Possible effects of climate change on induced defenses in tadpoles

By: Alyssa D'Alessandro

July, 2015

Director of Thesis: Heather Vance-Chalcraft, PhD

Major Department: Biology

Global climates are changing and the rate of change is expected to increase in the next century. Researchers predict that temporary ponds will be affected, resulting in shorter average hydroperiods (the amount of time a pond holds water). Species that live in these ponds will face increased stress due to these environmental changes, which may affect the timing of yearly events and alter community composition. For example, important pond predators, such as dragonfly nymphs, may emerge from ponds in the autumn instead of overwintering in the pond.

Amphibians are important components of temporary pond systems, and their tadpoles can exhibit a great amount of plasticity in morphology and life history traits due to environmental change. For example, tadpoles can alter their morphology in response to specific predators. These induced defenses have been found to increase survival with predators but to decrease fitness in the absence of predators; thus, the defenses are costly. This phenotypic plasticity also has limits, in that some species have not been found to use them and other tadpole species are limited in the circumstances in which they can induce changes.

I measured the morphological and life history responses (survivorship, larval period, and mass at metamorphosis) of tadpoles to simultaneous changes in hydroperiod and predator composition. I used Cope's gray treefrog tadpoles, *Hyla*

chrysoscelis, as prey in an experiment that crossed two hydroperiod length treatments (short, long) with four predator treatments (caged *Anax* dragonfly nymph presence/absence x caged *Belostoma* water bug presence/absence) to address two questions. First, do the two factors of hydroperiod and predator presence interact either synergistically or antagonistically? Secondly, do tadpole responses, or their costs, to each predator differ and do tadpoles respond to the more lethal predator (*Anax*) when both predators are present?

I did not see evidence of morphological induced defenses in Cope's gray treefrog tadpoles, nor did I observe effects of predator presence or shortened hydroperiod on tadpole developmental rate, size or survivorship. There was, however, an effect of hydroperiod on the within-tank variance in tadpole larval period and mass at metamorphosis. For both variables, longer hydroperiods significantly increased within-tank variance relative to shorter hydroperiods.

The lack of morphological changes in my experiment runs counter to my expectations, but highlights the fact that induced defenses are context-dependent. The concentration of predator cue present in the water, conspecific density, and abiotic stressors may all reduce tadpoles' ability to respond to predators. Thus, some tadpoles may not be able to perform well in conditions with shortened pond hydroperiods and changing community compositions.

Possible effects of climate change on induced defenses in tadpoles

A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of the Requirements for the degree

Master of Science in Biology

By

Alyssa D'Alessandro

July, 2015

Possible effects of climate change on induced defenses in tadpoles

By

Alyssa D'Alessandro

Approved By:

Director of Thesis: _____
Heather Vance-Chalcraft, PhD

Committee Member: _____
David Chalcraft, PhD

Committee Member: _____
Kyle Summers, PhD

Committee Member: _____
Kevin O'Brien, PhD

Chair of the Department of Biology: _____
Jeff McKinnon, PhD

Dean of the Graduate School: _____
Paul J. Gemperline, PhD

Acknowledgments

I would like to thank my advisor, Dr. Heather Vance-Chalcraft, for her assistance and guidance at every stage of this project, and for her guidance in navigating the intimidating world of graduate school and academia. I would also like to thank my committee members, Dr. David Chalcraft, Dr. Kyle Summers, and Dr. Kevin O'Brien, for their helpful suggestions and assistance in the editing process. Special thanks go out to Dr. Chalcraft, for the use of many of the materials for this experiment including the photochambers and cattle tanks. I would also like to thank Dr. Mike McCoy, who performed the analysis of the geometric morphometrics data and was also very helpful in the interpretation of the results.

I would like to thank several fellow graduate students, including Scott Jones, Rick Trone, Lauren McCarthy, Suelen Calado-Tullio, and Bevin Blake for their help during the experiment, including setting up and tearing down tanks (in the heat) and their advice about experiments and analyses. I was also lucky to have had the assistance of two detail-oriented, hardworking undergraduate assistants, Kyle Luke and Genesis Munoz, in the landmark-placement stage of the analysis; without them I might still be processing tadpole pictures today.

Finally, I would like to thank the ECU Biology Department, especially Dr. Terry West, for financial support throughout graduate school, and for always being helpful when I had questions about anything from classes to the graduation process.

Table of Contents **Page**

List of Tables

List of Figures

CHAPTER 1

Introduction

Over the next century, temperature is projected to increase from 2.5-5°C in the southeastern U.S. (USGCRP 2009).   Precipitation is predicted to have a less dramatic increase, but become more variable and episodic. The result will be more drought periods followed by downpour events, with fewer low-intensity rain episodes (USGRCP 2009, Brooks 2004). These changes will cause evapotranspiration to increase disproportionately over precipitation. Temporary ponds, also called vernal or ephemeral pools, dry periodically and are likely to be particularly sensitive to climate change because they are so strongly influenced by the balance between precipitation and evapotranspiration (Brooks 2009, Bauder 2005). Thus hydroperiod, or the amount of time throughout the year a pond holds water, in temporary ponds is very dependent on climatic factors, and is expected to shorten in some locations (Brooks 2004), but may lengthen or simply become more variable in others.

Even without climate change, pond hydroperiod is temporally and spatially variable (Welborn et al. 1996) and is a very important factor in determining the biological structure and function of temporary pond communities (Foti et al. 2012, Sim et al. 2013, Amburgey et al. 2012, Walls et al. 2013, Schriever & Williams 2013). The predator composition of ponds varies with pond hydroperiod, with fish inhabiting permanent ponds but being absent from more ephemeral water bodies (Welborn et al. 1996). Due to their complex life cycle, hydroperiod is especially important to amphibian larvae. For example, hydroperiod has been shown to impact density-dependent processes in tadpoles (Rogers & Chalcraft 2008). In addition, amphibian species have different development rates, but each species has a minimum duration for which a pool must be

flooded for the larvae to develop. Tadpoles have a great deal of plasticity in their speed

of development and while some tadpole species may be able to use plasticity in

development time to adjust to changing hydroperiods (Wilbur 1987, Denver et al. 1998,

Gomez-Mestre et al. 2013), these responses will likely result in smaller size at

metamorphosis (Gomez-Mestre et al. 2013) and may only be possible in the absence of

competitors or food limitations (Wilbur 1987). Moreover, some tadpoles may not be able

to metamorphose fast enough to escape shorter hydroperiods of ponds in the coming

years (Amburgey et al. 2012).

Climate changes will impact temporary pond species in other ways beyond

changing hydroperiods. The phenology of many species is already changing (reviewed

by Parmesan 2006) in response to increased temperatures and other climate changes,

with more drastic changes predicted for the future. Increased average temperature is

causing autumn-breeding amphibians to breed later and spring-breeding amphibians to

breed earlier (Todd et al. 2010). In addition, there is a large body of theoretical and

experimental research on the predicted effects of climate change on Odonates

(dragonflies), whose nymphs are important predators in temporary ponds. Temperature

has been shown to affect developmental rate, phenology, immune function, behavior,

and trophic interactions of dragonfly nymphs (reviewed by Hassall and Thompson

2008). Dragonfly spring emergence from ponds and developmental durations have also

been shown to change in response to climate changes in long-term survey data

(Hassall et al. 2007, Dingemanse and Kalkman 2008, Doi 2008), experimental

manipulations (McCauley et al. 2014), and models (Richter et al. 2008, Söndgerath et

al. 2012). As a result, the timing of the presence of dragonfly nymphs in ponds is expected shift as climate changes.

Changes in predator phenology caused by climate change will alter the predator composition of temporary ponds, for at least some parts of the year. For example, while species like dragonfly nymphs may alter the timing of their presence in ponds, other species, like giant water bugs (family *Belostoma*), may be constantly present. Thus, tadpole populations likely will need to respond to changing predator communities at the same time as changing pond hydroperiods. Just as tadpoles can sometimes adjust their development speed in the face of changing hydroperiod, most species also use morphological, behavioral, or other phenotypic changes in response to predators (Relyea 2003, Carfagno et al. 2011). This type of phenotypic plasticity, called an induced defense, has been shown to increase the fitness of induced morphs in the presence of predators but reduce the fitness of those morphs in the absence of predators (McCollum & Van Buskirk 1996, Van Buskirk et al. 1997, Van Buskirk & Relyea 1998, Laurila 2000, Relyea 2002b, Van Buskirk 2002, Kishida & Nishimura 2005). Costs of induced defenses by tadpoles may include decreased survivorship in the absence of predators (McCollum & Van Buskirk 1996), slower growth (Van Buskirk & Relyea 1998, Van Buskirk 2000, Skelly & Werner 1990), and lower fecundity. Competitive ability may also be reduced (Relyea 2002a). These costs may be exacerbated when food supplies are limiting (e.g. LaFlandra & Babbitt 2003).

Tadpole induced defenses may be general and effective against most predators, such as a decrease in activity and increase in refuge use (Chivers and Smith 1998), or specific to particular predators. If generalized defenses are always used, changing

predator composition will not change the induced defenses needed. However, studies

have shown that prey often match their defenses to specific predators. For example,

when Eastern gray treefrog (*Hyla* versicolor) tadpoles were raised in the presence of

either mud minnows, dragonfly nymphs, tiger salamander larvae, or giant water bugs,

tadpoles produced significantly different phenotypes in response to each of the four

predators (Relyea 2001). Defenses against one predator may not be as effective

against a second predator, as tadpoles that are induced by one predator have reduced

fitness when there are different predators present (Bernard 2006). Using specialized

defenses against specific predators may be costly, however, as it is necessary to

maintain chemical receptors for different types of predator cues (Van Buskirk &

McCollum 2000).

