METABOLIC, PHYSIOLOGICAL, AND BEHAVIORAL RESPONSES OF PREY TO PREDATION

By

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July 2015

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Predators are known to cause prey to alter their morphology, life history or behavior in ways that reduce the likelihood of the prey being consumed by the predator. Seldom considered, however, are the consequences of predators on internal morphology (e.g., gut length) or physiology of prey. Such consideration is important because these traits likely affect prey growth and could explain why prey often grow more slowly in the presence of predators. Furthermore, a history of exposure to predators may alter how strongly visual or chemical signals from predators affect prey physiology and behavior. I raised larval frogs in artificial ponds that either lacked or contained a caged fish predator and assessed whether rearing environment affected prey gut length, morphology, behavior, and metabolic rate. I also assessed whether the rearing environment affected the metabolic and behavioral response of larval frogs to either short-term visual or chemical signals from fish by measuring the metabolism and behavior of predator naïve and predator exposed larval frogs when exposed to short-term visual and/or chemical signals from fish.

Tadpoles raised with predators had shorter guts but long-term predator exposure had no effect on the metabolic rate of tadpoles, body mass, or survival. The effect of long-term predator exposure on tadpole shape depended on body size. Occurrence of predators caused tadpole shape to differ for both small and large tadpoles but not tadpoles of the average body size. Short-term exposure to chemical cues from predators altered the metabolic rate of naïve tadpoles but not tadpoles with prior exposure to predators. Smaller naïve tadpoles reduced their metabolic rate but larger naïve tadpoles enhanced their metabolic rate in response to short-term chemical cues. Chemical cues caused the metabolic rate of naïve tadpoles to be 24% greater than that observed in tadpoles that were reared with predators. Short-term visual cues did not influence the metabolic rate of any tadpoles. Prior exposure to predators did not cause tadpoles to differ in their activity levels or their likelihood to seek a refuge. Exposure to short-term chemical cues increased the number of naïve tadpoles seeking a refuge. Short-term visual cues resulted in more predator exposed tadpoles hiding in a refuge.

My results indicate that long-term exposure to predators may compromise the ability of prey to extract resources by causing prey to develop shorter guts. These results further suggest the greater activity of predator exposed tadpoles to be a result of a less efficient digestion system requiring increased foraging effort but the risk of increased activity in the face of predation may be mitigated to some degree by modifications to body shape. This study supports the idea that there are complex interactions among physiology, behavior, and morphology in predator-prey interactions.

METABOLIC, PHYSIOLOGICAL, AND BEHAVIORAL RESPONSES OF PREY TO PREDATION

A Thesis

Presented To the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

By

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July, 2015

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ACKNOWLEDGEMENTS

First and foremost I would like to thank all of the amazing creatures that sparked my interest in the natural world at a very young age and who have continued to hold my fascination all these years. On the human side of things my greatest thanks must go to a very supportive and patient wife who wishes to remain nameless. Without her guidance and encouragement to better myself none of this would have been possible.

I would also like to thank my brother Larry for his encouragement and support. I also wish to acknowledge my fellow graduate students who lent a hand in the field. I would also like to give special thanks to my lab mates Lauren McCarthy, Suelen Calado Tullio, and Scott Jones who helped me with statistics, the manuscript, or just served as a sounding board. I also thank Erik Jessen at Loligo Systems for his tremendous patience and support with the respirometry equipment and software. Erik was always prompt in responding to my cries for help through email and despite being based in Denmark never hesitated to call me on the phone to offer assistance. I doubt this project could have proceeded as planned without his assistance.

I would like to thank my mentor Dr. David Chalcraft, for his expertise in all things ecology and statistics as well as his patience. A big thank you also must go out to the staff and faculty of the biology department for their support and guidance. Finally, I would like to acknowledge my graduate committee: Dr. Krista McCoy, Mr. Jeff Hall, and Dr. Anthony Overton for their encouragement, assistance, and support of the project.

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INTRODUCTION

The possibility of being eaten by predators is an important threat to many species. Subsequently, many species of prey have evolved the ability to detect the presence of predators, which can reduce their risk of mortality. For example, prey animals are known to possess the ability to detect predator odor cues (kairomones) and alter their behavior and/or morphology in a way that allows prey to better escape being eaten (Harvell 1990, reviewed in Kats and Dill 1998). In addition to predator kairomones, prey animals may detect predators through the use of alarm signals generated from conspecifics that have encountered a predator (reviewed in Chivers and Smith 1998) and/or through predator diet cues, disturbance cues, or through visual means (Huryn and Chivers 1999, Chivers *et al*. 2000, Wisenden and Millard 2001, Goddard 2006, Ferrari *et al.* 2010).

A large body of work has demonstrated that many species of prey alter their morphology, behavior, or physiology under the threat of predation. The ability of some organisms to adjust their phenotype in response to changes in the environment (e.g., presence of predators) has been termed phenotypic plasticity (Harvell 1990, El Balaa and Blouin-Demers 2013). In response to predators, some taxa modify tail shape, grow spines, alter metabolism, change body shape, or simply reduce activity thereby decreasing the odds of a predator encounter (Ferrari *et al*. 2010). These responses demonstrate that prey can rapidly and accurately analyze their environments for the presence of predators (Steiner and Van Buskirk 2009). Responses of prey to the presence of predators (i.e., the non-consumptive or non-lethal effects of predators) can strongly influence prey species (Paterson *et al.* 2013). For example, prey exposed to predators have often been found to metamorphose at small sizes or grow more slowly than conspecifics not exposed to

predator cues and such predator-induced growth reduction may impact adult survival or reproduction (McPeek *et al*. 2001).

Morphological responses of some prey species to the non-lethal presence of predators are widespread and probably the most impressive type of plastic response as well as the best studied. Various zooplankton species develop spines and neck teeth in response to predators such as copepods (e.g., rotifers, *Daphnia*) (Walls and Ketola 1989, Kats and Dill 1998). *Mytilus* bivalves develop thicker and shorter byssal threads in response to crab predators (Cote 1995, Kats and Dill 1998), while some gastropods exhibit changes in their shells, which may include modifications in thickness or the development of teeth (Appleton and Palmer 1988, Palmer 1990, Kats and Dill 1998). Alterations to prey morphology may benefit prey by making them more efficient at escaping predators, more difficult to eat, or more cryptic, and may be combined with other types of plasticity that increase the odds of prey survival.

In addition to, or in place of morphological defenses, some prey species may modify their behavior as a means of reducing the mortality risk from predators. For example, some prey become less active and/or seek refuge to reduce the likelihood of predator detection. This common behavioral response has been shown to occur in gastropods, larval amphibians, amphipods, sticklebacks, and many others (Holomuzki and Hoyle 1990, Malmqvist 1992, Gelowitz *et al.* 1993, Stauffer and Semlitsch 1993). Other behavioral responses to predation can include prey leaving water that contains the predator (Hoffman and Weldon 1978), or simply avoiding areas that contain predator cues as is seen across many taxa. For example, mollusks, *Daphnia*, insects, echinoderms, and others are known to avoid areas with predators in favor of those without (Feder 1967, Mann *et al.* 1984, Dodson 1988, Malmqvist 1992).

Prey having these predator induced phenotypes should have higher fitness than prey without such traits when cohabitating with predators because predator induced plasticity should provide a survival advantage. In the absence of predators, however, prey with induced traits should have lower fitness because there are tradeoffs involved with plastic traits (Lima and Dill 1990). In order to grow a larger tail or body to facilitate escape from predators, energy used in growth or development must instead be diverted to morphological defenses. Shifting resources to predator defenses instead of growth is worthwhile so long as the impact on growth or development is not overly high. Prey that shuttle most of their resources into predator defense may suffer by maturing at a smaller size or by becoming less fecund as adults. Because predator-induced traits are not found in the absence of predators we know there must be costs in responding to predators, otherwise all prey would possess predator-induced adaptive traits at all times.

Costs associated with the development of morphological or behavioral defenses can manifest themselves as a trade-off for prey between efforts to better defend oneself against predators and to grow (Lima and Dill 1990, McPeek 2004). For example, a reduction in foraging time to minimize risk of mortality to prey can come at the cost of reduced food intake which may result in slower growth and development (Steiner 2007). However, a reduction in activity due to the risk of predation may not be the only explanation for reduced growth or development in prey. Studies involving anuran larvae indicate the tradeoff may develop not only due to reduced activity under predation risk but also because of a costly increase in metabolic rate in the presence of predators (Steiner and Van Buskirk 2009). Because prey may spend time hiding from predators and spending less time foraging for food resources, some aspects of prey

physiology may change to minimize any reduction in body growth (Richardson 2001, Barry and Syal 2013).

Physiological plasticity is less studied than morphological and behavioral plasticity. Physiological plasticity may occur in gut evacuation, digestion, and metabolism (McPeek *et al*. 2001, Relyea and Auld 2004, Stoks *et al*. 2005, Steiner 2007). Predator-induced plasticity in metabolism has been shown to occur in damselfly and amphibian larvae resulting in reduced growth (McPeek *et al*. 2001, Steiner and Van Buskirk 2009). Studies in physiological plasticity have been conducted on fish (Milano *et al* 2010), damselflies (McPeek 2001), and particularly on larval frogs (Steiner 2007, Steiner and Van Buskirk 2009, Barry and Syal 2013). Fish have been found to reduce oxygen consumption in the presence of predators though this response may be an artifact of reduced activity (Milano *et al*. 2010). Work on damselflies indicates that different species may respond to physiological stress caused by predators differently (McPeek 2001). McPeek (2004) found that one species of damselfly can more efficiently convert assimilated food into new biomass but was more active which lead to higher mortality from predators while a second species had a faster rate of growth despite consuming the same amount of resources. This work demonstrates the importance of physiological plasticity because it indicates similar species can coexist in the presence of predators due to different physiological responses to the risk of predation. Plasticity in physiology may increase prey survival by allowing prey to adjust their metabolic needs in response to predators or other environmental conditions. Prey species that typically hide from predators may have reduced time for effective foraging but this cost may be mitigated by an adjustment in metabolic rate (Barry and Syal 2013).

Predator induced changes in physiology of prey animals appears to be little studied outside of some studies on grasshoppers (Hawlena *et al*. 2011) and amphibian larvae (Relyea and

Auld 2004, Steiner 2007) in particular. Predators have been found to induce tadpoles to develop shorter guts and to grow and develop more slowly; possibly as the result of less efficient digestion (Relyea and Auld 2004, Steiner 2007). Relyea and Auld (2004) concluded that tadpoles can invest resources into growing a larger tail to escape predators or develop a longer gut for more efficient digestion but they could not do both. Steiner (2007) demonstrated that predator exposed tadpoles evacuated their guts at a faster rate and developed more slowly than tadpoles not exposed to predators despite having similar ingestion rates. Steiner (2007) suggested that the consequences of predator exposure on prey growth or survival was not associated with reductions in food intake by prey but likely due to changes in other aspects of prey physiology (e.g., metabolic rate, allocation of resources to morphological defense).

