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PREDATOR AND ABIOTIC EFFECTS ON HATCHING PHENOTYPE AND SURVIVAL OF ARBOREAL FROG EGGS WITH IMPLICATIONS FOR PHYTOPLANKTON

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PREDATOR AND ABIOTIC EFFECTS ON HATCHING PHENOTYPE AND SURVIVAL OF ARBOREAL FROG EGGS WITH IMPLICATIONS FOR PHYTOPLANKTON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE AT VIRGINIA COMMONWEALTH UNIVERSITY.

by

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Abstract

PREDATOR AND ABIOTIC EFFECTS ON HATCHING PHENOTYPE AND SURVIVAL OF ARBOREAL FROG EGGS WITH IMPLICATIONS FOR PHYTOPLANKTON

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A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Historically studies have focused on either the terrestrial or aquatic environments independently. However, these systems are inherently linked through numerous pathways including organisms with complex life cycles. Both abiotic factors and predators of these organisms can influence connections by changing the number of organisms moving across habitat boundaries and by changing their phenotype. When the focal organisms are primary consumers, these effects may have important implications for ecosystem processes. My study investigated how terrestrial predators and abiotic factors affect the number and phenotype of herbivorous tadpole inputs into a tropical forest pond. I found that predators and abiotic factors altered survival and timing of hatching and these effects varied temporally. Thus, temporal changes in the relative importance of these threats from abiotic sources and terrestrial predators on prey with complex life cycles may potentially have implications for connections with and food web dynamics in adjacent ecosystems.

INTRODUCTION

Studies in community ecology have typically focused on interactions within an ecosystem, often studying the terrestrial or aquatic environments independently (Polis et al. 1997). However, these systems do not exist in isolation, and are inherently linked through numerous pathways (Polis and Strong 1996, Anderson and Polis 1999, Nakano et al. 1999, Sabo and Power 2002) including organisms with complex life cycles (e.g., amphibians and aquatic insects; Baxter et al. 2005, Knight et al. 2005, Regester et al. 2006). When organisms with complex life cycles use both habitats sequentially through development (i.e., life stages are markedly separated by habitat), effects in early stages may have consequences for ecological processes in later stages and habitats. Both predator and abiotic effects on these organisms can influence connected ecosystems via two general mechanisms: 1) by changing the number of organisms moving across habitat boundaries (via direct mortality) and 2) by changing the phenotype of focal organisms (e.g., size, hatching time). Both density and phenotypic changes have important consequences for prey life history, development and feeding rates (Abrams et al. 1996, McPeek and Peckarsky 1998, Schmitz 1998, Vonesh and Bolker 2005) all of which have the potential to affect population and community dynamics (Altwegg 2002, Vonesh and De la Cruz 2002). When organisms moving between habitats are primary consumers, these effects may also cascade to influence ecosystem dynamics (e.g., primary productivity) due in large part to changes in size and density specific feeding rates. Furthermore, in natural

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populations organisms generally face threats from predator and abiotic sources simultaneously and the relative importance of these risks varies over time. For example, in times of drought, abiotic factors may outweigh any threats from predators (Touchon and Warkentin 2008). In this scenario, a study focusing only on predators may overlook the effects of drought (e.g., tadpoles increase development rate in response to pond drying regardless of predators; Laurila and Kujasalo 1999). Empirical studies throughout a breeding season may be able to better assess the relative importance of density and phenotypic effects of both predators and abiotic effects and how these vary over time.

Predators and abiotic factors can have strong effects on the density of focal organisms. In aquatic environments changes in density can have strong effects on predation (*sensu* Holling 1965) and intraspecific competition (Vonesh and De la Cruz 2002, Vonesh and Bolker 2005). Results from these studies show that if larval survivorship is density dependent, reduced egg stage survival may reduce larval competition for limiting resources, thus indirectly increasing larval growth (reviewed in Skelly and Kiesecker 2001) and survival rates (Wilbur 1976, 1977, VanBuskirk and Smith 1991, Vonesh and Bolker 2005). Additionally, predator effects on intermediate consumers can alter per capita resource demand by consumers, indirectly affecting basal resources (Wootton and Power 1993). Understanding the magnitude of changes in density specific consumption rates is key to evaluating the community and ecosystem-level effects of larval reductions due to predator and abiotic sources of mortality (Bolker et al. 2003, Werner and Peacor 2003). If the effect of abiotic factors and predators on inputs into the pond varies over the season,

intraspecific competition along with resource demand and consumption might also vary over the season.

Predators and abiotic factors can also have non-lethal effects on prey phenotype. Organisms have developed ways to assess and respond to various risks, including predation and abiotic factors, in ways that should maximize fitness while also reducing mortality (Werner and Gilliam 1984, Werner 1986, reviewed in Skelly and Werner 1990). Typical responses include altering behavior (e.g. reduced foraging, hiding; Schmitz et al. 2007), morphology (e.g. developing spines; Spitz 1991) or the timing of key life history switch points like hatching and metamorphosis (Wilbur 1996). However, avoiding one risk often carries unforeseen fitness consequences and plastic responses to risk frequently involve trade offs. For example, numerous studies have shown that decreased foraging in response to predation risk reduces prey growth and development, because prey switch to predator-free habitats that are often of lower feeding quality (Schmitz and Suttle 2001, Schmitz et al. 2007). Additionally, in organisms with complex life cycles (e.g., amphibians and aquatic insects), induced early hatching often means that prey are more vulnerable to predators in subsequent stages (Warkentin 1995, but see Vonesh and Bolker 2005). The phenotypic responses of amphibians to predators and abiotic factors are well understood from laboratory (Warkentin 1995, 1999, 2000, 2005, Warkentin et al. 2007) and controlled field (Gomez-Mestre and Warkentin 2006) manipulations. However, prey responses to a subset of cues in an experimental context may not always reflect responses to natural

predation (Warkentin 2007). Field studies with natural populations are needed to better understand the relative importance and adaptive value of this phenotypic response.

