RISK ASSESSMENT AND BEHAVIORAL CHOICES OF LARVAL ANURANS

(*LITHOBATES SPHENOCEPHALUS*)

by

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April 2011

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Major Department: Biology

Larval anurans assess risk and make behavioral choices to avoid predation. Since antipredator behaviors may reduce foraging opportunities, prey behavioral decisions can be constrained by a tradeoff between survival and growth. To improve our understanding of prey risk assessment, I asked whether *L. sphenocephalus* tadpoles make antipredator behavioral choices based on characteristics of predators such as their lethality, microhabitat use, or taxonomic group. To test this question, I ran an experiment in aquaria that included 13 treatments (6 predators x lethal/nonlethal plus a no-predator control), replicated eight times in a temporal block design. Three predators occupy benthic microhabitats (white crayfish, *Pachydiplax* dragonfly larvae, and pirate perch), and three occupy pelagic microhabitats (bluegill sunfish, broken-striped newt, and fishing spider). I made behavioral observations of each aquarium twice during each trial, and recorded the prey remaining at the end of each 20-hour trial. Prey antipredator behaviors differed when in the presence of predators from different microhabitats or different taxonomic groupings. When confronted with vertebrate predators (e.g., the fish and the newt) fewer proportions of tadpoles were outside of

refuges. The predator microhabitat usage impacted activity levels of tadpoles, as significantly fewer tadpoles were active when presented with a pelagic predator. Species-specific reactions appeared to play a role as large numbers of tadpoles avoided the benthos when sharing habitat with the crayfish. The proportions of visible and moving tadpoles were different between observation periods, which indicates that tadpoles are able to progressively gauge whether the presence of a predator is a threat, since tadpoles increased their visibility and movement levels during the second observation period in nonlethal treatments. Predator lethality did not impact which antipredator behavior was chosen by the tadpole, but it did appear to affect the strength of the response. Predator characteristics such as microhabitat use, taxonomic affiliation, and lethality influence tadpoles as they determine the potential threat of predation and the appropriate behavioral response.

RISK ASSESSMENT AND BEHAVIORAL CHOICES OF LARVAL ANURANS

(*LITHOBATES SPHENOCEPHALUS*)

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CHAPTER 1: BACKGROUND

Predation emerged as a significant and strong selective pressure around 550 million years ago, just prior to the Cambrian period (McNamara 1996). Researchers believe that predation was the motivating pressure behind the sudden calcification of plants and animals in the fossil record (Bengston 2002). The calcification likely served as a protective mechanism against organisms like the *Anomalocaris*, a predator of the Cambrian era nicknamed the "*Tyrannosaurus rex* of its time" (Stolzenburg 2008). And ever since then, predation has continued to be a selective pressure capable of driving speciation (Fox et al. 1976, Dawkins and Krebs 1979, Van Valkenburgh 1988, Richards et al. 1999, Stanley 2008, Genovart et al. 2010).

Until recently, the lethal processes of predation (i.e. consumptive effects) were the main focus of research concerning the ecological impacts of predation, and several prominent ecological hypotheses were developed within this focus. Hairston et al. (1960) hypothesized that the reason that the color green is one of the world's more prevalent shades was due to the effects of predation being transmitted throughout a food chain. They suggested that predation on herbivores controlled herbivore population sizes. The smaller herbivore populations thereby released primary producers from herbivory. With this reduction in grazing and browsing, the populations of primary producers could increase, covering the earth's surface and generating a generally green hue (Hairston et al. 1960). Appropriately, this classic idea was entitled the 'Green World Hypothesis'.

Predation has also contributed to our understanding of top-down trophic cascades on species' diversity. Paine (1966) experimented with a simple food web in

the rocky intertidal zone of the Pacific Northwest to illustrate the diversity promoting effects of top

predators (i.e., keystone predators). After removing the apex predator, Paine discovered that diverse areas quickly drifted towards a monotypic assemblage. He observed that without predators to disrupt the monopolies that competitively superior species held on limiting resources, superior species would competitively exclude other species and the assemblage would gradually decline into a monoculture (Paine 1966).

In addition to lethal effects, predation has nonlethal effects that can significantly alter species interactions and shape ecosystems. Species' abundances, space use, movement, resource acquisition, and competitive interactions are all subject to change as a result of the non-consumptive effects of predation (Smith 1983, Lima and Dill 1990, Lima 1998). In some cases, nonlethal responses to predators can detract from the prey organism's fitness by compromising the animal's health or increasing its vulnerability to a different predator. However other responses may help organisms survive and reproduce. The various traits and phenotypes that result in increased fitness in prey are selectively favored in nature. Examples of adaptive defenses include the development of cryptic coloration to hide from predators, or antithetically, the evolution of bright colors in an aposematic display to advertise unpalatability. Features that enhance escape or avoid capture, like the ability to defensively sever the tail if captured (i.e., caudal autonomy) found in the *Scincidae* family, can also be attributed to the strong selective pressure from the nonlethal threat of predation. Some animal species possess plastic morphological features, which are flexible changes that are induced when organisms encounter the particular conditions that trigger the change. For example, when tadpoles

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detect predators in their habitat, they are able to grow deeper tails and shorter bodies, presumably to aid escape or lure attention from the tadpoles' head towards the tail (Van Buskirk et al. 1997, Relyea 2003, Teplitsky 2004).

Behavioral responses and life history shifts are also triggered as a reaction to the threat of predation. Spatially avoiding predators, shifting temporal cycles, and altering activity levels are commonly utilized strategies (Skelly and Werner 1990, Jackson and Semlitsch 1993, Van Buskirk 1997, Relyea 2001a, Werner and Peacor 2003). Some antipredator responses can influence the entire life history of the prey species by varying reproductive maturity, reproductive frequency, and fecundity of the prey (Reznick and Endler 1982, Skelly and Werner 1990).

Nonlethal effects resulting from threat of predation have altered traditional models of predation. The historic data on the lynx and the hare that was collected by fur trappers in Canada in the early 20th century, a commonly used example of predator-prey fluctuations, demonstrates the population interplay between predator and prey (Elton and Nicholson 1942). Researchers recently revisited the example of the lynx and hare and demonstrated the possible impact of non-consumptive effects. During times of high lynx populations, hares altered foraging behavior and produced higher amounts of stress-related hormones. High levels of stress hormones can reduce fecundity by 25-30% or even cause complete infertility (Boonstra et al. 1998a). It is therefore unlikely that the observed predator-prey dynamics between the lynx and the hare are solely driven by the lethal impact of prey consumption. Rather, the non-consumptive and consumptive effects likely worked in concert to produce the observed vacillations of hare populations (Peckarsky et al. 2008).

Prey rely on optic, olfactory, tactile, auditory, and even gustatory senses to appraise the environment and detect predators. Most organisms, including copepods, insects, arachnids, mollusks, amphibians, reptiles, birds, and mammals are known to have the capacity to detect predators (Relyea 2003, Smith and Awan 2008, Poelman et al. 2008). Developing a sensitivity to predation risk is especially important when organisms utilize plastic antipredator defenses, given that these flexible responses would be evolutionarily useless if the prey lack the capability to accurately assess threats (Schoeppner and Relyea 2008, Thibert-Plant and Henry 2011).

Several studies show that organisms that are capable of plastic phenotypic variation also possess sensitive capabilities in detecting differences between various threat levels imposed by predators (Schoeppner and Relyea 2008, Thibert-Plante and Hendry 2011). Marine whelks can distinguish the difference in risk between a moving and non-moving predator, and respond accordingly (Rochette et al. 1997). Larval newts are capable of discriminating between predatory larvae (*Ambystoma tigrinum*) and non-predatory larvae (*Hyla chrysoscelis*) that occupy the same habitat (Mathis and Vincent 2000). Red squirrels distinguish between aerial and terrestrial predators and alert nearby squirrels with separate alarm calls for each (Greene and Meagher 1998). Predator size, when related to risk, is also detectable as studies have shown that prey respond more when presented with larger predators (Richards and Bull 1990, Pettersson et al. 2000, Kusch et al. 2004). One mayfly species, *Ephemerella subvaria*, exemplifies the sensitivity of fine-tuned risk-detection capabilities as it differentiates between three species of stonefly, two of which are predators, the third harmless (Peckarsky 1980).

Larval anurans can react with both morphological and behavioral plastic traits in response to the threat of predation. Tadpoles can develop deeper tails with shorter bodies in reaction to some predators. They use the tail as a lure to attract attention away from the critical head, or possibly as a mechanism to allow for extra propulsion in times of near-capture events (Teplitsky 2004, Van Buskirk et al. 1997, Relyea 2003). Larval anurans can also change their behavior as predators are detected. They lower activity levels (thus reducing foraging time), use shelters or refuge spaces, and spend time in areas of the water column that are less risky (Peterson et al. 1992, Feminella and Hawkins 1994, Semlitsch 1993, Turner et al. 1999, Horat and Semlitsch 1994, Relyea 2001a, Relyea 2001b, Smith and Awan 2008). Since antipredator behaviors may reduce foraging opportunities, prey behavioral decisions can be constrained by a tradeoff between survival and growth (Anholt et al. 1996, Steiner 2007). My research aims to increase our understanding of tadpole behavioral decisions given different predators within the confines of this evolutionary tradeoff.

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CHAPTER TWO: RISK ASSESSMENT AND BEHAVIORAL CHOICES OF LARVAL

ANURANS (*LITHOBATES SPHENOCEPHALUS*)


*INTRODUCTION:*

The impacts of predation can cascade throughout ecosystems, capable of

interrupting an individual's pursuit of resources and mating opportunities or even

modifying the entire species assemblage of a habitat (Hairston et al. 1960, Paine 1966,

Turner and Mittlebach 1990, Werner and Anholt 1996, Schmitz et al. 1997). The

majority of early predator studies have focused on the lethal impacts (e.g., consumptive

effects) of predation, and these have been integral in developing a basic understanding

of ecosystems (Hairston et al. 1960, Paine 1966). Recent research has demonstrated

that nonlethal impacts of predation have an immense influence on ecosystems as well

(Lima and Dill 1990, Lima 1998, Werner and Peacor 2003, Peckarsky et al. 2008).

Any response that is a reaction to the threat of predation can be classified as a

nonlethal effect of predation. Prey may respond to the mere presence of predators in

any number of ways, including changing their habitat use, increasing the use of refuges,

altering activity levels, shifting temporal cycles, and inducing plastic morphological

characters (Skelly and Werner 1990, Jackson and Semlitsch 1993, Van Buskirk 1997,

Relyea 2001a, Werner and Peacor 2003).  Collectively, nonlethal responses can alter

species' abundances, resource acquisition, and the competitive interactions throughout

the entire ecosystem (Smith 1983, Lima and Dill 1990, Lima 1998). These responses

typically reduce the rates of encounter, capture, and consumption by predators (Alvarez

and Nicieza 2009), and thus are beneficial because they reduce a prey's immediate risk

of predation.  However, antipredator responses may be costly and result in lowered

energy intake, compromised health, or increased risk associated with different predators

(Anholt and Werner 1998, Relyea 2003, Teplitsky et al. 2004). These negative

possibilities may translate to lower reproductive success or long-term survival

(Semlitsch 1993, Relyea 2003). For example, when hares are exposed to the threat of

predation by lynx, they produce higher levels of stress hormones, which lower their

fecundity (Boonstra et al. 1998a).