Recent studies have demonstrated that the presence of predator chemical cues over long and short term time periods (i.e., chronic and acute predator cue exposure) resulted in predator naïve anuran larvae lowering their metabolic rate (e.g., Steiner and Van Buskirk 2009, Barry and Syal 2013, Barry 2014). A reduction in metabolic rate in predator exposed prey may be a response by prey to minimize the costs of anti-predator defenses. Predator exposed tadpoles were found to have reduced growth or developmental rate in the presence of predators in two of these recent studies. Steiner and Van Buskirk's (2009) work suggests that changes in metabolic rate may not be connected to a reduction in growth because predator exposed tadpoles still incurred a cost in reduced growth. Barry (2014) found that the presence of predators caused prey to lower their overall metabolic rate and to be less active while food consumption by prey increased their metabolic rate. Predator exposed tadpoles suffered reduced growth compared with tadpoles not exposed to predators. It is unclear if anuran larvae can compensate in some way to prevent

impacts to growth under predation risk by lowering metabolic costs, nor is it clear if reduced activity is the cause of reduced growth.

To further elucidate the above interactions, I was interested in examining how long term (1.5 months) and short-term (< 1 hour) exposure of prey to non-lethal predators affects prey to advance our understanding of potential trade-offs between traits that reduce the risk of mortality to prey and traits that promote body growth in prey. I examined the consequences of predator exposure to prey on prey growth, morphology (internal and external), behavior, and physiology. To do this I conducted an experiment with larvae of the Southern Leopard Frog (*Rana sphenocephala* Cope 1886), and the predatory fish, Bluegill (*Lepomis macrochirus* Rafinesque 1819). Prior work has demonstrated that anuran larvae respond to the threat of predation from bluegill by altering their morphology and reducing activity (Eklov and Werner 2000, Smith *et al*. 2007). Leopard frogs were chosen for this study because both the Northern and Southern species are known to exhibit morphological and/or behavioral plasticity when exposed to predation cues (Collier *et al*. 2008, Relyea 2012, El Balaa and Blouin-Demers 2013). At the time of this writing, no study has examined the effects of predators on the metabolic rate of any species of leopard frog.

Two indicators of the presence of predators to prey in aquatic systems are visual detection and the detection of chemical signals from predators or their prey (Stauffer and Semlitsch 1993). Most research indicates that chemical alarm cues dispersed in the water to be the most common and reliable indicator of predators for larval frogs over other cues such as visual and tactile cues because many aquatic environments of anuran larvae are densely vegetated, turbid, and because these cues may travel over longer distances (Stauffer and Semlitsch 1993, Hickman *et al*. 2004, Mogali *et al*. 2012, Takahara *et al*. 2012). Comparatively

few studies have tested whether or not visual cues play a role in predator recognition by larval amphibians and most indicate visual cues to be of little importance (Hickman *et al*. 2004, Kiesecker *et al*. 1996, Takahara *et al*. 2012). I assessed how short-term exposure to both visual and chemical cues affects prey but focused on responses from long-term exposure to chemical cues. This was done because 1) most evidence suggests that chemical cues are the most important and reliable cues and 2) the logistics of conducting experiments assessing responses of both short-term and long-term exposure to multiple types of cues were not practical at this time.

I developed several predictions based on the results of previous studies. I predicted prey exposed to predators over the long-term would develop morphological defenses such as a larger tail, shorter body, and more tail muscle and would have reduced growth. I predicted predator exposed prey would alter their internal morphology and develop a shorter gut than prey not exposed to predators. Furthermore, I predicted that predator exposed prey would have a lower metabolic rate after long-term predator exposure in order to compensate for reduced activity and reduced foraging. I predicted that prey exposed to predators in the short-term would have a sharp increase in oxygen consumption to chemical predator cues and that visual cues from predators would have no effect on tadpole behavior or metabolic rate. I expected that short-term exposure to predators would cause naïve tadpoles to reduce their activity compared to treatments where no exposure has occurred.

METHODS

To examine the effect of long-term exposure to chemical cues from non-lethal predators on tadpole morphology, behavior, and physiology, I raised tadpoles in artificial ponds (mesocosms) at East Carolina University's West Research Campus near Greenville, NC. Mesocoms either contained or lacked a caged predator (Bluegill sunfish) placed within a cage that allowed prey to detect chemical but not visual or tactile signals of the predator. A subset of tadpoles from these mesocosms were then transferred to the lab 42 days later where I measured their size and assessed their behavior and physiology in respirometers. To assess how the behavior and physiology of tadpoles respond to short-term exposure to predators, a different subset of tadpoles were placed in respirometers where tadpoles received no signals that a predator is present, visual signals that a predator is present, chemical signals that a predator is present, or both visual and chemical signals that a predator is present. Lastly, I also measured tadpole gut length and body shape to evaluate whether exposure to predators causes tadpoles to develop a different internal and/or external morphology.

Study Design: manipulations of rearing environment

I raised larval *Rana* in outdoor artificial ponds (mesocosms) that either contained or did not contain a caged *Lepomis* with the goal of producing two different groups of tadpoles (predator exposed and predator naïve) from which I assessed how predator exposure affects prey growth, behavior, morphology, and physiology (Figure 1). Mesocosms allow for close simulation of natural ponds on a smaller scale, allow for processes that are important in both natural and artificial ponds, and allow for a reduction in variability among different experimental pond units (Morin 1983, Rowe and Dunson 1994, Wilbur 1997). Use of mesocosms is

appropriate because they ensure independent experimental units and may help control for confounding issues (i.e., factors other than those the experimenter is manipulating) (Chalcraft *et al.* 2005).

The mesocosms I utilized consisted of 1100 l stock tanks. Each tank was modified with a standpipe that is attached to the tank drain to prevent the mesocosm from overfilling during precipitation events. Standpipe openings were covered with fiberglass screen in order to keep experimental animals in and other organisms out. Mesocosms were also fitted with a tight fitting fiberglass screen shade cloth that prevents colonization of the tanks by other organisms. Twenty mesocosms were arranged in two adjacent rows of 10 each in an open field at the West Research Campus. I paired one mesocosm from one row with the mesocosm in the adjacent row that is closest to it to form a statistical block. This method produced 10 statistical blocks and one of the two treatments (presence or absence of a caged *Lepomis*) was randomly assigned to one mesocosm within each block (Figure 1). An additional 5 mesocoms were set up nearby to hold additional *Rana* tadpoles that would be used as food for the experimental *Lepomis*.

Mesocosms were cleaned and filled on 5-16-2014. Mesocosms received 500 grams of a mixed hardwood and pine leaf litter to act as a source of nutrients and refuge for anuran larvae. Pond water was collected from a local pond and used as a source of plankton and algae for an additional level of realism in the mesocosms. An aliquot (473 ml) of this pond water was added to each mesocosm on 5-19-2014 after it had been filtered to remove invertebrate predators. Plankton and leaf litter were added to mesocoms on a block by block basis and the assignment of plankton and leaf litter to each mesocosm within a block was randomly determined.

Collection, addition, and care of experimental organisms

Leopard frog egg masses were collected from local wetlands on 18-19 May 2014 and transported to the lab. On 26 May 2014, I added 100 *Rana* tadpoles to each mesocosm. Each holding tank received 500 tadpoles. *Lepomis* fish were collected in local ponds and added to mesocosms on 27 May 2014. Each fish was weighed and visually inspected to ensure fish of similar size were used in the experiment. Fish were randomly added to mesocosms assigned the predator treatment on the same day they were collected. Fish were housed in mesh-sided cages and placed into mesocosms. Mesocosms designated to the non-predator treatment received an empty predator cage. Mesocosms were checked daily and *Lepomis* fish were fed 3 *Rana* tadpoles 3 times per week. All predator enclosures, whether they did or did not contain a predator were manipulated in the same manner in all mesocosms in order to equalize disturbance across the two rearing treatments during predator feedings (see Relyea 2005).

Study Design: manipulations of environment where prey physiology and behavior is assessed

After raising tadpoles in mesocosms for 42 days I brought a subset of tadpoles from each mesocosm into the lab so that I could measure their body size, metabolic rate, behavior, and internal and external morphology. Behavior and metabolic rate of tadpoles were assessed after tadpoles were placed inside a 1 liter intermittently closed respirometer (Loligo Systems) that was itself placed within 15.8 l aquaria and filled with water from the rearing mesocosms. To investigate whether the behavioral and metabolic response of tadpoles to short-term visual and chemical signals from predators depended on whether predators were present in the tadpoles' rearing environment, I manipulated the occurrence of visual and chemical signals from predators in the aquaria. Specifically, I assessed the short-term metabolic rate and behavior of tadpoles

from both rearing environments when tadpoles were presented with 1) no cue, 2) visual cue, 3) chemical cue, and 4) a combination of visual and chemical cue. This produced a total of 8 different scenarios (i.e., treatments) for measuring metabolic demand and behavior of tadpoles (i.e., 2 levels of rearing environment x 4 levels of predator cue).

Long-term responses were assessed in the absence of any cues within respirometers and served as a baseline to compare to short-term exposures of tadpoles to predators. I could not measure the metabolic rate of all tadpoles on the same day, so I measured the metabolic rate of animals from two of the experimental blocks per day and completed all measurements of metabolic rate on 7-11 July 2014. The day prior to measuring behavior and metabolic rate of tadpoles, I placed 8 aquaria within each of two spatial clusters (i.e., clusters of aquaria rather than blocks so as not to confuse blocks of aquaria with blocks of mesocoms) within the same lab. Each cluster of aquaria was assigned tadpoles from a different block of mesocosms and the eight aquaria within each cluster corresponded to the 8 scenarios in which I wanted to assess behavior and metabolic rate. I completed all measurements within each of two mesocosm blocks in a day and reset aquaria on the same day that measurements were completed so that they could be used again for additional measurements on animals from two other mesocosm blocks the next day. The two clusters of aquariums corresponded to the two mesocosm blocks that were tested each day. It required five days to complete measurements on tadpoles derived from all 10 blocks in the mesocosm portion of the study.

All animals were weighed on a digital balance 1 hour before being placed into the respirometer chamber. After mass was recorded I created 8 groups of 5 tadpoles within each block where all tadpoles within a group were of similar size through size varied among groups. Each group of five tadpoles was randomly assigned to one of the 16 aquaria and only used to

obtain oxygen consumption data once. The 1 hour acclimation period was implemented because it has been shown that disturbances cause hyperventilation, tachycardia, and increased oxygen consumption in anuran larvae (Feder 1981). Previous studies indicate that flow-through respirometry is not overly stressful to experimental animals (Abel *et al*. 1992).

Before adding animals to the respirometer, I submerged the respirometer chamber into an aquarium which prevents fluctuations in the chamber water temperature and provides a source of oxygenated flush water for the chamber. One respirometer chamber was utilized to obtain oxygen consumption values. During times of oxygen measurement a recirculation pump attached to the chamber is used to keep water flowing over the oxygen probe for accurate measurements. Respiration rate measurements were measured on a group of 5 tadpoles added to the respirometer chamber where oxygen consumption was measured for measurement periods of 3 minutes alternated with 3 minute flush periods for a total trial time of 15 minutes. A trial therefore consisted of 3 three minute measurement periods and two flush periods. At the conclusion of the third measurement period the trial was stopped.

Each aquarium received one of the 4 cue treatments that differed in the kind of cue that prey may encounter to reveal the presence or absence of a predator (Figure 2). The treatment where prey will only be exposed to a visual cue of a predator was executed by placing a clear water filled chamber containing a *Lepomis* into the ambient aquarium next to the respirometer. The chamber containing the *Lepomis* was sealed so that no water was exchanged between the chamber and the ambient aquarium as water exchange would allow prey to also detect the chemical cues of the predator. In this scenario the ambient aquariums were filled with water from mesocosms that contained tadpoles only. The treatment where prey was only to be exposed to chemical cues was implemented by using water from a mesocosm that contained *Lepomis*

feeding on *Rana* into the ambient tank where the only cues that prey will receive are those chemical cues produced by predators that were previously present in the source water. The treatment with no cues did not have a predator or predator odor cues present in the ambient aquarium and was filled with water from a mesocosm containing only *Rana* tadpoles. The addition of water from a mesocosm lacking *Lepomis* ensures that it is not merely the addition of water from a mesocosm that elicits a reaction in the treatment where chemical cues are provided via the addition of water derived from mesocosms containing *Lepomis*. The treatment providing both visual and chemical cues received both a clear chamber with a *Lepomis* and water from a mesocosm containing *Lepomis*.