Herbivorous tadpole larvae can be the most abundant vertebrates in aquatic habitats throughout the neotropics (Stebbins and Cohen 1995, Duellman 1999, Ranvestel 2004) and as such, they have the potential to affect the aquatic food web (i.e. many are important algal consumers). Several studies have shown that tadpoles, many of which are primary consumers, can influence food web dynamics in temperate ponds (Seale 1980), rivers (Kupferberg 1997), and both temperate (Kiffney and Richardson 2001) and neotropical streams (Ranvestel et al. 2004, Whiles et al. 2006). The majority of these studies have primarily focused on the presence or absence of tadpoles. However, only a small subset have considered how natural variation in tadpole densities or size over the breeding season might influence the aquatic food web (but see Flecker et al. 1999). Changes in both the density and phenotype of herbivores have been shown to influence ecosystem processes in markedly different ways (Krivan et al. 2004, Peckarsky et al. 2008, reviewed in Schmitz et al. 2008). Previous laboratory work has shown that early-hatched tadpoles develop faster, begin feeding earlier and have higher larval growth rates than late-hatched tadpoles (Warkentin 1999, Vonesh unpubl).

In this study I investigated the effects of terrestrial predators and abiotic sources of mortality and phenotypic changes in the red-eyed treefrog, *Agalychnis callidryas*. My study consists of two main components 1) assessing how predator and abiotic effects

influence the hatching success and timing of arboreal eggs throughout the breeding season and 2) exploring how these effects could influence ingestion rates which might potentially alter aquatic ecosystem processes. Here, I focused on the arboreal eggs and aquatic larvae of the red-eyed treefrog, *Agalychnis callidryas. Agalychnis callidryas* lays its eggs on vegetation overhanging small ponds. Hatchlings then drop into the aquatic environment below where they remain until metamorphosis. The arboreal eggs of red-eyed treefrogs are vulnerable to desiccation, submergence, and a number of terrestrial predators, including several species of tree snakes, wasps, and parasitoid flies (Warkentin 2005). These predators consume embryos (often in large numbers; Warkentin 1995, Gomez-Mestre and Warkentin 2007) thus reducing the input of tadpoles to the pond (e.g., direct density effect; Vonesh and Osenberg 2003, Vonesh 2005, Vonesh and Bolker 2005). In addition, predators and abiotic factors can also induce early hatching. *Agalychnis callidryas* are hatching competent after 4 days (Gosner stage 23); however if undisturbed, they will delay hatching usually until between day 6 and day 8 (Warkentin 2002). Immediately after hatching early hatched tadpoles are less developed and ~30% smaller than hatchlings from undisturbed clutches (e.g., phenotypic effect; Warkentin 1995, 2000, 2005).

Study Site

This study was conducted at Ocelot pond, located in Soberanía National Park, near the Smithsonian Tropical Research Institute in Gamboa, Panamá (9° 7' 0 N and 79° 42' 0 W). Surveys were conducted during the rainy season from June – November of 2008. Ocelot pond is a small sized (ranging: 117 – 1,513 m^2), lowland tropical pond surrounded by evergreen, wet, tropical forest. The dominant vegetation in the riparian zone and surrounding forest includes various species of Palms, *Psidium sp.* (Guava)*, Ficus* sp. (Fig), and *Bursera simaruba (Gumbo-Limbo)* with little, if any, emergent vegetation in the pond itself.

Field Surveys

To characterize how terrestrial predator effects vary both temporally over the breeding season, and how the magnitude of these effects vary relative to abiotic effects, I collected detailed data on clutch density, survivorship and sources of mortality. I then used this information to estimate tadpole inputs into Ocelot pond. I conducted two types of surveys, a density survey where I counted all visible clutches and a more detailed clutch monitoring survey where I closely monitored the fate of a subset of clutches from the day they were laid until all the embryos had either hatched or died.

Clutch density surveys (9 in total) were conducted twice-monthly along eight, 9m,

randomly placed, pond-side transects. For each clutch I recorded the height above water, substrate type (e.g., leaf or tree type) position on leaf (top or bottom), and whether it was laid over water or over land. One-way ANOVAs were used to examine temporal variation in clutch density.

To quantify clutch survivorship, and percentage of clutches with different sources of mortality, I used systematic visual encounter survey methods (Donnelly and Guyer 1994, Heyer et al. 1994) with the pond divided into four sampling quadrants and alternated start locations and surveyors to reduce observer biases. My sampling was limited to clutches within 0-2m (i.e., the ones we were able to reach and monitor without inducing hatching). However, I make the assumption that these data are reflective of the entire population of clutches. Each clutch was checked twice daily (early morning and late afternoon). I marked each new clutch with numbered flagging tape on adjacent vegetation and recorded the clutch number, developmental stage (according to Gosner 1960) and any signs of damage or mortality. Before clutches became hatching competent, I suspended a water-filled plastic cup beneath the clutch to capture new hatchlings. Small holes were drilled in the side to prevent cups from overflowing, but small enough so that no hatchlings could escape (Hayes 1983, Warkentin 1995, Lips 2001, Vonesh 2005). All hatchlings were released into the pond after counting. Proportion survival for each clutch was calculated as the ratio of the initial number of eggs to the final number of hatchlings.

Because major arboreal predators and other mortality sources leave characteristic and obvious signs on the remaining egg mass (Warkentin 1995, 2000, Lips 2001,Vonesh

2005), I was able to attribute sources of mortality for each clutch. For example, snakes generally consume all of the eggs and leave behind the gelatinous matrix of the clutch. Wasps tend to consume only one embryo at a time and often return repeatedly to the same clutch. Parasitic flies deposit their eggs in the clutch, which then hatch into clearly distinguishable larvae. In dehydrated clutches the matrix surrounding the egg mass is less turgid. Flagging tape on the above vegetation marks the location of missing submerged clutches. For submerged clutches this method presents an obvious challenge, because the clutch is no longer visible. Previous laboratory studies have shown that hatching competent (> 4d) *A. callidryas* respond to hypoxic conditions (e.g., resulting from submergence due to flooding) by inducing early hatching (Warkentin 2002). Clutches that were submerged after 4d, were counted as hatched, for those that were submerged prior to 4d, they were counted as dead. Based on previous work and this season's field data, I was able to identify 5 major sources of mortality: 1) wasps (e.g., *Polybia rejecta;* Warkentin 2000)*,* 2) fly larvae (e.g., *Megaselia scalaris*; Roberts 1994), 3) several snakes (e.g., *Leptodira* spp. and *Leptophis spp*.; Warkentin 2007), 4) non-developed eggs and 5) abiotic sources: desiccation and submergence due to rapid increases in the water level of the pond (Touchon and Warkentin 2008). Clutches with multiple sources of mortality rarely occurred (12 total, 8%), however, in these instances I classified clutches according to the most obvious and substantial source of mortality.

For all tests, proportional data were arcsine-square-root-transformed prior to analysis to improve normality and homogenize variances (Anscombe 1948, Gotelli and Ellison 2004).