When multiple predator species are present, they may induce similar or opposite

trait responses in their prey (Relyea 2003). In both cases, there may be a hierarchical

response according to the perceived dangerousness of the predator (Relyea & Werner

1999, Relyea 2000, reviewed by Relyea 2003). For example, when *Rana* tadpoles were

exposed to the nonlethal presence of fish and *Aeshna* dragonfly nymphs separately,

they induced different morphs (Teplitsky et al. 2004). When simultaneously exposed to

both nonlethal predators, they induced the same morphology as in the *Aeshna*

dragonfly treatment, which was the more lethal predator (Teplitsky et al. 2004). If

climate change impacts the presence of the most lethal predator in temporary ponds,

prey may induce different morphs from what was historically present.

I used Cope's gray treefrog tadpoles as prey in an experiment that crossed two

hydroperiod length treatments (short, long) with four predator treatments (caged *Anax*

4

dragonfly nymph presence/absence x caged *Belostoma* water bug presence/absence). I chose these predators because Anax is likely to change the timing of its presence in ponds as climate changes, while Belostoma is not. By comparing tadpole morphological differences and potential costs (i.e., on survivorship, larval period, and mass at metamorphosis) associated with any morphological changes, I addressed two questions. First, do the two factors of hydroperiod and predator presence have opposing effects on tadpoles? Secondly, do tadpole responses, or their costs, to each predator differ and do tadpoles respond to the more lethal predator (*Anax*) when both predators are present?

   If hydroperiod shortens, tadpoles will be under more pressure to develop quickly and metamorphose before the pond dries. Therefore, it could reduce the likelihood of tadpoles producing induced defenses, or impose a greater cost to these defenses. Thus, I hypothesized that tadpoles in short hydroperiod tanks would be less likely to show morphological change in the presence of predators than those in long hydroperiod tanks, and that tadpoles with induced defenses in long hydroperiod tanks would be smaller at metamorphosis and have lower survivorship than uninduced morphs. Larval period should be under conflicting pressures since short hydroperiod has been shown to shorten (e.g. Amburgey et al. 2012) and predator presence has been shown to lengthen (e.g. Hettyey et al. 2011) larval period in tadpoles. Therefore, shortened hydroperiod combined with predator presence may interact antagonistically and produce effects on larval period in treatments with shortened hydroperiods and predators that are comparable to those in control tanks (long hydroperiod and no predators).

Only a few studies have examined morphological responses of Cope's gray treefrogs to *Anax*. They found deeper, more brightly colored tails in response to *Anax*, as well as lower survival of induced morphs in the absence of predators (McCollum & Van Buskirk 1996, Richardson 2006). The responses of Cope's gray treefrogs to *Belostoma* have not been examined to my knowledge. However, closely related Eastern grey treefrogs produce shallower tails with deeper tail muscle, and wider bodies when exposed to *Belostoma* (Relyea 2001). I expected that Cope's grey treefrogs would produce deeper tails and shorter bodies in response to *Anax*, and shallower tails in response to *Belostoma*. When both predators are present, I expected that tadpoles would respond to *Anax*, typically the more dangerous predator (Relyea 2003), and have similar morphology to tadpoles raised with *Anax* only.

Methods

Study System

This experiment was conducted at East Carolina University's West Research Campus, using 1100-liter cattle tanks to simulate natural ponds. Each tank had a screen top to prevent colonization by other organisms, and rotatable stand pipes to adjust the water level. The tanks each received a 400 mL inoculation of plankton from a local pond as well as 750 g of leaf litter of mixed composition: mainly pine (*Pinus sp.*), with some ash (*Fraxinus sp.*), oak (*Quercus sp.*), and holly (*Ilex sp.*). The tanks were filled with 50 cm of well water and left for three weeks to allow the plankton to establish.

Cope's gray treefrog (*Hyla chrysoscelis*) was chosen as the prey species for this experiment because it is an abundant tree frog species in eastern North Carolina that commonly oviposits in temporary pools and has been shown to induce morphological

defenses (McCollum and Van Buskirk 1996, McCollum and Leimberger 1997, and Richardson 2006). Pairs of amplexed gray tree frogs were collected from three sites near Winterville, NC. 15 clutches of tadpoles were counted out and evenly distributed among the 40 experimental units, so that each tank received 200 tadpoles comprised of individuals from all 15 clutches. The tadpoles were added to the tanks on June 16, 2014.

The predators that were used for the experiment were dragonfly nymphs (*Anax sp.*) and giant water bugs (*Belostoma sp.*). *Belostoma* were collected from a large pond in Greenville, NC, and *Anax* were collected from the Croatan National Forest. Predators were weighed and measured (length for *Belostoma,* length and head width for *Anax*). All predators were caged, and were added to the tanks on June 14, 2014. Predators of similar size were grouped into the same block.

Experimental Design

There were two hydroperiod treatments crossed with four predator treatments for a total of 8 treatments. Each of the eight treatments was replicated five times in a randomized block design for a total of 40 tanks. Blocks were used to account for differences in predator sizes and all experimental procedures were carried out on a block-by-block basis. The two hydroperiod treatments were short (manipulated) and long (unmanipulated). The short hydroperiod tank water levels were lowered according to a nonlinear drying schedule that is similar to what would occur in a natural NC pond (Wilbur 1987). The target drying date was chosen to be approximately the minimum number of days required for gray tree frog tadpoles to develop, according to historical observations (50 days) (Wright 1932). When the water reached 10 centimeters, the

tanks were destructively sampled (July 26-28, 2014), and any remaining tadpoles were weighed and released. The long hydroperiod tanks' water levels were maintained at 50 cm. After five days in a row with no metamorphs following peak metamorphosis, all long hydroperiod tanks were also destructively sampled (August 16-17, 2014) and any remaining tadpoles weighed and released. Thus, short hydroperiod treatments ended before the long hydroperiod treatments. Tanks were checked daily throughout the course of the experiment. As tadpoles metamorphosed, they were removed from the tanks and taken back to the lab until their tails were completely resorbed. On that day, they were weighed and then released within 48 hours near the sites where their parents were collected.

The predator treatments were: no predators, one *Anax*, one *Belostoma*, and both predators (one individual of each species). Predators in tanks were caged (each species caged separately), and tanks that did not have a predator had empty cages. The *Anax* cages were composed of a section of PVC pipe with window screen on the ends so that water (and olfactory cues) could circulate in and out of the cages. The *Belostoma* cages were constructed of a floated plastic cup with holes so that water could circulate. Both types of cage included a piece of vegetation to act as a predator perch. Predators were fed three gray tree frog tadpoles every three days from different clutches than the experimental tadpoles, but of the same age. Some of the dragonflies emerged during the experiment; these were released and then replaced within 48 hours.

Response variables recorded during the experiment were larval period (number of days from egg hatching to tail resorption), mass at metamorphosis, survival, and

periphyton levels. Periphyton samples were taken from each tank at the end of the short

hydroperiod treatments to compare standing stock of algae. Periphyton levels were

measured using a periphytometer (sections of flagging tape submerged inside the

mesocosm). The periphyton was analyzed by removing the periphyton on an 8cm area

of the flagging tape, collecting the periphyton via filtration on glass filters, freezing filters,

and then using fluorometric analysis to determine chlorophyll-a levels (Arar and Collins

1997). It is hypothesized that tadpoles under the risk of predation will forage less

because they will be less active (Van Buskirk 2000), so there may be higher algal

contents in predator treatments.

To determine shape, tadpoles were photographed twice during the experiment

using a digital camera and photochamber (designed as in Van Buskirk & McCollum

2000, Davenport & Chalcraft 2013). Tadpoles were haphazardly selected from each

tank and sedated using a dilute solution of MS-222, a common aquarium fish

anesthetic, using standard procedures (Wingerter 2010, AVMA Guidelines 2013).

Tadpoles were returned to their tanks after recovery from the anesthetic. The first round

of pictures was taken about halfway through development, (21-25 days) and the second

round was taken near the end of development (35-37 days).

Analysis

Both rounds of digital images of tadpoles were imported into Image J software

(Rasband, National Institute of Health), and analyzed using geometric morphometrics, a

technique that is able to capture more of the variation in tadpole morphology than

simple linear measurements (Arendt 2010). 14 landmarks were placed on pictures of

ten tadpoles from each tank, from both rounds of pictures. Landmarks (Figure 1) were

placed in similar locations to previous studies (Dayton et al. 2005). The (x,y)

coordinates of the 14 points were imported into R (The R Core Team 2015), and

analyzed using the Geomorph package (Adams & Otarola-Castillo 2013). Generalized

Procrustes Analysis (GPA), a standardization method, was used to superimpose the

shapes of the tadpoles and remove differences in orientation and position, and scale the

tadpoles to the same size, so that all that remained were differences in shape. Centroid

size, the sum of squared distances of the 14 landmarks from their centroid

(mathematical center), was calculated for each individual tadpole. The procrustes

distances from the GPA were analyzed using a Procrustes ANOVA with 999

permutations, with centroid size as a covariate and *Anax*, *Belostoma*, and hydroperiod

as main factors, along with all possible interactions. Size interactions were found to be

nonsignificant and were removed from the model.

Variation in the mass at metamorphosis among individuals within a tank was best

described by a lognormal distribution. Thus, I estimated the average mass at

metamorphosis for individuals within a tank as the geometric mean mass at

metamorphosis for individuals within the tank. I estimated the instantaneous mortality

rate of tadpoles in tanks as the natural log of the proportion of individuals that survived

during the experiment. Total survivorship includes the total number of metamorphs that

came out of each tank plus the number of tadpoles that were left at the end of the

experiment. Another response variable, "realistic survivorship", does not include the

tadpoles that were left in the short hydroperiod tanks when they were taken down,

because in nature, these tadpoles would have died when the pond dried.