Each respirometer chamber was removed from the ambient aquaria after each trial, animals removed, and rinsed with tap water before being placed into the next aquarium for a different trial. At the end of each day, all aquariums and equipment were rinsed for the next day's work. I performed a total of 80 trials resulting in 8 treatment replicates. After measuring oxygen consumption in the respirometer, experimental animals used in the respirometry trials were euthanized via overdose of MS 222 (tricaine methanesulfonate) and preserved in a 10% formalin and water solution.

Quantification of metabolic rate

I estimated the combined metabolic rate for each group of tadpoles placed into the respirometer for each of the 3 measurement periods. Mass specific oxygen consumption (MO_2: measured in $mgO_2/kg*hr$) was estimated as:

$$MO_2 = (V/W)*m \quad \text{Equation (1)}$$

where V is the volume (1000 mL) of the respirometer chamber and associated tubing, W refers to the wet weight of organisms in the respirometer chamber, and m refers to the slope (linear rate at which the amount of dissolved O_2 present in the chamber declines through time) (Loligo Systems 2011). The rate of oxygen decline during each measurement period was assessed by regressing dissolved oxygen concentration in the chamber against the length of time in which the chamber was closed off from the ambient aquarium and the flush pump was off.

I inspected plots depicting how the amount of oxygen present changed through time during each measurement period. I noted that in most cases the 0_2 values tended to decline linearly as assumed by equation (1) for the first minute in each of the 3 measurement periods in a trial but the values often leveled off or increased during the remaining two minutes of the measurement periods. Equation (1) requires a slope estimate from a linear regression that best fits the decline in oxygen over time (Loligo Systems 2011). Regression models describing how oxygen declined over time described changes better when the models only described changes in oxygen for the first minute of a measurement period than when models described changes across the full 3 minutes of a measurement period (Median R^2 for models describing changes in O_2 during first minute=74%; Median R^2 for models describing changes in O_2 during full three minutes=50%). In addition to describing changes in oxygen levels through time less well, models assessing changes in oxygen over three minutes were more likely to produce positive slope estimates (suggesting that tadpoles produce oxygen rather than consume it) than models assessing changes over 1 minute (# positive estimates for 3 minutes=58 and # positive estimates for 1 minute=24). Consequently, I estimated metabolic rates on the basis of using slope estimates obtained during the first minute of each observation period.

I also noticed that measurements during the first measurement period were more likely to produce a positive slope estimate than measurements made in either the second or third measurement period (positive slopes in the first measurement period=16, positive slopes in the second measurement period=5, positive slopes in the third measurement period=3). This may be due to mixing of water at the start of the trial (see Steiner and Van Buskirk 2009). Consequently, I obtained an estimate of metabolic rate for each subset of tadpoles by averaging the metabolic rate estimates for that particular subset of tadpoles that were made for the last two measurement periods.

Quantification of gut length and morphology

I removed and measured the gut length of a randomly chosen subset of 5 tadpoles from each mesocosm. Each gut was carefully straightened and measured with the use of digital calipers to the nearest 0.01 mm. (Relyea 2002, Unrine *et al*. 2005).

To facilitate the description of the external shape of animals that were raised in either the presence or absence of a predator, I took digital images of 30 randomly selected tadpoles from each mesocosm that were preserved in formalin. Digital images of tadpoles were obtained through the use of a photo chamber equipped with a fluid filled reservoir that allowed tadpoles to suspend naturally. These digital images were later utilized to obtain landmark coordinates based on common morphology (Figure 3) with the use of ImageJ software (version 1.49j). Landmark data were utilized in a geometric morphometrics approach to evaluate for differences in tadpole shape both quantitatively and visually. Geometric morphometrics is a more powerful approach over traditional methods of analyzing shape because it allows for later visualization of shape by preserving geometrical shape differences (Rohlf and Marcus 1993, Ruehl and DeWitt 2005). The

ability to more readily visualize differences in shape is a significant advantage of geometric morphometrics over traditional distance measurements, which focus on a few predefined aspects of shape (Rohlf and Marcus 1993, Klingenberg 2013).

To quantify tadpole shape, Procrustes superimposition was performed on the 13 landmark coordinates taken from each individual tadpole (Dayton *et al*. 2005, Klingenberg 2013) utilizing MorphoJ software (Version 1.06c). Procrustes analysis provided coordinates for all individual tadpoles used in the analysis. Superimposition techniques adjust coordinates to a common scale and account for image orientation (i.e., remove variation in position of coordinates due to rotation or translation of the landmark configuration) (Klingenberg 2013). A principal component analysis using a covariance matrix was performed on the subsequent coordinates after calculating the average of each landmark across all animals within each mesocosm. I retained the first four PCA axes as a scree plot indicated that additional axes only contribute toward minor (<5%) increases in the cumulative amount of variation in shape. The first four axes explained (87%) of the variation in coordinate values.

Quantification of tadpole behavior

I was also interested in how the perceived risk of fish predation affected tadpole behavior. In order to observe and record tadpole behavior during respirometry trials, I outfitted the respirometry chamber with a refuge consisting of a small patch of artificial plants in which tadpoles could hide. This refuge was adhered to the bottom of the respirometer chamber in such a manner as to not interfere with the flow of water or tadpole movement.

I measured 4 aspects of tadpole behavior while tadpoles were located within the respirometer: number of tadpoles in the refuge, number of tadpoles active, time active, and time

in refuge. Number of tadpoles in the refuge and number of tadpoles active (i.e., # that are actively swimming) in a chamber were quantified instantaneously at 30 seconds into the first minute of each of the 3 measurement periods in each trial. I observed a randomly chosen focal tadpole in each aquarium for 1 minute during the second minute of each measurement period to quantify how much time the animal was active. I observed a different focal animal for the final minute during each measurement period to quantify how much time it spent in the refuge. Given that I obtained 3 estimates of each behavior for each aquarium (1 for each measurement period), I averaged responses across each of the 3 measurement periods in a trial to obtain one independent estimate of each behavior for each aquarium.

Statistical Analyses

All statistical tests were conducted utilizing SAS Version 9.4® and SAS Enterprise Guide 6.1®. To evaluate the effect of rearing environment on mean tadpole mass in each mesocosm and survivorship I utilized linear mixed models (i.e., PROC MIXED in SAS) that included rearing environment treatment (i.e., predator or no predator) as a fixed effect and mesocosm block as a random effect.

I conducted an analysis of covariance (ANCOVA) to determine if rearing environment affected gut length after controlling for differences in tadpole body size. In addition to including rearing environment as a fixed effect, the ANCOVA included a random block effect, body size as a covariate, and a term (interaction between rearing environment and body size) to account for possible differences in the relationship between gut length and body size between rearing environments. I found that the slope of the relationship between gut length and body size did not differ with rearing environment ($p=0.4757$) so the ANCOVA was performed again with the

exclusion of the interaction term. Visual inspection of graphs supported the idea that the slope of the relationship between gut length and body size was similar for the two rearing environments.

I conducted a split-plot ANOVA using PROC MIXED in SAS to assess how metabolic rate varied across my experimental conditions to detect differences in metabolic rate in response to my treatments. A split-plot ANOVA is appropriate because my experimental design includes both a whole-plot factor (rearing treatments) and four subplot factors (chemical cue, visual cue, no cue, and chemical/visual cues). The model included chemical cue (CC), visual cue (Vis), mean body mass of tadpoles in the respirometer, rearing environment, the two-way and three-way interactions involving experimental factors (CC x RE x Vis, CC x Vis, and CC x RE x Vis), and the four-way interaction (Mass x CC x RE x Vis) to evaluate whether the slope of the relationship between metabolic rate and body size varied among treatments. Random effects included Date, Block nested within Date, the interaction between RE and Block(Date), and the interaction between rearing environment and Date. Date refers to the particular day on which metabolic rate and behavior were assessed. Visual inspection of graphs suggested that slopes varied among treatments and a rather low ($p=0.1455$), but not statistically significant p value associated with the four-way interaction (Mass x CC x RE x Vis) indicated that the relationship between metabolic rate and body size varied among experimental treatments. A similar method was performed on behavioral data but any terms involving size were excluded from the model because preliminary analyses and inspections of graphs suggested that behavior did not vary predictably with size in any treatment. I performed 10 different contrasts to evaluate how experimental conditions affected metabolic rate of tadpoles for each of three body sizes; mean body size (4.54 g), large body size (mean +1 standard deviation=5.85 g) and small body size (mean –1 standard deviation= 3.23 g). I performed the same 10 contrasts for tadpole behavioral

data but unlike for the metabolic rate data no size references were included. For each contrast, I adjusted p values by controlling for the false discovery rate (FDR) for performing the same hypothesis test at different body sizes (for metabolic rate responses) or for different kinds of behavior. False discovery rates were estimated independently for physiological responses and behavioral responses.

Contrasts consisted of the following: 1) what is the effect of rearing environment when tadpoles are not presented with either visual or chemical cues during measurement of metabolic rate and behavior?, 2) do chemical cues alter the metabolic rate or behavior of predator naïve tadpoles?, 3) do chemical cues alter the metabolic rate or behavior of tadpoles raised with predators?, 4) do visual cues alter the metabolic rate or behavior of predator naïve tadpoles?, 5) do visual cues effect the metabolic rate or behavior of tadpoles raised with predators?, 6) does the interaction of visual and chemical cues affect the metabolic rate or behavior of predator naïve tadpoles?, 7) does the interaction of visual and chemical cues affect the metabolic rate or behavior of tadpoles raised with predators?, 8) do tadpoles reared in different environments differ in metabolic rate or behavior when exposed to chemical cues?, 9) do tadpoles reared in different environments differ in metabolic rate or behavior when exposed to visual cues?, and 10) do tadpoles reared in different environments differ in metabolic rate or behavior when exposed to both chemical and visual cues? The Kenward-Roger approximation was employed to calculate degrees of freedom in all ANOVA's used in analyzing the metabolic rate and behavior data.

I evaluated whether rearing environment affected tadpole shape by performing a MANCOVA on the four PC axes that described variation in tadpole shape. The MANCOVA included rearing environment, mean centroid size, the interaction between rearing environment

and centroid size, and block as independent variables. Centroid size is the measure of the spread of landmarks around their center of gravity and provides an indicator of the size of an animal (Klingenberg 2013). I found that differences in tadpole body shape between rearing environments depended on centroid size ($F_{5, 3}$=43.4, p=0.0048) so I specified contrasts to compare body shape between rearing environments when centroid size was held constant at either mean centroid size, small (1 standard deviation below mean centroid size) and large (1 standard deviation above mean centroid size). I used F-tests associated with Wilk's lambda for assessing treatment effects.