One-way ANOVAs were used to examine if clutch size, proportion survival and the mean day of hatching varied among clutches with different sources of mortality and among different months. To test for interactions among proportion survival or mean day of hatching and month I used separate two-way ANOVAs. When ANOVAs revealed significant differences, I conducted Tukey's Post Hoc analyses. Since I expected the major source of mortality to change over the course of the season (e.g., due to variation in patterns of rainfall and predator natural history), I conducted separate repeated measures ANOVAs to examine how the relative importance of each source of mortality varied over the course of the season. The proportion of clutches with different fates (undisturbed or with different sources of mortality) was the response variable and mortality source and time were the predictor variables. To obtain a more conservative estimate and to better approximate assumptions of the repeated measures design, I adjusted the degrees of freedom by aggregating each of the individual fate categories into three broad categories (undisturbed, abiotic and biotic; Scheiner and Gurevitch 2001). Each monthly clutch monitoring survey was conducted over the same quadrant, therefore, for the repeated measures analyses, quadrant is the unit of replication to provide a conservative estimate and account for any potential autocorrelation among clutches (Gotelli and Ellison 2004). All statistical analyses were performed in R version 2.8.0 (Ihaka and Gentleman 1996).

Tadpole input and hatchling phenotype over the season
Terrestrial predators reduce tadpole hatchling inputs into the pond by directly consuming them. Hatchling inputs are also reduced by abiotic sources of mortality (desiccation and

submergence). To estimate the percent reduction due to predators and abiotic sources of mortality for each month, I compared the change in hatchling input for the entire pond over each 9-day (0-8d) clutch monitoring period from clutches with a given fate using the following equations for ambient inputs and inputs in the absence of predators and abiotic sources of mortality. To illustrate this approach, I provide an example for estimating the effect of snakes on hatchling inputs in June (month 6):

Equation 1:

$I_{Ambient6} =$

$(D_6 * h_{u6} * S_6 * V_{u6}) + (D_6 * h_{f6} * S_6 * V_{f6}) + (D_6 * h_{s6} * S_6 * V_{s6}) + (D_6 * h_{w6} * S_6 * V_{w6})$
$+ (D_6 * h_{a6} * S_6 * V_{a6})$

Equation 2:

$I_{S6'} = (D_6 * h_{u6} * S_6 * V_{u6}) + (D_6 * h_{f6} * S_6 * V_{f6}) + (D_6 * h_{s6} * S_6 * V_{u6}) +$
$(D_6 * h_{w6} * S_6 * V_{w6}) + (D_6 * h_{a6} * S_6 * V_{a6})$

Equation 3:

$I_{NS6} = (I_{S6'} - I_{Ambient6}) / I_{S6'}$

For the overall effect of predators only:
Equation 4:

$I_{NPx} = I_{NFx'} + I_{NSx'} + I_{NWx'}$

For the overall effect of abiotic factors only:
Equation 5:

$I_{NAx} = (I_{Ax'})$

Where D_x = the number of clutches per pond for a given month x, U_x is the proportion of undisturbed clutches for month x, S is mean clutch size for month x, V_{nx} is proportion survival for clutches in a given fate category (V_{ux} = Undisturbed, V_{fx} = Fly, V_{sx} = Snake, V_{wx} = Wasp, V_{ax} = Abiotic) in month x, h_{nx} is the proportion of clutches in a given fate category ($h_{ux,}$ = Undisturbed, h_{fx} = Fly, h_{sx} = Snake, h_{wx} = Wasp, h_{ax} = Abiotic) for month x. For example, I_{NS6} is the estimated hatchling inputs in the absence of snake predation in June, where proportion survival is equal to survival in undisturbed clutches $V_{u6.}$ For each month x, $I_{Ambientx}$ is estimated input given undisturbed clutches and all sources of mortality, I_{NPx} is estimated hatchling input in the absence of predators and I_{NAx} is estimated hatchling input in the absence of abiotic sources of mortality. I used parallel methods for flies, wasps and abiotic factors. Each density survey should capture one cohort, so these estimates yield hatchling inputs for the pond for a 6d (based on average hatching day) cohort period.

Terrestrial predators and abiotic sources of mortality also alter the phenotype of hatchlings entering the pond. To evaluate the phenotypic effect of predators and abiotic sources, I used one way ANOVAs to compare the mean day of hatching from undisturbed clutches to that of clutches experiencing different sources of mortality. Embryos of *A. callidryas* are able induce early hatching after day 4, therefore if predators attack a clutch prior to this window, clutches will have no behavioral defense. To better assess the importance of this particular response over the season (i.e., do most disturbance events occur prior to when the clutches are hatching competent?), I recorded the age of the clutch when the damage

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first occurred. Fly damage is most accurately detectable only after the larvae are visible, making it difficult to know exactly when the adult fly visited the clutch therefore, I focus on predators leaving the most obvious signs of predation, snakes and wasps.

Feeding Trials

Hatching success and timing alter the density and phenotype of tadpoles entering the pond, and previous work has shown that these phenotypic changes affect tadpole development and the onset of feeding (Warkentin 1999). *Agalychnis callidryas* rely on yolk stores immediately after hatching and the onset of active feeding depends on their developmental stage (Warkentin 1999). Early hatched tadpoles develop faster and begin feeding earlier resulting in higher larval growth rates than late-hatched tadpoles (Warkentin 1999, Vonesh unpubl). Based on these results, early-hatched tadpoles appear to be on a different growth trajectory than those tadpoles hatching later (i.e., 30 days post hatching early hatched tadpoles are larger than late hatched tadpoles). Therefore, throughout the breeding season differences in hatching time should result in differences in tadpole sizes. To begin to understand the relationship between tadpole size and their ability to consume aquatic resources, I conducted short term (24-hr.) feeding trials (Seale and Wassersug 1979). First, I held density constant (1 tadpole/400ml) and used different sizes of tadpoles (Mean ± SD; small: 2.09 ± 0.21 cm to large: 4.04 ± 0.29 cm). Second, I examined the effects of density on per capita tadpole foraging rates by holding size constant and manipulating density levels (1 – 4 tadpoles). By measuring ingestion rates for tadpoles over a range of sizes and densities, these studies will provide insight into how, for example, initial reductions in

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tadpole density might persist and possibly magnify as tadpoles become larger and ingestion rates change.