Larval anurans can react with both behavioral and morphological plastic traits in

response to the threat of predation. To reduce encounter rates with predators, larval

anurans reduce movement and foraging (Relyea 2001a, Relyea 2001b, Smith and

Awan 2008). Tadpoles may also spatially avoid predators or seek safety within a refuge

(Peterson et al. 1992, Feminella and Hawkins 1994, Horat and Semlitsch 1994, Relyea

2001a). Shelters offer physical protection and help avoid detection and capture by

predators (Turner et al. 1999). To enhance the possibility of escape after detection,

tadpoles may form shorter bodies and deeper tails (Teplitsky 2004, Van Buskirk et al.

1997, Relyea 2003). The deeper tail allows for a quick burst of swimming to evade the

predator's grasp. The shortened body reduces the target size of the vital organs as the

tail lures the predator's focus away from the critical viscera (Van Buskirk 2003, Teplitsky

2004, Relyea 2001a). If these responses fail to allow the tadpole to escape capture,

some species may yet avoid consumption via non-plastic constitutive defenses involving

noxious skin chemicals that reduce palatability (Laurila et al. 1997, Jara and Perotti

2010).

As with other animals, antipredator responses have benefits and costs for larval anurans. While defenses improve the larval anuran's short-term survival, they are often associated with a decrease in energy intake that can result in a slower growth or a smaller size at metamorphosis (Collins and Wilbur 1973, Skelly 1992, Anholt and Werner 1995, Rose 2005). For anurans, a large size at metamorphosis is positively correlated with breeding success and fecundity as an adult (Smith 1983, Newman 1988, Semlitsch et al. 1988, Lima and Dill 1990, Laurila et al. 1997, Relyea 2003, Steiner and Pfeiffer 2007). Models and experiments suggest that in optimal habitats with a steady food supply, low competition for resources, and low risk, tadpole metamorph size is large with a shortened larval period (Wilbur and Collins 1973, Travis 1984). But it is much more likely that tadpoles will deal with sub-optimal habitat conditions that may include high competition, low resource availability, pond drying, and predators. Due to this environmental heterogeneity, tadpoles must rely upon behavioral or morphological responses to mitigate the potential risks of staying in an ephemeral or dangerous habitat while exploiting a potentially limited food supply to attain the optimal size at metamorphosis (Wilbur and Collins 1973, Collins 1979, Travis 1984, Rose 2005).

To maximize the benefits and minimize the costs associated with antipredator defenses, it is necessary for larval anurans to accurately determine risk of predation. Tadpoles use chemical, visual, and tactile cues to detect predator presence (Petranka et al. 1987, Eklov 2000, Fraker 2009, Ferland-Raymond et al. 2010). The chemical cues anurans perceive are combinations of predator kairomones and the digested waste products of consumed prey (Eklov 2000, Schoeppner and Relyea 2008, Saidapur et al. 2009). Kairomones are communicative chemicals that are produced by one

organism that gives an advantage to the recipient organism (usually of a different species). Kairomones convey information about the predator, its location relative to the prey, and its recent diet. Tadpoles detect kairomones released by predators in aquatic environments and use them to recognize potential risk (Laurila 1997, Pettersson et al. 2000).

Previous studies have supported that tadpoles display generalized responses to the risk of predation (Semlitsch and Gavasso 1992, Teplitsky et al. 2003), but recent hypotheses suggest that tadpoles respond differently to different predators in a species-specific manner (Relyea 2003, Schoeppner and Relyea 2008, Fraker 2009).

To measure risk, tadpoles may simply gauge the levels of cue present and use that information as an indication of predation risk. It is more likely, however, that tadpoles use the presence of the cue with other pieces of information to determine the strength of risk. As suggested by recent studies (Semlitsch and Gavasso 1992, Anholt et al. 2000, Mathis and Vincent 2000, Relyea 2001a, Relyea 2001b, Relyea 2003, Teplitsky et al. 2003, Teplitsky et al. 2004, Schoeppner and Relyea 2008), it is possible that larval anurans recognize specific predator identities and gauge risk accordingly. However, species composition in freshwater systems can be very diverse, variable, and unpredictable, and utilizing this strategy would likely leave the tadpole undefended and vulnerable against a foreign or new predator. Due to this environmental heterogeneity, it is unlikely that prey use a species-specific risk assessment strategy. In a study that presented tadpoles with native and non-native fish, naïve tadpoles displayed general antipredator responses to all of the fish (Teplitsky et al. 2003), lending support to the hypothesis that tadpoles may detect predator types rather than specific predator

identities. Few studies have focused on determining general criteria about the predators themselves that larval anurans may assess as they respond to their surroundings. Understanding anuran predator detection and response may allow for accurate predictions of behavior given different predator assemblages, a necessary tool in the conservation of habitats and the species' diversity therein. The ability to determine general conclusions about predator characteristics may help us better predict the consequences of a particular predator that is added to (via invasion) or lost from (via local extinctions) systems. Finally, it is practical to uncover similar responses based on characteristics so that experiments do not have to be run on every species individually.

To improve our understanding of prey risk assessment, I observed patterns of tadpole behavioral choices in the presence of various predators, which represented a range of predator characteristics. The predator characteristics assessed were the lethality of each predator, microhabitat usage of the water column (benthic/pelagic), and two taxonomic groupings (invertebrate/vertebrate predators, and fish/non-fish predators). I also assessed whether there are correlations between prey antipredator behavior and predator lethality.

Each of these predator characteristics has been hypothesized to impact the potential levels of risk that the tadpoles endure in their habitats. Efficient and deadly predators with a large appetite are hypothesized to evoke large responses from prey (Semlitsch and Gavasso 1992, Anholt et al. 2000, Mathis and Vincent 2000, Relyea 2001a, Relyea 2001b, Relyea 2003), probably due to high cue concentrations in water with high prey mortality (Teplitsky et al. 2003, Fraker 2009). However, studies have shown that it is not just lethality and cue alone that determines risk and subsequent

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response, as several other variables such as water temperature, dissolved oxygen content, and habitat complexity can alter the perceived risk levels for tadpoles (Babbitt and Tanner 1997, Moore et al. 1998, Turner et al. 1999, Schoeppner and Relyea 2008).

The second predator characteristic, microhabitat usage of the predator, may be important for two reasons. Tadpoles spend the majority of their time foraging along the benthos. Therefore tadpoles are likely to have higher encounter rates with benthic predators, which may indicate that benthic predators are perceived as more risky to tadpoles (Relyea 2001a). Secondly, the cue delivery from pelagic predators at farther distances may be weaker or potentially more degraded, which may communicate less risk to the tadpoles (Turner and Montgomery 2003).

The third characteristic group, taxonomic affiliation, teases apart the different predator assemblages that are typically found across the gradient of permanent to ephemeral ponds. In ephemeral ponds there are likely to be high concentrations of invertebrate predators as well as the occasional semi-aquatic vertebrate such as the Red-striped Newt (Wellborn et al. 1996). In permanent systems, the dominant predators are generally fish, with lower abundances of invertebrate predators (Wellborn et al. 1996). Determining some of the differences in tadpole behavior between these two assemblages is likely to provide information on expected behavioral responses in permanent vs. ephemeral habitats.

*Study System:*

I used Southern Leopard frog tadpoles (*Lithobates sphenocephalus*) and six predator species to test prey risk assessment and antipredator behaviors. Larval anurans in freshwater ecosystems are ideal candidates to study predator-prey

interactions as their aquatic habitats provide discrete, diverse communities with the added benefit of experimental tractability. While several studies have indicated that prey identity is as important as predator identity in regards to antipredator strategy choice (Relyea 2001a, Werner and McPeek 1994, Gregoire and Gunzburger 2008), *L. sphenocephalus* tadpoles have been shown to respond in a manner similar to that of several other species including *Hyla versicolor, Lithobates sylvatica,* and *Lithobates catesbeiana* (Relyea 2001a).

Six predator species, known to consume and co-occur with *L. sphenocephalus,* were chosen to represent a range of predator characteristics. Three of the predator species occupy a primarily benthic microhabitat: dragonfly larvae (*Pachydiplax longipennis*), the white Crayfish (*Procambarus acutus*), and pirate perch (*Aphredoderus sayanus*). The remaining three predators occupy primarily pelagic microhabitats: the bluegill sunfish (*Lepomis macrochirus*), the broken-striped newt (*Notophthalmus viridescens viridescens*), and the fishing spider (*Dolomedes triton*). These predators can be taxonomically divided into vertebrate (pirate perch, bluegill, and newt) and invertebrate (dragonfly, crayfish, and spider), as well as fish (pirate perch and bluegill) and non-fish groups.

The specific identities of the predators were chosen primarily due to their ubiquity and ability to be grouped into the previously mentioned groups. As I wished to have a study with as broad of an inference as possible, I decided that responses to common predators might allow for more general interpretation into a variety of freshwater systems along the coastal plains of the southeast United States.  In addition, we chose two fish that we expected may differ substantially in their effects on prey.  Sunfish are

known to be voracious predators of tadpoles (Eklov 2000, Gallie et al. 2001, Smith and Awan 2008) and even cause frogs to avoid ovipositing in their presence (Binckley and Resetarits 2002). In contrast, frogs do not avoid ovipositing in ponds with pirate perch (Binckley and Resetarits 2002), a fish known for the movement of its anus to its throat during ontogeny (Boltz and Stauffer 1993).

*METHODS*:

*Experimental Methods:*

*Lithobates sphenocephalus* tadpoles and all six predator species are commonly found throughout freshwater ecosystems along the coastal plains of North Carolina. The predators were collected from the Croatan National Forest, North Carolina and Bray Hollow in Grifton, North Carolina. The tadpoles were collected as newly laid egg-mass clutches in the Croatan National Forest and from small wetlands surrounding Greenville, North Carolina.

The experiment consisted of 13 treatments, each replicated once in each of eight temporal blocks. Six treatments included each predator species with the prey (lethal treatments); the other six treatments had caged predators such that the prey were only impacted by the visual and chemical predator cues (nonlethal treatments). The final treatment was a no-predator control. Within each temporal block, each of the 13 treatments was randomly assigned to an aquarium (30.48 cm x 30.48 cm x 60.96 cm). Barriers between aquaria prevented visual interactions between organisms in different tanks. Aquaria were marked 7.6 cm above the bottom, marked again 7.6 cm above that line, and were filled with 22.86 cm of fresh tap water and treated with TopFin® Tap

Water Dechlorinator to form three distinct regions in the water column. Ten grams of fresh mixed deciduous and pine leaf litter was added to each tank as refuge for the prey. A clear, porous container was added to each aquarium to hold the predator in a nonlethal treatment or to serve as a control for the addition of the cage in the lethal treatments. Each container (15.24 cm x 15.24 cm x 25.40 cm) had two opposing sides removed and replaced with a fine mesh screen.

To begin each temporal block, twenty naïve *L. sphenocephalus* tadpoles were haphazardly selected, placed into each tank, and allowed to acclimatize for approximately an hour. One individual of each predator species, were haphazardly selected and randomly assigned to each lethal and nonlethal treatment. The predators were weighed and measured prior to initiating the trial (Table 1). Predators were starved for 36 hours prior to the start of the trial. The predators in the nonlethal treatments were each fed five tadpoles in their cages at the beginning of each block to ensure the presence of strong conspecific cues.

I observed tadpole position and activity level twice in each tank during each temporal block. Approximately two hours after the predators were added, the first observation period began. The first observation period always occurred in the late afternoon after the start of the experiment, and the second occurred the following morning prior to the block's conclusion. At thirty second intervals, I recorded the number of tadpoles moving on the bottom third of the tank, the number of tadpoles not moving but visible on the bottom third of the tank, the number of tadpoles present in the middle third, and the number of tadpoles present in the top third of the tank. Twenty hours after the predators were added to the aquaria, they were removed from the tanks, and

survivorship of the prey was recorded. All contents of the tank were emptied and each tank was thoroughly cleaned.