To visualize variation in shape I produced sets of wireframe graphs that depicted the body shape of predator reared and predator naïve tadpoles for small, mean, and large size tadpoles. I obtained coordinates for each of the landmarks in a set of wireframe graphs by using the canonical axis associated with the contrast in my MANCOVA that tested the hypothesis of whether there are differences in body shape for tadpoles in different rearing environments for the size of tadpole represented by the wireframe graph. The canonical axis describes the axis of variation in the multidimensional shape space where the greatest differences in shape between rearing environments occurs after controlling for all other terms in the MANCOVA (e.g., rearing environment differences in the slope of relationship between shape and body size). Scores along this axis represent how different mesocosms are in the shape of tadpoles they contain – scores that are more different indicates that the mesocosms contain tadpoles that are more different in their shape. I obtained the score representing the location of each mesocosm along this canonical axis by:

$$CS_x = \sum_{i=1}^{i=4} r_{PC_i,CS} \times PCS_{i,x} \quad \text{Equation (2)}$$

where r refers to correlation between canonical scores and scores along PC axis i, PCS refers to PC score on axis i for mesocosm x, and CS_x refers to canonical score for mesocosm x.

I then determined the mean canonical score across all mesocosms within each rearing environment for a particular tadpole body size (i.e., small, mean or large size) and then back transformed these scores into landmark locations for each rearing environment. The back transformation was completed by first producing weighted scores of each shape variable (i.e., principal component) by multiplying the mean canonical score for mesocosms in a particular rearing environment to the correlation coefficient between the canonical axis and the principal component axis. This step of the back transformation provided estimates of the mean shape for each rearing environment in terms of the shape variables produced by the Principal Components Analysis of the Procrustes coordinates while recognizing that differences between rearing environments along some shape variables are more or less important (determined by magnitude of correlation between canonical axis and principal component axis) than other shape variables. I then transformed these weighted shape scores for each treatment back into landmark locations by first determining how much each landmark coordinate for a particular rearing environment and tadpole size deviates (in both x and y dimensions) from the mean landmark coordinate for all tadpoles regardless of size and rearing environment. This was done by:

$$DC=\sum_{i=1}^{i=4} wPC_i \times E_i \quad \text{Equation (3)}$$

where DC represents a vector describing how each of the 13 x and y coordinates deviates from the mean coordinate configuration of all tadpoles, wPC is the weighted shape (PC) score, and E is the eigenvector describing the correlations between each of the 13 x and y coordinates with PCi. The landmark coordinates for tadpoles of a particular size and rearing environment were

then obtained by summing DC to a vector describing the mean x and y coordinates for the 13 landmarks of tadpoles from all mesocosms.

RESULTS

Fitness components and morphology

Rearing environment did not significantly alter either survival ($F_{1, 9}$ =0.20, p=0.6623) (Figure 4) or tadpole mass ($F_{1, 9}$=1.32, p=0.2807) (Figure 5). Overall, tadpole survivorship was high (86%). The presence of a fish predator caused tadpoles to develop shorter guts ($F_{1, 19}$ =8.38, p=0.0201) (Figure 6).

The effect of rearing environment on the external morphology of tadpoles depended on body size ($F_{5, 3}$=46.4, p=0.0048) as measured by centroid size from Procrustes superimposition. Long-term exposure of predators to tadpoles caused small tadpoles to develop longer tail muscles and proportionately smaller bodies for their size than small tadpoles that did not receive long-term exposure to predators ($F_{5, 3}$=60.74, p=0.0033) (Figure 7 A). Differences in the shape of averaged size tadpoles reared in the presence or absence of predators were similar to that observed for small sized tadpoles (Figure 7 B) but these differences were not statistically significant ($F_{5, 3}$=2.34, p=0.2574). Long-term exposure to predators caused large tadpoles to have taller tail muscles and fins and proportionately larger bodies for their size than large tadpoles that did not receive long-term exposure to predators ($F_{5, 3}$=19.24, p=0.0174) (Figure 7 C).

Metabolic rate

Though the mass specific metabolic rate of tadpoles generally declined with increasing body size of tadpoles ($F_{1, 63.8}$=15.36, p=0.0002), a visual inspection of graphs for each treatment indicated that the slope of the relationship between mass specific metabolic rate and body size varied among the experimental treatments even though these differences were not statistically

significant ($F_{7, 61.8}$=1.62, p=0.1455). Rearing environment had no effect on the metabolic rate of tadpoles when measured in the absence of predator cues regardless of body size (p values for contrast 1: p≥0.5597 for all body sizes) (Figure 8) (Table 1). Neither short-term exposure to visual or chemical cues nor their interaction affected the metabolic rate of tadpoles of average size and these effects did not depend on the rearing environment in which tadpoles were raised (p values for contrasts 2-9: ≥0.1069) (Table 1). There was however a trend which indicated that predator exposed and predator naïve tadpoles of average size differed in how they responded to the combined influence of short-term chemical and visual cues (p value for contrast 10: p=0.062). Short-term exposure to chemical cues caused small predator naïve tadpoles to slow their metabolic rates (p value for contrast 2: p=0.0348) (Table 1) but did not affect the metabolic rate of small tadpoles reared in the presence of predators (p value for contrast 3: p=0.8121) (Figure 8 B). In contrast, large predator naïve tadpoles enhanced their metabolic rate in response to the short-term occurrence of chemical cues from predators (p value for contrast 2: p=0.0251) (Table 1) while large tadpoles raised in the presence of predators did not alter their metabolic rate in response to short-term chemical cues from predators (p for contrast 3: p=0.4225) (Table 1). Furthermore, large tadpoles that were reared with predators and predator naïve tadpoles differed in their metabolic rate when measured with the short-term chemical cues of predators present in the water (p value for contrast 8: p=0.0185) (Figure 8 A) (Table 1). The metabolic rate of small predator naïve tadpoles and small tadpoles reared with predators was similar when these tadpoles were exposed to short-term chemical cues from predators (p value for contrast 8=0.2368) (Figure 8 B) (Table 1).

Short-term exposure to visual cues from predators did not affect the metabolic rate of small tadpoles and this effect did not depend on whether tadpoles were reared with predators or

not (p values for contrasts 4,5,9: ≥0.4910) (Table 1). Furthermore, the presence of short-term visual cues did not alter the effect of chemical cues on the metabolic rate of small tadpoles and this result was not dependent on the rearing environment of the tadpole (p values for contrasts 6,7,10: ≥0.0924) (Table 1). Though there was a trend for the occurrence of visual cues in the short-term to increase the metabolic rate of large tadpoles reared with predators by 53% this difference was not statistically significant (p value for contrast 5: p=0.1196) (Table 1) and tadpoles from different rearing environments, regardless of size, did not differ in how they altered metabolic rate in response to the presence of short-term exposure to visual cues (p values for contrast 9: ≥0.2157) (Table 1). Short-term exposure to visual cues did not appear to have a large effect on the metabolic rate of large predator naïve tadpoles (p value for contrast 4: p=0.5795) nor did it alter how large tadpoles (either predator naïve or predator exposed) altered their metabolic rate in response to the occurrence of short-term chemical cues from predators (p values for contrasts 6,7,10: ≥0.2571) (Table 1).

Behavior

Long-term exposure of tadpoles to predators induced tadpoles to spend more than twice as much time active as predator-naïve tadpoles but this difference was not statistically significant (contrast 1 for mean time active: p=0.2455) (Figure 9) (Table 2) and activity level of the predator exposed tadpoles constituted a very small fraction of the overall time frame over which activity was measured (i.e., on average, predator exposed tadpoles only spent 8 seconds out of 60 actively moving). Long-term exposure to predator cues had even less noticeable effects on other behaviors (p values for contrast 1 for mean # in refuge: p=0.496, for mean time in refuge: p=0.8611, for mean # active: p=0.8441) (Table 2). Exposure to short-term chemical cues

increased the likelihood that naïve tadpoles sought a refuge (p value for contrast 2: p=0.0126) but the same effect was not found for other measures of behavior (Table 2). Predator exposed tadpoles did not respond as strongly to short-term exposure of chemical predator cues (p values for contrast 3: $p \geq 0.149$) (Figure 10) (Table 2).

Short-term exposure to visual predator cues did not affect any measure of naïve tadpole behavior (p values for contrast 4: $p \geq 0.1106$) (Table 2) but increased the number of predator exposed tadpoles that sought refuge (p value for contrast 5: p=0.0296) (Figure 10) (Table 2). Though short-term visual cues appeared to cause the active number of tadpoles raised with predators to be reduced by 81% this effect was not statistically significant (p values for contrast 5: p=0.0801) (Figure 11) (Table 2) in part because very few animals were active during the time in which this behavior was assessed. Short-term visual cues had no influence on the amount of time that predator exposed tadpoles were active or the amount of time that predator exposed tadpoles spent in a refuge (p values for contrast 5: $p \geq 0.454$). The effect of short-term visual cues on the behavior of predator naïve tadpoles was not influenced by the occurrence of chemical cues from predators (p values for contrast 6: $p \geq 0.2648$). Though the effect of short-term visual cues on most behaviors of predator exposed tadpoles did not depend on the occurrence of chemical cues from predators, the number of predator exposed tadpoles in refuge was significantly lower than expected when tadpoles were exposed to both visual and chemical cues (p value for contrast 7: p=0.0098) and there was an effect on exposed tadpoles when exposed to visual cues alone (p value for contrast 5: p=0.0296) (Figure 10) (Table 2).

There was a trend for predator naïve tadpoles to spend less time in a refuge than expected (p value for contrast 6: p=0.0617) (Figure 12) (Table 2). The average time spent in a refuge when no cues are present (p value for contrast 1: p=0.8611) is not different than when

both cues are present (p value for contrast 10: p=0.4848). However, this same interaction did not affect the average number of naïve tadpoles in a refuge (p values for contrast 6: p=0.3366) (Figure 11) (Table 2). The presence of short-term visual cues did not alter the effect of the exposure to short-term chemical cues on the number of tadpoles active (Figure 11) nor on the average time tadpoles were active (Figure 9) and this result was not dependent on the rearing environment of the tadpole (p values for contrasts 6,7,10: p>0.05) (Table 2). Short-term visual cues caused exposed and naïve tadpoles to differ in their refuge use (p value for contrast 9: p=0.0126) (Figure 12) (Table 2). Predator exposed tadpoles spent 103% more time active in the presence of both short-term visual and chemical cues than did predator naïve tadpoles though this effect was not statistically significant (p value for contrast 10: p=0.0755) (Figure 10) (Table 2).

DISCUSSION

Long term responses of prey to the presence of predators

Long-term exposure to predators caused tadpoles to alter their internal and external morphology but had no measurable effect on size, baseline behavior, or baseline metabolic rate (i.e., behavior and metabolic rate of animals when measured in the absence of any predator cues). Long-term predator exposure has been found to reduce the metabolic rate of prey in other studies (Holopainen *et al*. 1997, Steiner and Van Buskirk 2009, Barry and Syal 2013) but the tadpoles in my study did not appear to differ in baseline metabolic rate at the time sampled. Predator exposure over the long-term had no measurable effect on baseline metabolic rate or behavior in my study and this may be because tadpoles ignore signals from predators such that there is no long-term effect. I found no difference in baseline metabolic rate after long-term exposure to predators and this may be because predator exposed tadpoles have adjusted their metabolic rate over the long-term to a level similar to that of naïve tadpoles such that any difference was no longer detectable. Measurements of predator exposed tadpoles through time may have found evidence of a decrease in metabolic rate as it occurred (see Barry and Syal 2013).

