Possible effects of climate change on induced defenses in tadpoles

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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

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By
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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 (USGCRCP 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*...
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*Belostoma $p=0.0840$, 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, Schoepchner & 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
McCoy et al. 2015). I used one individual of each species of predator per tank (0.92
predator per m³ in the long hydropertiod treatments; short hydropertiod 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
Anax per m³ to induce defenses in Cope’s gray treefrogs. Since I did not see induced
responses in the short hydropertiod 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,
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


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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Round 1</th>
<th></th>
<th>Round 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>F(1,31)</td>
<td>P-value</td>
<td>R²</td>
</tr>
<tr>
<td>Size</td>
<td>0.1836</td>
<td>7.9286</td>
<td><strong>0.001</strong></td>
<td>0.3639</td>
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<tr>
<td>Anax</td>
<td>0.0140</td>
<td>0.6062</td>
<td>0.961</td>
<td>0.0130</td>
</tr>
<tr>
<td>Belostoma</td>
<td>0.0136</td>
<td>0.5871</td>
<td>0.938</td>
<td>0.0052</td>
</tr>
<tr>
<td>Hydroperiod</td>
<td>0.0188</td>
<td>0.8139</td>
<td>0.672</td>
<td>0.0119</td>
</tr>
<tr>
<td>Anax*Belostoma</td>
<td>0.0128</td>
<td>0.5539</td>
<td>0.954</td>
<td>0.0093</td>
</tr>
<tr>
<td>Anax*Hydroperiod</td>
<td>0.0130</td>
<td>0.5616</td>
<td>0.952</td>
<td>0.0144</td>
</tr>
<tr>
<td>Belostoma*Hydroperiod</td>
<td>0.0109</td>
<td>0.4725</td>
<td>0.988</td>
<td>0.0057</td>
</tr>
<tr>
<td>Anax<em>Belostoma</em>Hydroperiod</td>
<td>0.0152</td>
<td>0.657</td>
<td>0.883</td>
<td>0.0179</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mass</th>
<th>Larval Period</th>
<th>Total Survivorship</th>
<th>Realistic Survivorship</th>
<th>CV, Larval Period</th>
<th>CV, Mass</th>
<th>Chlorophyll-a</th>
</tr>
</thead>
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<tr>
<td><em>Anax</em></td>
<td>0.6834</td>
<td>0.2897</td>
<td>0.8198</td>
<td>0.2967</td>
<td>0.7468</td>
<td>0.3461</td>
<td>0.4139</td>
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<tr>
<td><em>Belostoma</em></td>
<td>0.2359</td>
<td>0.3931</td>
<td>0.5546</td>
<td>0.4780</td>
<td>0.0852*</td>
<td>0.7430</td>
<td>0.8978</td>
</tr>
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<td>Hydroperiod</td>
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<td>0.1333</td>
<td>0.1636</td>
<td>0.1148</td>
<td><strong>0.0118</strong></td>
<td><strong>0.0427</strong></td>
<td>0.2922</td>
</tr>
<tr>
<td><em>Anax</em>Belostoma</td>
<td>0.0840*</td>
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<td>0.3949</td>
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<td>0.2611</td>
<td><strong>0.0425</strong></td>
<td>0.3994</td>
<td>0.4951</td>
<td>0.7332</td>
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<tr>
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<td>0.9992</td>
<td>0.7213</td>
<td>0.2975</td>
<td>0.8140</td>
<td>0.4924</td>
<td>0.4598</td>
<td>0.6488</td>
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</table>
**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

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 Hydropedest 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

SMjd

Enclosure