*Data Analysis:*

Variables were formed from the raw data collected during observation periods to represent antipredator behavior in *L. sphenocephalus* tadpoles. The proportion of visible tadpoles was calculated by adding the number of visible tadpoles, including both moving and non-moving in each section of the tank and dividing by the total number of tadpoles present. Visible tadpoles were not seeking shelter in refuges (e.g., leaf litter); therefore the number visible served as a proxy for refuge-seeking behavior. The proportion of moving tadpoles was calculated by dividing the number of moving tadpoles by the total number of tadpoles visible. Since it is assumed that tadpoles continually forage as they move (Wassersug and Hoff 1979, Anholt and Werner 1995, Relyea 2002), the movement variable served as a representation of foraging activity. To determine whether prey were altering their microhabitat use in response to a specific predator, I calculated the number of visible tadpoles (both moving and nonmoving) that were spending time along the bottom third and divided that sum by the total number of visible tadpoles. Few tadpoles used the upper sections of the tanks, so data from the two upper-most sections were summed and then divided by the total number visible to quantify the proportion of visible tadpoles in the pelagic regions of the aquarium.

A correction was applied to lethal treatments to correct for consumptive effects when comparing lethal treatments to nonlethal treatments. The lethal treatments are expected to have fewer prey exhibiting each behavior than the nonlethal treatments due

to tadpole consumption throughout the trial. Therefore I used the exponential model of decay ($N_t = N_o e^{-rt}$) to first calculate the rate of prey removal (-r), and secondly to estimate the mean number of tadpoles present for each predator at the time of each observation period ($N_t$) (Table 2). I divided the mean number of tadpoles present at each observation period by twenty, the initial number of tadpoles, to determine the mean percent consumed for each predator for each observation period. To determine the number of expected tadpoles in the lethal treatments for each predator and observation period, I multiplied the percent consumed by the original total (20 tadpoles) and subtracted that result from the original total (20 tadpoles). Then, to correct the behavioral data and transform each number into a proportion, I carried out the following calculation for each predator and each observation period. I took the raw behavioral variable (number of tadpoles moving, number of tadpoles visible, number of tadpoles on top, number of tadpoles on bottom) and divided by the previously calculated total number of expected tadpoles.

The software used for statistical analyses was PASW 18® and SAS 9.2®. The first two analyses determined differences in survivorship among the lethal predator treatments and the no-predator control. Since the no-predator control had no variance (every trial had 100% tadpole survivorship), to determine if the predator treatments were different from the no-predator control, I used a one-sample T-test comparing survivorship against the fixed value of 1. I then used a univariate ANOVA (excluding the no-predator control) followed by a comparison of all pairwise combinations of treatments using Ryan-Einot-Gabriel-Welsch (REGW) range test to determine differences in survivorship among the lethal predator treatments.

The four tadpole behavioral variables (e.g., the total proportion visible, the proportion moving, the proportions visible on the bottom, and the proportions visible on top) were all analyzed using separate repeated-measures ANOVAs. A repeated-measures approach was necessary due to the non-independence of the two observation periods on a single treatment within each temporal block. Two sets of repeated-measures ANOVAs were calculated using Proc MIXED in SAS. In these analyses, all factors were considered fixed except for blocks which were considered random. The blocks represented specific points in time that are potentially interchangeable with other points in time and represent only a subset of the possible set of blocks that could have been used.

The first set of repeated-measures ANOVAs looked for differences among each predator/lethality combination, the two observation periods, and the interaction between predator/lethality combination and observation period for each behavioral variable. Each of these repeated-measures ANOVAs was followed by a planned contrast comparing the behavior in lethal treatments to the no-predator control as well as a planned contrast comparing the behavior in nonlethal treatments to the no-predator control. If a significant interaction between predator/lethality combination and observation period was uncovered, separate planned contrasts were used for each observation period. Because of the multiple tests, I used the Simulate option in SAS to adjust the p-values to account for experiment-wise error rates.

The second set of repeated-measures ANOVAs were factorial and determined the effects of predator identity, lethality, observation period, and all the two-way and three-way interactions between these three factors. The repeated-measures ANOVAs

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were followed by planned comparisons for the different predator characteristic groups (i.e., benthic vs. pelagic, vertebrate vs. invertebrate, and fish vs. non-fish). The no-predator control was excluded from these analyses. All of the above analyses included a block effect.

A linear regression was used to determine if the strength of each antipredator behavior was related to the survivorship of the prey. Regressions were run on the average prey survivorship and behavioral variables for each of the six lethal predators treatments. A separate linear regression was calculated for each observation period.

## *RESULTS:*

There was no mortality of predators, and no loss of prey in the nonlethal treatments or control. Survivorship in the six lethal predator treatments was significantly less than one ($t_{47}$ = -9.712, $p < 0.001$). The six lethal predator treatments were also different from one another ($F_{5,42}$ = 5.296, $p = 0.001$, Fig. 1). The crayfish and the newt were the two most lethal predators; the bluegill, pirate perch, spider, and dragonfly larvae removed intermediate proportions of tadpoles that were not different from the crayfish, but were different from the newt.

There were no significant differences in the proportion of visible tadpoles among the predator/lethality combinations ($F_{12,84}$ = 1.50; $p$= 0.1388) (Fig 2), but there were significant effects of observation period ($F_{1,7}$ = 10.54; $p$= 0.0141) and the interaction between these effects ($F_{12,84}$ = 2.67, $p = 0.0044$). The proportion of visible tadpoles in the lethal treatments was significantly lower than the no-predator control during the first observation period ($F_{1,84}$ = 7.50, $p = 0.0075$), while the proportion of visible tadpoles in

the nonlethal treatments in the first observation period was not significantly lower than the no-predator control, but displayed a trend ($F_{1,\,84}$ = 3.05, p = 0.0844). During the second observation period, the proportion of visible tadpoles in both lethal and nonlethal treatments did not differ from the no-predator control ($F_{1,\,84}$ = 0.01, p = 0.9193; $F_{1,\,84}$ = 1.36, p = 0.2472, respectively).

The proportion of tadpoles that were visible did not significantly differ among predators ($F_{5,\,35}$ = 1.84, p = 0.1308) (Fig. 3) and no differences were detected in the proportion of visible tadpoles between the lethal and nonlethal treatments ($F_{1,7}$ = 0.09, p = 0.7742). There was a significant difference in the proportion visible between the two observation periods ($F_{1,\,7}$ = 8.84, p = 0.0207). The only significant interaction was between lethality and time ($F_{1,7}$ = 7.07, p = 0.0325). Benthic and pelagic predators did not invoke significant differences in tadpole visibility ($F_{1,\,35}$ = 0.09, p = .7707) (Fig. 3A). Fish, when compared with non-fish predators, were associated with fewer tadpoles visible ($F_{1,\,35}$ = 7.33, p = 0.0104) (Fig. 3B). Similarly, vertebrate predators were associated with fewer visible tadpoles than invertebrate predators ($F_{1,\,35}$ = 5.15, p = 0.0296) (Fig. 3C). There was a significant positive relationship between the proportion of tadpoles visible and prey survivorship during the first observation period, but no significant relationship during the second observation period (first observation period: $F_{1,5}$ = 22.91, p = 0.008; $R^2$ =0.821); second observation period: $F_{1,5}$ = 2.140, p = 0.203; $R^2$ = 0.300) (Fig. 4).

The predator/lethality combinations significantly differed in the proportion of prey moving ($F_{12,84}$ = 2.17, p = 0.0202) (Fig 5), but there were no significant differences between observation periods ($F_{1,7}$ = 0.15, p = 0.7067) or the interaction ($F_{12,84}$ = 0.77,

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p = 0.6782).  The proportion of prey moving differed from the control in both the lethal

and nonlethal treatments (lethal: $F_{1,84}$ = 11.01, p = 0.0013; nonlethal: $F_{1,84}$ = 8.01, p =

0.0058). The total proportion of moving tadpoles was different with different predator

identities ($F_{5,35}$ = 2.78, p = 0.0324) (Fig. 6). No differences were detected between the

lethal and nonlethal treatments ($F_{1,7}$ = 0.99, p = 0.3539), between observation periods

($F_{1,7}$ = 0.45, p = 0.5242), or with any of the interactions.  Fewer prey were active when

confronted with a pelagic predator than when confronted with a benthic predator ($F_{1,35}$

= 11.13, p = 0.0020) (Fig. 6A). Fish predators were not significantly different from non-

fish predators ($F_{1,35}$ = 0.00, p = 0. 9459) (Fig. 6B) and the movement of tadpoles with

vertebrate predators was not significantly different from invertebrate predators ($F_{1,35}$ =

1.70, p = 0.2003) (Fig. 6C). There was no significant correlation between survivorship

and tadpole movement levels in either observation period (first observation, $F_{1,5}$ =

3.760, p = 0.110, $R^2$ = 0.429; second observation, $F_{1,5}$ = 6.588, p = 0.059; $R^2$ = 0.569)

(Fig. 7).

There were significant differences among predator/lethality combinations in the

proportion of tadpoles on the bottom ($F_{12,84}$ = 2.22, p = 0.0174) (Fig 8).  There were not

significant differences in the proportion of prey on the bottom between the two

observation periods ($F_{1,7}$ = 0.30, p = 0.6024) and there was no significant interaction ($F_{12,84}$ = 1.12, p = 0.3558).  There were not significantly different proportions of tadpoles

along the bottom in the lethal treatments or nonlethal treatments when compared to the

no-predator control (lethal; $F_{1,84}$ = 0.14, p = 0.7046, nonlethal; $F_{1,84}$ = 0.03, p =

0.8737).

The total proportion of tadpoles on the bottom varied according to predator identity ($F_{5, 35}$ = 4.52, p = 0.0028) (Fig. 9). No differences were detected between the lethal and nonlethal treatments for the proportion of tadpoles found along the bottom ($F_{1,7}$ = 0.17, p = 0.6960), nor between observation periods ($F_{1,7}$ = 0.18, p = 0.6832), or any interactions. There was no difference in the proportion of tadpoles along the bottom when confronted with a benthic or pelagic predator ($F_{1,35}$ = 1.08, p = 0.3064) (Fig. 9A). When classified by taxonomy, a larger proportion of visible tadpoles were found along the bottom of the tank when confronted with a fish predator ($F_{1,35}$ = 6.30, p = 0.0168) (Fig. 9B). The proportion of visible tadpoles located along the benthos when a vertebrate predator was present was not different than when presented with an invertebrate predator ($F_{1,35}$ = 1.60, p = 0.2149) (Fig. 9C). There was a significant positive correlation between the proportions of tadpoles found along the bottom of the tank against tadpole survivorship for the first observation period, but there was no association in the second observation period (First observation period: $F_{1,5}$ = 7.822, p = 0.038, $R^2$=0.610; second observation period: $F_{1,5}$ = 0.317, p = 0.598, $R^2$ = 0.060) (Fig. 10).

There were significant differences among the predator/lethality combinations ($F_{12,84}$ = 2.22, p = 0.0174) (Fig. 11) in the proportion of prey at the top of the water column, but not between the observation periods ($F_{1,7}$ = 0.29, p = 0.6045) or the interaction ($F_{12,84}$ = 1.12, p = 0.3566). There were no significant differences between the lethal treatments or the nonlethal treatments and the no-predator control in the proportion of prey found at the top of the water column (lethal; $F_{1,84}$ = 0.14, p = 0.7045: nonlethal; $F_{1,84}$ = 0.02, p = 0.8752). The total proportion of tadpoles that were present in the upper

portions of the aquaria differed according to predator identity ($F_{5, 35}$ = 4.52, p = 0.0028) (Fig. 12). There was no difference in the proportion of tadpoles that spent time at the top of the aquaria between the lethal and nonlethal treatments ($F_{1, 7}$ = 0.17, p = 0.6934) or between observation periods ($F_{1,7}$ = 0.18, p = 0.6854), or with the interactions.

