially responded to their relatively higher risk, and reduced their growth while *D. ebraccatus* did not.

Competition for resources and resource dynamics may have also played a role in *A. callidryas’* response to competitors and predators, as there was a significant predator by competitor interaction on their size. In the absence of predators, the presence of *D.*
*ebraccatus* slightly reduced *A. callidryas* size at both time points, while in the presence of predators *D. ebraccatus* had a much greater positive effect on their length. Thus the presence of caged predators reversed the sign of their interaction, for competition to facilitation. Since predators increased periphyton, regardless of tadpole treatments, it is possible they simply reduced competition for that resource. Similarly, competition is expected to reduce nonlethal effects of predators on prey growth when there is competition for resources, because prey can not “afford” to reduce their foraging (Luttbeg et al. 2003, Peacor & Werner 2001, Relyea 2004). As previously mentioned, reduced tadpole foraging in response to predators under high competition/low resource conditions can actually increase total resource levels, which can result in a net positive effect of predators on prey growth (Peacor 2002), regardless of their nutrient based facilitation of resources. Alternatively, *D. ebraccatus* could have improved *A. callidryas* growth in the presence of predators because of the higher overall density of tadpoles. Due to our additive design, it is possible *A. callidryas* is responding to their relative risk. In the presence of more heterospecifics, we might expect their responses to predators to be reduced. Higher densities of conspecifics has been shown to reduce the negative effects of predators on the growth rates of *A. callidryas* and other tadpole species (McCoy 2007, Van Buskirk et al. 2011, Vonesh et al. in press), but this has not been addressed with heterospecifics. Although it is not possible to distinguish among these mechanisms it is likely that they are all occurring simultaneously and are contributing to our results.
Another explanation of *D. ebraccatus*’ facilitation of *A. callidryas*’ growth in the presence of predators is the amount of predator cue concentrations in the mixed species versus the single species tadpole treatments. Predators in mixed species treatments were fed the same total number of tadpoles but half as many of each species than the single species treatments. Previous work has shown the growth response of *A. callidryas* to predators is an asymptotic function of prey biomass consumed, and increasing prey biomass while holding prey number constant elicits a smaller asymptotic response than increasing both the biomass and the number of prey consumed (McCoy et al. in review). So this could explain how focal species’ responses to predators in mixed species treatments may be reduced. We did not see this with *D. ebraccatus*, but we did with *A. callidryas*. This scenario is unlikely however, given that the biomass of individual feeder tadpoles increased over time enough to surpass the biomass required to reach the asymptote of the phenotypic response of *A. callidryas* (~0.2g). Furthermore, tadpoles have been shown to respond similarly to chemical cues from predators feeding on other tadpole species (Schoeppner & Relyea 2005) so it is unlikely the amount of predation cues can explain our results.

For *D. ebraccatus*, size was affected by a competitor by time interaction in which *A. callidryas* initially had no affect on their size, but by the end of the experiment their TL was reduced by 13%. Similarly, *A. callidryas* had a significant negative effect on their growth rates (~44%), but only in the second half of the experiment. This is most likely due to an increase in exploitative competition for periphyton over time, as *A.*
Callidryas has been shown to be a more efficient periphyton grazer per unit biomass (Costa, MS thesis, Chapter 1). This is also similar to previous work that showed D. ebraccatus’ growth was 13% slower with A. callidryas than with the same number of conspecifics (Gonzalez et al. 2011). However, this previous experiment was much shorter in duration (8 days), supplemented food, and used a substitutive design, making straightforward comparisons between the two studies difficult.

Despite the strong effects of predators and competitors on tadpole growth, total tank biomass was only affected by tadpole composition, due to the fact that D. ebraccatus treatments were lower than A. callidryas and mixed species treatments. Given that mixed species treatments had twice as many tadpoles, per individual biomass levels then decreased in these treatments as total tadpole biomass appears to be limited by interspecific competition. Similarly, a previous study showed that the total tank biomass of both species did not change despite the doubling of intraspecific competitors (Costa, MS thesis, Chapter 1), showing that both intra- and interspecific competition can limit total tank biomass production. Within each species however, D. ebraccatus was not affected by any explanatory variables, while A. callidryas’ total tank biomass was reduced only by the presence of predators, although this effect was marginal. For A. callidryas, it appears that the initial decrease in growth caused by predators was able to affect their final biomass, despite the positive effect of predators in the second half of the experiment. Conversely, D. ebraccatus’ biomass was not affected by competitors or
predators which decreased and increased their growth, respectively, but only in the second half of the experiment.

Predator and competitor effects on tadpole growth and resources could also be attributed to indirect effects due to their interactions with zooplankton. Predators tended to increase copepods while tadpoles decreased total zooplankton and rotifers. Because predators increased phytoplankton, their facilitation of copepods could be due to an increase in this resource. It is not clear exactly how tadpoles interact with these different zooplankton groups, but previous work in this system has shown A. callidryas can reduce copepods, their younger instars, nauplii, and total zooplankton levels (Costa, MS thesis, Chapter 1). However, it appears these effects can be variable across experiments and over time, so these results may not reflect biologically relevant interactions and it is unlikely tadpole and predator interactions with zooplankton are indirectly contributing to the effects of competitors and predators on tadpole growth and basal resources.