In addition, I calculated the coefficient of variance (CV) for larval period and mass at metamorphosis for each tank to examine levels of within tank variability. Variation around the mean as a response variable has been historically neglected in ecological research, yet there have been suggestions in the literature that interpreting variation within populations will be a key factor in predicting their responses to environmental variation, such as will occur with global climate change (e.g. Benedetti-Cecchi 2003). Effects of treatments on the geometric mean of mass at metamorphosis for each tank, mean time to metamorphosis per tank, CV of mass at metamorphosis, CV of time to metamorphosis, natural log of total and realistic survivorship, and chlorophyll-a content were analyzed by a mixed model factorial ANOVA in SAS Enterprise Guide ® (v. 6.1, © 2013) using hydroperiod, *Anax*, and *Belostoma* as fixed effects and block as a random effect.

Results

There was a significant effect of size on tadpole shape (Round 1: $F_{1,31} = 7.9286$, $p = 0.001$; Round 2: $F_{1,31} = 20.1880$, $p = 0.001$). However, there were no significant effects of hydroperiod (Round 1: $F_{1,31} = 0.8139$, $p = 0.672$; Round 2: $F_{1,31} = 0.5175$, $p = 0.794$), *Anax* (Round 1: $F_{1,31} = 0.6062$, $p = 0.961$; Round 2: $F_{1,31} = 0.7202$, $p = 0.630$), or *Belostoma* (Round 1: $F_{1,31} = 0.5871$, $p = 0.938$; Round 2: $F_{1,31} = 0.2896$, $p = 0.951$). There were also no significant interactions between *Anax* and *Belostoma*, *Anax* and hydroperiod, *Belostoma* and hydroperiod, or all three factors (Table 1). None of the factors (besides tadpole size) explained more than 2% of the variation in tadpole morphology in either round of pictures (Table 1).

There were no significant effects of *Anax*, *Belostoma*, or hydroperiod on tadpole mass at metamorphosis (Figure 3), larval period (Figure 4), total survivorship (Figure 5), or realistic survivorship (Figure 6) (Table 2). There was a significant interaction between *Belostoma* and hydroperiod for realistic survivorship (p = 0.0425, Table 2), in which realistic survivorship was lower in short hydroperiod tanks than in long hydroperiod tanks only when *Belostoma* were present (Figure 6). There was also a trend for the mass at metamorphosis to be larger in the presence of Belostoma alone, but not when both predators were together (Anax*Belastoma p=0.0840, Table 2, Figure 3).

The amount of within-tank variance (i.e., CV) for larval period (p = 0.0118, Figure 7) and mass at metamorphosis (p = 0.0427, Figure 8) was significantly lower in short than in long hydroperiod treatments. Furthermore, presence of *Belostoma* caused a nearly-significant reduction in within-tank variation in larval period (p = 0.0852, Table 2) compared to tanks without *Belostoma* present (i.e., *Anax* and no predator tanks). None of the remaining factors or interactions accounted for significant differences (Table 2).

There were no significant effects of any of the three factors on the chlorophyll-a concentrations from periphyton samples in the tanks (Figure 9) and none of the interactions explained a significant amount of variation in chlorophyll-a concentration (Table 2).

Discussion

My research combines three "hot topics" in current research: wetlands, amphibians, and climate change. Temporary ponds, because of their dependence on precipitation, will be increasingly vulnerable to climate change as precipitation patterns become more variable. In addition, climate changes may drive changes in the

phenology of temporary pond species, in which species like dragonfly nymphs may no longer be present in ponds at the same times as they have been historically. I predicted that combining changes in pond hydroperiod and predator composition would affect gray treefrog tadpole morphology and life history traits since some species of tadpoles are capable of changing these traits in response to predators (Relyea 2001, 2003, 2004) and hydroperiod (Loman 2002) separately. However, in this experiment, I did not see evidence of morphological induced defenses in Cope's gray treefrog tadpoles, nor did I observe any costs of predator presence or shortened hydroperiod in tadpole developmental rate, size or survivorship.

The lack of morphological changes in my experiment runs counter to my expectations, based mainly on cited morphological changes in a closely related species, Eastern gray treefrogs. Only a few prior studies have examined the effect of *Anax* on Cope's gray treefrog (McCollum & Van Buskirk 1996, Richardson 2006), and they found induced responses in the form of a deeper tail and differing color pattern on the tadpoles' tails. I am unable to quantify color pattern due to lighting differences among the tadpole photographs, however I found no support for deeper tails in the presence of *Anax*. Either Cope's gray treefrog populations differ in their likelihood of producing induced responses or their responses are context-dependent.

One important context known to impact the likelihood of tadpoles inducing morphological changes is the amount of cue present. There appears to be a dosage-response relationship between the concentration of cue in the water and the degree of morphological response exhibited by tadpoles (Van Buskirk & Arioli 2002, Schoeppner & Relyea 2008, Schlichting & Pigliucci 1998). Several experiments have shown that

tadpoles respond more strongly to an increased predator density (Relyea 2004, Van Buskirk & Arioli 2002, Schoeppner & Relyea 2008) or higher rates of predation (Anholt et al. 1996, Van Buskirk & Arioli 2002, Schoeppner & Relyea 2008, McCoy et al. 2012, McCoy et al. 2015). I used one individual of each species of predator per tank (0.92 predator per m^3 in the long hydroperiod treatments; short hydroperiod tanks varied with water volume) and although many studies have used only one predator per tank to induce defenses, those studies used smaller mesocosms (e.g. Relyea 2003, Schoeppner & Relyea 2005, Relyea & Werner 1999) or aquaria (e.g. McCollum & Leimberger 1997). Most studies that used comparatively sized mesocosms used multiple predators per tank (McCollum & Van Buskirk 1996, Davenport & Chalcraft 2013, Maher et al. 2013). For comparison, McCollum and Van Buskirk (1996) used 3.33 *Anax* per m^3 to induce defenses in Cope's gray treefrogs. Since I did not see induced responses in the short hydroperiod treatments, where the cue would have been stronger since the water level was lower, my results may not be driven by the amount of cue available. Moreover, I did not see induced defenses in the tanks containing higher levels of cue due to the presence of two predators (one Anax, one Belostoma). However, using two different predators in these treatments is different from using twice the density of a single predator, so I cannot rule out that the amount of cue present was too low to induce a response.

A factor known to influence growth and foraging levels in tadpoles, and therefore may have influenced the likelihood of producing induced defenses, is intraspecific competition among tadpoles. For example, in green frogs (*Rana clamitans*) and wood frogs (*Rana sylvatica*), impacts of predators on growth costs are only observed at low

densities (Peacor & Werner 2000, Van Buskirk & Yurewicz 1998, respectively). In wood

frogs (*Rana sylvatica*), competitors and predators have opposite effects on growth rate,

activity level, body size, tail length (Relyea 2002, Relyea 2004) and foraging

morphology (Relyea & Auld 2005). Other studies have also confirmed that predators

and (intra- and interspecific) competitors have conflicting effects on induced defenses in

multiple species of larval amphibians (Davenport & Chalcraft 2013, Wilbur 1987, McCoy

2007) including Eastern grey treefrogs (*Hyla versicolor*) (Relyea & Hoverman 2003).

Furthermore, a general meta-analysis of 40 publications found that the strength of

predator-prey interactions is affected by competition (Bolnick & Preisser 2005). I used

200 tadpoles per tank, which reflects an ecologically relevant density for North Carolina

ponds (Resetarits et al. 2004) but which is sufficiently high to produce intraspecific

competition in the tanks. For example, Resetarits et al. (2004) used a comparable

density of newly hatched tadpoles in his experiment to address questions related to

intraspecific competition.

Abiotic stressors, such as high temperatures, also may influence tadpoles' ability

to induce defenses to predators. My experimental tanks were in the sun during a very

hot summer, in which the local average monthly temperature during the experiment was

24.8° C, with average daily maximum temperatures of 29.8° C (The Weather Channel

2015). A prior study found that tadpoles raised at temperatures as similar as 20º and

23º C had significantly different foraging rates (Warkentin 1992). Field studies have

shown that most of the variation in time to metamorphosis for multiple species of

tadpoles is explained by temperature; and that the tadpoles metamorphose faster and

at a smaller size when it is warm (Harkey & Semlitsch 1988, Alvarez & Nicleza 2002,

Loman 2002, Reading 2003). In this experiment, tadpoles began metamorphosing from

both hydroperiod treatments within 3 weeks of hatching, which is faster than was

expected based on the information in the literature. Perhaps the temperatures were so

warm in the tanks that all of the tadpoles were focusing most of their energy on

developing quickly and escaping the ponds, regardless of treatment. Even if water

temperatures differed between short and long hydroperiod tanks due to water depth

differences, temperatures could have imposed such stress in all of the treatments that

the tadpoles could not respond to treatment effects. Support for this idea includes an

experiment with neotropical treefrog tadpoles in which those reared at warmer

temperatures produced fewer defenses to predators than those reared at lower

temperatures (Touchon & Warkentin 2011).

Even though my treatments did not affect the among-tank variation in mass at

metamorphosis and larval period, they did affect the within-tank variation in these two

traits. Shortening hydroperiod decreased the within-tank variation in tadpole mass and

larval period, so it is possible that current variation in developmental strategies (i.e. fast

development with small mass at metamorphosis versus slow development with larger

mass at metamorphosis) will be reduced with climate change. Thus, instead of having

multiple developmental strategies available to them, tadpoles in these ponds may be

restricted to developing fast enough to escape a rapidly-drying pond or dying. Similarly,

Belostoma nearly decreased within-tank variation in tadpole larval period, suggesting

that all the prey in the presence of *Belostoma* cue converged on a narrower range of

larval periods relative to those raised without predator cues. Like hydroperiod, predator

cues may create an environment in which a particular developmental strategy must be

closely matched to maximize fitness, whereas environments without predators do not require such tight adherence to a particular strategy.

My experimental results do not mean that climate change will not affect tadpoles and temporary pond systems. Since pond hydroperiods may become more variable, and some ponds may disappear altogether (Brooks 2004, Brooks 2009, Bauder 2005), the predators and prey that live in these ponds may also be disrupted. Amphibians are of special concern due to the unprecedented rates of extinction that have been occurring in the recent past (McCallum 2007). Since some amphibians are unable to respond to shorter hydroperiods (Amburgey et al. 2012), and in other cases the presence of competitors precludes them from responding to shortened hydroperiods (e.g. Rogers and Chalcraft 2008), it is likely that some species will not be able to "keep up" with climate change and the associated variability in hydroperiod. The IUCN (2008) cites climate-driven habitat loss (such as the drying up of temporary ponds) as one of several major causes of the increased extinctions and recent declines in amphibian populations. Often there is a delicate balance in predator-prey interactions, and habitat loss due to climate change could disrupt this balance. A better understanding of the potential interactions between multiple effects of climate change on tadpoles will be invaluable in our effort to conserve and manage amphibian species and wetland habitats before many of them disappear forever.

LITERATURE CITED

Adams, D.C., and E. Otarola-Castillo. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution. 4:393-399.

Alvarez, D., and Nicieza, A. G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. *Functional Ecology, 16*(5), 640-648.

Amburgey, S., Funk, W. C., Murphy, M., and Muths, E. 2012. Effects of hydroperiod duration on survival, developmental rate, and size at metamorphosis in boreal chorus frog tadpoles (*Pseudacris maculata*). *Herpetologica*, *68*(4), 456-467.

Anholt, B. R., Skelly, D. K., and Werner, E. E. 1996. Factors modifying antipredator behavior in larval toads. *Herpetologica, 52*(3), 301-313.

Arar, E.J. and Collins, G.B. 1997. Method 445.0: *In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence. U.S. Environmental Protection Agency. pp.1-22

Arendt, J. 2010. Morphological correlates of spring swimming speed in five species of spadefoot toad tadpoles: comparison of morphometric methods. *Journal of Morphology, 1(*9), 1044-1052.

Bauder, E. T. 2005. The effects of an unpredictable precipitation regime on vernal pool hydrology. *Freshwater Biology*, *50*, 2129-2135.

Benedetti-Cecchi, L. 2003. The importance of the variance around the mean effect size of ecological processes. *Ecology, 84*(9), 2335-2346.

Bernard, M. F. 2006. Survival tradeoffs between two predator-induced phenotypes in pacific treefrogs (*Pseudacris regilla*). *Ecology*, *87*(2), 340-346.

Bolnick, D. I., and Preisser, E. L. 2005. Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta-analysis. *Ecology, 86*(10), 2771-2779.

Brooks, R. T. 2004. Weather-related effects on woodland vernal pool hydrology and hydroperiod. *Wetlands*, *24*(1), 104-114.

Brooks, R. T. 2009. Potential impacts of global climate change on the hydrology and ecology of ephemeral freshwater systems of the forests of the northeastern United States. *Climatic Change*, *95*, 469-483.

Carfagno, G. L. F., Carithers, J. M., Mycoff, L. J., and Lehtinen, R. M. 2011. How the cricket frog lost its spot: the inducible defense hypothesis. *Herpetologica, 67*(4), 386-396.

Chivers, D. P., and Smith, R. J. F. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Ecoscience, 5*(3), 338-352.

Davenport, J. M., and Chalcraft, D. R. 2013. Increasing conspecific density weakens the ability of intermediate predators to develop induced defences to top predators. *Freshwater Biology, 59,* 87-99.

Dayton, G. H., Saenz, D., Baum, K. A., Langerhans, R. B., and DeWitt, T. J. 2005. Body shape, burst speed and escape behavior of larval anurans. *Oikos, 111*(3), 582-591.

Denver, R. J., Mirhadi, N., and Phillips, M. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat dessication. *Ecology, 79*(6), 1859-1872.

Dingemanse, N. J., and Kalkman, V. J. 2008. Changing temperature regimes have advanced the phenology of Odonata in the Netherlands. *Ecological Entomology, 33*(3), 394-402.

Doi, H. 2008. Delayed phenological timing of dragonfly emergence in Japan over five decades. *Biology Letters, 4,* 388-391.

Foti, R., del Jesus, M., Rinaldo, A., and Rodriguez-Iturbe, I. 2012. Hydroperiod regime controls the organization of plant species in wetlands. *PNAS*, *109*(48), 19596-19600.

Gomez-Mestre, I., Kulkarni, S., and Buchholz, D. R. 2013. Mechanisms and consequences of developmental acceleration in tadpoles responding to pond drying. *PLoS One, 8*(12), e84266.

Harkey, G. A., and Semlitsch, R. D. 1988. Effects of temperature on growth, development, and color polymorphism in the Ornate Chorus frog *Pseudacris ornate*. *Copeia, 1988*(4), 1001-1007.

Hassall, C., and Thompson, D. J. 2008. The effects of environmental warming on Odonata: A review. *International Journal of Odonatology, 11*(2), 131-152.

Hassall, C., Thompson, D. J., French, G. C., and Harvey, I. F. 2007. Historical changes in the phenology of British Odonata are related to climate change. *Global Change Biology, 13*(5), 933-941.

IUCN. 2008. *Amphibians on the IUCN red list: Summary of key findings*. Retrieved from http://www.iucnredlist.org/initiatives/amphibians/analysis

Kishida, O., and Nishimura, K. 2005. Multiple inducible defences against multiple predators in the anuran tadpole, *Rana pirica*. *Evolutionary Ecology Research, 7*(4), 619-631.

LaFlandra, E. M., and Babbitt, K. J. 2003. Predator induced phenotypic plasticity in the pinewoods tree frog *Hyla femoralis*: necessary cues and the cost of development. *Oecologia, 138*(3), 350-359.

Laurila, A. 2000. Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria*. *Oikos, 88*(1), 159-168.

Leary, S. et al. 2013. S7.3 Captive Amphibians and Reptiles. In *AVMA guidelines for the euthanasia of animals: 2013 edition*, 76-78. Shaumburg, IL: 2013.

Loman, J. 2002. Temperature, genetic and hydroperiod effects on metamorphosis of brown frogs *Rana arvalis* and *Rana temporaria* in the field. *Journal of Zoology, 258*(1), 115-129.

Maher, J. M., Werner, E. E., and R. J. Denver. 2013. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proceedings of the Royal Society B, 280*, 20123075.

McCallum, M. L. 2007. Amphibian decline of extinction? Current declines dwarf background extinction rate. *Journal of Herpetology*, *41*(3), 483-491.

McCauley, S. J., Hammond, J. I., Frances, D. N., and Mabry, K. E. 2014. Effects of experimental warming on survival, phenology and morphology of an aquatic insect (Odonata). *Ecological Entomology, 40*(3), 211-220.

McCollum, S., and Van Buskirk, J. 1996. Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis. Evolution 50:583–593.*

McCollum, S., and J. Leimberger. 1997. Predator-induced morphological changes in an amphibian: Predation by dragonflies affects tadpole shape and color. *Oecologia 109*, 615–621.

McCoy, M. W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. *Oecologia, 153*(4), 871-878.

McCoy, M. W. 2012. Prey responses predator chemical cues: disentangling the importance of the number and biomass of prey consumed. *PLoS ONE 7*(10), e47495.

McCoy, M. W., Wheat, S. K., Warkentin, K. M., and Vonesh, J. R. 2015. Risk assessment based on indirect predation cues: revisiting fine-grained variation. *Ecology and Evolution,* doi: 10.1002/ece3.1552.

Millennium Ecosystem Assessment. 2005. *Ecosystems and human well-being: Wetlands and Water Synthesis*. World Resources Institute, Washington, DC.

Parmesan, C. 2006. Evolutionary and ecological responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics, 37,* 637-669.

Peacor, S. D., and Werner, E. E., 2000. Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. *Ecology, 81*(7), 1998-2010.

Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014.

Reading, C. J. 2003. The effects of variation in climatic temperature (1980-2001) on breeding activity and tadpole stage duration in the common toad, *Bufo bufo*. *Science of the Total Environment, 310*(1-3), 231-236.

Relyea, R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predators. *Ecology, 81*(8), 2278-2289.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology, 82*(2), 523-540.

Relyea, R. A. 2002a. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs, 72*(4), 523-540.

Relyea, R. A. 2002b. Costs of phenotypic plasticity. *The American Naturalist, 159*(3), 272-282.

Relyea, R. A. 2003a. How prey respond to combined predators: a review and empirical test. *Ecology, 84*(7), 1827-1839.

Relyea, R. A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology, 85*(1), 172-179.

Relyea, R. A. 2007. Getting out alive: how predators affect the decision to metamorphose. *Oecologia, 152*(3), 389-400.

Relyea, R. A., and Auld, J. R. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology, 86*(7), 1723-1729.

Resetarits Jr, W. J., Rieger, J. F., and Binckley, C. A. 2004. Threat of predation negates density effects in larval gray treefrogs. *Population Ecology, 138*(4), 532-538.

Richardson, J. L. 2006. Novel features of an inducible defense system in larval tree frogs (*Hyla chrysoscelis*). *Ecology 87*(3), 780–787.

Richter, O., Suhling, F., Müller, O., and Kern, D. 2008. A model for predicting the emergence of dragonflies in a changing climate. *Freshwater Biology, 53*(9), 1868-1880.

Rogers, T. N., and D. R. Chalcraft. 2008. Pond hydroperiod alters the effect of density-dependent processes on larval anurans. *Canadian Journal of Fisheries and Aquatic Sciences 65*, 2761-2768.

SAS software, Version 6.1 of the SAS System for Windows. Copyright © 2013 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

Schlichting C. D., and Pigliucci, M. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer Associates Inc., Sunderland, Massachusetts, USA.

Schoeppner, N. M. and Relyea, R. A. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecology Letters, 8*(5), 505-512.

Schoeppner, N. M. and Relyea, R. A. 2008. Detecting small environmental differences: risk-response curves for predator-induced behavior and morphology. *Oecologia, 154*(4), 743-754.

Schriever, T. A., and Williams, D. D. 2013. Influence of pond hydroperiod, size, and community richness on food-chain length. *Freshwater Science*, *32*(3), 964-975.

Sim, L. L. et al. 2013. The influence of changing hydroregime on the invertebrate communities of temporary seasonal wetlands. *Freshwater Science*, *32*(1), 327-342.

Skelly, D. K. and Werner, E. E. (1990). Behavioral and life-historical responses of larval American toads to an Odonate predator. *Ecology, 71*(6), 2313–2322.

Söndgerath, D., Rummland, J., and Suhling, F. 2012. Large spatial scale effects of rising temperatures: Modelling a dragonfly's life cycle and range throughout Europe. *Insect Conservation and Diversity, 5*(6), 461-469.

Teplitsky, C., Plenet, S., and Joly, P. 2004. Hierarchical responses of tadpoles to multiple predators. *Ecology, 85*(10), 2888-2894.

Touchon, J. C., and Warkentin, K. M. 2011. Thermally contingent plasticity: temperature alters expression of predator-induced colour and morphology in a Neotropical treefrog tadpole. *Journal of Animal Ecology, 80*(1), 79-88.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

The Weather Channel, LLC. 2015. Weather Underground: Weather History for Greenville, NC. Retrieved from: http://www.wunderground.com/history/airport/KPGV/2014/6/11/MonthlyHistory.html.

Todd, B. D., Scott, D. E., Pechmann, J. H. K., Gibbons, J. W. 2010. Climate change correlated with rapid delays and advancements in reproductive timing in an amphibian community. *Proceedings of the Royal Society of Biological Sciences, 278*(1715), 2191-2197.

U.S. Global Change Research Program 2009. *Global climate change impacts in the United States, 2009 report*. Retrieved from http://nca2009.globalchange.gov/southeast

Van Buskirk, J. 2000. The costs of an inducible defense in anuran larvae. *Ecology, 81*(10), 2813-2821.

Van Buskirk, J. 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *The Ameriacan Naturalist, 160,* 87-102.

Van Buskirk, J. and Arioli, M. 2002. Dosage response of an induced defense: how sensitive are tadpoles to predation risk? *Ecology, 83*(6), 1580-1585.

Van Buskirk, J., and McCollum, S. A. 2000. Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *Journal of Evolutionary Biology, 13,* 336-347.

Van Buskirk, J. and Relyea, R. A. 1998. Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biological Journall of the Linnean Society, 65*(3), 301-328.

Van Buskirk, J., and Yurewicz, K. L. 1998. Effects of predators on prey growth: relative contributions of thinning and reduced activity. *Oikos, 82*(1), 20-28.

Van Buskirk, J., Anderwald, P., Lüpold, S., Reinhardt, L., and Schuler, H. 2003. The lure effect, tadpole tail shape, and the target of dragonfly strikes. *Journal of Herpetology, 37*(2), 420-424.

Van Buskirk, J., McCollum, S. A., and Werner, E. E. 1997. Natural selection for environmentally induced phenotypes in tadpoles. *Evolution, 51*(6), 1983-1992.

Walls, S. C., Barichivich, W. J., Brown, M. E., Scott, D. E., and Hossack, B. R. 2013. Influence of drought on salamander occupancy of isolated wetlands on the southeastern coastal plain of the United States. *Wetlands, 33*, 345-354.

Warkentin, K. M. 1992. Effects of temperature and illumination on feeding rates of Green frog tadpoles (*Rana clamitans*). *Copeia, 1992*(3), 725-730.

Welborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms Creating Community Structure Across a Freshwater Habitat Gradient. *Annual Review of Ecology and Systematics 27*, 337-363.

Wilbur, H. M. 1987. Regulation of structure in complex systems: experimental
temporary pond communities. *Ecology, 68*(5), 1437-1452.

Wingerter, K. 2010. Aquarium Fish: Use of MS-222 (Tricaine Methanesulfonate) to
Induce Sedation and Anesthesia in Ornamental Fishes. Retrieved from:
http://www.advancedaquarist.com/2010/11/fish.

Wright, A.H. 1932. Life Histories of the Frogs of the Okefinokee Swamp, Georgia. North
American Salientia (Anura) Number 2. Macmillan Press, New York.

TABLES

Table 1: Procrustes ANOVA (using 999 permutations) results from the morphology analysis of both rounds of pictures. Significant differences are shown in bold.

Factor	Round 1			Round 2		
	R^2	$F_{(1,31)}$	P-value	R^2	$F_{(1,31)}$	P-value
Size	0.1836	7.9286	**0.001**	0.3639	20.188	**0.001**
Anax	0.0140	0.6062	0.961	0.0130	0.7202	0.630
Belostoma	0.0136	0.5871	0.938	0.0052	0.2896	0.951
Hydroperiod	0.0188	0.8139	0.672	0.0119	0.5175	0.794
Anax*Belostoma	0.0128	0.5539	0.954	0.0093	0.6599	0.705
Anax*Hydroperiod	0.0130	0.5616	0.952	0.0144	0.798	0.618
Belostoma*Hydroperiod	0.0109	0.4725	0.988	0.0057	0.3134	0.941
Anax*Belostoma*Hydroperiod	0.0152	0.657	0.883	0.0179	0.9944	0.484

Table 2: P-values from mixed-model factorial ANOVAs performed for seven response variables: mass at metamorphosis, larval period, survivorship, realistic survivorship, coefficient of variation (CV) for mass, CV for larval period, and periphyton chlorophyll-a concentration. Nearly-significant values are marked with an asterisk (*) and significant values are in bold.

Factor	Response Variable						
	Mass	Larval Period	Total Surviv-orship	Realistic Surviv-orship	CV, Larval Period	CV, Mass	Chloro-phyll-a
Anax	0.6834	0.2897	0.8198	0.2967	0.7468	0.3461	0.4139
Belostoma	0.2359	0.3931	0.5546	0.4780	0.0852*	0.7430	0.8978
Hydroperiod	0.6622	0.1333	0.1636	0.1148	**0.0118**	**0.0427**	0.2922
*Anax*Belostoma*	0.0840*	0.6445	0.6261	0.3328	0.1663	0.1779	0.2412
*Anax*Hydroperiod	0.1039	0.1790	0.3949	0.7529	0.5745	0.4022	0.4935
*Belostoma** Hydroperiod	0.2860	0.1092	0.2611	**0.0425**	0.3994	0.4951	0.7332
*Anax*Belostoma* Hydroperiod	0.9992	0.7213	0.2975	0.8140	0.4924	0.4598	0.6488

FIGURES
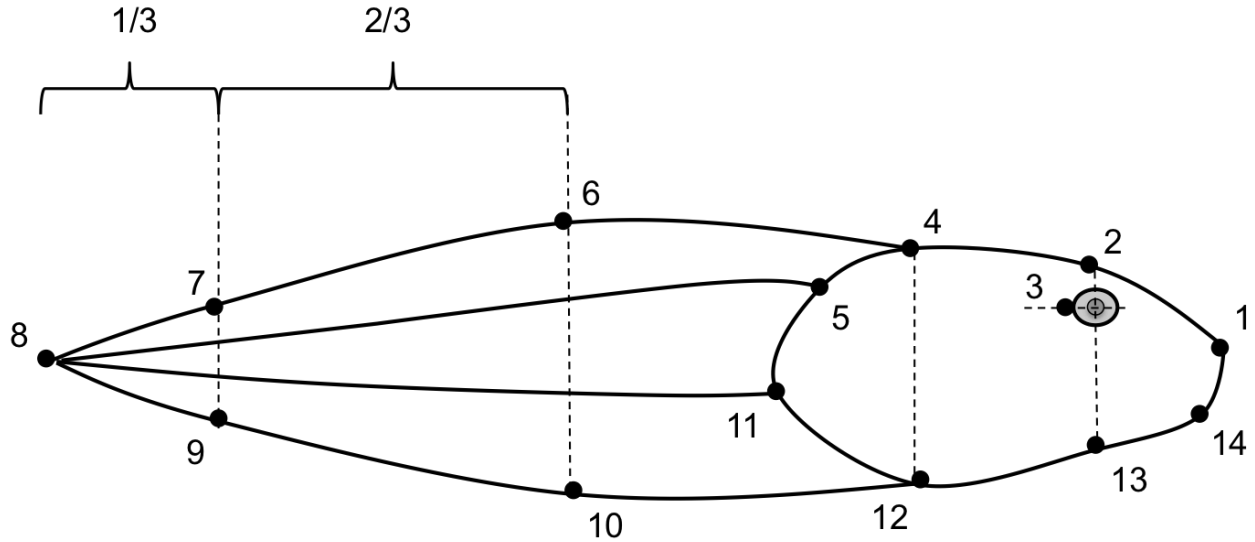
Figure 1: Landmarks used for the morphological analysis, based on those used by

Dayton et al. (2005). Each point represents the mean location of that landmark for all

individuals. Landmarks were placed on photos in the order shown by numbers.