No significant differences were found between benthic and pelagic predators ($F_{1,35}$ = 1.09, p = 0.3046) (Fig. 12A), or between vertebrate and invertebrate predators ($F_{1,35}$ = 1.59, p = 0.2160) (Fig. 12C). However there were significantly fewer tadpoles found along the top of aquaria when presented a fish predator compared to non-fish predators ($F_{1,35}$ = 6.28, p = 0.017) (Fig. 12B). There was an significant correlation between prey survivorship and the amount of time spent in the upper portions of the tank during the first observation period, but no significant association during the second observation period (First observation period: $F_{1,5}$ = 7.822, p = 0.038, $R^2$=0.610; second observation period: $F_{1,5}$ = 0.317, p = 0.598, $R^2$ = 0.060) (Fig. 13).

### *DISCUSSION:*

The plasticity of behavioral responses in larval *L. sphenocephalus* is an adaptation to deal with a variable environment (Ferland-Raymond et al. 2010). Plastic traits are selectively favored when the ability to flexibly respond to heterogeneous conditions allows the organism to persist longer. Plastic traits are typically behavioral or morphological responses within a single generation. However, if the ability to be plastic is maintained throughout generations, we can infer that ability to be morphologically and behaviorally plastic is an adaptation in itself. This serves as another example of a survival strategy induced and maintained by the selective pressure of predation.

Larval anurans make use of a suite of plastic behavioral adaptations to reduce predation. While previous studies suggest that antipredator adaptations are context and species-specific and therefore unpredictable, my experiment has revealed general patterns of tadpole behaviors that may be expected when confronted with different predator types.

My results revealed interesting patterns concerning the information assessed by tadpoles as they detect risk and respond to risk. The tadpoles did not appear to choose the behavioral strategy solely as a reaction to the lethality of the predator, but rather chose the behavioral response according to various predator characteristics. The taxonomy of the predator affected the visibility of tadpoles while the microhabitat of the predator affected prey movement patterns. Qualities unique to individual predator species appeared to drive antipredator reactions as well, as the crayfish induced a much higher spatial avoidance reaction compared to the other five predators. The strength of the spatial avoidance appeared to be driven by the higher lethality of the crayfish. Each of the behavioral responses that I observed has been recorded in other tadpole/predator experiments (Hopper 2001, Relyea 2001a, Relyea 2003, Teplitsky et al. 2003, Gregoire and Gunzburger 2008).

Changes in tadpole movement appear to be prompted by the predator's microhabitat. When confronted with a predator that resided in the same benthic microhabitat where they are generally located, significantly more tadpoles were active. It is possible that the close proximity of the predators along the benthos induced escape movement away from the predator (Turner et al. 1999). But if escape were the motivator, the largest proportion of tadpoles should have responded to the crayfish

since the crayfish was the most mobile and disruptive of the benthic predators (Albecker, *personal observation*). The crayfish, however, produced the smallest proportion of moving tadpoles among the benthic predator group (Fig. 6). Another explanation is that tadpoles may more easily detect pelagic predators swimming above them whose body form is outlined by shadow when the sun shines light from above. Prey may be more likely to cease activity and rely on camouflage coloration to blend into the leafy benthos of their habitat if they detect a predator swimming above. Tadpoles that share the benthos with a predator may be able to determine whether they are within a risky proximity to the predator and may exercise less caution and increase movement when they are out of range of a benthic predator. Verification that benthic prey have greater ability to detect predators in the pelagic zone than the benthic zone, and respond accordingly, requires additional research.

Predator taxonomy was the main driver of changes in tadpole visibility. When presented with a fish or newt predator (the vertebrates), the proportion of visible tadpoles was significantly lower when compared to an invertebrate predator. The tadpoles may be utilizing refuge space to avoid detection by the fish and newt, as tadpoles are not capable of out-swimming these agile predators (Hopper 2001, Hossie and Murray 2010). Additionally, refuges are good predator avoidance strategies only for predators that lack the dexterity or are too large to access the shelters like the fish or newt (Turner et al. 1999). Predators such as the crayfish and dragonfly larvae are able to access and extract prey from these shelters. This indicates that anuran larvae may recognize the protective limitations of refuges and choose a different antipredator behavior accordingly. An earlier study supports this hypothesis by determining that prey

survival increased as refuge efficiency increased from partial to complete protection (Persson and Eklov 1995). Future experiments could manipulate refuge size and shape to determine if prey use refuges more in the presence of predators that are unable to access the refuges relative to predators that can access the refuges.

The hunting style of the fish and newt predators may also play a role in the reduced numbers of visible tadpoles. Sit-and-wait predators tend to rely on prey movement to detect prey, while pursuing predators seek out their prey actively (Teplitsky et al. 2005). Since both fish species and the newt are predators that hunt actively, tadpoles may stay generally hidden from the pursuing predators (Teplitsky et al. 2005, Preisser et al. 2007, Hossie and Murray 2010). In that case, the predator's hunting strategy may combine with the predator's agility and cause tadpoles to disregard information on microhabitat usage, as sharing habitat with a fast and agile predator is risky regardless of the predator's microhabitat usage.

Significantly more tadpoles were found along the bottom portions of the tank when the prey were confronted with fish or newts compared to the other predators, but this spatial pattern seems likely to be driven specifically by the movement of tadpoles to the top of the water column in the presence of the crayfish. The tadpoles in the aquaria with the crayfish greatly reduced their concentrations along the benthos, while tadpoles in the other five predator treatments were not very different from the no-predator control in regards to spatial positioning (Fig. 9). This response to the crayfish is consistent with what one may expect from exposure to such an active predator. Leaf litter is a poor shelter against a predator like the dexterous crayfish that is capable of overturning and disrupting leaf litter with ease. With no safe refuge available on the benthos, the

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tadpoles likely avoided the crayfish by spending the majority of their time in the upper portions of the aquaria. Spatial avoidance is an important predator avoidance technique, and several studies have demonstrated that tadpoles will utilize different areas of the water column to avoid confrontation with predators (Anholt and Werner 1995, Relyea 2001a, Hammond et al. 2007, Smith and Awan 2008).

My results revealed an interesting pattern in the movement and visibility patterns of *L. sphenocephalus* tadpoles in the treatments between the observation periods. The positive correlation between survivorship of prey and the strength of the behavioral response may indicate that the prey are indeed using the predator's lethality as an indicator of risk. However, lethality appears to affect the strength of the behavior utilized, not which behavior is chosen. The differences in the strengths of reactions according to survivorship are likely due to the higher concentrations of chemical cues in aquaria with higher tadpole lethality (Eklov 2000, Schoeppner and Relyea 2008, Ferland-Raymond et al. 2010). In two of the behavioral responses (proportion visible and proportion moving), I detected a larger difference in response when compared to the no-predator aquaria during the first observation period than the second observation period. This supports the supposition that tadpoles likely have the ability to progressively modify the strength of the behavior according to perceived risk through time.  Previous studies have recorded that larval anurans change their defensive behaviors as they consider things like food availability, cue composition, and other environmental factors (Burks and Lodge 2002, Relyea 2004, Schoeppner and Relyea 2008).   It is possible that as the trials progressed, the tadpoles were able to determine that while there was a predator present, they were not in immediate danger and thus

resumed higher levels of visibility or activity. These observations also maintain the hypothesis that tadpoles use many pieces of information to assess risk rather than simply perceiving the quantity and quality of chemical cues. Future behavioral experiments ought to account for these potential changes in behavioral response through time by making multiple observations at different times throughout the trials.

My experiment demonstrates that predators that occupy a pelagic microhabitat may be expected to cause a reduction in the visibility of tadpoles as higher proportions of prey seek refuge. Tadpoles may decrease activity levels after detecting a fish or newt predator. As fish predators are found only in permanent aquatic systems, lower activity levels by tadpoles may lead to less resource acquisition for tadpoles that are oviposited in permanent systems (Werner and McPeek 1994), and thus result in smaller metamorphs emerging from permanent ponds. This may be a contributing factor in the selective pressure behind the strong preference of certain species of female frogs to avoid ovipositing in pools that contain fish (Binckley and Resetarits 2002, Reiger et al. 2004, Gregoire and Gunzburger 2008).

My research suggests that tadpoles respond differently to different predator types. In situations where tadpoles are repeatedly exposed to similar predator assemblages, disruptive selection may occur over time. In that case, the heterogeneous predator communities may drive differences among prey species that could eventually lead to speciation.

Previous studies have been noted that bluegill sunfish (*Lepomis macrochirus*) are voracious predators to tadpoles, capable of consuming entire populations (Eklov 2000, Gallie et al. 2001, Gregoire and Gunzburger 2008, Smith and Awan 2008).

Surprisingly, the bluegill consumption patterns that I observed were very different than these studies and did not result in the differences in consumption between the pirate perch and bluegill that we expected based on the results of prior oviposition site selection studies (Binckley and Resetarits 2002). Only two of the eight lethal trials housed bluegill that consumed a majority of the tadpoles. The remaining six sunfish only consumed an average of 10% of the available prey. All predators were food-deprived for 36 hours prior to the start of each trial, so each sunfish ought to have been hungry at the time of the trial. In addition, each of our fish appeared healthy and there was no mortality in the bluegill that were held in the lab before or after a block.

Since bluegill ontogenically shift diets from zooplankton to larger prey, there is a chance that I collected bluegill on each side of that dietary preference, with the planktivorous bluegill yielding very low tadpole consumptions. However in a comparison of the bluegill sizes to consumptive behavior, I found that the two sunfish that consumed 90% of the prey were actually two of the three smallest sunfish, so this hypothesis is unlikely. Another potential explanation may be that the fish with low appetites were actually gravid females, who have traditionally been observed to eat less during egg development. Since the fish were returned to ponds shortly after being used in a trial, I cannot say whether the fish were gravid females. Finally, the sunfish were held together in a holding tank before a block began and I did not observe the sunfish as they were fed in the holding tank. It is possible that the larger fish consumed all or most of the tadpoles that were placed in the tank as food. This may have caused the smaller sunfish to be hungrier than the larger sunfish when placed into an experimental tank, resulting in higher tadpole consumption rates by smaller sunfish. While this idea is

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circumstantially supported by the high consumption rates of the smaller sunfish, I am unable to determine the definite mechanism.

I purposefully conducted this study at a small scale, as behavioral observations are more precise in aquaria. Larger tanks and natural systems may have weaker cue presence as the cues dissipate throughout the system, possibly resulting in a weaker response by prey. There may also be fewer encounters between predator and prey as there is more space to move. Finally, my experiment only offered a single prey type whereas natural systems typically offer a host of different prey items to predators. Given these limitations, the strength of the prey response may change given larger spatial scales, but the underlying patterns of detection and response are likely to be maintained (Semlitsch et al 1996, Resetarits and Fauth 1998). The consumption rates for the different predators used in my study (with the exception of the bluegill) were similar to consumption results published in previous studies (Lawler 1989, Relyea 2001a, Chalcraft and Resetarits 2003, Kerby and Sih 2005, Gunzburger 2005). Care was given to ensure that the aquaria mimicked natural environments as much as possible and previous studies indicate that mesocosm studies translate well to field settings (Wilbur 1989, Semlitsch et al 1996, Resetarits and Fauth 1998, Chalcraft et al. 2005).