Rearing environment also had no effect on behavior in the long-term, tadpoles exposed to predators may be able to compensate for long-term predator exposure by altering their behavior. In many studies, prey, including tadpoles, have been found to reduce activity in the presence of predators (Holomuzki and Hoyle 1990, Malmqvist 1992, Stauffer and Semlitsch 1993, Gelowitz *et al.* 1993), but I did not find this in predator exposed tadpoles after long-term exposure to predator cues. Modifications to external morphology in predator exposed tadpoles (e.g., tail muscle, tail fin, body shape) may help mitigate the risk of being active in the presence of

predators by giving tadpoles increased ability to escape predators through improved burst speed (Dayton *et al*. 2005).

Exposure to predators over the long-term induced prey to develop shorter guts suggesting that prior exposure to predators may compromise the ability of prey to extract energy from food. Plasticity in the gut of grasshoppers (Yang and Joern 1994) and tadpoles (Relyea and Auld 2004) has been found to involve an increase in gut length when food is scarce or of low quality. In my study, predator exposed and predator naïve tadpoles were raised in identical conditions so the type or quality of available food should not have been a factor in my findings. Additionally, I raised tadpoles at low densities negating any effects of crowding on tadpole physiology. Gut plasticity has also been shown to occur in anuran larvae in response to both predators and competitors. Tadpoles kept at high density have been found to develop longer bodies and guts due to competition but a shorter body and gut when exposed to predators (Relyea and Auld 2004, Steiner 2007). The simplest explanation for these findings is that a smaller body results in a shorter gut, but my results do not support this idea because only small predator exposed tadpoles had a smaller body. A smaller body may serve to present a smaller target to predators for small tadpoles while the larger tail fins and increased tail muscle may help larger tadpoles escape predators. Despite exposed tadpoles having a shorter gut they did not suffer reduced growth. Perhaps gut physiology is decoupled from growth, shorter guts are actually more efficient, or shorter guts have no impact on tadpole growth (Steiner 2007).

The effects of long term predator exposure on tadpoles in this study suggest that tadpoles can compensate for the costs of induced defenses with modifications to metabolic rate, behavior, and morphology which may help explain the lack of growth anomalies between predator exposed and predator naïve prey (Barry and Syal 2013). Predator exposed tadpoles appear to have the

ability to adjust their metabolic expenditure during long-term exposure to predator cues which lessens the costs of predator response. An elevated metabolic rate in exposed prey would likely result in excess energy expenditure leading to reduced growth or growth rate. Tadpoles exposed to predator cues over the long-term were overall more active probably due to the fact they had developed morphological defenses that increased their odds of being able to escape potential predators. It is unclear what effects a shorter gut has on predator exposed tadpoles in the larval stage.

Short term responses of prey to additional predator cues

In my study the responses of prey to predator cues in the short-term depended on tadpole body size (for metabolic rate) and whether or not predators had been present in the rearing environment (for metabolic rate and behavior). Predator exposed tadpoles did not alter their metabolic rate when exposed to additional predator cues in the short-term likely because they are habituated to the presence of these cues in the environment; a sudden increase in metabolic rate would be costly and unnecessary because there is already a constant perception of threat. Predator exposed prey were more active when exposed to predator chemical cues than were naïve prey probably because predator cues did not represent a novel threat and they were equipped with morphological defenses to help mitigate the threat. Predator naïve tadpoles did respond to predator chemical cues in the short-term with changes to their metabolic rate and body size dictated the response. Small naïve tadpoles reduced their metabolic rate in response to predator cues possibly because their baseline metabolic rate was already high and they were more likely to reduce activity levels as they entered a refuge. In contrast, large naïve tadpoles enhanced their metabolic rate in order to prepare themselves to flee the threat (i.e., flight

response). This is logical because larger tadpoles are already faster swimmers due to their larger body size (Dayton *et al*. 2005). Naïve tadpoles were more likely to utilize a refuge and may ramp up their metabolic rate in order to flee to such a refuge. In general, predator naïve tadpoles were more likely to seek a refuge when exposed to chemical cues or the combined effects of chemical and visual cues and were overall less active in the presence of chemical cues. Seeking a refuge would be beneficial for these animals because my results indicate that naïve tadpole's lack the morphological defenses that predator exposed tadpoles developed after long-term exposure to predators.

In aquatic amphibian larvae, most research indicates that chemical alarm cues dispersed in the water to be the most common and reliable indicator of predators compared to other cues such as visual and tactile cues (Hickman *et al*. 2004, Saidapur *et al*. 2009) but my results are not entirely consistent with this. Short-term visual cues had no effect on tadpole metabolic rate but did have an effect on the behavior of both naïve and exposed tadpoles. Visual cues may be used alone or in conjunction with other types of cues and my results support this argument. A reliance on chemical cues is logical if the larval environment is densely vegetated and/or turbid, or this reliance may be due to a lack of visual acuity in some species of tadpoles (Stauffer and Semlitsch 1993, Mogali *et al*. 2012). Because visual cues affected tadpole behavior my results suggest visual cues do play some role in predator detection in leopard frog larvae at least when water conditions allow for visual detection.

Conclusions

Predator exposed tadpoles were more active than naïve tadpoles possibly because they required more food resources due to having a shorter and less efficient digestive system. An

increase in activity should equal an increase in foraging time allowing exposed tadpoles to reduce the costs of predator exposure (e.g., a shorter gut) allowing for similar growth rates as naïve tadpoles. Predator exposed tadpoles at small and large body sizes developed morphological defenses, which would help them mitigate some of the risk of being more active in the presence of predators.

Predator exposed tadpoles did not suffer reduced size because they are able to rapidly assess the environment for the presence of predators and adjust their behavior and metabolic rate over both the short and long-term in order to keep the costs of predator response low. Some have argued that tadpoles exposed to predators can develop a larger tail to better escape predators or a longer gut for more efficient digestion but they could not do both (Relyea and Auld 2004) and my results support this idea. My results however do not support the idea that predator exposed prey are smaller because they shift resources that could have been used in growth into making morphological defenses; tadpoles in my study exposed to long-term predation risk altered their morphology but did not suffer reduced growth. Whether or not some impact of a shorter gut would manifest itself later in life (i.e., at metamorphosis) is unknown but risk of predation in the larval stage has been found to result in larger limbs and narrower bodies for prey exposed to predators in the larval stage (Relyea 2001). Being raised in the presence of predators has also been shown to result in a smaller size at metamorphosis (Lardner 2000, Kiesecker *et al*. 2002, Benard 2004). Some have argued however that these predator effects are altering larval development time and are not due to morphological changes that occurred to prey in the larval stage (Relyea 2001). My results indicate that predator exposed and predator naive tadpoles did not differ in ways that would affect growth in the larval stage. It is perplexing though as to why gut length did not affect growth in predator exposed prey. The differences I found in gut length

do not appear to be related to overall size therefore I am left to wonder if these differences would manifest themselves at later life stages in some way.

In conclusion, this study supports the idea that there are complex interactions among physiology, behavior, and morphology in predator-prey interactions (Steiner and Van Buskirk 2009). My study demonstrates that predator exposure is not always connected to a reduction in growth. It may be that reduced growth in the presence of predators is not a generalized response in anuran larvae. Because predator driven non-lethal effects have such a large influence on various aspects of prey they are likely to affect community structure, ecological patterns, and drive the dynamics of prey populations, therefore expanding our knowledge in this area of research is important (Peacor and Werner 2001, Alexander *et al*. 2013, Paterson *et al*. 2013). This study adds to our knowledge of the interactions between predators and their prey as well as the costs involved in predator defense and how prey can mitigate these costs. My study has extended this work to another species and demonstrates that predator-induced growth reduction in anuran larvae is more complicated than traditional views suggest (e.g., a reduction in activity leads to a reduced growth under predation risk). How prey respond to their predators may not be as generalized as some suggest (Barry and Syal 2013) and much is surely yet to be discovered.

Table 1. Raw p values and p values after adjusting for the false discovery rate (FDR) for the ten contrasts used to evaluate tadpole metabolic rate at three body sizes.

Metabolic Rate	**Small Size**			**Mean Size**			**Large Size**		
Contrast	t value	Raw p value	FDR adjusted p value	t value	Raw p value	FDR adjusted p value	t value	Raw p value	FDR adjusted p value
1. Effect of rearing environment when tadpoles are not presented with either visual or chemical cues.	-0.27	0.7903	0.8249	0.22	0.8249	0.8249	0.59	0.5597	0.8249
2. Do chemical cues alter the metabolic rate of predator naïve tadpoles?	-2.16	0.0348	0.0522	0.73	0.4679	0.4679	2.29	0.0251	0.0522
3. Do chemical cues alter the metabolic rate of tadpoles raised with predators?	-0.24	0.8121	0.8121	-0.77	0.4431	0.6647	-0.81	0.4225	0.6647
4. Do visual cues alter the metabolic rate of predator naïve tadpoles?	-0.56	0.5752	0.8693	0.02	0.9879	0.9879	0.56	0.5795	0.8693
5. Do visual cues effect the metabolic rate of tadpoles raised with predators?	-0.69	0.4910	0.6716	0.43	0.6716	0.6716	1.58	0.1196	0.3588
6. Does the interaction of visual and chemical cues affect the metabolic rate of predator naïve tadpoles?	-0.33	0.7403	0.7403	1.64	0.1069	0.1604	2.00	0.1503	0.1509
7. Does the interaction of visual and chemical cues affect the metabolic rate of tadpoles raised with predators?	-0.24	0.8150	0.8417	-0.32	0.7498	0.8417	-0.20	0.8417	0.8417
8. Do tadpoles reared in different environments differ in metabolic rate when exposed to chemical cues?	1.19	0.2368	0.2368	-1.24	0.2206	0.2368	-2.42	0.0185	0.0555
9. Do tadpoles reared in different environments differ in metabolic rate when exposed to visual cues?	-0.37	0.7109	0.7109	0.64	0.5235	0.7109	1.25	0.2157	0.6471
10. Do tadpoles reared in different environments differ in metabolic rate when exposed to both chemical and visual cues?	1.71	0.0924	0.1386	1.90	0.0620	0.1386	1.14	0.2571	0.2571

Table 2. Raw p values and p values after adjusting for the false discovery rate (FDR) for the ten contrasts used to evaluate the four measures of tadpole behavior.