The growth of each of these tadpole species was affected by both competitors and predators, but depending on the timing during ontogeny, both the magnitude and the direction of these effects changed. This is important because the size and growth of larval amphibians can determine their fitness because it can affect time to metamorphosis, juvenile growth, size at maturity, and future egg production (Berven & Gill 1983, Semlitsch et al. 1988, Smith 1983, Van Allen et al. 2010). Although I did not quantify behavior in this study, previous work in the lab and field has shown that the responses of these two species to the presence of predators and competitors does not
appear to be attributable to changes in behavior per se (Costa unpub, Vonesh & Warkentin 2006). Instead, differences between the species in their responses to predators and competitors are most likely due to their perception of relative predation risk, their different resource requirements, and the complex interplay among the costs of avoiding predation, resource responses to altered foraging and predator nutrient cycling, and their effects on intra- and interspecific competition. Thus, future studies examining predator induced trait-mediated interactions should monitor resources and growth responses over time in order to disentangle the effects of ontogeny and resource levels on the trait-mediated impacts of predators on prey and their communities.
References


Table 1: Results of two-way ANOVAs on the effects of tadpole species identity and density on the abundances of zooplankton morphospecies.

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Table 2: Main effects and the significant interactions of time, predators and competitors on the total length, growth rates and final biomass of *A. callidryas*.

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Table 3: Main effects and the significant interactions of time, predators and competitors on the total length, growth rates and final biomass of *D. ebraccatus*.

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Table 4: Main effects and significant interactions among time, predators, and tadpole treatments on the abundances of zooplankton morphospecies.

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</tbody>
</table>
Figure 1. Mean (±SE) of tadpole size (A), growth rates (B), and total tank biomass (C) in which we manipulated the abundances, high (50) and low (25), of Ac (A. callidryas) and De (D. ebraccatus) tadpoles. Results of two-way ANOVA looking at effects of tadpole species identity and density. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Letters indicate statistically different treatments (Tukey HSD, p < 0.05)
Figure 2. Mean (±SE) of water column chlorophyll a (A) and dried periphyton biomass (B) in experimental treatments. Results of two-way ANOVA looking at effects of tadpole species identity and density. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Letters indicate statistically different treatments (Tukey’s HSD, p < 0.05)
Figure 3. Mean (±SE) of zooplankton morphospecies abundances in experimental treatments. Results of two-way ANOVA looking at effects of tadpole species identity and density. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. Letters indicate statistically different treatments (Tukey’s HSD, p < 0.05)
Figure 4. (A) Mean (±SE) of water column chlorophyll a after being filtered through different sized Nitex, (B) periphyton chlorophyll a abundances inside and outside of tadpole exclosures, and (C) zooplankton abundances in which we manipulated the presence and absence of 50 *A. callidryas* tadpoles. Results of ANOVA looking at effects of treatment, and in the case of periphyton, results of a two-way ANOVA looking at treatment and exclosure side effects. * = p < 0.05, ** = p < 0.01. Letters indicate statistically different treatments (Tukey’s HSD, p < 0.05)
Figure 5: Mean (±SE) of phytoplankton chlorophyll a for A. Predator presence and B. Tadpole composition over time. Main effects and interactions of time, predator presence and tadpole composition are shown * = p < 0.05, ** = p < 0.01, *** = p < 0.001
Figure 6: Mean (±SE) of periphyton chlorophyll a for A. Predator presence and B. Tadpole composition over time. Main effects and interactions of time, predator presence and tadpole composition are shown * = p < 0.05, ** = p < 0.01, *** = p < 0.001
Figure 7: Mean (±SE) of tadpole length for A. (Agalychnis callidryas) and B. (Dendropsophus ebraccatus) by treatment over time. Main effects and interactions of time, predator presence and competitors are shown * = p < 0.05, ** = p < 0.01, *** = p <0.001.
Figure 8: Mean (±SE) of tadpole growth rates for A. (*Agalychnis callidryas*) and B. (*Dendropsophus ebraccatus*) by treatment over time. Growth rates are calculated as Final: log(Final TL/Mid TL) and Mid: (Mid TL/Initial TL). Main effects and interactions of time, predator presence and competitors are shown * = p < 0.05, ** = p < 0.01, *** = p <0.001.
Figure 9: Mean (±SE) of zooplankton numbers per liter for A. Total zooplankton B. Copepods and C. Rotifers. Main effects and interactions of time, predator presence and tadpole composition are shown * = p < 0.05, ** = p < 0.01, *** = p < 0.001
Zacharia J. Costa was born on 8 February 1985 in Santa Rosa, California. He graduated from the University of California, Davis in 2007 with a Bachelor of Science in Evolution and Ecology. After working in a plant ecology lab and then living in San Francisco, CA, he realized only riding bikes and getting hyph was not the life he envisioned it to be. Going to graduate school was a great opportunity to explore the world and to keep himself mentally stimulated, so he joined Dr. James Vonesh’ lab at Virginia Commonwealth University in 2009. Now enamored with research, he hopes to return to Latin America to do research while pursuing a Ph.D. in aquatic ecology.