Future studies should focus on collecting data on the behavioral responses of additional prey species' against predator characteristics along environmental gradients and should also incorporate these findings into systems that contain multiple predators. In circumstances with multiple predators, it is hypothesized that tadpoles will respond hierarchically according to threat – choosing to react primarily to the most dangerous predator present (Teplitsky et al. 2004). But in some cases, responding to one predator

has been shown to enhance the risk of consumption by another predator (Turner et al.

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Table 1.  The mean length (in mm), standard deviation of the length (in mm), mean mass (in g), and standard deviation of the mass (in g) of all predators used during the experiment.  The average length of the fish predators (bluegill sunfish and pirate perch) is the standard length from the tip of the snout to the end of the caudal peduncle. The length of the cephalothorax and abdomen comprise the average length of the fishing spider. The dragonfly larvae were similarly measured from its head to the end of the abdomen. The length of the white crayfish was measured from the cephalothorax and abdomen (excluding the telson). The length of the red striped newt is the length from the snout to the vent. The mean mass is the average weight of each predator.

| | Mean Length (mm) | Std.Dev. (mm) | Mean Mass (g) | Std.Dev.(g) |
|---|---|---|---|---|
| Bluegill sunfish | 53.03 | 6.06 | 4.38 | 1.8 |
| Pirate perch | 60.29 | 7.96 | 5.18 | 1.95 |
| Fishing spider | 11.12 | 1.20 | 0.73 | 0.31 |
| White crayfish | 59.33 | 5.32 | 8.24 | 1.69 |
| Dragonfly Larvae | 20.17 | 2.88 | 0.42 | 0.13 |
| Red Striped Newt | 35.96 | 5.38 | 2.12 | 0.32 |

Table 2. Predator-specific information for the exponential model of decay used to estimate the amount of prey consumption that occurred per hour, and by each observation period (2 hours, 20 hours), in the lethal treatments. The full details of the calculations are available in the "Methods" section.