At the start of each experiment, 0.0200 ± 0.0002g ($\pm$ SD) of Seramicron® (finely ground algal food; Opitz et al. 2005, McCoy et al. 2007) was carefully added to each beaker along with 400mL of filtered, aged tap-water. To clear the gut of the majority of food particles all tadpoles were collected (all from "Experimental" Pond), individually separated according to size and held overnight without food. Tadpoles were allowed to acclimate in grazing chambers for 1 hr. before the trial began and temperature was maintained at approximately $25 \pm 3°C$ (Seale and Wassersug 1979). Room temperature measurements were recorded as well as dissolved oxygen and water temperature for a random subset (~10) of grazing chambers at the beginning and end of each feeding trial. At the end of 24 hours, tadpoles were removed from beakers and photographed for later size analysis using ImageJ image processing software (http://rsbweb.nih.gov/ij/) and then returned to the pond. At the end of each trial a subsample of the remaining water from each replicate was filtered onto a pre-ashed and weighed glass fiber filter (0.45 mm). Filters were dried at 60ºC for 48 hours, weighed and then re-dried for another 24 hours and weighed again to the nearest 0.0001g to obtain changes in total suspended solids (i.e., food concentration).

Ingestion rates for each treatment were calculated as the difference between the initial and final concentration of food (Frost 1972, Ranvestel et al. 2004). Controls were used to detect random variation when no tadpoles were present. Regression analyses were used to examine the relationship between ingestion rates and tadpole size and density.

Clutch Density and Oviposition Characteristics

Over the course of the study (June-November) I censused a total of 605 clutches. Clutch density varied temporally ($F_{4, 35}$ = 6.54, P < 0.001, Fig. 1). Mean clutch density was highest in July (mean ± SE, N = 8 transects throughout; 4.05 ± 1.21 m^{-1}) and lowest in November (0.15 ± 0.03 m^{-1}). During June, oviposition information was not recorded for all clutches; therefore, the following percentages are out of 598. The majority of clutches (79%) were laid over water, 16% over land and 5% unknown. The majority of clutches (57%) were laid on the bottom of the leaf, 36% on the top of the leaf and 6% were laid directly on the trunk or branch of the plant. Clutch height above water averaged 174 ± 3.76 cm (mean ± SE). I monitored the fates of 148 clutches, approximately 5,000 developing embryos and approximately 2,600 hatchling tadpoles. Average clutch size was 35 ± 16.71 eggs clutch^{-1} (mean ± SD) and varied temporally ($F_{4, 143}$ = 2.86, P = 0.03, Fig.2A). Average clutch size was greatest in September (40 ± 14.92) and lowest in June (29 ± 15.16). Neither oviposition characteristics (the subset measured for monitoring surveys: position over water or land, substrate top and position on leaf. Height above water was only measured in density surveys) nor clutch size were important for clutch success, mean day of hatching or occurrence of mortality (all P > 0.05).

Proportion of Clutches with Different Sources of Mortality

I examined if clutches experienced different sources of mortality (undisturbed, biotic and abiotic sources of mortality) and whether particular types of mortality varied throughout the breeding season. Over the study, the total proportion of clutches with different fates varied significantly (Repeated Measures ANOVA, $F_{2,67} = 5.46$, $P < 0.01$, Figs. 3,4). There was also a significant month by clutch fate interaction ($F_{8,67} = 6.70$, $P < 0.001$). The proportion of undisturbed clutches varied temporally ($F_{4,9} = 5.39$, $P = 0.02$) and the highest proportion was in September ($64 \pm 27\%$ Fig. 4A). Clutches with abiotic sources of mortality varied temporally ($F_{4,9} = 9.45$, $P < 0.001$, Fig. 4B). The mean number of clutches with abiotic sources of mortality was highest in July ($54 \pm 43\%$, Fig. 4B) and was entirely driven by submerged clutches. Snake predation also varied temporally ($F_{4,9} = 7.73$, $P < 0.01$) and was highest in October ($77 \pm 20\%$, Fig. 4E). The proportion of clutches with fly and wasp predation did not vary over the course of the season ($F_{4, 10} = 3.06$, $P = 0.07$, Fig. 4F) and ($F_{4, 9} = 7.73$, $P = 0.67$, Fig. 4D), respectively. Non-developed clutches were excluded from analyses do to low sample sizes (n=4; Fig. 4C).

Within Clutch Survival

Over the course of the breeding season, within clutch survival varied among clutches with different sources of mortality. Clutch survival also varied through time ($F_{4,39} = 2.18$, $P < 0.0001$, Fig. 2B). There was also an interaction between month and the source of mortality ($F_{13, 122} = 2.51$, $P < 0.01$). Proportion of embryonic survival was significantly different among clutches with different sources of mortality ($F_{4, 139} = 35.16$, $P < 0.001$; Fig. 3B).

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Mean proportion survival was greatest in healthy clutches (mean ± SE throughout; 79 ± 0.03%), followed closely by clutches with flies (74 ± 0.04%) and wasps (55 ± 0.07%). Survival was lowest in clutches with snake predation (15 ± 0.04%) and abiotic sources of mortality (25 ± 0.09%).

Predator and Abiotic Effects on Hatchling Phenotype

In addition to reducing the density of tadpole inputs, predators and abiotic factors also changed the phenotypes of hatchlings going into the pond. Clutches with different sources of mortality varied in the mean day of hatching ($F_{4, 104} = 9.91$, $P < 0.0001$, Fig. 3C). Clutches with no predation hatched on day 6. Embryos from clutches with snake predation (n = 37) hatched 13% earlier than undisturbed clutches. The mean age of clutches attacked by wasps was 3.10 ± 1.19d (mean ± SD, n = 23) and snakes (2.68 ± 1.27). The largest effects on hatchling phenotype came from abiotic sources (24% earlier). Specifically, submerged clutches (n = 11) hatched 23% earlier and dehydrated clutches (n = 8) hatched 29% earlier than undisturbed clutches (Fig. 3C). The mean day of hatching also varied by month, however ($F_{4, 104} = 4.26$, $P < 0.01$), there was no interaction between the source of mortality and month ($F_{12,88} = 0.80$, $P = 0.65$).