Behavior	**Mean # active**			**Mean time active**			**Mean # refuge**			**Mean time refuge**		
Contrast	t value	p value	FDR adjusted p value	t value	p value	FDR adjusted p value	t value	p value	FDR adjusted p value	t value	p value	FDR adjusted p value
1. Effect of rearing environment when tadpoles are not presented with either visual or chemical cues.	-0.20	0.8441	0.8611	1.17	0.2455	0.8611	-0.68	0.496	0.8611	-0.18	0.8611	0.8611
2. Do chemical cues alter the behavior of predator naïve tadpoles?	1.18	0.2403	0.3204	-0.08	0.9336	0.9336	-2.57	0.0126	0.0504	-1.30	0.1971	0.3204
3. Do chemical cues alter the behavior of tadpoles raised with predators?	0.20	0.8441	0.8441	-0.53	0.6006	0.8008	-1.20	0.2353	0.4706	-1.46	0.149	0.4706
4. Do visual cues alter the behavior of predator naïve tadpoles?	-0.00	1.0000	1.000	-1.51	0.1366	0.2732	-0.34	0.7332	0.9776	-1.62	0.1106	0.2732
5. Do visual cues effect the behavior of tadpoles raised with predators?	1.78	0.0801	0.1602	0.75	0.454	0.6053	-2.23	0.0296	0.1184	-0.46	0.6455	0.6455
6. Does the interaction of visual and chemical cues affect the behavior of predator naïve tadpoles?	0.28	0.7809	0.7809	-1.12	0.2648	0.4488	-0.97	0.3366	0.4488	-1.90	0.0617	0.2468
7. Does the interaction of visual and chemical cues affect the behavior of tadpoles raised with predators?	0.98	0.3319	0.5448	0.61	0.5448	0.5448	-2.66	0.0098	0.0392	-0.82	0.4132	0.5448
8. Do tadpoles reared in different environments differ in behavior when exposed to chemical cues?	-1.18	0.2403	0.4806	-1.61	0.112	0.4448	0.68	0.496	0.6613	-0.33	0.7403	0.7403
9. Do tadpoles reared in different environments differ in behavior when exposed to visual cues?	1.58	0.1189	0.2378	1.09	0.2805	0.3308	-2.57	0.0126	0.0504	0.98	0.3308	0.3308
10. Do tadpoles reared in different environments differ in behavior when exposed to both chemical and visual cues?	-0.39	0.6942	0.6942	-1.81	0.0755	0.3020	1.20	0.2353	0.4706	-0.70	0.4848	0.6464

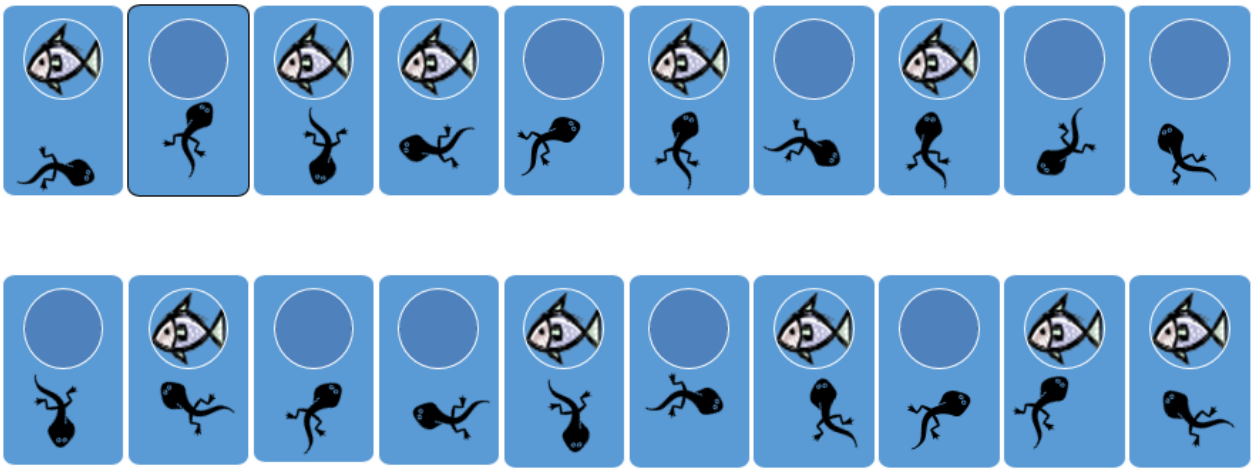

Figure 1. Experimental design of mesocosm rearing tanks. One mesocosm from one row was paired with the mesocosm in the adjacent row that is closest to it to form a statistical block. This method produced 10 statistical blocks with one each of the two rearing treatments (presence or absence of a caged predator) that allowed me to assess the effects of long term predator exposure on prey.

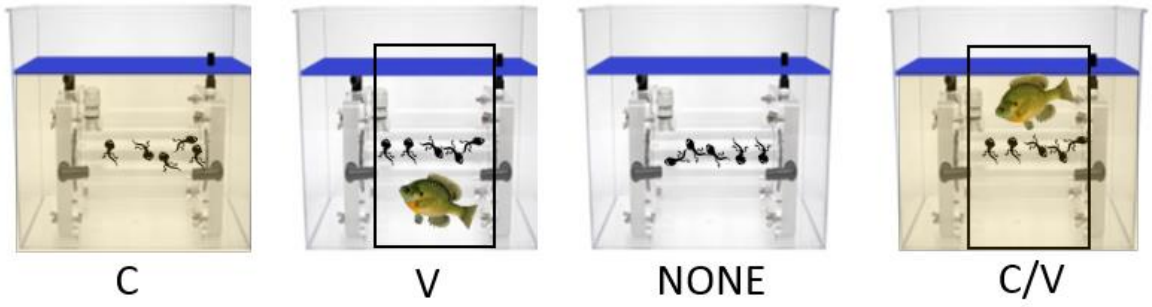

Figure 2. I measured metabolic rate of tadpoles in respirometers that varied in the presence of either visual (V) or chemical (C) cues from predators, both chemical and visual cues (C/V), or no cues at all (NONE). Black boxes represent enclosures placed within aquaria to contain fish.

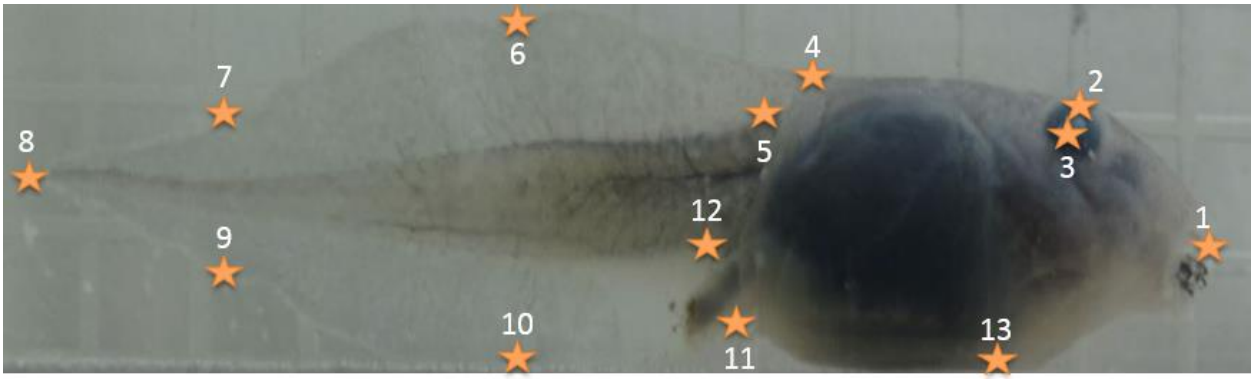

Figure 3. Location of the 13 landmarks utilized to quantify tadpole morphology. Landmarks were chosen based on common morphology.

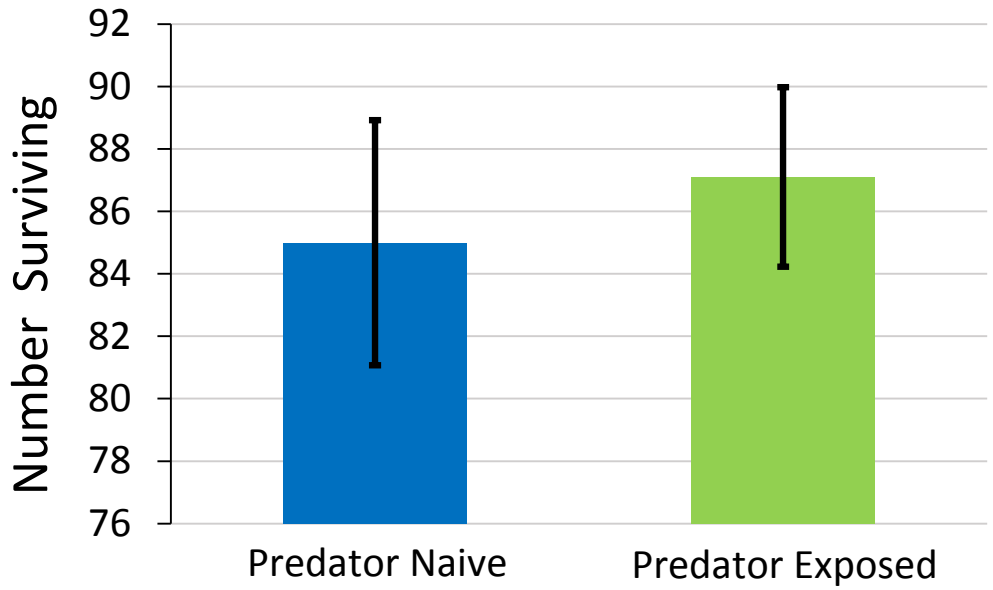

Figure 4. Least square mean (± 1 SE) of the number of tadpoles surviving in treatments where tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed). Each mesocosm started with 100 tadpoles.

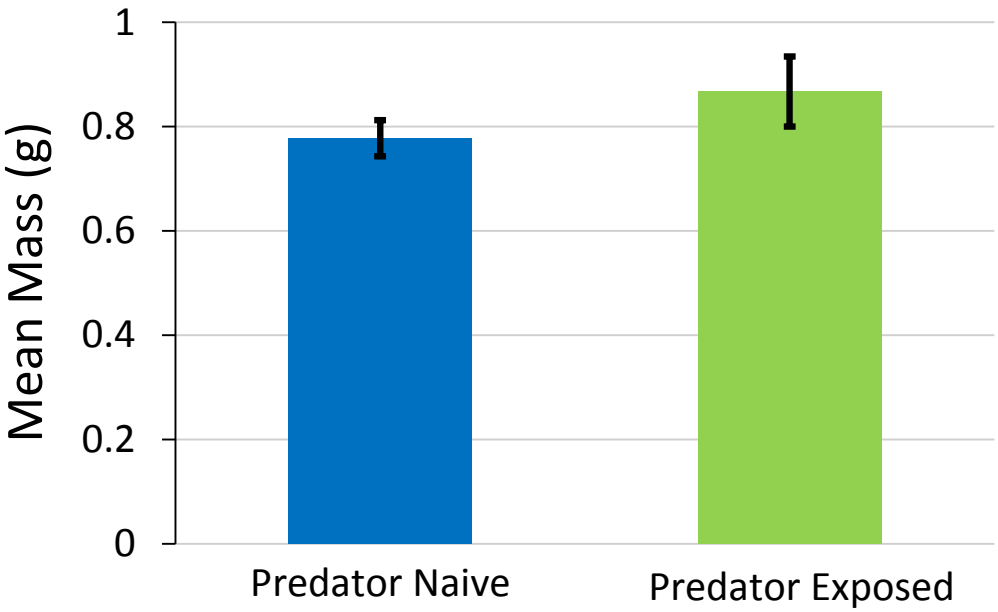

Figure 5. Least square mean (± 1 SE) for the mean mass of tadpoles in treatments where tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed).