| | Rate of Decay (% removed per hour) | % Removed at 2 hr time | % Removed at 20 hr time |
|---|---|---|---|
| White crayfish | -0.036 | 7.9 | 55.6 |
| Dragonfly Larvae | -0.044 | 3.2 | 27.5 |
| Pirate perch | -0.045 | 3.1 | 26.9 |
| Bluegill sunfish | -0.045 | 3.1 | 26.9 |
| Red Striped newt | -0.029 | 11.7 | 71.3 |
| Fishing spider | -0.043 | 3.6 | 30.0 |
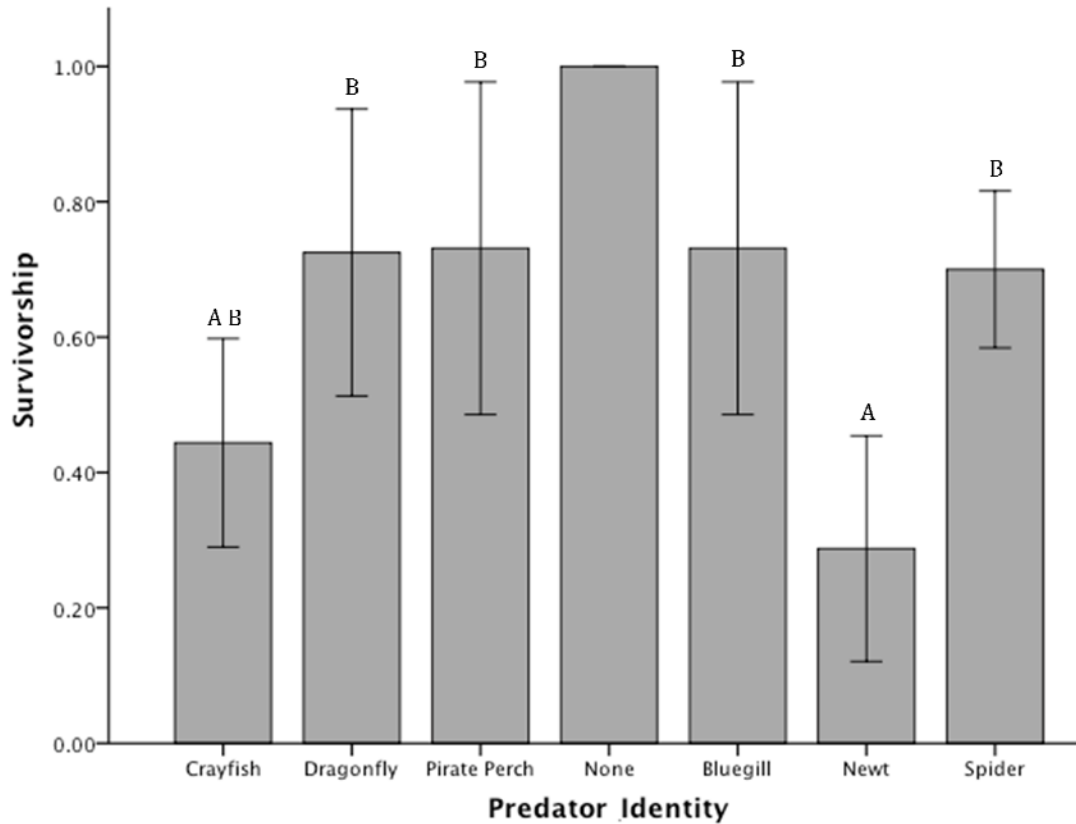
Fig. 1.  The survivorship of *Lithobates sphenocephalus* tadpoles with each lethal predator, ($F_{5,42}$ = 5.296, p= 0.001). Bars are means ±1 SE.  Bars that share the same letter are not significantly different from one another, as determined by a univariate ANOVA followed by a comparison of all pairwise combinations of treatments using Ryan-Einot-Gabriel-Welsch (REGW) range test.
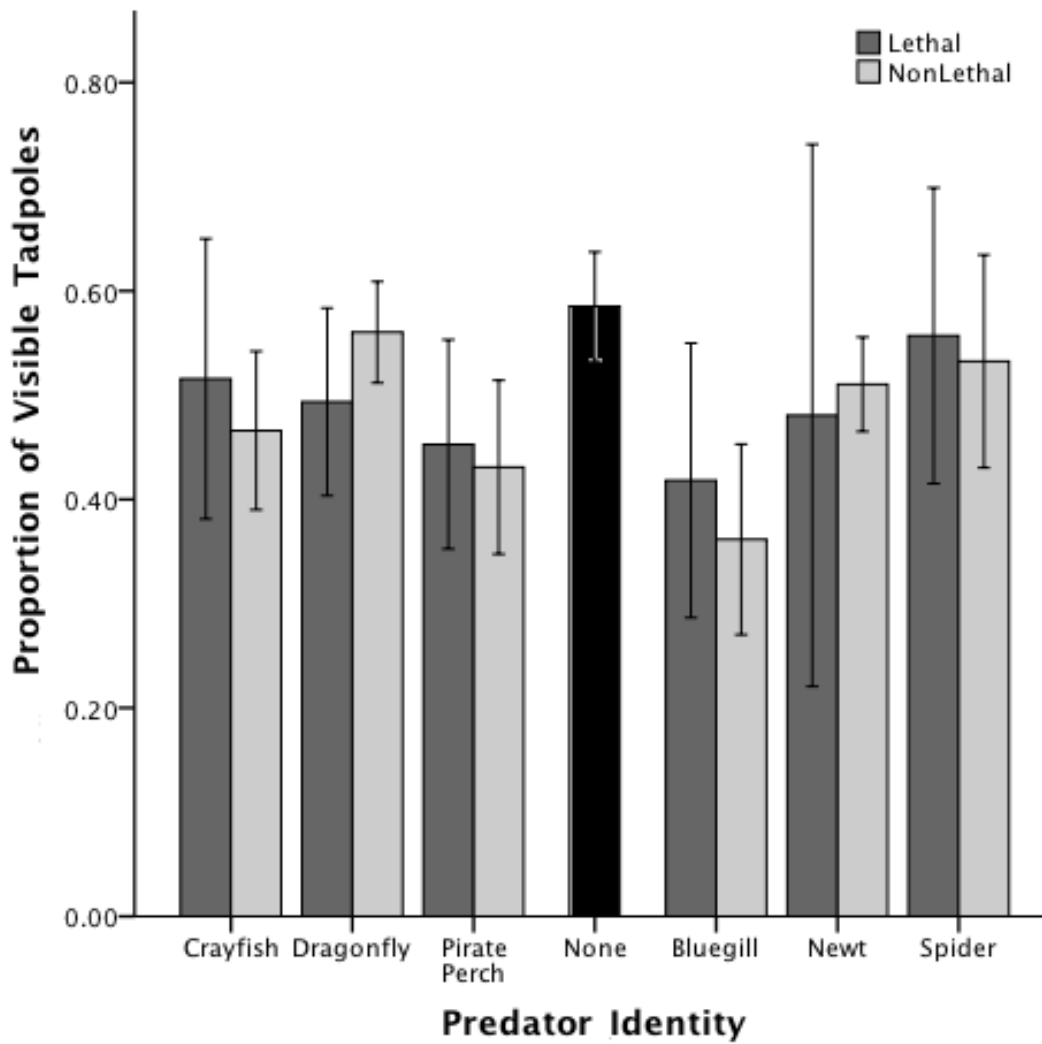
Fig. 2. The proportion of visible tadpoles separated by treatment (lethal and nonlethal) for each predator during both observation periods ($F_{12, 84}$ = 1.50, p = 0.1388 ). In all cases, bars are means ±1 SE.
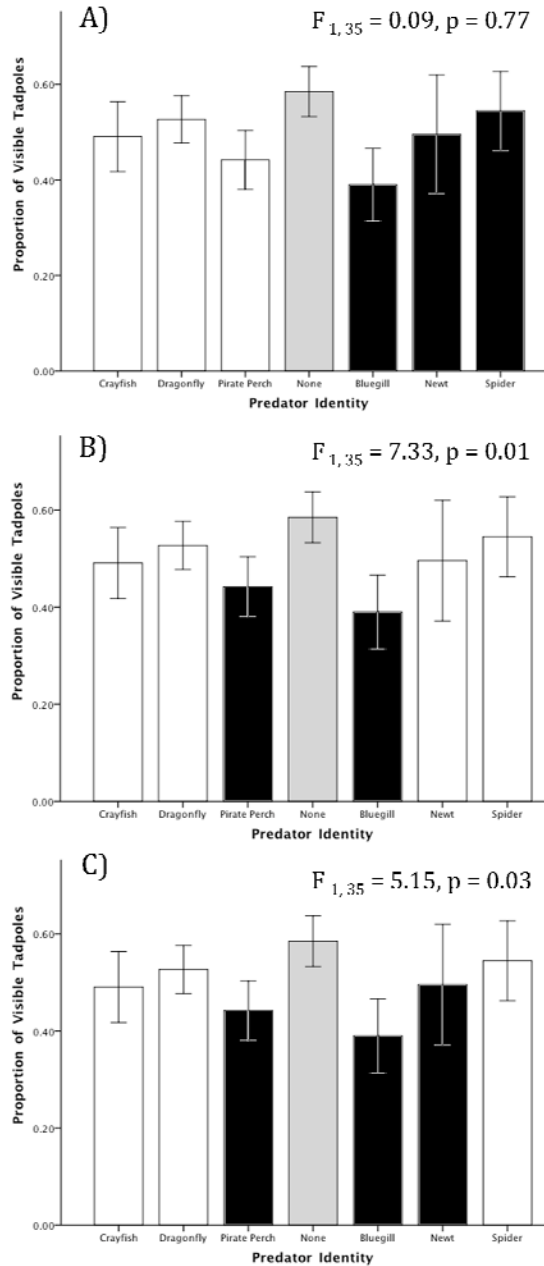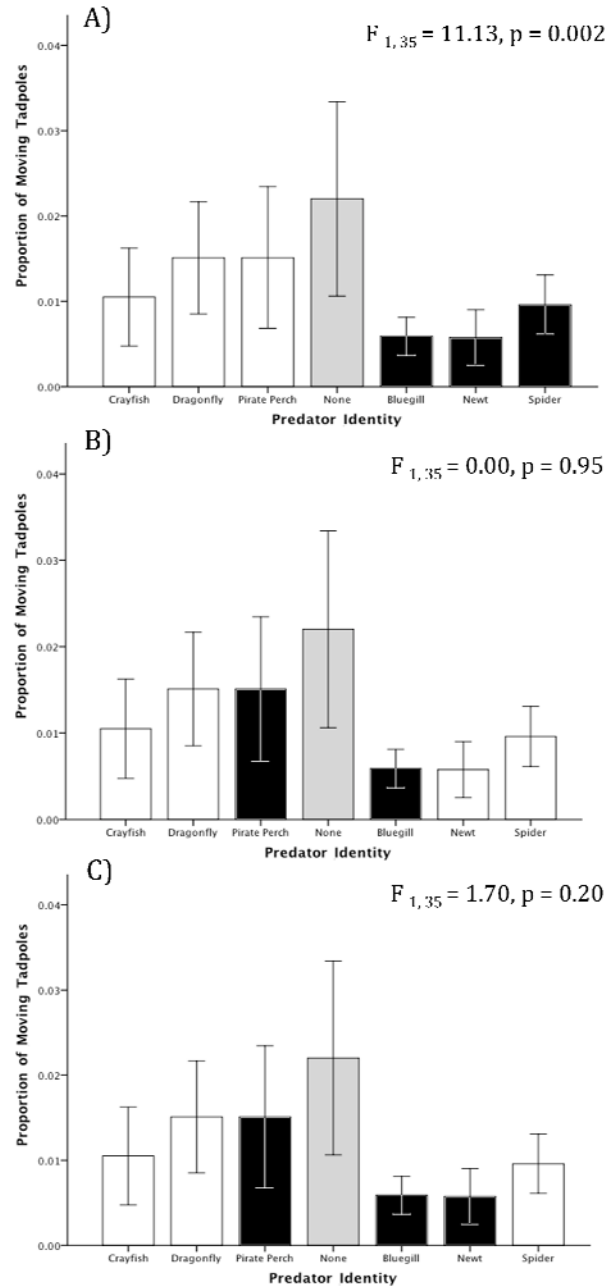
Fig. 3. The proportion of tadpoles visible in the presence of each predator (lethal and caged) during both observation periods ($F_{5, 35} = 1.84$, p = 0.1308). The data is the same in all three panels (A, B, C) but the shading differs to emphasize the planned contrast of interest. A) Comparison of benthic (open bars) vs. pelagic predators (black bars), B) Comparison of fish (black bars) vs. non-fish predators (open bars), and C) Comparison of vertebrate predators (black bars) vs. invertebrate predators (open bars). In all cases, bars are means ±1 SE.
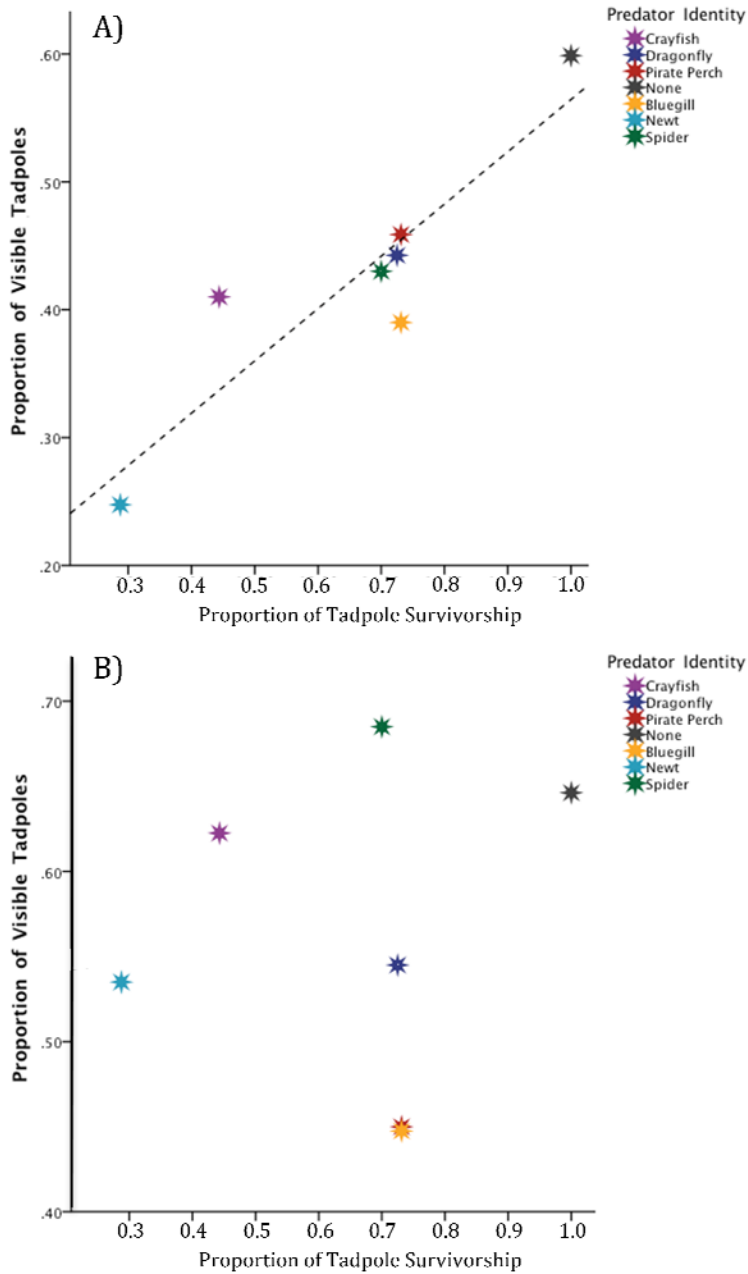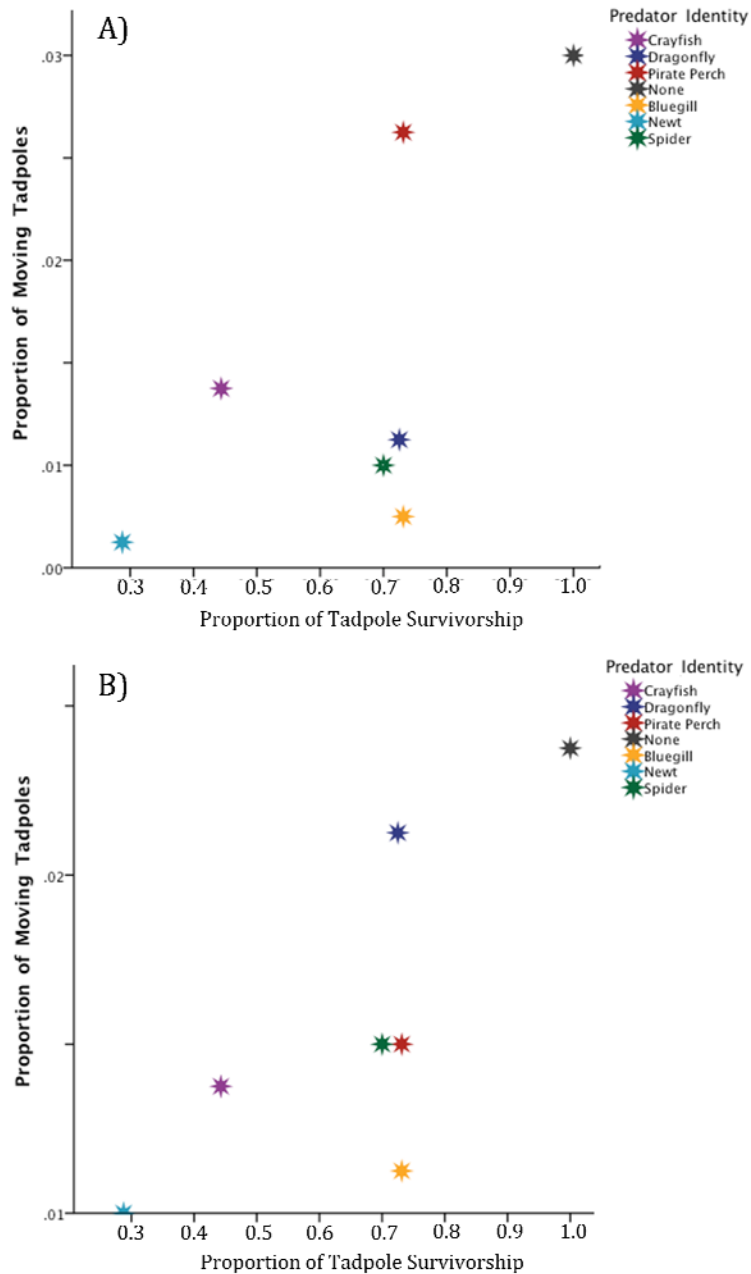
Fig. 4. The relationship of the proportion of visible *L. sphenocephalus* tadpoles against the mean survivorship of tadpoles with each predator. Graph A represents the first observation period ($R^2$= 0.821, p= 0.005). Graph B represents the second observation period ($R^2$= 0.30, p = 0.203).
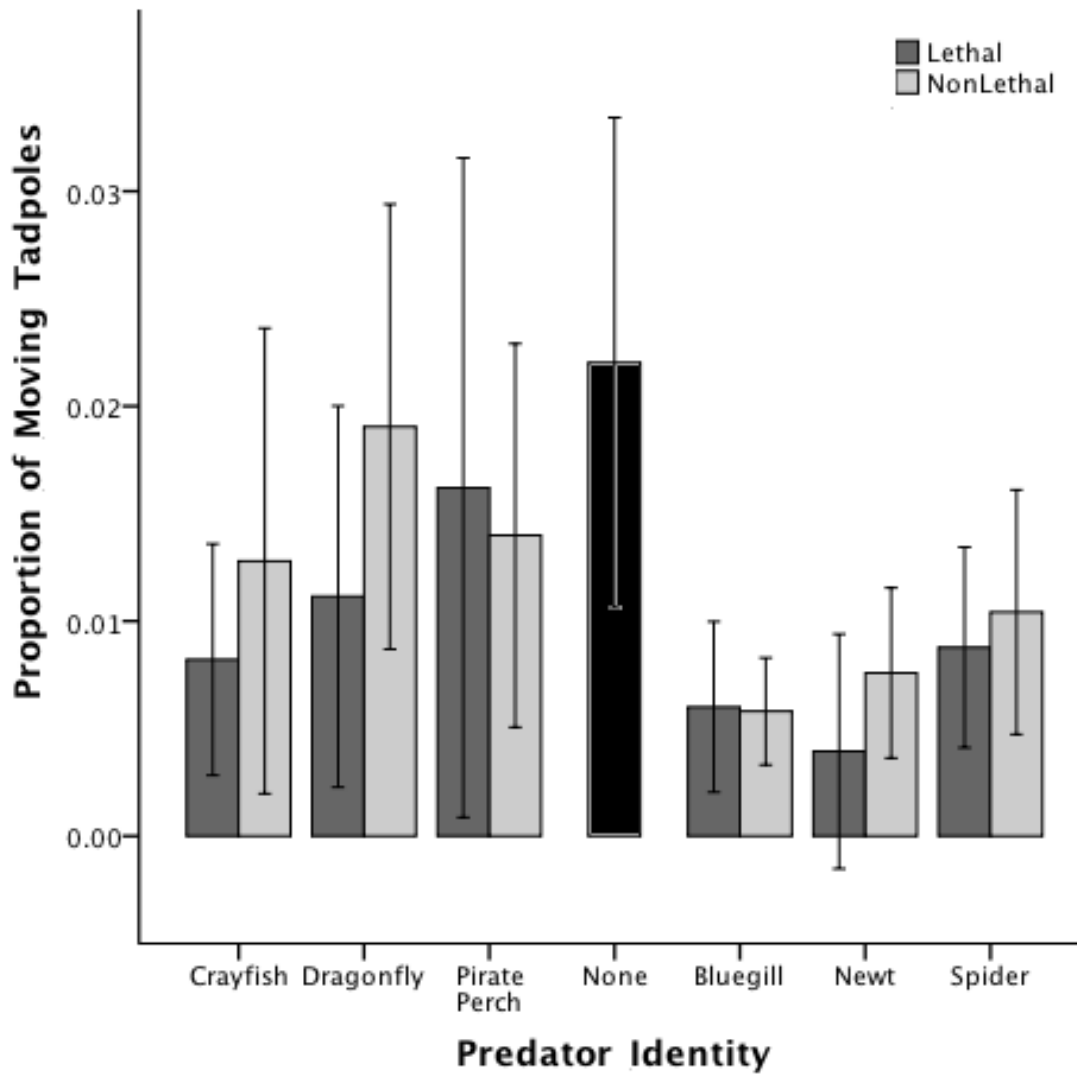
Fig. 5. The proportion of moving tadpoles separated by treatment (lethal and nonlethal) during both observation periods for each predator ($F_{12,84}=2.17$, p = 0.0202). In all cases, bars are means ±1 SE.