Predator and Abiotic Effects on Hatchling Inputs

Predator effects on hatchling inputs into the pond varied over the rainy season. In order to estimate tadpole inputs into the pond for each month, I used data on clutch density (Fig. 1),

mean clutch size (Fig. 2A), proportion of clutches (Fig. 4) and proportion survival (Fig. 3B) for clutches in each fate category. Peak ambient inputs occurred in August (4,471 hatchlings pond^{-1} 6d^{-1} cohort Fig. 5), followed by July (3,330 hatchlings pond^{-1} 6d^{-1} cohort Fig. 4). Inputs were lowest in October (225 hatchlings pond^{-1} 6d^{-1} cohort Fig. 5). In October the estimated reduction in hatchling inputs due to predators alone was 89% followed by 29% in July. In October this decrease was primarily driven by snakes (Fig. 4E), whereas in July, the effect was primarily due to wasps (Fig. 3F). Abiotic sources of mortality also varied temporally and were estimated to reduce tadpole inputs by 48% in July (Fig. 5) when heavy rains increased the perimeter of the pond by ~ 11m.

Laboratory Feeding Trials

Results from feeding trials show a positive relationship between both size and density and ingestion rate. However, in a few replicates, ingestion values were negative or zero, suggesting that tadpoles were perhaps stressed or unhealthy and consumed no food over the 24-hr. trial. I analyzed results with and without these values, and results were largely unaffected. Therefore, I present results without the negative values. Per capita ingestion rates increased with tadpole length ($F_{1,64}$ = 39.93, P < 0.0001, r^2 = 0.38, Fig. 6A) and decreased with increasing density ($F_{1,51}$ = 18.52, P < 0.0001, r^2 = 0.27, Fig. 6B). For density trials, mean tadpole length was 2.24 cm ± 0.03 (mean ± SE) and was not significantly different between density treatments ($F_{3,52}$ = 1.86 0, P = 0.1479).

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DISCUSSION

I found that terrestrial predators and abiotic factors influence the hatching success and timing of arboreal frog eggs. The most important factors that influenced hatching success were snakes and submergence (Fig. 3B) and these factors varied in their relative importance over the rainy season (Fig. 4). Early in the season submergence was the major source of mortality, and inputs were reduced by 48% when heavy rains resulted in drastic increases in the water level of the pond. Later in the season, predation became much more important and snakes reduced inputs by an estimated 77%. In addition to reducing the input of tadpoles into the pond, predators and abiotic factors also altered the timing of hatching. Again, these effects were largely driven by submerged clutches early in the season and clutches with snake predation later in the season (Fig. 4). The effect on the timing of hatching was greatest in submerged clutches, which hatched on average 18% earlier than undisturbed clutches (Fig. 3C). These results suggest that the changes in tadpole phenotype (i.e., size) are more important early in the season whereas, changes in tadpole density are more important later in the season.

Induced early hatching often serves as an effective means for *A. callidryas* to escape embryonic predators including wasps and snakes (Warkentin 1995, 2000, 2007). However, my results suggest that surprisingly, survival was very low in clutches that were attacked by snakes (14%) because snakes were extremely adept at consuming the entire clutch. It is

possible that snakes displaced leaves outside of the cup during an attack so that the hatchlings would not be caught and that my results overestimate mortality due to snakes. However, I was very careful to note displaced leaves and in the few cases where this did occur, I excluded the clutch from analyses. A more probable explanation is that snake attacks generally occurred on day 3 ± 1.52d, earlier than the window where embryos are capable of inducing early hatching. These results suggest that in this system, the magnitude of the density effects of snake predators may be greater than those caused by their phenotypic (non-lethal) effects.

In my study flies did not appear to cause any significant reduction in tadpole inputs. This result is surprising because dipteran larvae are often the major predator of arboreal eggs throughout the Old and New World (Villa 1980, Vonesh 2000). There are 2 species of flies Phoridae and Psychodidae, which are difficult to distinguish in the field. Laboratory studies have shown that extremely high infestations of *M. scalaris* (Phoridae) can cause substantial mortality (Hughey unpubl.). It is possible that the different species of fly might differ in their degree of damage or that fly densities might reach extremely high levels in some seasons or under certain conditions (e.g., prolonged drought), or that I missed clutches with flies that were located higher in the canopy. However, throughout the course of this study, proportion survival in clutches with flies was not significantly different from undisturbed clutches (Fig. 3B).

Implications for Later Life-stages

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Previous work has shown that prey phenotypic responses to predators in one life stage can have important implications for later life stages of organisms with complex life cycles (Vonesh and Osenberg 2003, Vonesh and Bolker 2005, Vonesh 2005, Vonesh and Warkentin 2006). Theory suggests (Werner and Gilliam 1984, Werner 1986) and previous studies have shown (reviewed in Skelly and Werner 1990) that the optimal timing of hatching is maintained by a tradeoff between predation risk in the embryonic and larval environments because larval size often confers a size refuge depending on the type of aquatic predators (Kaplan 1992, Warkentin 1995, Warkentin 1998, Vonesh and Bolker 2005). My study focused on terrestrial sources of mortality however, hatched tadpoles fall into the water below where they face an entire suite of aquatic predators. Short-term predation trials show that older hatchlings are larger, more developed and less susceptible to 5 out of 6 aquatic predators (Vonesh and Warkentin unpubl). Additionally, both density and size have important implications for intraspecific competition (Wilbur 1996, Vonesh and Osenberg 2003). For example, if larval survivorship is density dependent, reduced egg stage survival may reduce larval competition sufficiently resulting in the same number of metamorphs as those found at higher larval densities (Vonesh and De la Cruz 2002). Because the affect of abiotic factors and predators on the size and density of tadpole inputs into the pond varies over the season, the risk associated with aquatic predators and intraspecific competition should also vary over the season.

Potential Across System Implications

Organisms with complex life cycles represent significant energy transfers between the terrestrial and aquatic environments (Wassersug 1975, Burton and Likens 1975, Seale 1980, Morin 1981, Baxter et al. 2004, Regester et al. 2006,). Studies have shown that tadpoles can significantly affect aquatic food web dynamics in a wide range of temperate (Seale 1984, Kupferberg 1997, Kiffney and Richardson 2001) and neotropical habitats (Flecker et al. 1999, Ranvestel et al. 2004, Whiles et al. 2006).

By affecting the success and timing of hatching, terrestrial predators and abiotic factors altered both the density and phenotype of tadpoles entering the pond. Early in the season (July) submergence induced early hatching, resulting in relatively smaller, less-developed tadpoles entering the pond. Previous work has shown that early-hatched tadpoles develop faster and begin feeding earlier than late-hatched tadpoles (Warkentin 1999). Furthermore, differences in hatching of only half a day were enough to cause significant differences in the onset of feeding in early-hatched *A. callidryas* due to differences in morphological development (Warkentin 1999). Results from my feeding trials show that for every 1 cm increase in tadpole length, per capita ingestion increased by 3.50 mg/ind/d (Fig. 6A). In addition, July also had the highest density of clutches (Fig. 1). Results from the feeding trials show that per capita consumption rates are non-additive and decrease with increasing density (Fig. 6B). These results suggest that early in the season, when submergence is the primary threat, mortality is low because of induced early hatching. Therefore, density is high, tadpoles begin feeding earlier and develop faster, resulting in high resource demand. Later in the season, when predation is high, fewer tadpoles enter the pond, which could

reduce algal resources demand. However, if the larval environment is density dependent, lower densities could reduce competition for resources, thus each tadpole should be growing maximally, and overall ingesting more phytoplankton.