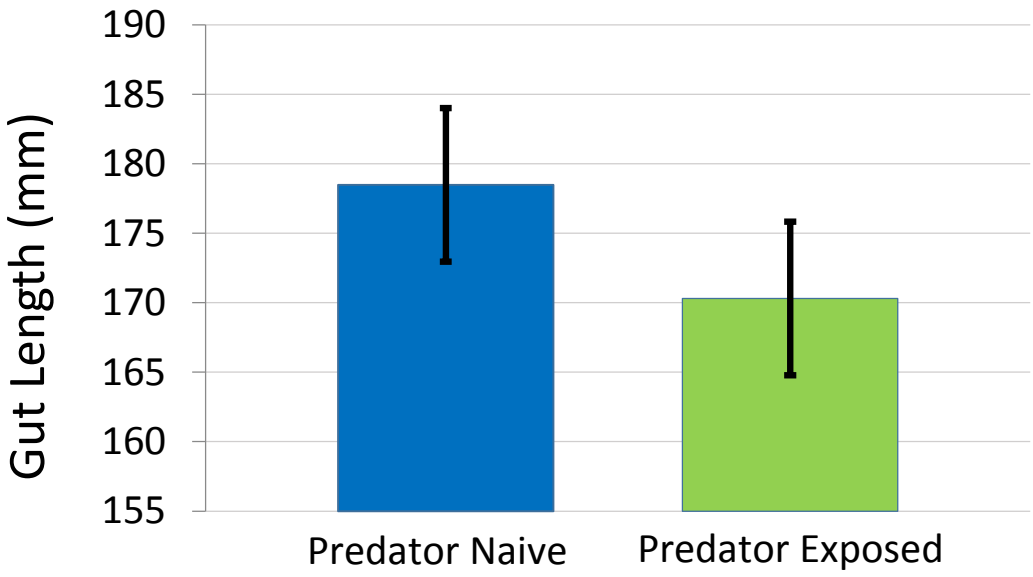

Figure 6. Least square mean (± 1 SE) of tadpole gut length in treatments where tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed).

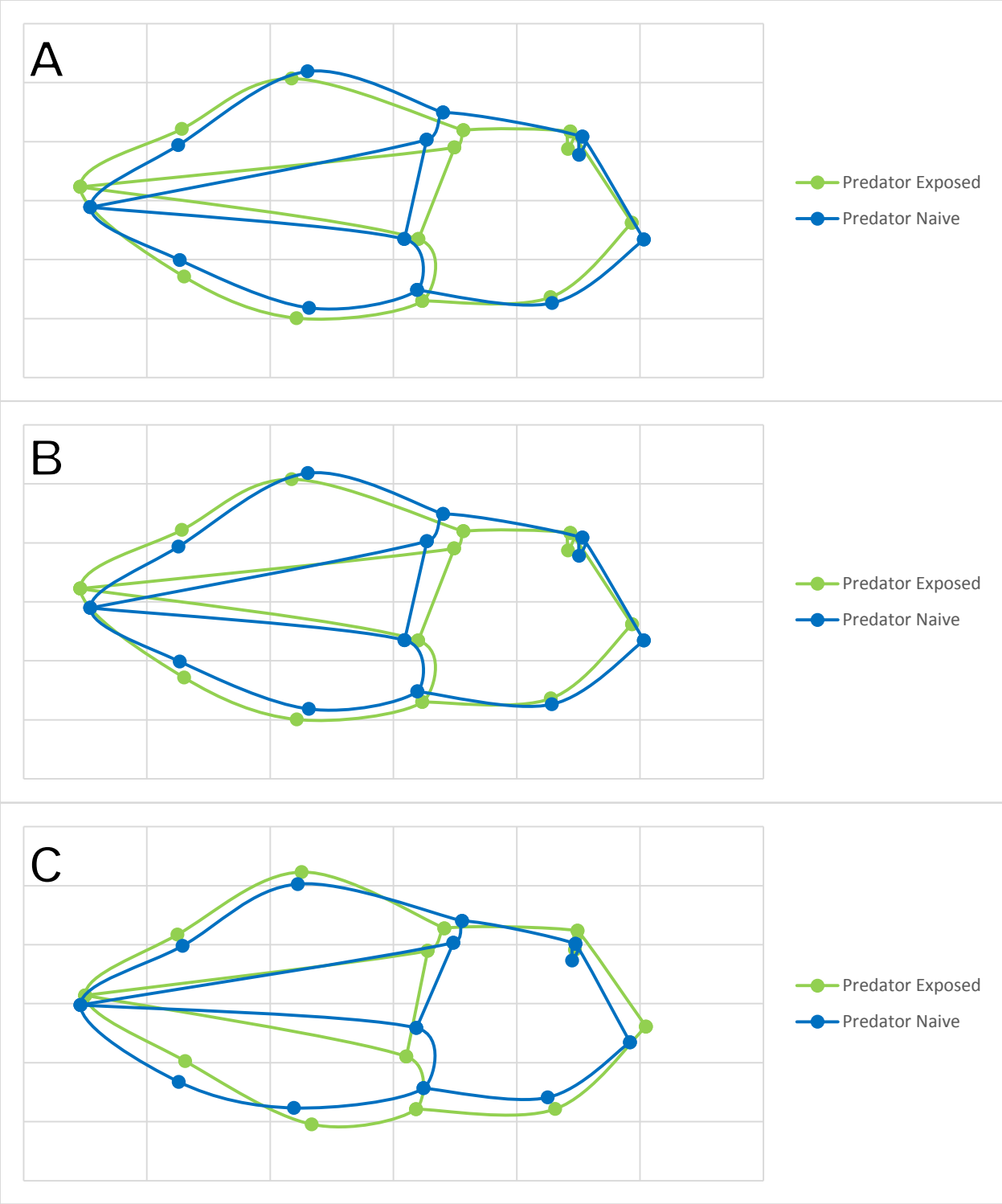

Figure 7. Wireframe graphs each with two wireframes: green for predator exposed tadpoles and blue for tadpoles not exposed to predators. One graph is represented for each tadpole body size: A) small body size B) mean body size C) large body size.

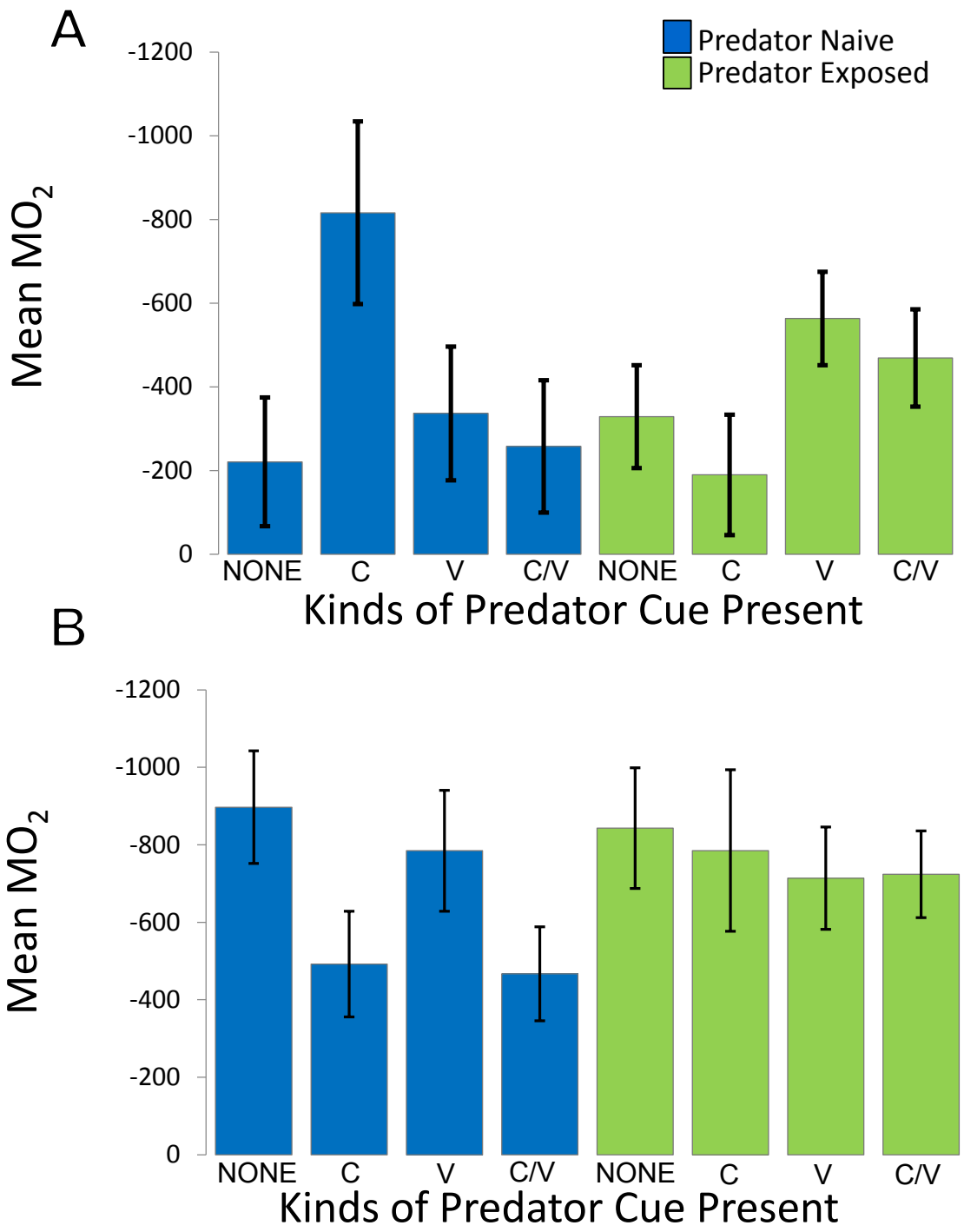

Figure 8. Least square mean (± 1 SE) for mass specific oxygen consumption for A) large (5.84 g) tadpoles and B) small (3.21 g) tadpoles. Tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed) and received a short-term exposure to no (NONE), chemical (C), visual (V), or both chemical and visual cues (C/V) from predators when metabolic rate was assessed.

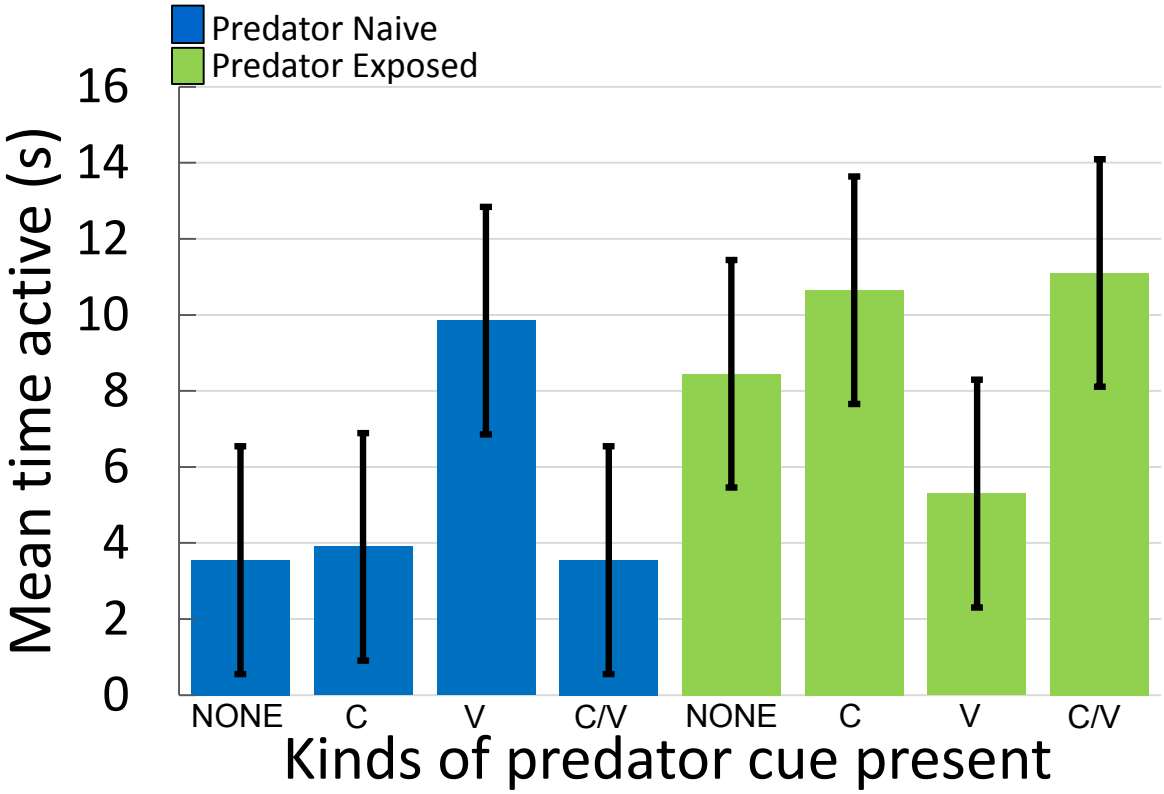

Figure 9. Least square mean (± 1 SE) for mean time tadpoles were active (seconds). Tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed) and received a short-term exposure to no (NONE), chemical (C), visual (V), or both chemical and visual cues (C/V) from predators when mean time active was assessed.

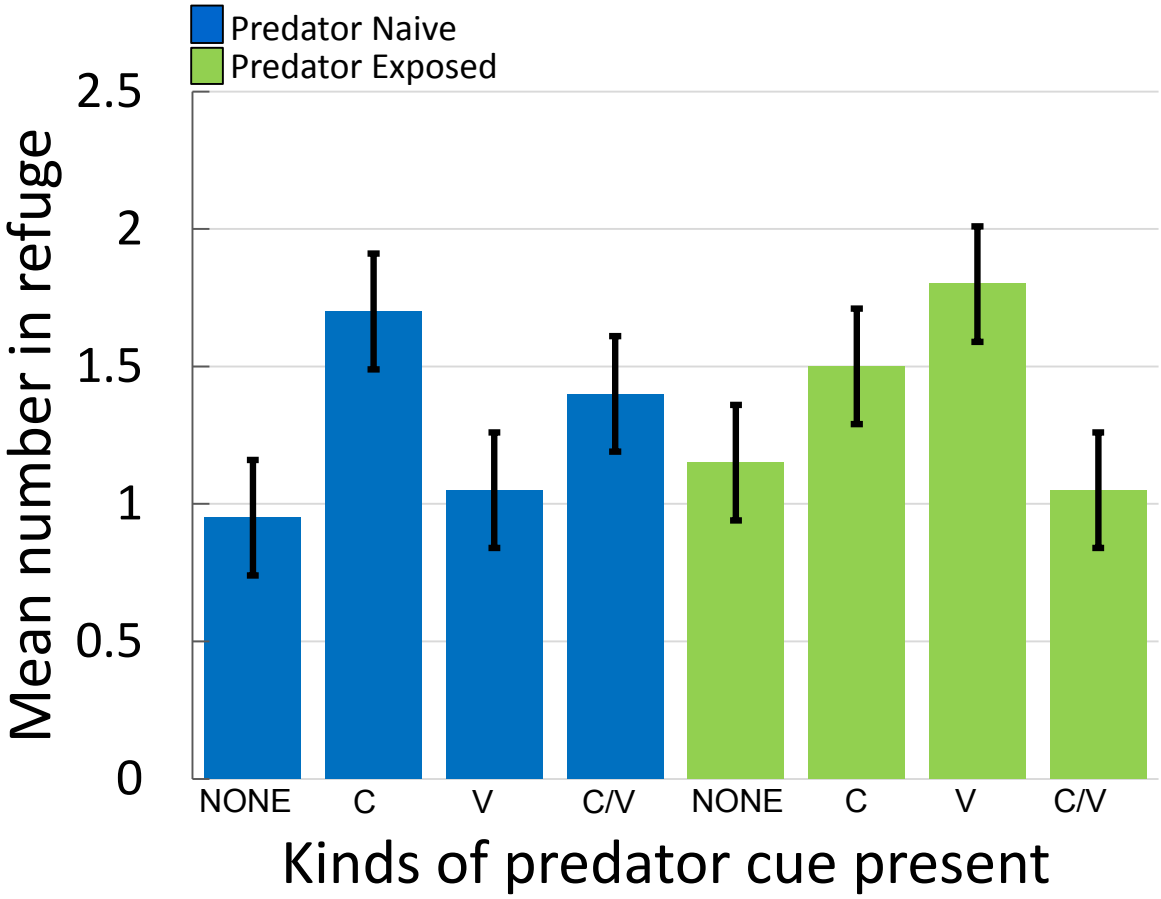

Figure 10. Least square mean (± 1 SE) for the mean number of tadpoles in a refuge. Tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed) and received a short-term exposure to no (NONE), chemical (C), visual (V), or both chemical and visual cues (C/V) from predators when the mean number of tadpoles in a refuge was assessed.

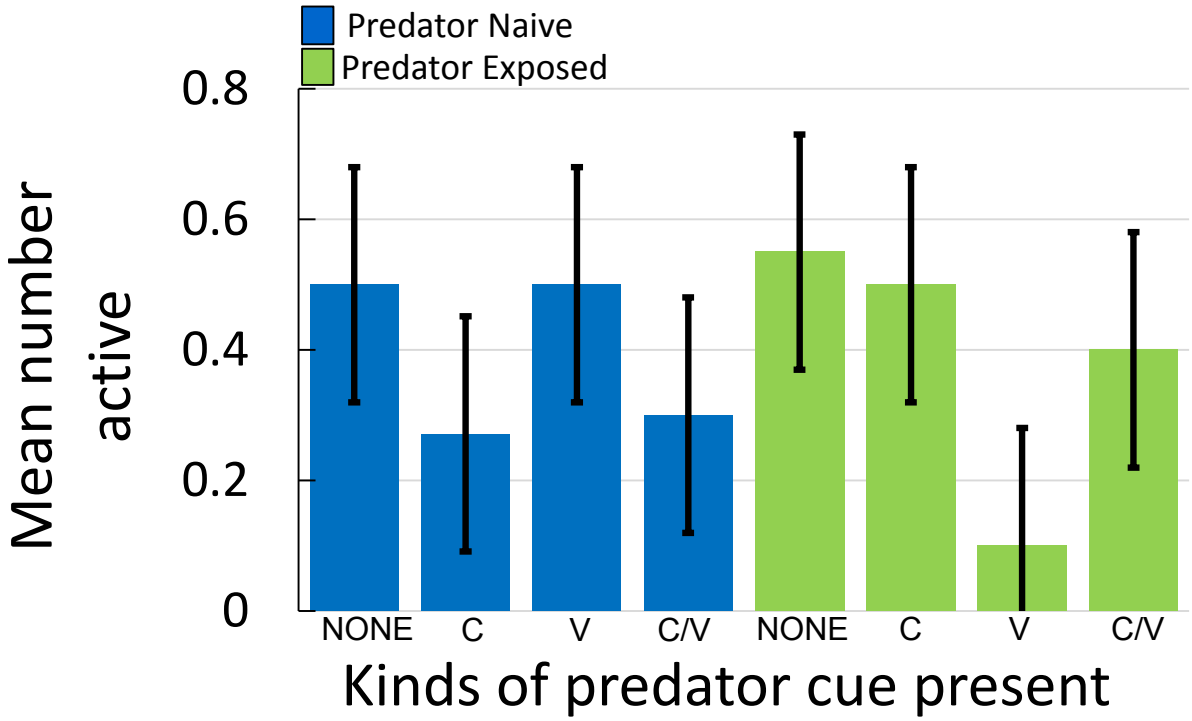

Figure 11. Least square mean (± 1 SE) estimates for the mean number of active tadpoles. Tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed) and received a short-term exposure to no (NONE), chemical (C), visual (V), or both chemical and visual cues (C/V) from predators when the mean number of tadpoles active was assessed.

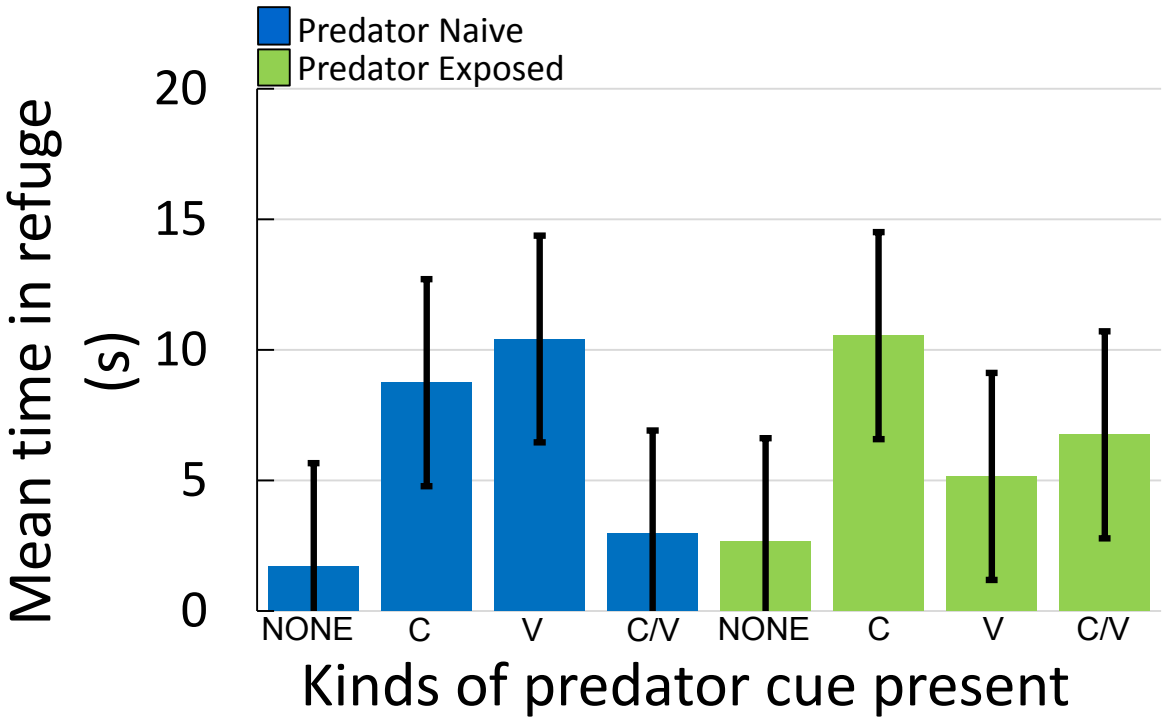

Figure 12. Least square mean (± 1 SE) estimates for mean time (seconds) that tadpoles spent in a refuge. Tadpoles were either reared in the presence or absence of predators (blue for predator naïve and green for predator exposed) and received a short-term exposure to no (NONE), chemical (C), visual (V), or both chemical and visual cues (C/V) from predators when time spent in a refuge was assessed.

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APPENDIX: IACUC APPROVAL LETTER

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834
252-744-2436 office
252-744-2355 fax

May 13, 2014

David Chalcraft, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Chalcraft:

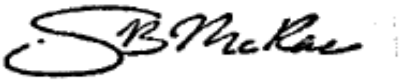
Your Animal Use Protocol entitled, "Physiological Responses of Anuran Larvae to Predation Cues" (AUP #D315) was reviewed by this institution's Animal Care and Use Committee on 5/13/14. The following action was taken by the Committee:

"Approved as submitted"

NOTE: An administrative change was made on page 28, section C.

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

Sincerely yours,



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

SM/jd

Enclosure