Fig. 6. The proportion of tadpoles moving in the presence of each predator (lethal and caged) during both observation periods ($F_{5, 35}$ = 2.78, p = 0.0324). The data is the same in all three panels (A, B, C) but the shading differs to emphasize the planned contrast of interest. A) Comparison of benthic (open bars) vs. pelagic predators (black bars), B) Comparison of fish (black bars) vs. non-fish predators (open bars), and C) Comparison of vertebrate predators (black bars) vs. invertebrate predators (open bars). In all cases, bars are means ±1 SE.

Fig. 7. The relationship of the proportion of moving *L. sphenocephalus* tadpoles against the mean survivorship of tadpoles with each predator. Graph A represents the first observation period ($R^2$= 0.43, p= 0.110). Graph B represents the second observation period ($R^2$= .57, p=0.057).
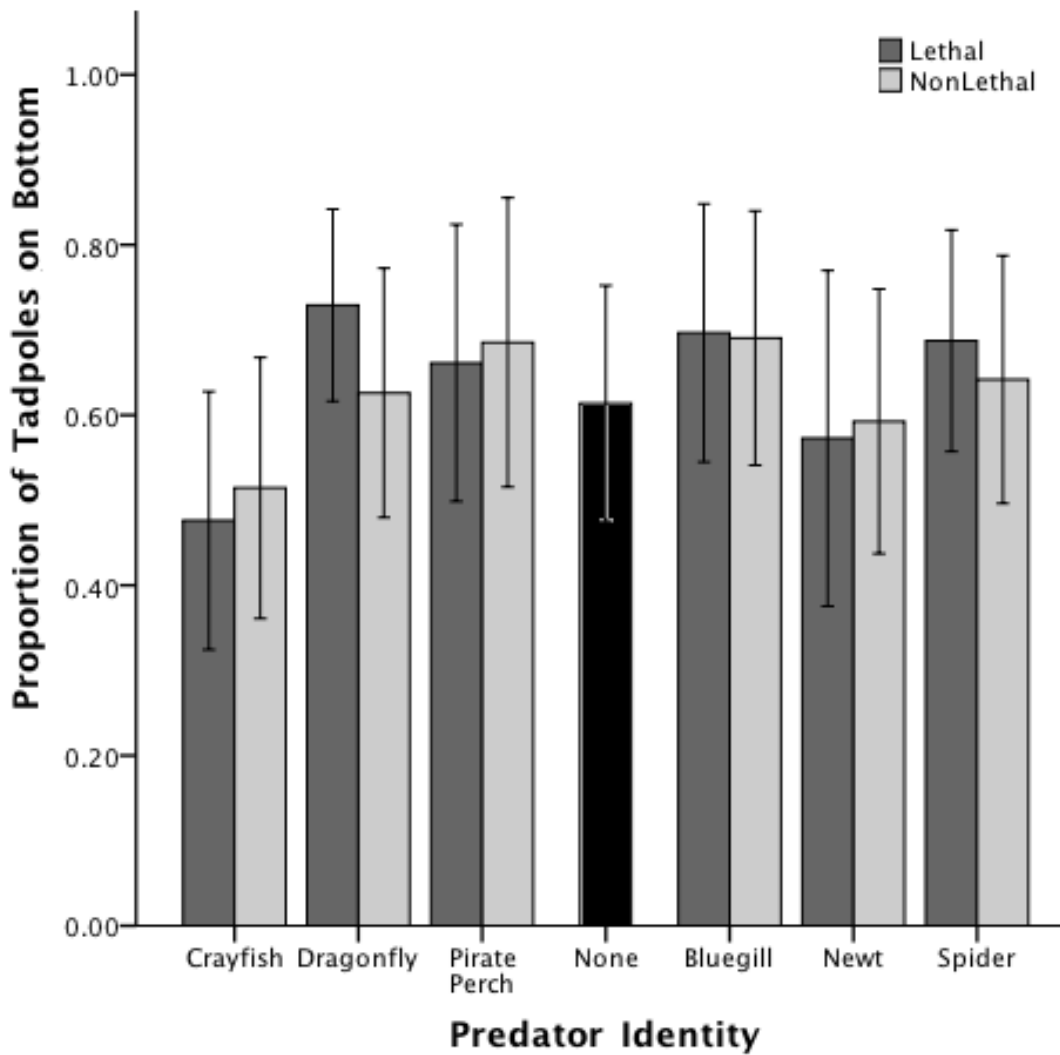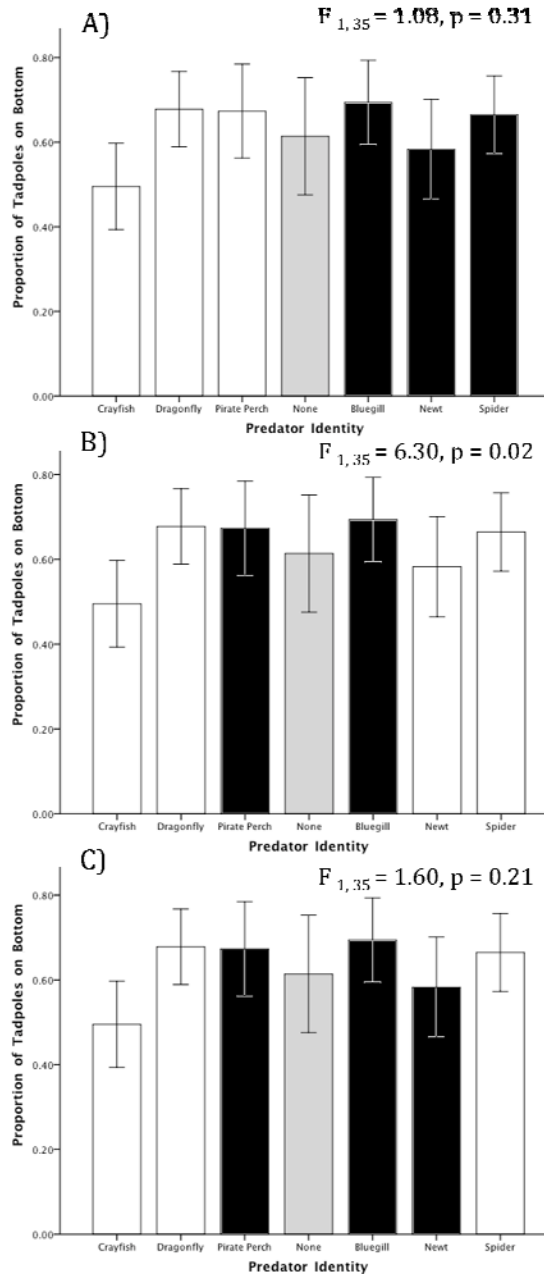
Fig. 8. The proportion of tadpoles along the bottom of the aquaria separated by treatment (lethal and nonlethal) for each predator during both observation periods (F $_{12,84}$ = 2.22, p = 0.0174). In all cases, bars are means ±1 SE.