Combined, both the high density and early onset of feeding suggest that early in the season, tadpole ingestion should be large with subsequent effects on the aquatic food web. Later in the season, snake predation reduced the density of tadpoles entering the pond. Over the course of the season, the mechanisms underlying the effects of predator and abiotic effects on tadpole inputs vary. Changes in size and density also affect tadpole ingestion in different ways and suggest that both may counter intuitively increase tadpole effects on the aquatic foodweb. These results suggest that the interaction between predators and abiotic factors have important effects on initial reductions in tadpole size and density that might persist and possibly magnify as tadpoles become larger and ingestion rates change.

Conclusions

Ingestion rates for *A. callidryas* (mean ± SD, 10.8 ± 4.35mg/ind/d, Fig. 6) are comparable to ingestion and filtration rates for various species of zooplankton (reviewed in Reynolds 1984, Lampert et al. 1986) and zebra mussels, *Dreissena polymorpha* (Roditi et al. 1996), which are known to cause dramatic changes in aquatic food webs (Brooks and Dodson 1965, Carpenter and Kitchell 1987, DePinto and Narayanan 1997). Ultimately, understanding the effects of terrestrial predators on aquatic resources mediated through

prey with complex life cycles will require integrating the effects on all larval ontogeny. However, taken together these results illustrate that understanding the temporal variation in the relative importance of density and phenotypic (trait-mediated) predator and abiotic effects can help us understand important underlying mechanisms that link aquatic and terrestrial food webs.

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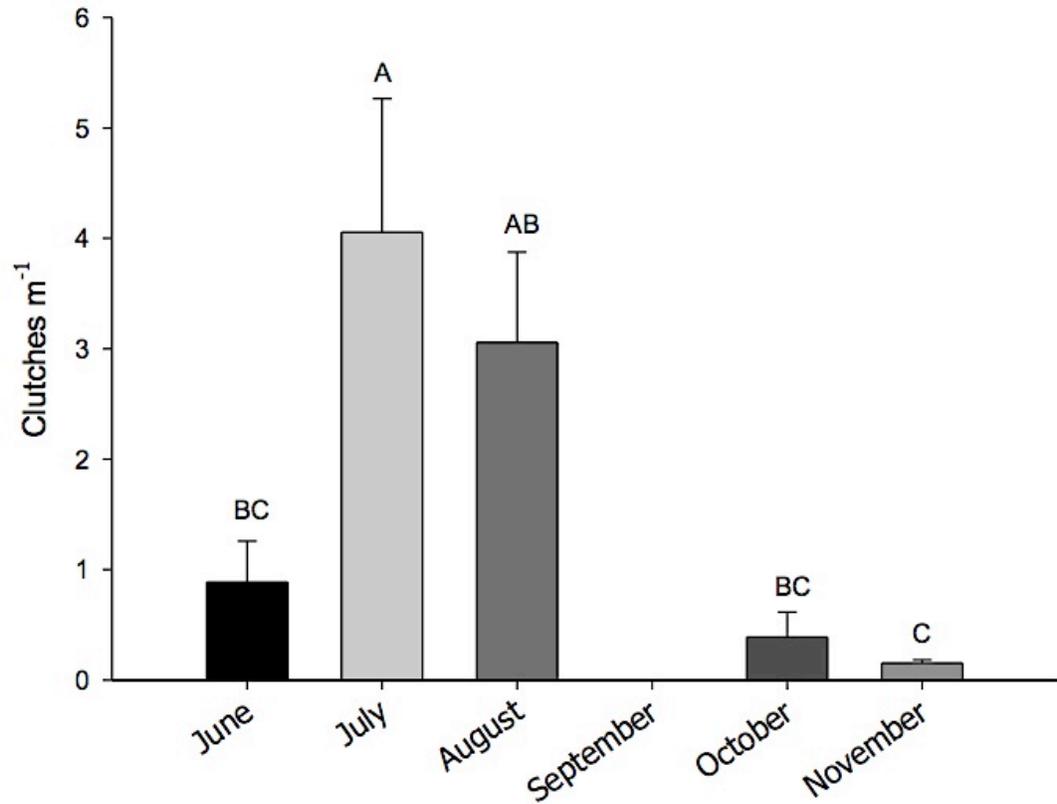
Figure 1: Clutch density for *A. callidryas* through rainy season. Mean ± SE
for: A) Clutches m^{-1} from June-August and October-November. "*" Low
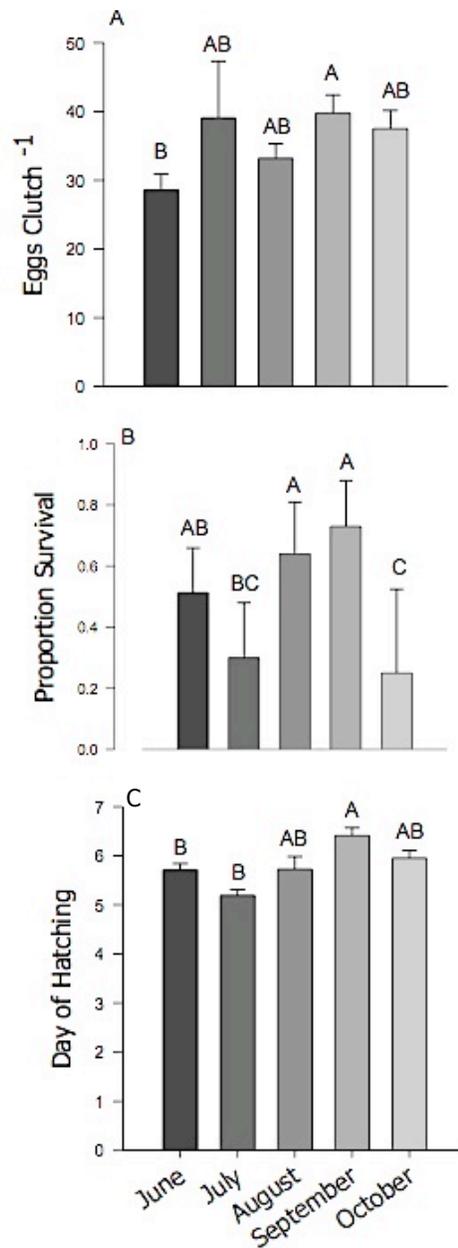levels of rainfall at the beginning and end of September precluded density surveys,
n = 9 transects.

Figure 2: Clutch characteristics for *A. callidryas* through rainy season. Mean ± SE for: A) Number of eggs per clutch, B) Proportion survival from June-October, C) Mean day of hatching from June-October, n = 605 clutches. Proportional survival data were arcsine-square root transformed prior to analysis. Different letters above bars represent statistically significant differences based on post hoc Tukey's HSD.
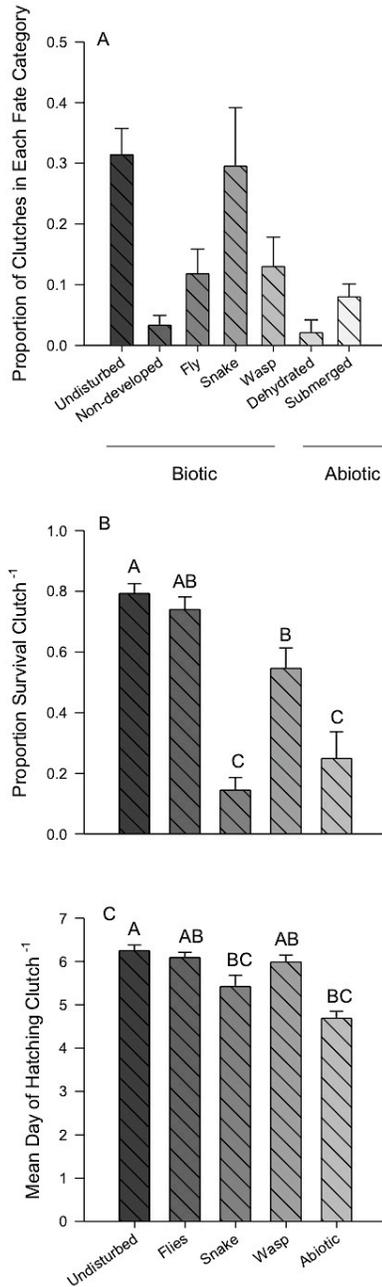
Figure 3: Factors affecting clutch survival and hatchling phenotype. Means ± SE for: A) Proportion of all clutches in each mortality category, B) Proportion survival and C) Mean day of hatching in clutches with different sources of mortality, n = 4 quadrants. Bars indicate means ± SE. Bars with different letters identify statistically significant differences based on post hoc Tukey's HSD.
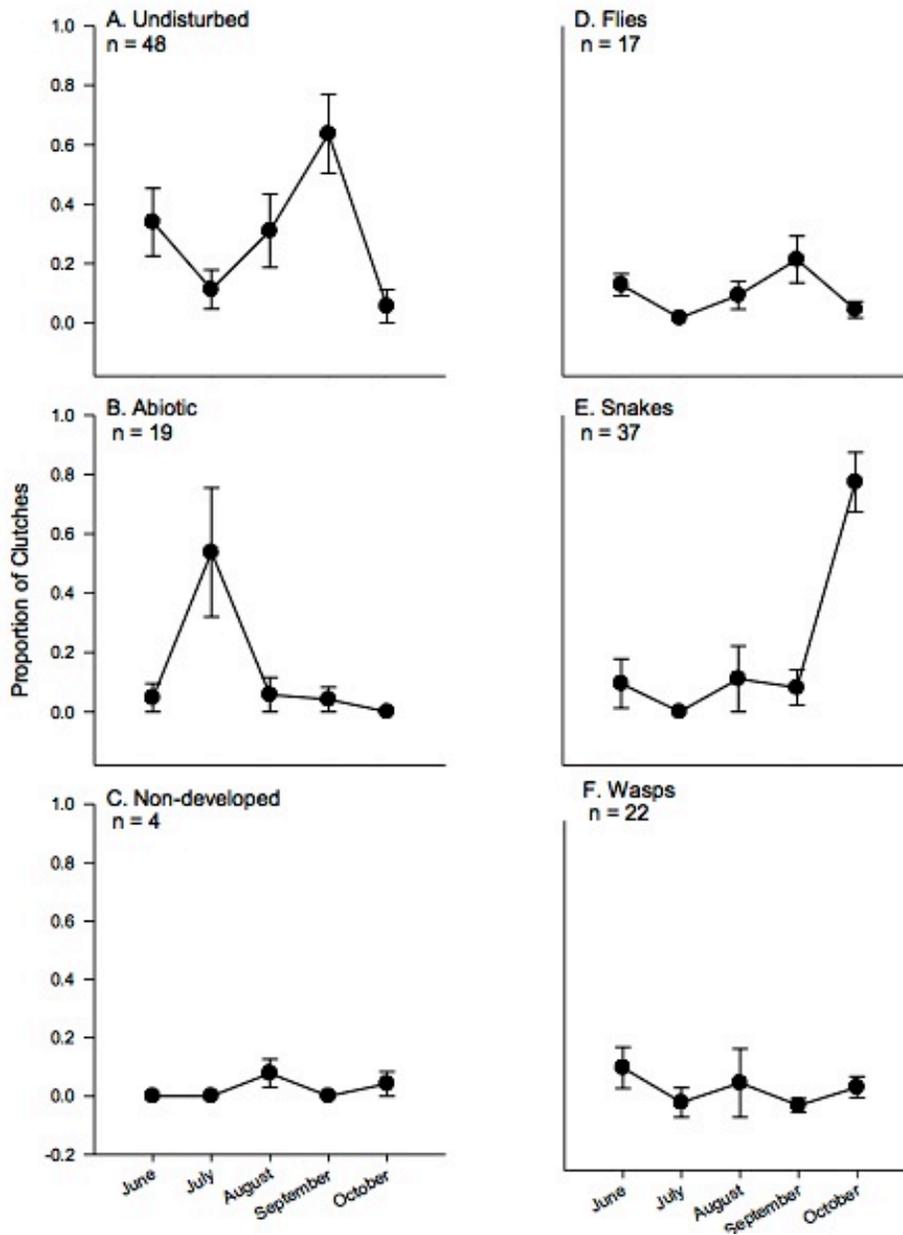
Figure 4: Variation in the proportion of clutches in each fate category throughout the study. Mean proportion ± SE for: A) Undisturbed clutches, B) Clutches with abiotic sources of mortality (primarily due to submerged clutches), C) Non-developed clutches, D) Fly larvae, E) Snakes and F) Wasps. Based on replicate quadrants for June and July n = 2 for August n = 3, September and October n = 4.
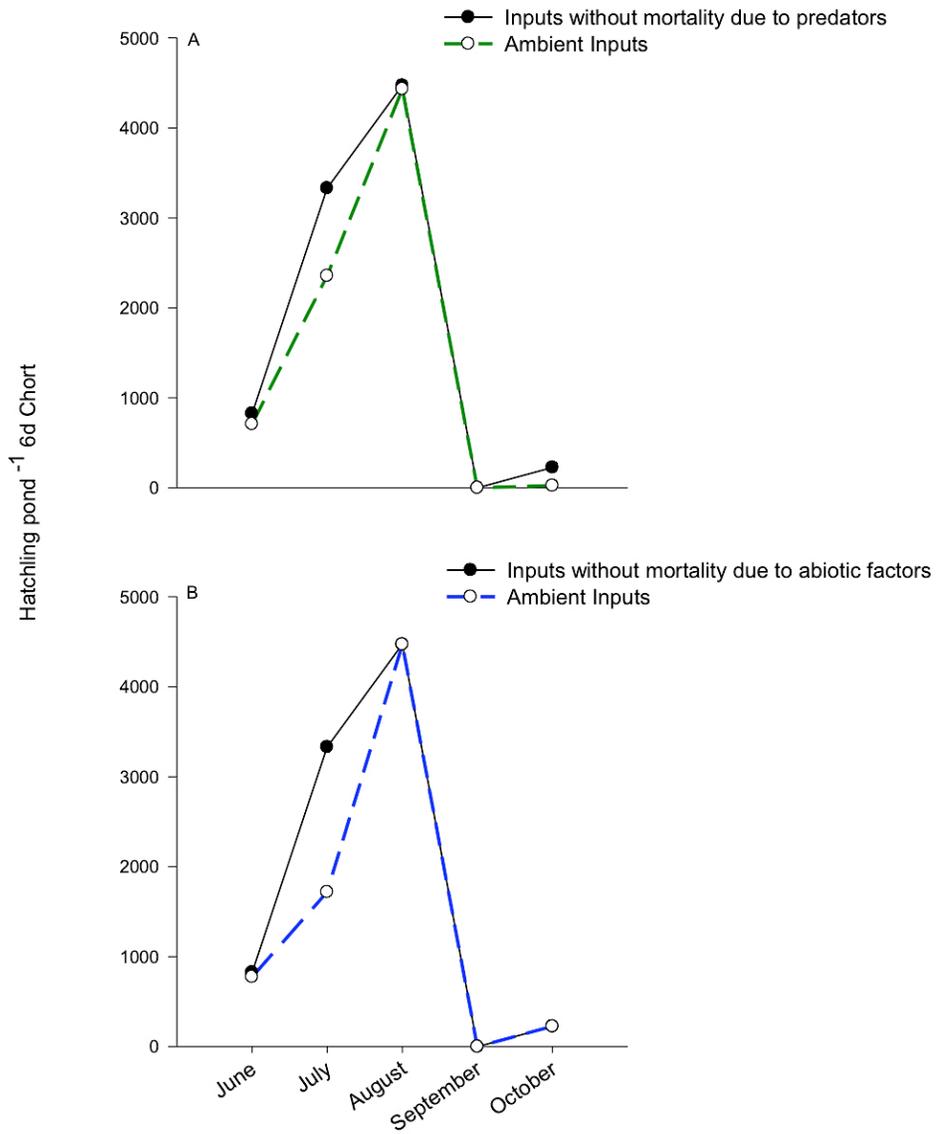
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Figure 5: Overall estimated reductions in hatchling input for Ocelot pond over each monitoring session due to mortality from A) predators and B) abiotic factors.
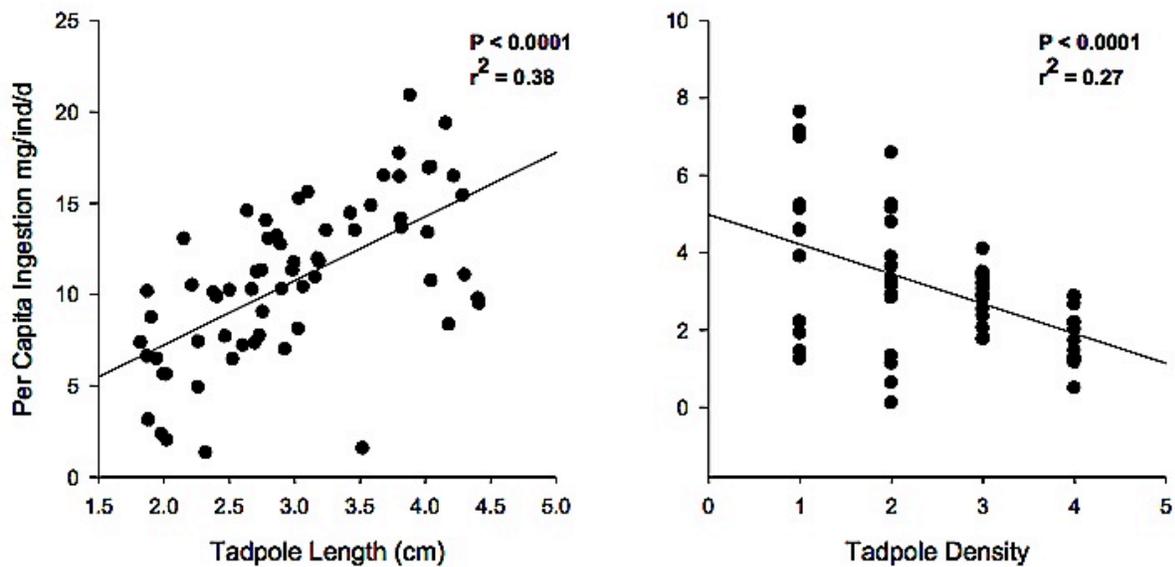
Figure 6: Ingestion rates for individuals of different size classes A) n = 72 and densities B) n = 53, from laboratory feeding trials conducted over 24 hours. Negative values have been removed to account for spurious results possibly due to tadpole stress, illness or lack of motivation.

VITA

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