Landmark 3 was placed directly behind the pupil of the eye. Pairs of landmarks (2 and

13, 4 and 12, 6 and 10, 7 and 9) were aligned vertically along dotted lines. Landmarks

6 and 10 were placed at the widest part of the tail. Landmarks 7 and 9 were placed 2/3

of the way from the widest part of the tail to the tip of the tail.
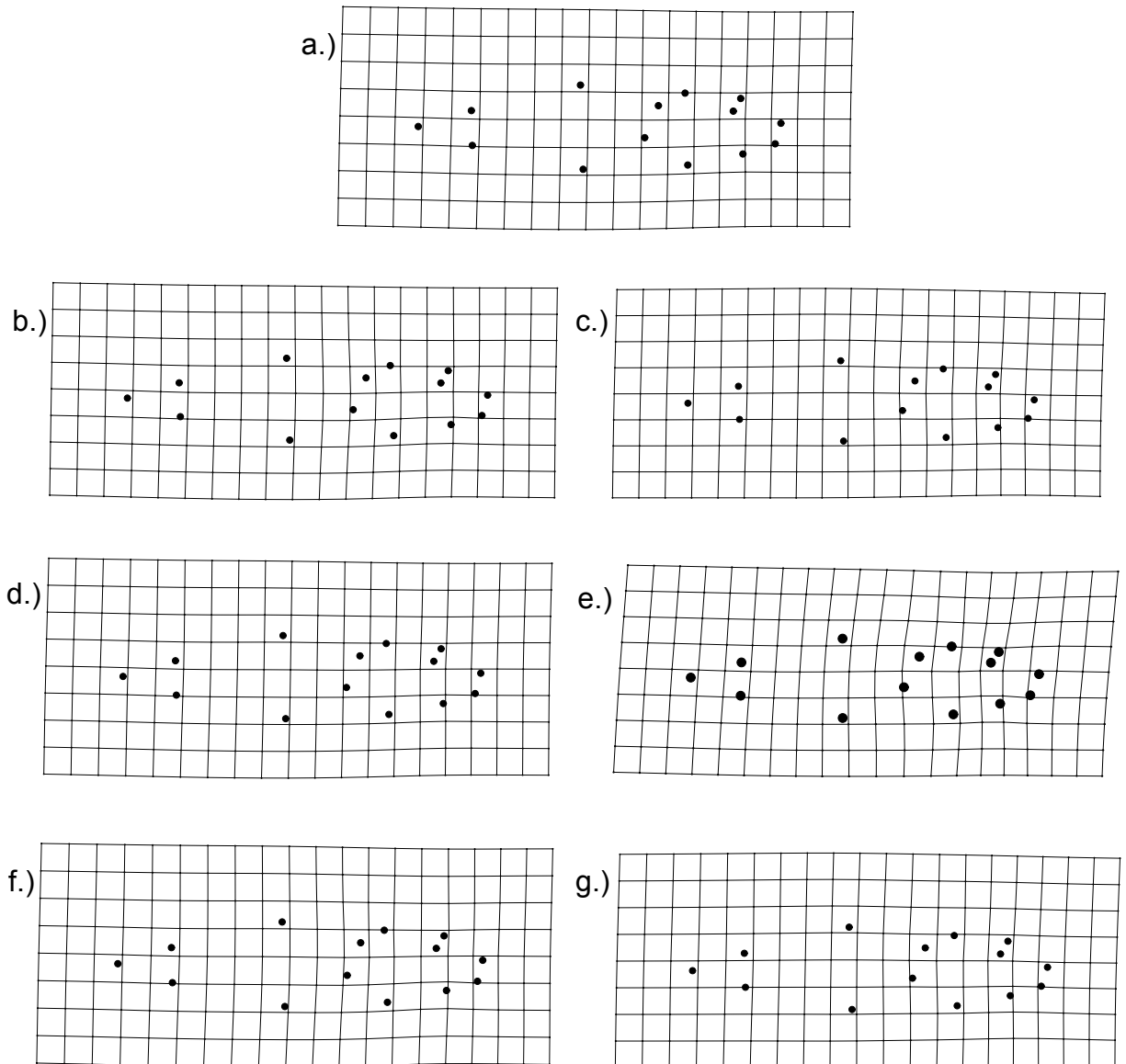
Figure 2: Relative warp diagrams generated from the geometric morphometrics analysis. Each diagram depicts the mean shape change from the control treatment (long hydroperiod, no predator) to treatments with: a.) short hydroperiod, no predator, b.) long hydroperiod, *Belostoma*, c.) short hydroperiod, *Belostoma*, d.) long hydroperiod, *Anax*, e.) short hydroperiod, *Anax*, f.) long hydroperiod, both predators, and g.) short hydroperiod, both predators.
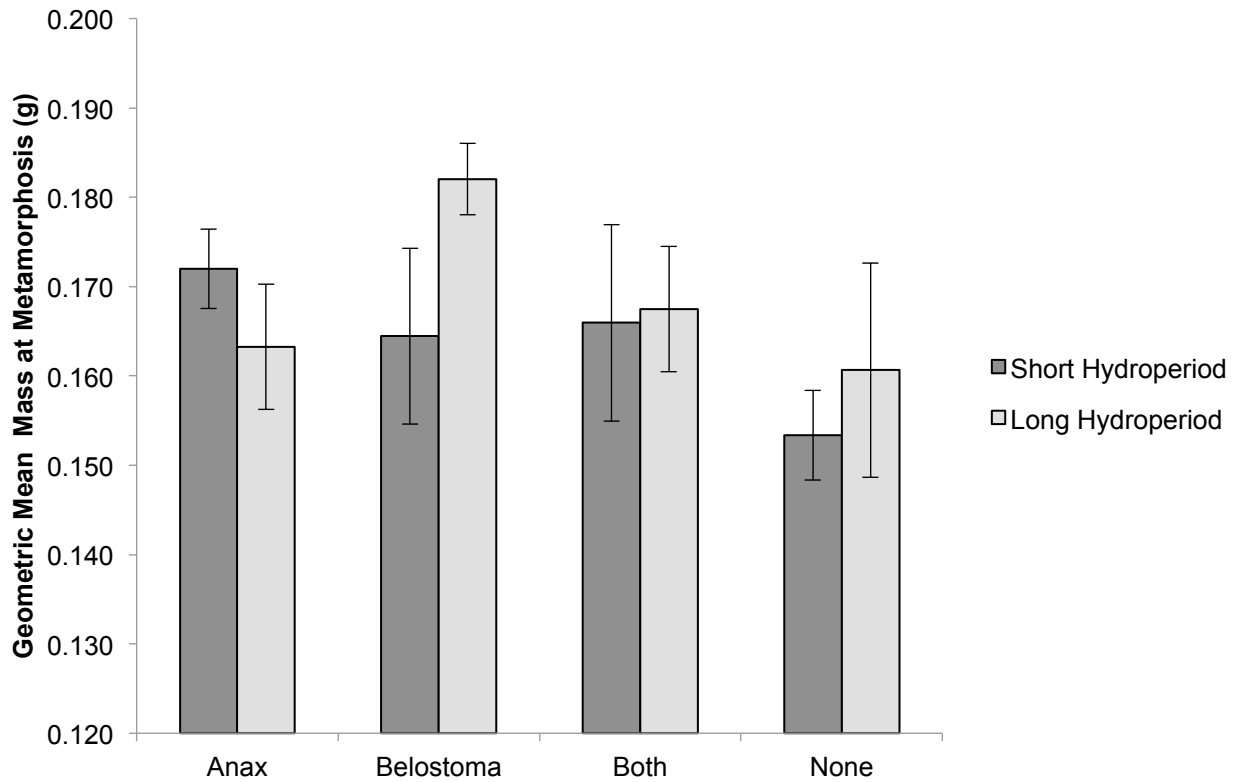
Figure 3: Geometric means of tadpole mass for each treatment are shown. Darker

bars represent short hydroperiod treatments and light bars represent long hydroperiod.

Error bars represent standard error of the mean.
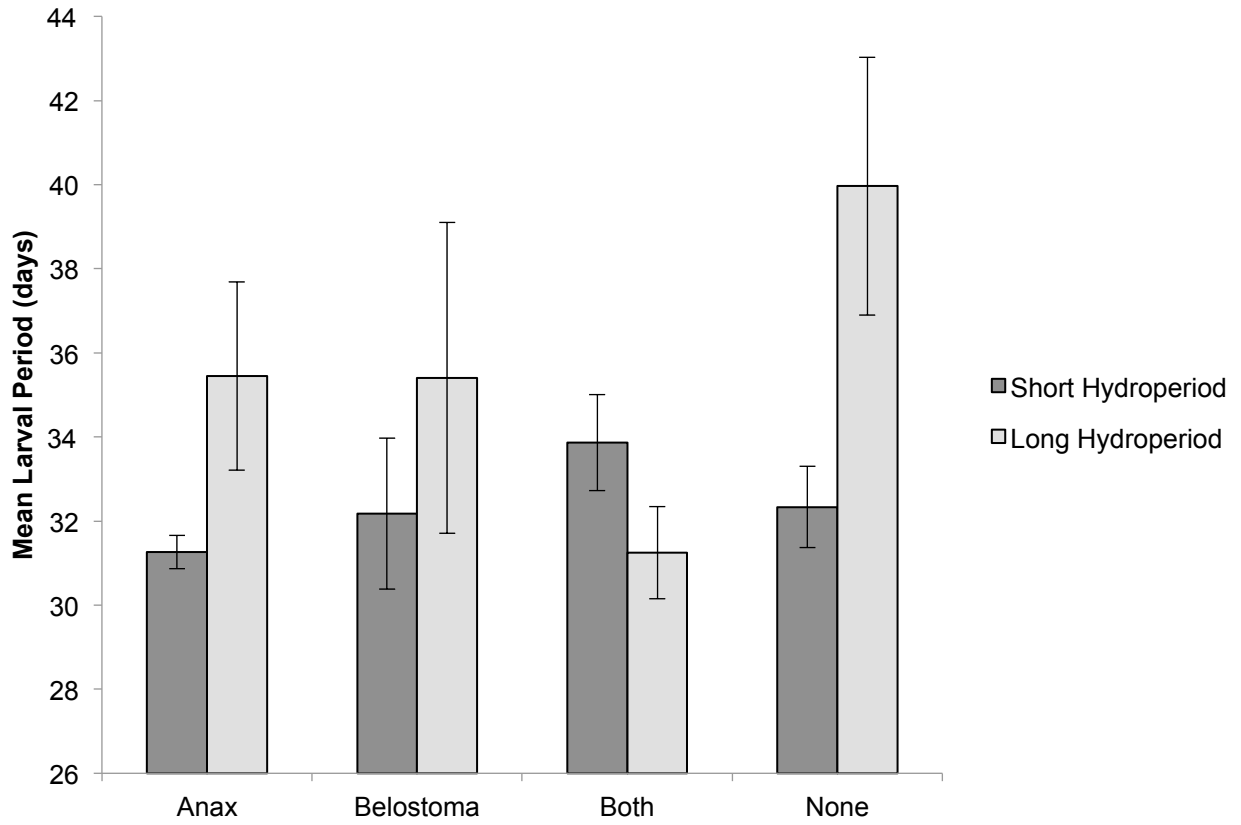
Figure 4: Mean larval period for each treatment is shown. Dark bars represent short hydroperiod treatments and light bars are long hydroperiod. Error bars represent standard error of the mean.
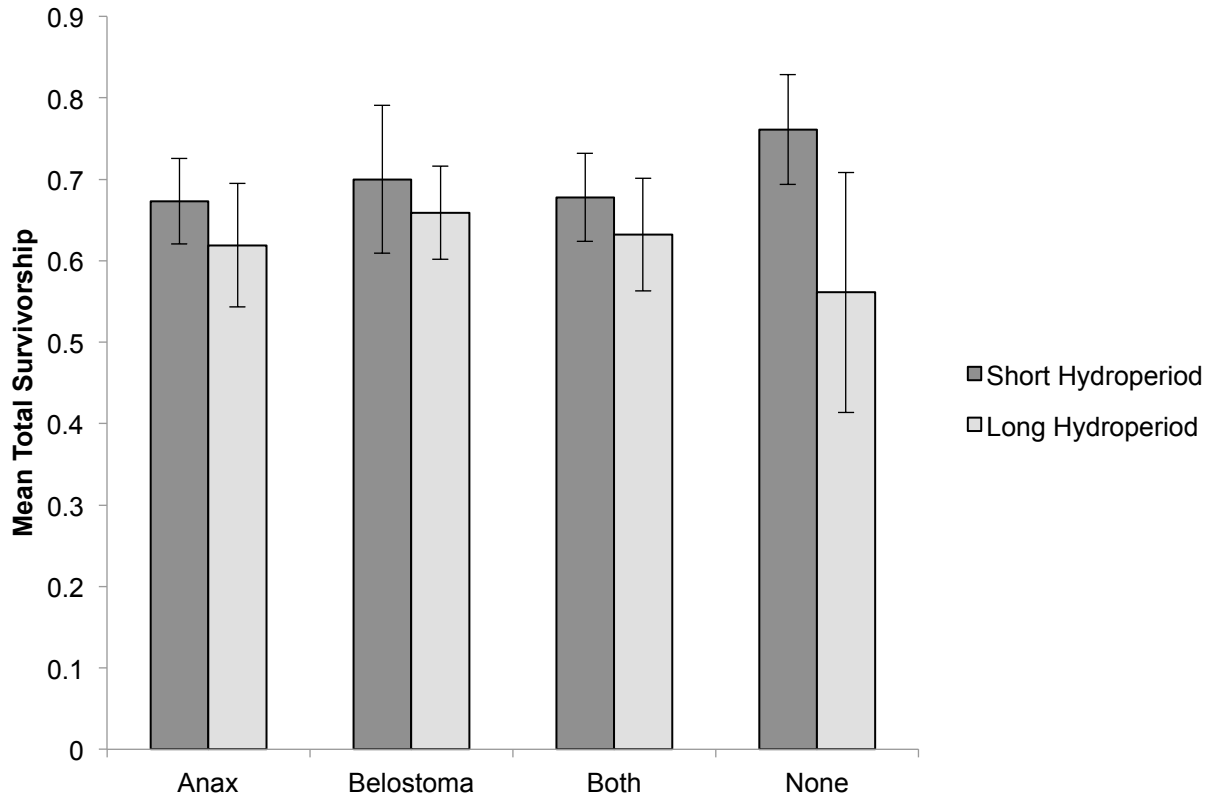
Figure 5: Data shown are untransformed mean proportion of total surviving individuals (metamorphs plus tadpoles remaining at the end of the experiment) for each treatment; error bars represent standard error of the mean. Dark bars represent short hydroperiod and light bars represent long hydroperiod treatments.
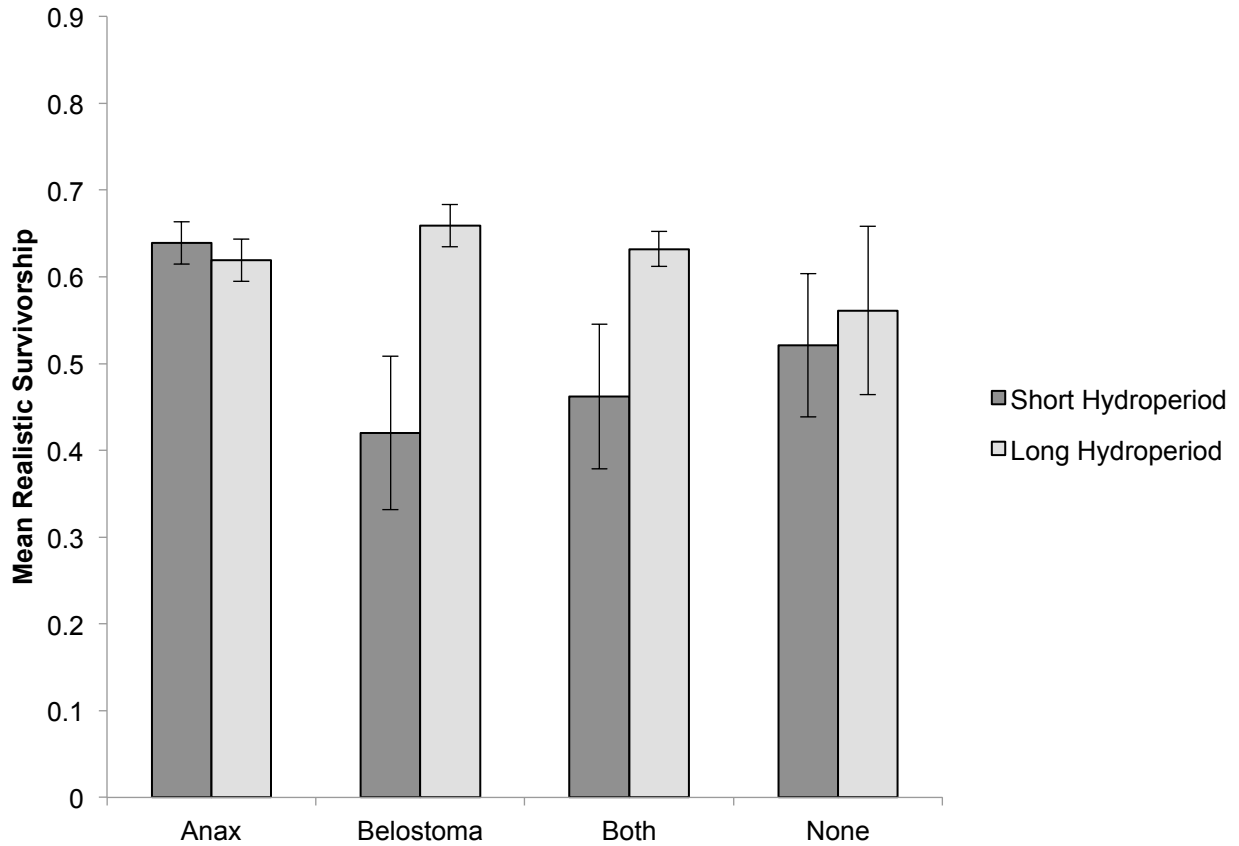
Figure 6: Data shown are untransformed mean proportion of individuals that would have survived in nature for each treatment. This metric includes tadpoles remaining at the end of the experiment in the long hydroperiod treatments plus individuals surviving to metamorphosis. It does not include tadpoles remaining in the short hydroperiod treatments at the end of the experiment because those individuals would have died in nature when those ponds dried. Dark bars represent short hydroperiod treatments and light bars represent long hydroperiod treatments. Error bars represent standard error.
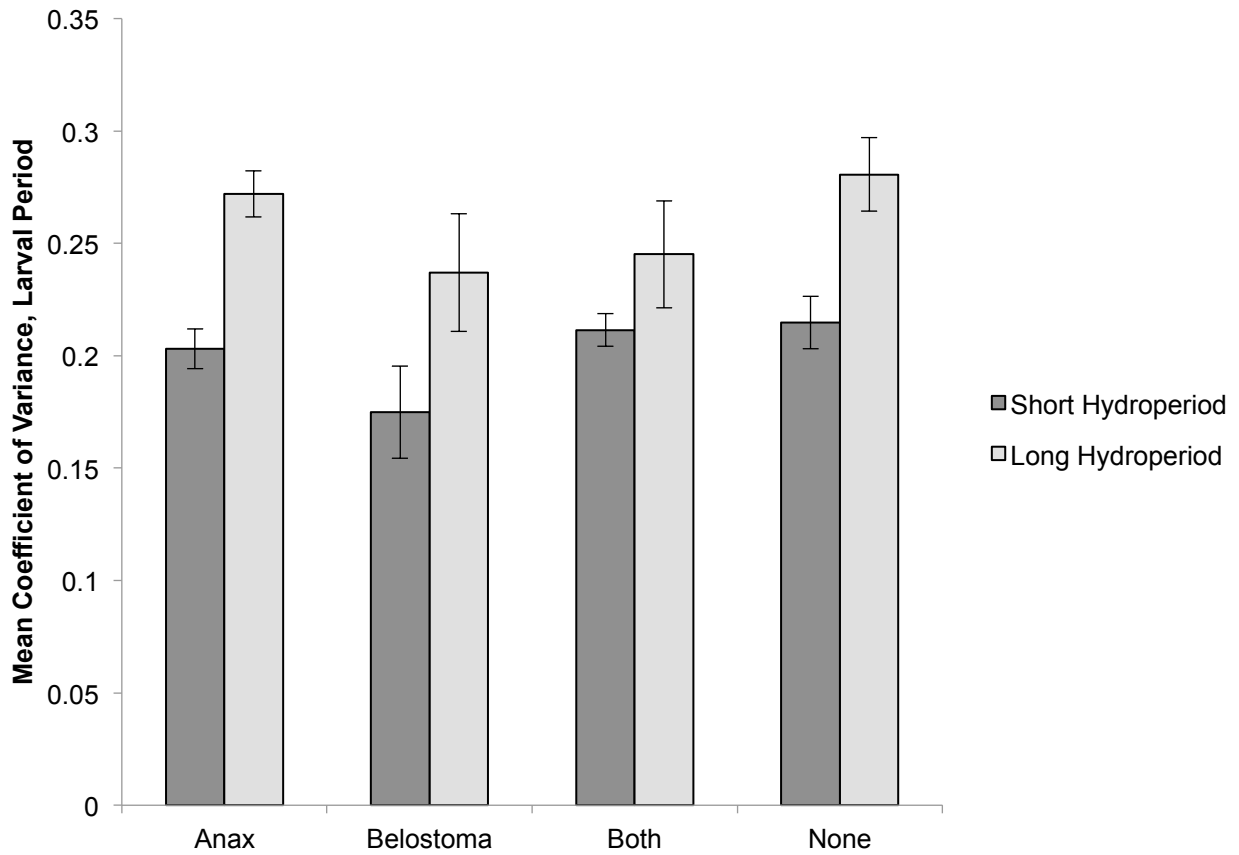
Figure 7: The mean coefficient of variance of larval period for each treatment is shown.

Dark bars represent short hydroperiod treatments and light bars represent long
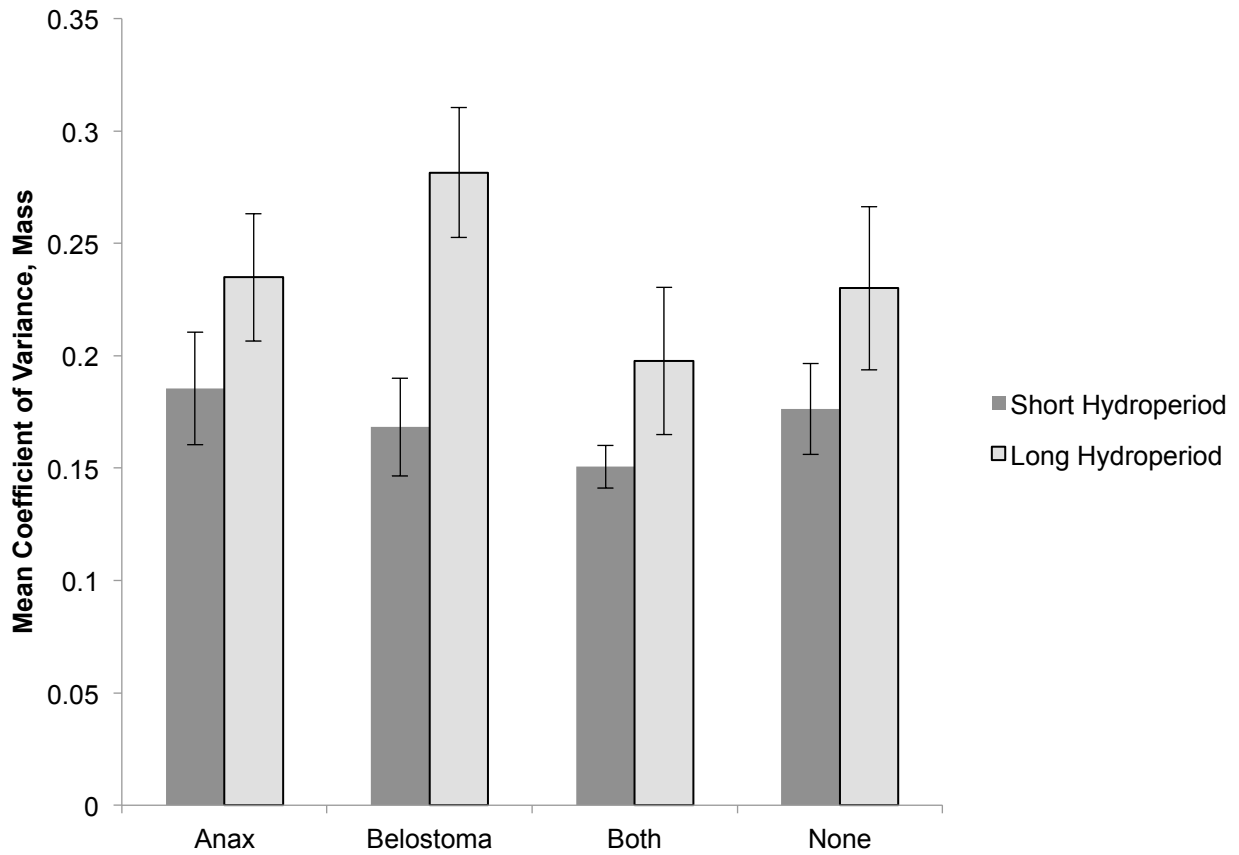
hydroperiod. Error bars represent standard error.

Figure 8: The mean coefficient of variance of tadpole mass for each treatment is shown. Dark bars represent short hydroperiod treatments and light bars represent long hydroperiod. Error bars represent standard error.
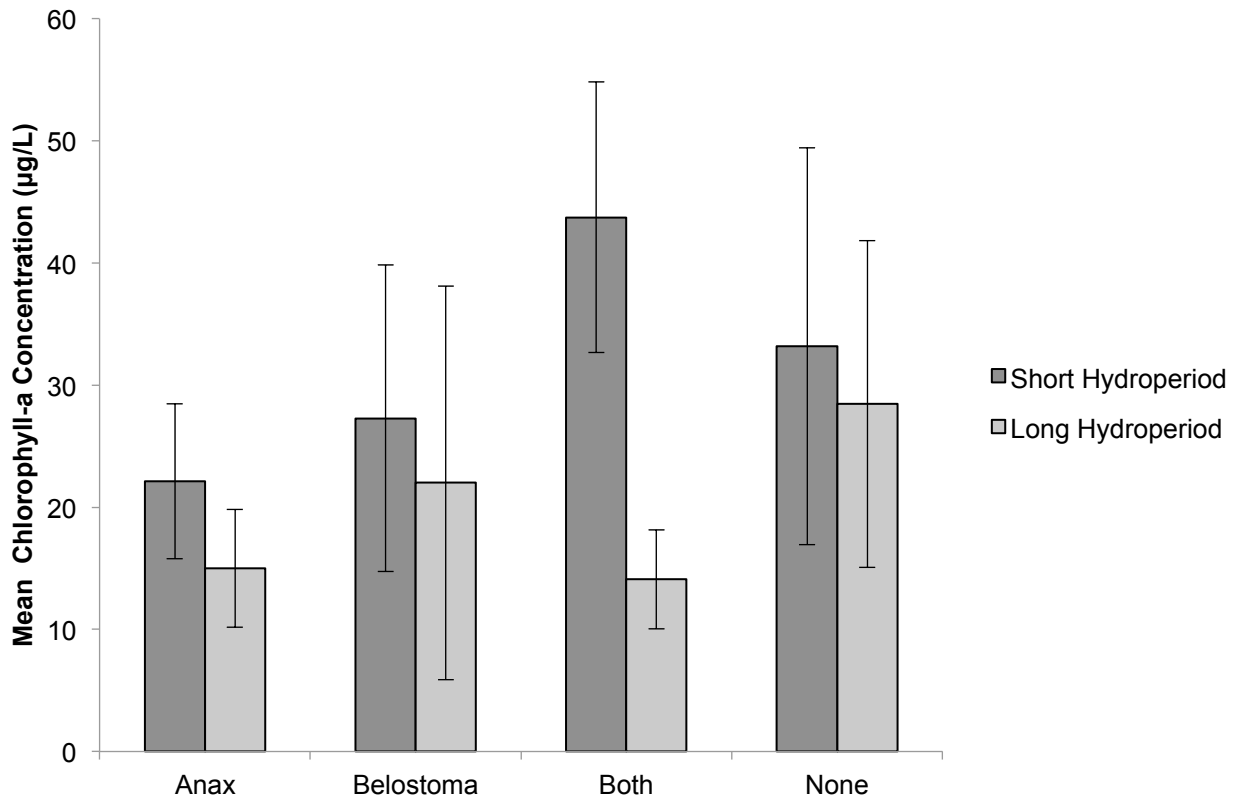
Figure 9: The mean chlorophyll-a concentration isolated from periphyton in each treatment is shown. Dark bars represent short hydroperiod and light represent long hydroperiod treatments; error bars represent standard error of the mean.

APPENDIX: Animal Care and Use Approval

m East Carolina University.

**Animal Care and
Use Commitee**

212 Ed Warren Life
Sciences Building

East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

May 19, 2014

Heather Vance-Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Vance-Chalcraft:

Your Animal Use Protocol entitled, "The Effect of Hydroperiod and Predator Arrival on
the Cost of Induced Defenses in Tadpoles" (AUP #D316) was reviewed by this
institution's Animal Care and Use Committee on 5/19/14. The following action was taken
by the Committee:

    "Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal
procedures must be conducted as described in the approved Animal Use Protocol.
Modifications of these procedures cannot be performed without prior approval of the
ACUC. The Animal Welfare Act and Public Health Service Guidelines require the
ACUC to suspend activities not in accordance with approved procedures and report such
activities to the responsible University Official (Vice Chancellor for Health Sciences or
Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure
that all personnel associated with this protocol have access to this approved copy of
the AUP and are familiar with its contents.**

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

Enclosure