Fig. 9 The proportion of tadpoles present at the bottom of the aquaria in the presence of each predator (lethal and caged) during both observation periods ($F_{5, 35}$ = 4.52, p = 0.0028). The data is the same in all three panels (A, B, C) but the shading differs to emphasize the planned contrast of interest. A) Comparison of benthic (open bars) vs. pelagic predators (black bars), B) Comparison of fish (black bars) vs. non-fish predators (open bars), and C) Comparison of vertebrate predators (black bars) vs. invertebrate predators (open bars). In all cases, bars are means ±1 SE.
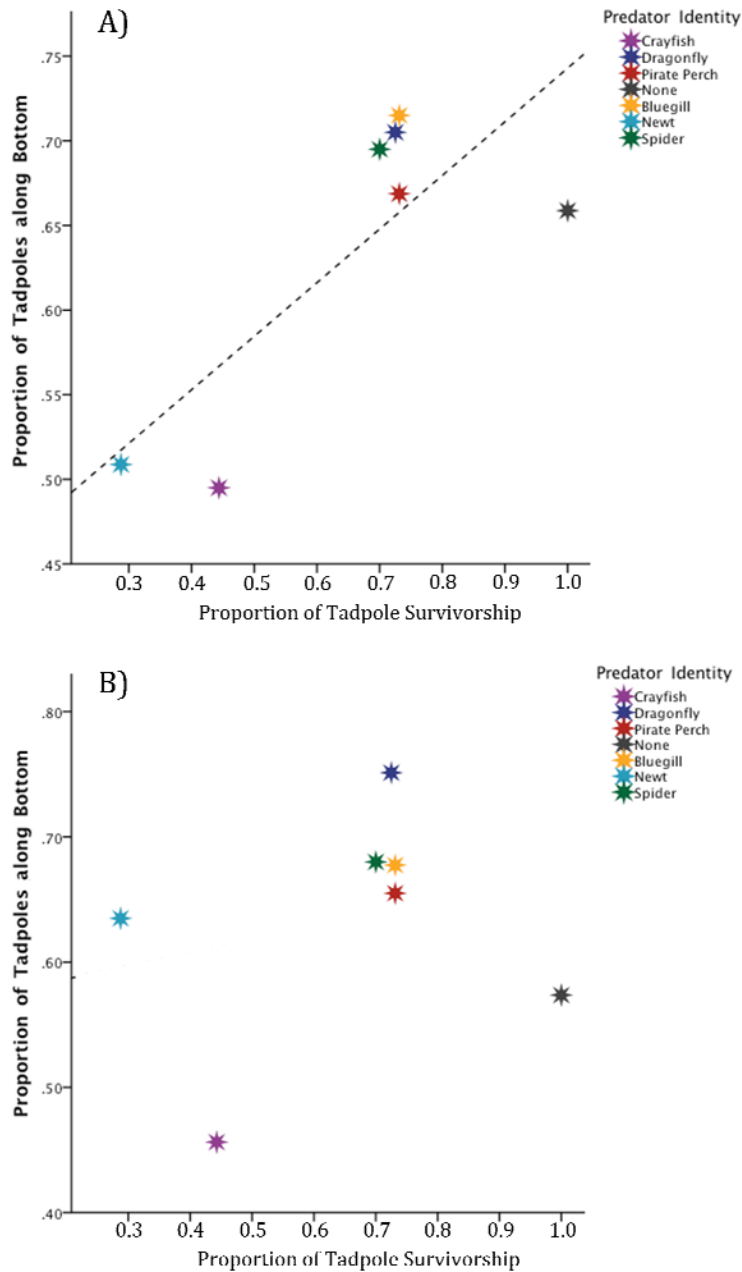
Fig. 10. The relationship of the proportion of *L. sphenocephalus* tadpoles along the bottom of aquaria against the mean survivorship of tadpoles in the presence of each predator. Graph A represents the first observation period ($R^2= 0.610$, $p= 0.038$). Graph B represents the second observation period ($R^2= 0.06$, $p= 0.0598$).
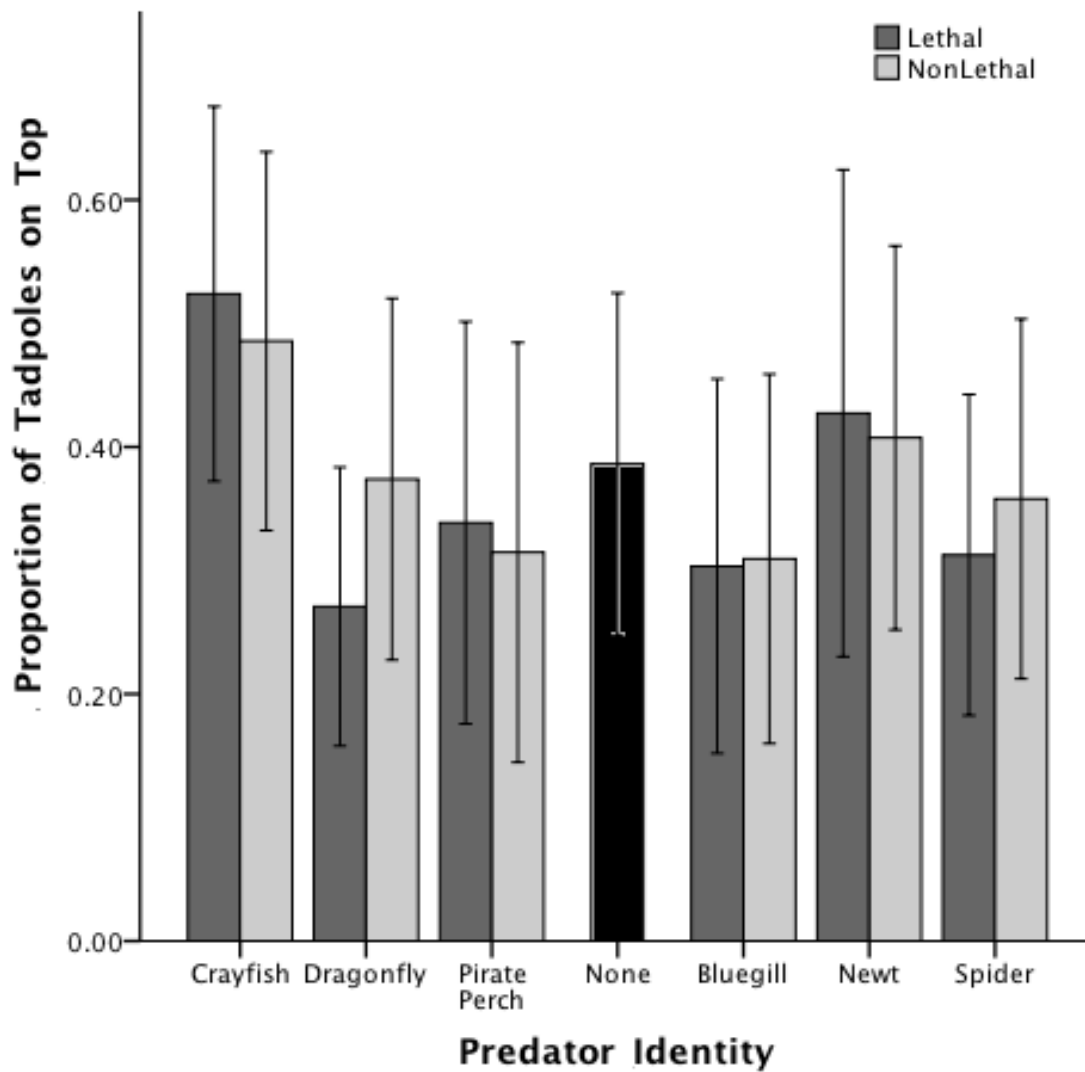
Fig. 11. The proportion of tadpoles along the top of the aquaria separated by treatment (lethal and nonlethal) for each predator during both observation periods ($F_{12,84}$ = 2.22, p = 0.0174). In all cases, bars are means ±1 SE.
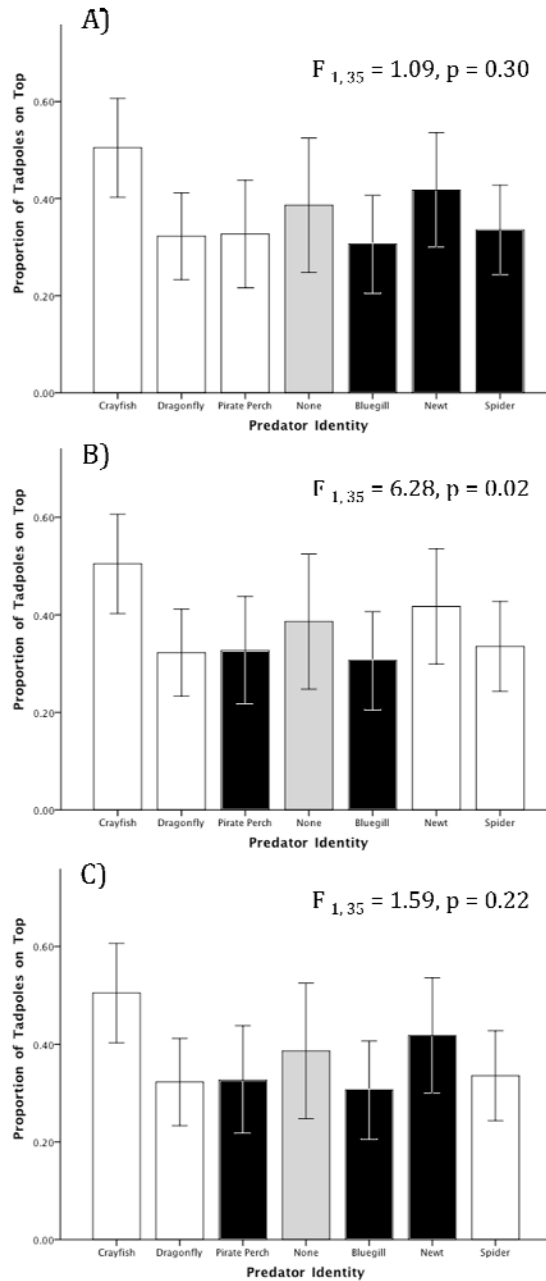
Fig. 12. The proportion of tadpoles present at the top of the aquaria in the presence of each predator (lethal and caged) during both observation periods ($F_{5, 35} = 4.52$, p = 0.0028). The data is the same in all three panels (A, B, C) but the shading differs to emphasize the planned contrast of interest. A) Comparison of benthic (open bars) vs. pelagic predators (black bars), B) Comparison of fish (black bars) vs. non-fish predators (open bars), and C) Comparison of vertebrate predators (black bars) vs. invertebrate predators (open bars). In all cases, bars are means ±1 SE.
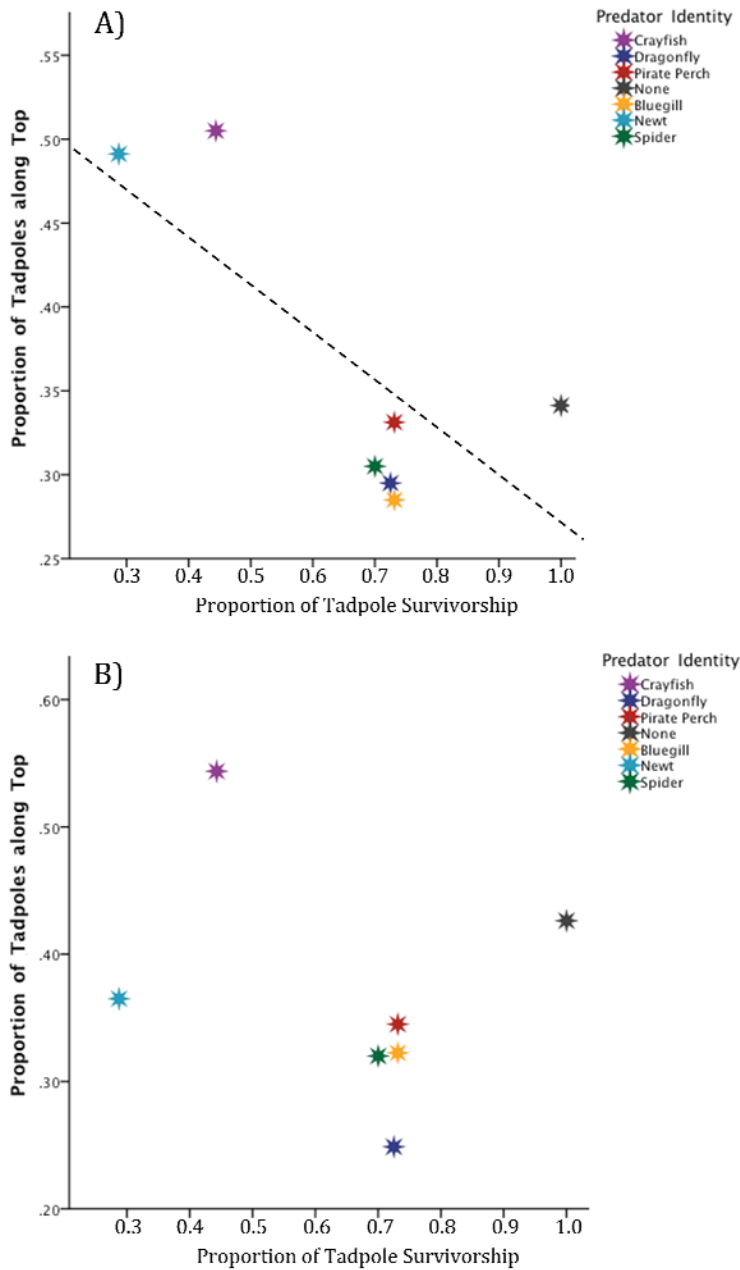
Fig. 13. The relationship of the proportion of *L. sphenocephalus* tadpoles along the top of aquaria against the mean survivorship of tadpoles in the presence of each predator. Graph A represents the first observation period ($R^2$= 0.610, p= 0.038). Graph B represents the second observation period ($R^2$= 0.06, p= 0.598).

APPENDIX A:  ANIMAL USE PROTOCOL APPROVAL

# 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

June 23, 2010

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

Dear Dr. Vance-Chalcraft:

Your Animal Use Protocol entitled, "Predator Efficiency and its Effect on Antipredator Behavior in Larval Anurans," (AUP #D246) was reviewed by this institution's Animal Care and Use Committee on 6/23/10. 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.

Sincerely yours,

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure