LATENT EFFECTS OF LARVAL EXPERIENCE: THE PERVASIVE EFFECTS OF TADPOLE PREDATION RISK ON FROG PHENOTYPE

By

LYNNE E. BEATY

Bachelor of Science in Wildlife Science
State University of New York College of Environmental Science and Forestry
Syracuse, New York
2010

Master of Science in Biology
Texas Tech University
Lubbock, Texas
2012

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2017

LATENT EFFECTS OF LARVAL EXPERIENCE: THE PERVASIVE EFFECTS OF TADPOLE PREDATION RISK ON FROG PHENOTYPE

Dissertation Approved:

Dr. Bernard Luttbeg

Dissertation Adviser

Dr. Scott McMurry

Dr. Craig Davis

Dr. Michi Tobler

ACKNOWLEDGEMENTS

I am very fortunate to have been supported both financially and emotionally throughout my PhD and while I wish I could write something heartfelt to every person and organization that has helped me along the way, I am frankly too exhausted to think of eloquent words to adequately express my thanks. So, while these acknowledgements may not sufficiently express my gratitude, I hope that I can show each of you my thanks in person or publication before time tears us apart.

I would like to first thank my funding sources, without which the research described in this document would not have been possible - the Department of Defense National Defense Science and Engineering Graduate Fellowship, the Animal Behavior Society, Prairie Biotic Research Inc., Sigma Xi, OSU's Graduate College, OSU's Department of Integrative Biology, OSU's Women's Faculty Council, and the Southwestern Association of Naturalists. I hope I have made you proud.

Next, I would like to thank my advisor, Barney Luttbeg. You took me into your lab as a stranger, kept and supported me as I struggled through my first year, and guided me to successfully completing my PhD. I hope I bring honor to the Luttbeg lab name and I will strive to provide the same sort of welcoming, helpful, and compassionate environment for my students when I have a lab of my own.

I would also like to thank my committee – Scott McMurry, Michi Tobler, and Craig Davis for their thought-provoking questions and comments during my comprehensive exams and private dissertation defense. Your feedback has helped to shape and improve this document and I am eternally grateful for it.

A number of people have helped the research described in this document happen with relatively few glitches. I would like to thank Jennifer Grindstaff and Jason Belden for the use of their laboratory spaces to conduct the corticosterone assay described in chapter 4. I would also like to thank Lizzie VanErt for her help scoring tadpole behavior videos in chapter 3 – man, that was a crazy amount of work. In addition, I would like to thank the following people for their help in the lab and field - Justin Agan, Medhavi Ambardar, Adam Simpson, Bill Mausbach, Madeleine Naylor, Maggie Bliss, Molly Byrne, Sally Beaty, Becca Atherton, Kyle Alvarez, Syndey Marouk, and Kelsey Lovell. You all helped to keep both me and the frogs healthy and happy.

The frogs used in these experiments also deserve my thanks. While they did not have a choice to participate in these experiments, I appreciate them more for it and I treated

them as well as I could and I hope that was enough. The research described in this document was approved by Oklahoma State's Institutional Animal Care and Use Committee, protocols #: AS-13-1 and AS-16-4.

I would also like to thank my roommates and friends for their emotional support during my PhD. I would like to thank Bill Mausbach, Madeleine Naylor, and, in particular, Medhavi Ambardar for letting me be a part of your lives and for being there to pick me up when I thought I was stuck in the gutter for good. In this, I would also like to include Ron Van Den Bussche and Meredith Hamilton who have been like parents to me during my time in Stillwater. You all are some of the most amazing people I have ever known and your support meant and still means the world to me.

I would like to thank my fur and human families for their support. I thank my cat, Monster, and my dog, Anders, for being there to absorb my stress squeezes and for being willing to go on walks and hikes to relieve stress when I needed to. I thank my parents and sisters for their support and understanding as I have been a grumpy, stressed person for the past five years and I am sure I have not been a joy to be around. Your spontaneous treat packages have made these last few months bearable.

Last, and most importantly, I would like to thank my fiancé, Adam, for putting up with me. You are my rock and I would not have been able to do this without you. I only hope that I can do the same for you.

Name: LYNNE BEATY

Date of Degree: MAY 2017

Title of Study: LATENT EFFECTS OF LARVAL EXPERIENCE: THE PERVASIVE EFFECTS OF TADPOLE PREDATION RISK ON FROG PHENOTYPE

Major Field: ZOOLOGY

Abstract: Predation risk influences prey phenotype. Predator-induced changes in prey behavior and morphology are hypothesized to be adaptive, as long as predation risk is constant. This restriction does not hold for organisms like frogs with complex life histories, however, because each life stage experiences different predators that may require conflicting changes in phenotype. Further, metamorphosis is not necessarily a new beginning and early life experiences can continue to influence the phenotype of subsequent life stages. To better understand how tadpole predation risk influences frog phenotype throughout development, I conducted four experiments aimed at quantifying how tadpole predation risk influences 1) tadpole behavior, 2) the dynamics of metamorphosis, and 3) juvenile frog behavior and behavioral carryovers across metamorphosis, as well as 4) examining the potential to use noninvasive methods to quantify glucocorticoid stress hormones, the primary physiological response exhibited by vertebrates to predator stress, in tadpoles. For all studies, I collected naïve Blanchard's cricket frog (*Acris blanchardi*) tadpoles from two sites around Stillwater, OK that differ in, among many factors, their history of fish predation and exposed them to cues from fish and/or dragonfly predators throughout tadpole development. After recording tadpole activity, age, size, and duration of metamorphosis, and I quantified the activity of juvenile frogs for two months after metamorphosis. To develop a non-invasive alternative to lethal whole body corticosterone (CORT) collection, I also collected waterborne CORT samples from a subset of tadpoles and compared them to tadpole whole body CORT levels and activity levels. Overall, the effects of tadpole predation risk and site of origin were pervasive – tadpole behavior, duration of metamorphosis, and juvenile phenotype were affected. In addition, I was also able to successfully assay tadpole CORT from water samples, but found CORT to not be related to tadpole activity levels. My studies are the first to show that fish can have lasting impacts on frog populations by altering frog transition during – and behavior after – metamorphosis. Furthermore, my results highlight under-studied linkages between aquatic and terrestrial ecosystems and help to develop techniques to repeatedly quantify physiological traits in small-bodied amphibians.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

CHRONIC PREDATION RISK CANALIZES TADPOLE BEHAVIOR

Abstract: Phenotypic variation exists in a hierarchy across multiple levels of biological organization ranging from major taxonomic groups to individuals. Labile traits, like behavior, exhibit an additional level of variation within individuals. To date, little is known about how different environmental factors influence within-individual variation, though there is evidence that predation risk and social environment may affect individual behavior. To elucidate the effects of chronic predation risk and conspecific density on the degree of behavioral variation between- and within-individuals, I reared Blanchard's cricket frog (*Acris blandchardi*) tadpoles in the presence and absence of chronic predation risk and three levels of conspecific density (low, medium, and high), and quantified their effect on individual variation in tadpole activity levels. Tadpoles reared in predator treatments were, on average, less active than tadpoles from control treatments while tadpoles from different density treatments did not differ in activity. In addition, I found that individual tadpoles reared in predation risk exhibited less variation between- and within-individuals (i.e. had more repeatable and predictable activity levels). However, there was no evidence for an effect of conspecific density on the degree of behavioral variation exhibited by individual tadpoles. These results have implications for predator-prey interactions by suggesting that past experiences with predation risk can alter the probability of future predator encounters

and highlights the idea that ecologically-induced changes in trait variation may influence the way we view the relationship between phenotypic plasticity and evolutionary processes.

Phenotypic variation exists in a hierarchy that spans from individuals to higher order taxa. Levels of phenotypic variation do not exist in isolation, however, and often influence each other. For example, directional selection on individual behavior may lead to a reduction in population-level variation. It is less obvious, however, how environmental factors influence variation at lower levels of biological organization.

Behavior, being one of the most labile phenotypic traits, has an additional level of variation resulting from differences in expression between instances of occurrence (i.e. within-individuals, intra-individual variation or IIV; Bell et al. 2009, Stamps et al., 2012). Within-individual variation is being increasingly incorporated into evolutionary thinking (e.g., Dingemanse and Dochtermann, 2013; Biro and Adriaenssens, 2013), including insights into its potential role in the complex relationship between phenotypic plasticity and the evolution of plastic traits (Snell-Rood 2013; Stamps 2015). This has led to discussions on effective statistical approaches (Westneat et al. 2015) and terminology (Stamps 2015) to ask and address questions about the causes and consequences of differences within individuals. Still, gaps still exist in our knowledge of the factors that affect within-individual variation.

One factor known to influence several aspects of individual behavioral variation is predation risk. Predators have a profound effect on the behavior of prey (Lima and Dill 1990; Sih 1987). To date, predators have been shown to alter mean levels of population and individual behavior (Lima 1998), the degree of behavioral consistency exhibited by individuals (e.g. Bell and Sih, 2007; Urzsán et al., 2015), and the amount of within-individual variation expressed by prey in the presence and absence of predators (Briffa 2013; Hugie 2003). In these studies, chronic and acute exposure to predation risk had contrasting effects on behavioral variation. Chronic predation risk, experienced either as a relic of

site differences or during development resulted in greater repeatability, i.e. a reduction in within-individual variation (Urszán et al. 2015), while individuals exposed to acute predation risk exhibited a greater degree of within-individual behavioral variation (Briffa 2013; Hugie 2003). A gap remains, however, in our understanding of the effect of experience with chronic predation risk on within-individual behavioral variation and how this relates to previously observed differences between predator-exposed and predator-naïve individuals.

In addition to predation risk, there is some evidence that social environment alone, and in interaction with predation risk, can have an impact on individual-level behaviors. For example, when reared without chemical cues of conspecifics, agile frog (*Rana dalmatina*) tadpoles exhibit reduced repeatability in behavior, but, in the presence of cues indicating predation risk and conspecifics, they demonstrated increased repeatability in behavior (Urszán et al. 2015). This interactive effect of conspecific presence and predation risk is likely based in the costs and benefits of the competition for resources and diluted *per capita* risk of predator attack associated with group living (Krause and Ruxton 2002).

Using variation in activity levels of tadpoles, my study examines the developmental plasticity of within- and between-individual variation in response to predation risk. To do this, I reared tadpoles in the presence or absence of cues indicating predation risk and repeatedly quantified their activity levels in arenas containing predator-free water. Overall, exposure to chronic predation risk had canalizing effects on tadpole behavior both within- and between-individuals, resulting in tadpoles that were more consistently less active than tadpoles reared in the absence of predation risk.

Methods

Species Description

Blanchard's cricket frogs (*Acris blanchardi*, Gamble et al. 2008) are wide-spread, small (1.6-3.8 cm) hylids that occur from north of the Ohio River to west of the Mississippi River in the southern United States (Gray et al. 2005). Blanchard's cricket frog tadpoles are small (0.01-0.6 g) and serve as prey

for a number of vertebrate and invertebrate predators (e.g. Caldwell 1982), particularly dragonfly nymphs (Carfagno et al. 2011). Cricket frogs can metamorphose and be of sexually-mature size by the end of the summer in which they hatched (Bayless 1969; Gray et al. 2005), but, given the low survival rates of juvenile and adult cricket frogs, female cricket frogs likely only breed once in their lifetime (Lehtinen and MacDonald 2011).

Site Description

I collected Blanchard's cricket frog eggs from two sites that differ in average hydroperiod and predator regime: Sanborn Lake and Oklahoma State University's Aquatic Ecology Research Area. Sanborn Lake, Stillwater, OK (Latitide: 36.155, Longitude: -97.078), is a permanent waterbody that contains vertebrate (e.g. fish) and invertebrate (e.g. dragonfly nymph - *Anax*) predators. In contrast, the Aquatic Ecology Research Area, Stillwater, OK (36.134287, -97.190065) is a series of relatively small, temporary to semi-permanent water bodies that contain only invertebrate predators, predominantly *Anax* nymphs. These sites will hereafter be referred to as SANB and EXPO, respectively.

Tadpole Collection and Treatment

To collect the tadpoles used in this experiment, I captured amplexed couples of Blanchard's cricket frog at night at both sites between 15 May and 11 June 2015 and placed them in 26 L plastic bins filled with 1.5 L of dechlorinated tap water overnight to deposit their eggs. The following morning, I released the adults, and brought the eggs back to a laboratory facility on Oklahoma State University's main campus. This was done to ensure that all eggs were naïve to chemical cues of predation prior to the start of the experiment and to allow me to account for clutch differences in my analyses (SANB: 7 clutches, EXPO: 6 clutches; Total: 13 clutches).

Shortly after hatching, tadpoles were placed in 5.7 L clutch-specific replicates of one of six treatments that represented all possible combinations of density (low: 5 tadpoles, medium: 10

tadpoles, high: 20 tadpoles) and predation risk (presence or absence of waterborne cues from a dragonfly nymph). All tadpoles were kept at a 14:10 light:dark cycle at 25.6 ± 1.2 °C and 61 ± 11 % humidity (mean ± SD). I did complete water changes and fed tadpoles ground algae wafers (Hikari Inc., Hayward, CA, USA) three times a week throughout the experiment. The amount of food each tank of tadpoles was fed depended on tadpole age and density such that each tank was given 0.01g food/week old/individual. For the predator cue treatments, 2 mL of dragonfly predator cues were added 3 times a week – every 48-72 hrs – immediately after a water change. For the control treatments, 2 mL of dechlorinated water was added in place of predator cues.

To make dragonfly predation cues, I collected six green darner (*Anax junius*) nymphs by dip net from Teal Ridge Wetland (36.100505, -97.080084) and housed them in individual 0.5 L plastic containers filled with dechlorinated tap water and plastic vegetation. To generate predator cues, I starved each nymph for 48 hrs, placed it in an individual 0.25 L plastic container filled with 150-710 mL dechlorinated tap water and a piece of plastic vegetation. Each nymph was fed 0.07 - 0.61g (1-3 individuals) tadpoles and allowed to feed for 1 hour resulting in a predator cue concentration of 1.1 ± 0.06 mg/mL. The water in which each nymph fed was homogenized, passed through a coffee filter, and immediately frozen so that it could be later thawed for predator cue application. At the same time, I froze dechlorinated water in 2 mL aliquots to thaw for control treatments.

Tadpole Activity Scores

Once a week after tadpoles were placed into treatments I recorded the Gosner stage (Gosner 1960) and weight of five focal tadpoles per clutch-treatment combination. I kept track of individual tadpoles by taking photographs of their unique, black saddle patterns on the dorsal portion of their tail once a week. These markings only shifted slightly during development and varied sufficiently between individuals to be able to reliably identify one individual out of a tank of twenty siblings.

Every other week I scored the activity levels of each focal tadpole. I recorded tadpole activity in white, opaque, gridded plastic bins (5.7 L) that had been divided into three zones, each 7 cm in

length. Each activity trial lasted 15 minutes during which I recorded the tadpole position (i.e. which zone it was in) every 30 s. I then quantified activity level as the probability that a tadpole changed zones between position checks by dividing the number of tadpole movements by the total number of position checks. I conducted activity trials until tadpoles were 6 weeks old, resulting in three activity trials per tadpole. Focal tadpoles that died or metamorphosed before three trials could be completed were removed from the analysis, leaving me with 285 individuals.

Statistical Analysis

Between-individual variation – To determine if the degree of variation between individuals differed as a result of tadpole density or exposure to chronic predation risk, I calculated individual average activity levels. Average activity scores were then $\log_{10}+1$ transformed to achieve normality and compared within sites and between treatments using a Brown-Forsythe tests for unequal variance. This test was run using the "lawstat" package (Gastwirth et al. 2017) in R Statistical Software.

Within-individual variation - I calculated two metrics to quantify within-individual variation: predictability and repeatability. Predictability is defined as the residual individual standard deviation (riSD), and I derived these values for each tadpole using random regression on individual activity (Briffa 2013; Stamps et al. 2012). Repeatability is defined as the coefficient of relative plasticity (Réale and Dingemanse 2010). These variables are similar, but different, and akin to behavioral precision (predictability – activity levels may vary linearly with trial number) and accuracy (repeatability – activity levels vary around a constant level). Because they are both measures of variation, lower values indicate more predictable and more repeatable behaviors.

To determine how tadpole density, chronic predation risk, and the interaction between the two influenced tadpole size, development, and within-individual behavioral variation, I used path analysis, ran separately for each site, and compared support for the competing path arrangements using Akaike's Information Criterion (AIC). Models with pretending variables (i.e. models in which the

addition of a variable increases the AIC score of ~2) were removed before interpretation of results. Tadpole weight and development stage were highly correlated (Pearson's $r = 0.92$, $p<0.0001$), so I only included development rate in each of the paths. The interaction term between predation risk and density was calculated as a separate variable prior to path construction and this singular interaction term was included in some of the paths. The most-complex path had tadpole density, predation risk presence, and the interaction between the two directly and indirectly, via tadpole development rate, influencing repeatability and predictability. A covariance term between predictability and repeatability was present in all models. All competing paths were constructed using the "lavaan" package (Rosseel 2012) in R Statistical Software (R Core Team 2016).

Results

The degree of variation between individual tadpoles differed between sites and was influenced by predation risk, but not conspecific density (EXPO: Predation risk: $F_{1,130} = 5.464$, $p=0.02$; Density: $F_{2,129} = 0.4812$, $p=0.62$; SANB: Predation risk: $F_{1,151} = 0.2317$, $p=0.63$; Density: $F_{2,150} = 1.985$, $p=0.14$). Individuals that were exposed to chronic predation risk from both sites were, on average, less active, than tadpoles from control treatments, and predator-reared tadpoles from EXPO had less variation between individuals (EXPO: Control: probability of activity = 0.14 ± 0.09 Predator: 0.10 ± 0.07, mean ± SD; $F_{1,130} = 8.22$, $p = 0.004$; SAND: Control: probability of activity = 0.13 ± 0.06 Predator: 0.09 ± 0.06, mean ± SD; $F_{1,151} = 16.14$, $p < 0.0001$; Figure 1.1).

Within-individual variation in tadpole activity was influenced by exposure to chronic predation risk during development. Tadpoles from the EXPO site that were exposed to chronic predation risk exhibited more predictable and repeatable behaviors, while predator-exposed tadpoles from SANB only exhibited more repeatable behaviors and had no difference in predictability (Table 1.1, Figure 1.2; EXPO: Control: repeatability = 1.21, predictability = 0.08; Predator: repeatability = 0.72, predictability = 0.05; SANB: Control: repeatability = 0.95, predictability = 0.06; Predator:

repeatability = 0.60, predictability = 0.06). In contrast, there was little evidence that density influenced within-individual variation, especially measures of predictability (Table 1.1).

Discussion

I found that the within- and between-individual behavioral variation of tadpoles was developmentally plastic – that is, developing in the absence or presence of predation risk altered the degree of variation that tadpoles exhibited. Overall, tadpoles from the Experimental Ponds that developed in the presence of cues from a dragonfly nymph predator exhibited more predictable and repeatable behaviors and had less variation between individuals while tadpoles from Sanborn Lake that were developed in predator cues had more repeatable behaviors, but no significant difference in predictability or variation between individuals.

A reduction in within-individual behavioral variation in response to predation risk has been found in other studies and underscores the importance of experience with stressors as a driver of behavioral variation. Similar to Urszán et al. (2015), I found that exposure to predation risk yielded tadpoles with a reduction in within-individual variation in behavior. This canalization of within-individual behavioral variation as a result of exposure to predation risk could reflect a reduction in organismal error as individual tadpoles became more certain of the state of their environment (i.e. this environment is risky – reduce activity; Sih, 1992; Stamps and Krishnan, 2017). Tadpoles from control treatments, on the other hand, may have exhibited increased behavioral variation due to greater uncertainty in how to appropriately respond. This finding again highlights the conflicting effects of acute and chronic exposure to predation risk on within-individual behavioral variation. This discrepancy may be due, in part, to differences in the types of phenotypic plasticity examined and their associated costs and benefits (Stamps 2015). Briffa (2013) and Hugie (2003) quantified contextual plasticity, i.e. changes in behavioral variation in response to acute exposure to predator cues, while the study presented here examined developmental plasticity (Stamps 2015). Given that the costs and benefits of plasticity likely differ by type of plasticity (e.g., Snell-Rood, 2013; Stamps,

2015), it is possible that predation risk may have divergent effects on within-individual behavioral variation depending on the time frame over which they are experienced. To fully examine how temporal variation in predation risk drives within-individual variation in behavior, future studies could examine how the duration, timing, and concentration of predator cue exposure influences individual behavior.

Site of origin had an effect on the degree to which tadpole predation risk altered the amount of behavioral variation between individuals. Tadpoles from the Experimental Ponds that were reared in the presence of predator cues exhibited less between-individual variation in activity levels than their control counterparts while tadpoles from Sanborn Lake did not differ in between-individual variation. There are many potential differences between the Experimental Ponds and Sanborn Lake to which cricket frog tadpoles could have become locally adapted (e.g. predator regime, hydroperiod), so this observed difference between sites may reflect the influence of a number of possible site-specific selective factors. Future studies should sample and score the behavioral variation of cricket frog tadpoles from a variety of sites to better understand the relationship between variation in tadpole behavior and abiotic/biotic factors.

There was little evidence for an effect of conspecific density on the degree of behavioral variation exhibited between- and within-individuals. It is possible that no density effect was observed for two reasons: 1) in my experiment, food was adjusted with tadpole density so the relative competition for food was consistent across density treatments, and 2) tadpole activity was assayed alone, not in varied densities. In the absence of direct competitor stress or immediate cues of conspecifics during activity trials, it is possible that prior experience in different social environments was insufficient to induce changes in behavior.

The data presented here demonstrate the influence of predation risk on the behavioral variation of a sexually immature life stage of an organism with a complex life history. Given that tadpoles are not yet reproductively active, the adaptive value and evolutionary consequences of the observed behavioral canalization must be made with caution. While it appears that some aspects of tadpole

experiences transcend metamorphosis (e.g. Barbasch and Benard, 2011; Trokovic et al., 2011), including consistent difference in behavior (Wilson and Krause 2012), it is yet unknown how tadpole experience influences within-individual behavioral variation after metamorphosis. To examine this, future studies should quantify behavioral variation of individuals as tadpoles, reared in the presence and absence of predator cues, and after metamorphosis. This approach would also help to determine the sensitive period, if such exists (e.g. Relyea, 2003), for predator-induced differences in behavioral variation for species with complex life histories.

A reduction in behavioral variation as a result of exposure to chronic cues of predation has implications for the dynamics of predator-prey interactions and represents an additional indirect effect of predators on prey phenotype. Tadpoles that were exposed to chronic cues of predation were less active and exhibited less within-individual variation in activity levels than tadpoles from control treatments. Given that a reduction in activity levels in tadpoles is considered an adaptation to reduce detection by predators, and even relatively small changes in activity can lead to increased survival (e.g. Azevedo-Ramos et al. 1992) it is possible that more experienced tadpoles (i.e. those that developed in the presence of predator cues) are less likely to be detected and therefore have a survival advantage compared to inexperienced tadpoles. In other words, past experiences with predation risk may mediate prey likelihood of future encounters with predators by altering the degree of behavioral variation that prey exhibit.

Predator-induced changes in within-individual individual behavioral variation has implications for our understanding of the role of phenotypic plasticity in evolutionary processes. In this case, the temporal disconnect between the plasticity-inducing ecological factor (predation risk) and the resulting change in the expression of a labile trait may alter the way in which we examine the effect of ecological processes on evolution (DeWitt et al. 1998; West-Eberhard 2003) and the evolution of plastic traits (Houston and McNamara 1992; Pigliucci 2001; Via and Lande 1985). In particular, the following questions remain - what is the ratio of costs to benefits of behavioral canalization following chronic exposure to predation risk? What effect may this behavioral canalization have on the

evolution of plasticity? To determine these effects, future studies should quantify the heritability and fitness consequences of reductions in developmental plasticity like the one observed in this study.

Acknowledgements

I would like to thank M. Bliss and K. Lovell for their help with metamorph maintenance and A. Simpson for their assistance scoring behavioral trials. In addition, I would like to thank the Department of Defense's National Defense Science and Engineering Graduate Fellowship (to LEB) for financial support. All research was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (protocol: AS-13-1).

Table 1.1. AIC table of alternative path models for the influence of density, chronic predation risk, and the interaction between the two on measures of within-individual variation in tadpole activity levels for both the Experimental Ponds and Sanborn Lake sites. Models presented were supported by the data given that they did not contain pretending variables and had $\Delta AIC < 7$.

Model	df	ΔAIC
Experimental Ponds		
Predation risk directly influences both predictability and repeatability	**9**	**0.0**
Predation risk influences predictability	**8**	**2.34**
Null	**7**	**2.86**
Sanborn Lake		
Predation risk influences repeatability	**8**	**0.0**
Predation risk * density influences predictability	**8**	**1.39**
Density influences predictability	**8**	**2.89**
Null	**7**	**3.10**

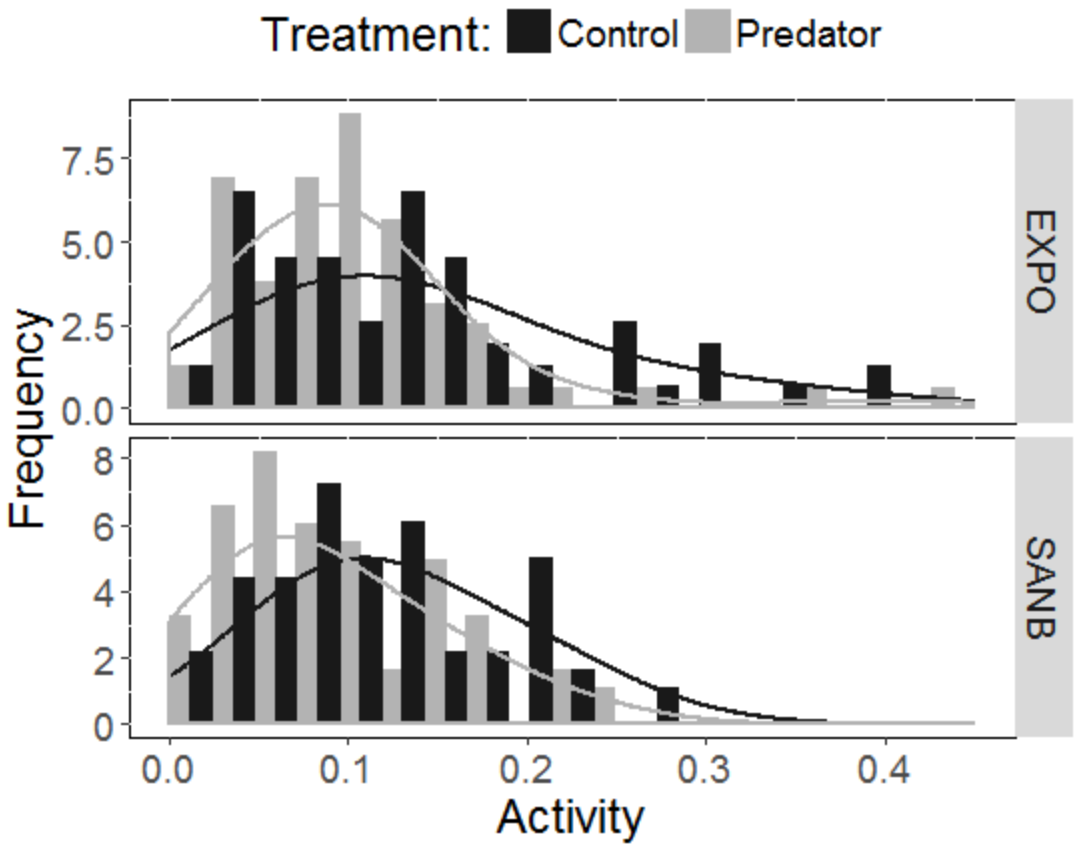

Figure 1.1. The distribution of average activity scores (i.e. probability that a tadpole moved between grid zones in a behavioral area) for tadpoles reared in control and predator treatments from the Experimental Ponds (EXPO) and Sanborn Lake (SANB). Smoothed curves represent kernel density estimates of each treatment's frequency distribution.

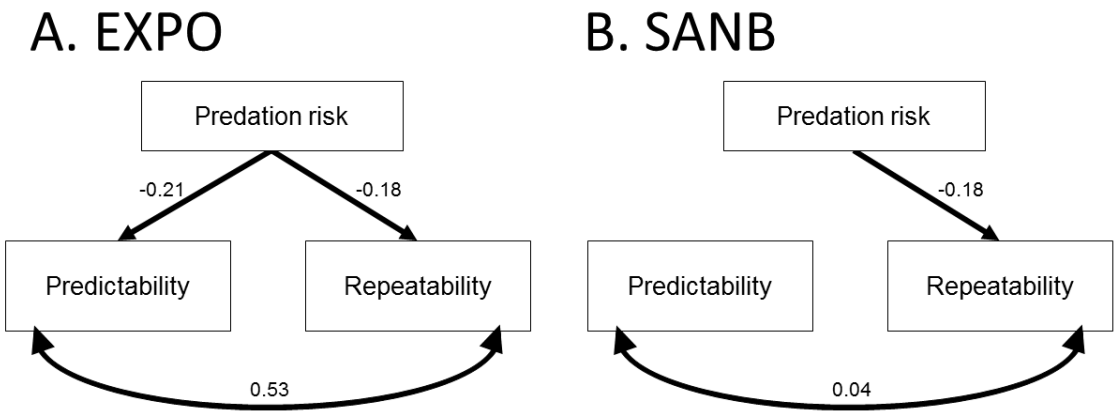

Figure 1.2. The influence of chronic predation risk on within-individual variation (i.e., predictability and repeatability) of tadpole activity levels from (A) the Experimental Ponds (EXPO) and (B) Sanborn Lake (SANB). Values represent partial correlation and covariance estimates. Because both predictability and repeatability are measures of variation, reductions in these values indicate individuals with more repeatable and predictable behaviors.

CHAPTER II

POPULATION HISTORY WITH-AND TADPOLE EXPOSURE TO-PREDATION RISK INFLUENCE THE AGE, SIZE, AND DYNAMICS OF ANURAN METAMORPHOSIS

Abstract: Anuran tadpoles exhibit plastic behavioral and morphological responses to predation risk that vary with predator type and site history. Tadpoles are not the only aspect of frog life history known to be variable, however, and frogs often alter life history transitions, especially metamorphosis, based on previous experience with predation risk. To date, clear results on the effect of current and past tadpole experience with predation risk on metamorph size and duration are lacking. I examined how site history and exposure to predation risk from dragonfly (*Anax junius*) and fish (bluegill – *Lepomis macrochirus*) predators affected the age, size, forearm emergence, and duration of metamorphosis in Blanchard's cricket frog (*Acris blanchardi*). In my analyses, I examined both the effect of predator presence and absence, as well as predator type on these measures of metamorphosis. I found that tadpoles from a site with a permanent water body and fish predators as well as tadpoles exposed to fish predator cues were smaller at metamorphosis while tadpoles exposed to dragonfly predators were larger metamorphs. Tail resorption rate was influenced by the interaction of site history and the presence of predator cues. Forearm emergence and weight change over the metamorphic period was not affected by site or treatment. My study is the first to show that site history can influence the duration of

metamorphosis and broadens our understanding of the pervasive effects of tadpole experience with predation risk, and especially fish predators, on frog life history.

Predators impose strong selection on prey and can alter prey phenotype simply by being present (Lima and Dill 1990; Sih 1987). These predator-induced modifications are particularly strong in larval amphibians, whose responses can be predator-, population-, and species-specific (Benard 2006; Buskirk and Saxer 2001; Hossie et al. 2016; Relyea 2001; Relyea 2002). These anti-predator modifications are often absent or reduced in individuals not exposed to the threat of predation, likely because of their costs (Van Buskirk 2000).

In aquatic systems, where most larval amphibians develop, predator presence is heterogeneous over space and time, such that predator composition can vary between proximate bodies of water. As a result of this variation in predator regime, many amphibians with low dispersal or high site fidelity exhibit a high degree of local adaptation to predators (Berven and Grudzien 1990; Relyea 2002; Storfer and Sih 1998). In some cases, local adaptation of tadpole morphology to different predator suites can occur on very small spatial scales (0.3-8 km; Relyea 2002). Predators of larval amphibians not only have an effect on tadpole phenotype, however, they also influence aspects of metamorphosis and juvenile phenotype (Laurila et al. 2006).

Metamorphosis, while not a new beginning, has many plastic facets that respond to predation risk (Pechenik et al. 1998; Touchon et al. 2013). Perhaps the most-studied plastic attributes of metamorphosis are the timing of – and size at – metamorphosis. The presence of predation risk is known to influence the time to metamorphosis (Benard 2004; Relyea 2007), with theory predicting that tadpoles exposed to predation risk should metamorphose earlier, with the cost of a smaller body size (Werner 1986; Werner and Gilliam 1984; Wilbur and Collins 1973). Empirical evidence for the effect of predation risk on metamorphosing frogs is conflicting, with studies finding negative, neutral, and positive effects of predator presence on the frog size and age at metamorphosis (Chivers et al. 1999; Gordon et al. 2016; Kiesecker et al. 2002; Laurila et al.

2006). This inconsistency is perhaps due to broader geographic patterns in predation pressure (Laurila et al. 2008) or differences in predator characteristics (Davenport et al. 2014).

A gap remains in our understanding of how predation risk influences the rate of morphological change during metamorphosis. While the duration of metamorphosis was once presumed to be minimized due to the increased risk of predation during that stage (Wassersug and Sperry 1977; Williams 1966), it is now known to be quite variable with evidence that predation risk can alter the duration of metamorphosis by 4-7%, depending on temperature (Walsh et al. 2008a). The influence of predation risk on the changes that occur during metamorphosis is an understudied aspect of predator-induced plasticity (Downie et al. 2004), potentially due to inconsistent definition of the end of the larval stage (Walsh 2010). Furthermore, forelimb emergence, which is generally viewed as the start of metamorphic climax, is often asynchronous and highly variable (Malashichev 2002; Zechini et al. 2015). To my knowledge, however, the influence of biotic factors, like predation risk, on the synchrony and symmetry of forelimb emergence is unknown.

To date, there have been only a few studies looking at the effect of tadpole predation risk on the duration and rate of morphological change in anuran metamorphosis, and still there is no consensus on the direction or magnitude of predator-induced effects. There is evidence that tadpole exposure to predation risk reduces the duration of metamorphosis (Walsh et al. 2008b), increases the duration of metamorphosis (Touchon et al. 2015), and influences rate of weight change, but not tail resorption or the rate at which tadpoles pass through metamorphosis (Van Buskirk and Saxer 2001). In addition to this conflicting evidence, no study has examined the influence of site history with predation risk on the duration or rate of morphological change in anuran metamorphosis, even though amphibians exhibit a high degree of local adaptation to predators.

My study aims to address this gap in our understanding of how population history and tadpole exposure to predation risk influences the dynamics of anuran metamorphosis. I exposed naïve

Blanchard's cricket frog (*Acris blanchardi*) tadpoles from two sites to cues of fish and dragonfly predation throughout development. After noting which forearm emerged first, I recorded individual metamorph body size and tail length daily for the duration of the metamorphic period. My results suggest that both site of origin and tadpole experience with predation risk influence many aspects of metamorphosis.

Methods

Study Species

Blanchard's cricket frogs (*Acris blanchardi*, formerly *A. crepitans blanchardi*; Gamble et al. 2008) are wide-spread, small (1.6-3.8 cm), terrestrial and semi-aquatic hylids that occur around waterbodies that vary greatly in hydroperiod – from lentic, permanent lakes to semi-permanent wetlands – and can be found from north of the Ohio River to west of the Mississippi River in the southern United States (Caldwell 1982; Gray et al. 2005; Lehtinen and Skinner 2006). In Oklahoma, Blanchard's cricket frogs breed from April to July and produce tadpoles that are prey for a number of predator species. Blanchard's cricket frog tadpoles possess an inducible, black tail spot that is present in sites with high dragonfly predation risk (Caldwell 1982) and lost in sites with high fish predation (Carfagno et al. 2011), supposedly conferring a survival advantage against both predator types. Tadpoles metamorphose in ~35-90 days into small ~1-1.5 cm juveniles that can reach a sexually-mature body size (~2 cm) by the end of the summer in which they hatched (Bayless 1969; Gray et al. 2005). While it is possible for some individuals to breed in less than a year, most individuals over-winter in cracks in the soil or crayfish burrows and breed in the following spring. Given that survival rates of juvenile and adult cricket frogs are very low, most individuals, at least females, breed only once in their lifetime (Lehtinen and MacDonald 2011).

Site Description

I collected Blanchard's cricket frog eggs from two sites that differ in hydroperiod and predator regime: Sanborn Lake and Oklahoma State University's Aquatic Ecology Research Area. Sanborn Lake, Stillwater, OK (Latitude: 36.155, Longitude: -97.078), is a permanent waterbody surrounded by a small tract of woodland. The lake contains a number of vertebrate and invertebrate predators (e.g., largemouth bass - *Micropterus salmoides*, sunfish – *Lepomis* spp., crappie – *Pomoxis* spp., dragonfly nymphs – *Anax* spp., water scorpions - Nepidae, and giant water bugs - Belostomatidae). In contrast, the Aquatic Ecology Research Area, Stillwater, OK (Latitude: 36.134, Longitude: -97.190) is a series of small, temporary to semi-permanent water bodies that contain a diversity of invertebrate predators (e.g. dragonfly nymphs –*Anax* spp., predaceous diving beetles - Dytiscidae, and giant water bugs - Belostomatidae), but no fish. These sites differ in a number of abiotic and biotic factors, but, because the main difference in tadpole predation risk between our two sites is the presence/absence of fish predators, I will hereafter refer to Sanborn Lake and the Aquatic Ecology Research Area as the "Fish" and "No Fish" sites, respectively.

The maximum reported dispersal by a cricket frog is 1.3 km (Burkett 1984). Given that our Fish and No Fish sites are > 11.3 km apart and have few connecting waterways, there is likely little gene flow between the populations of Blanchard's cricket frog at each of my sites. Thus, my two populations reflect distinct entities to examine for any effect of local adaptation/site history on our measures of metamorphic dynamics.

Tadpole Collection and Treatment

To acquire the tadpoles used in this experiment, I collected amplexed couples of Blanchard's cricket frog at night at the Fish and No Fish sites between 8 May and 14 June 2016 and placed them in 5.7 L plastic bins filled with 1.5 L of dechlorinated tap water overnight to deposit their eggs. The following morning, I released the adults at their site of capture, and brought the eggs

back to a laboratory facility on Oklahoma State University's main campus. This was done to ensure that all eggs were naïve to chemical cues of predation prior to the start of the experiment and to allow me to account for clutch differences in my analyses (Fish site: 7 clutches, No Fish site: 6 clutches, Total = 13 clutches).

Shortly after hatching, tadpoles were placed in 5.7 L clutch-specific replicates of either control, dragonfly, or fish treatments. All tadpoles were kept at a 14:10 light:dark cycle at 25.7 ± 0.7 °C and 52 ± 39 % humidity (mean ± SD). I did complete water changes and fed the tadpoles a mixture of equal parts ground algae wafers (Hikari Inc., Hayward, CA, USA), shrimp pellets (Aqueon, Franklin, WI, USA), and fish flakes (TetraCichlid cichlid flakes; Tetra, Blacksburg, VA, USA) three times a week throughout the experiment. The amount of food each tank of tadpoles was fed depended on tadpole age and density, such that each tank was given 0.01g food/week old/individual. For each of the predator cue treatments, I applied chemical cues of predation, i.e. damage-released chemical cues, which are known to elicit behavioral and morphological responses in prey (reviewed in Chivers and Smith 1998 and Ferrari et al. 2010). In each predator treatment, 2 mL (dragonfly cue) or 10 mL (fish cue) were added 3 times a week – every 48-72 hrs – immediately after a water change. The volume of predator cue added was lower for the dragonfly cue than the fish cue because the dragonfly predator cues were more concentrated than the fish predator cues (see below). Adding 2 mL and 10 mL of dragonfly and fish predator cues resulted in approximately the same final concentration of predator cue in each tadpole treatment tank. I added 6 mL of dechlorinated water to each control treatment tank to imitate the mechanical disturbance caused by the addition of predator cues in the other treatments.

To make dragonfly predation cues, I collected four green darner (*Anax junius*) nymphs by dip net from Teal Ridge Wetland (Latitude: 36.100, Longitude: -97.080). While green darner nymphs are present at both the Fish and No Fish sites, they are relatively more abundant and easier to catch at Teal Ridge. Each nymph was housed in an individual 0.5 L plastic container filled with

dechlorinated tap water and plastic vegetation. To generate predator cues, each nymph was starved for 48 hrs, placed in an individual 0.25 L plastic container filled with dechlorinated tap water and a piece of plastic vegetation from which to stalk prey. Each nymph was fed 0.06 - 0.15g (1-2 individuals) tadpoles and allowed to feed for 1 hour. Any tadpoles that were not consumed within that hour were removed and weighed to determine the resulting predator cue concentration (0.26 ± 0.07 mg/mL). After the dragonfly nymph was returned to its home container, the water in which each nymph fed was mixed together, passed through a coffee filter, and immediately frozen so that it could be later thawed for predator cue application.

To make fish predation cues, I collected four bluegill sunfish (*Lepomis macrochirus*) by seine from Sanborn Lake in May 2016 (15 ± 5 cm total length). Each fish was housed individually in a 10 gal tank, and fed fish flakes *ad libidum* (TetraCichlid cichlid flakes; Tetra, Blacksburg, VA, USA). For cue generation, one of the fish was starved for 48 hrs, placed in a covered and visually-isolated 9.5 L tank filled with 7.6 L of dechlorinated water. After an hour of acclimation, I allowed the fish to feed on 0.15 – 1.2 g (2-11 individuals) tadpoles for an hour. Any tadpoles that were not consumed during that time were removed and weighed to determine the resulting concentration of the predator cue (0.08 ± 0.07 mg/mL). The fish was then returned to its home tank and the water was immediately filtered and frozen so it could be thawed later for predator cue application.

Measures of Metamorphic Dynamics

Twice a day (between 7:00-10:00 and 18:00-22:00), I examined tadpole tanks for individuals with forearms present (Stage 42; Gosner 1960). If only one forearm had erupted at the time of discovery, I noted which forearm it was, and placed that metamorph in individual housing. Once a day, until there was < 3 mm of tail remaining, I measured the snout-vent length (SVL), tail length, and weight of these individuals. For nearly all metamorphs, it took four days to complete this process. Metamorphs that took less than four days to resorb their tail had both forearms

emerged at the time of discovery and were likely found after the tail resorption process had begun. These individuals were removed from the analysis ($N_{removed} = 12$), leaving a sample size of 346 individuals. In addition, I measured tibiofibula length of the right leg of each individual on the fourth day.

Statistical Analysis

To determine if site history and tadpole experience with predation risk influenced frog phenotype before and during metamorphosis, I analyzed the following metrics for site and treatment effects: forelimb emergence (right or left), initial tadpole tail length, age at metamorphosis (days), and SVL, weight, and tibiofibula length at the end of tail resorption. In addition, I estimated the residual leg length, rate of weight change, and tail resorption rate for each individual. Residual leg length was calculated as the difference between tibiofibula length and the values predicted by a linear model fit to the regression between SVL and tibiofibula length. To estimate the rate of weight change and tail resorption rate over the metamorphic period, I first compared the fit of linear and negative exponential models to the daily measures of the proportion of weight and tail length remaining using Akaike's Information Criterion (AIC). The linear model was a better fit for weight change over the metamorphic period ($\Delta AIC = 791$), while a negative exponential model was a better fit for the proportion of tail remaining ($\Delta AIC = 5958$). I then used these models to generate estimates of the rate of weight and tail loss per day and performed model selection on linear models fit to these estimates.

I used model selection approaches to analyze my data, comparing the AIC values of alternative linear or generalized linear (for forearm emergence) mixed effect models (LMM and GLMM, respectively), with clutch as a random effect and tadpole treatment, site of origin, and their interaction as fixed effects. In all analyses, tadpole treatments were coded in two ways: 1) predator presence with both predator treatments pooled (i.e., control vs. predator treatments), and 2) expanded to include predator identity (i.e., control vs. dragonfly vs. fish treatments). No model

contained both the predator presence and predator identity treatment effects. For the analysis of forearm emergence, I only used individuals that had either a right or left forearm detected. Individuals with both arms detected at first emergence were excluded, leaving me with 144 individuals. Within that subset, forearm emergence was treated as a binary variable (1 = right forearm, 0 = left forearm) and fit with a binomial GLMM with logit link function. Models receive support from the data when $\Delta AIC \leq 7.0$. I also excluded models with ΔAIC greater than a simpler version of the model, because this indicates that the model included a pretending variable, i.e. a variable that does not explain much of the variation and has a parameter estimate near zero (Anderson 2008; Richards 2008).

Results

Both site of origin and predator identity of tadpole treatment influenced age at metamorphosis, weight, SVL, and rate of tail resorption. In contrast, tibiofibula length was only affected by predator identity of the tadpole treatment, and residual leg length was only influenced by site of origin. Initial tail length, weight change throughout the metamorphic period, and forearm emergence were not influenced by site of origin or tadpole treatments.

Overall, cricket frog tadpoles were 43.7 ± 11 (mean ± SD; range = 28-105; Table 2.1) days old at the onset of metamorphosis. The best supported model for age at metamorphosis contained site of origin and tadpole predator identity, but not the interaction between the two (Table 2.2; Figure 2.1A). The model with only site of origin was also supported. None of the other models were well supported with them either being more complicated versions of the better supported models or having $\Delta AIC > 7$. Tadpoles reared from eggs collected from the Fish site metamorphosed, on average, 14 days earlier than tadpoles reared from eggs collected from the No Fish site. Tadpoles from both sites that experienced dragonfly and fish cues morphed out 2 and 0.2 days earlier than controls, respectively.

Most tadpoles (~58%) were discovered with both (B) forearms erupted (Table 2.1). Of the remaining 42% of tadpoles with a single forelimb emerged, most had their right forelimb erupt first (right: 130/346, left: 14/346). There was no site of origin or tadpole treatment effect on which forearm erupted first (Table 2.2).

Tadpole tail length at the start of metamorphosis was influenced by the identity of the tadpole predator (Table 2.2). Metamorphs that experienced cues of fish predators during tadpole development had shorter tails at the start of metamorphic climax than metamorphs from other treatments (Table 2.1; Figure 2.1B).

The best-supported model for weight at metamorphosis contained site of origin, predator identity, and their interaction (Table 2.2; Figure 2.1C). Tadpoles exposed to fish cues and those from the Fish site weighed less at metamorphosis than No Fish controls, while tadpoles from the Fish site that experienced dragonfly predator cues weighed more at metamorphosis (Table 2.1).

Metamorph size (SVL) was influenced by site and predator identity such that exposure to – or history with – fish predation resulted in smaller individuals while exposure to dragonfly predation caused individuals to be bigger (Table 2.2; Figure 2.1D). Metamorphs that experienced fish cues or came from the Fish site were shorter than those from the No Fish site or controls while metamorphs that experienced dragonfly cues were longer (Table 2.1).

The top model for tibiofibula length contained only predator identity (Table 2.2; Figure 2.1E), with metamorphs that experienced fish and dragonfly cues having shorter and longer legs than controls, respectively. After correcting for body length, relative leg length was best explained by site with metamorphs from the Fish site having relatively longer legs than those from the No Fish site (Table 2.2; Figure 2.1F).

All metamorphs lost weight during metamorphosis (48 ± 5% of starting body weight; mean ± SD). There was no evidence that site of origin or tadpole treatment influenced the rate at which metamorphs lost weight during metamorphosis (Table 2.2).

Metamorph tail resorption rate was influenced by the presence of predator cues and site of origin and their interaction, with some evidence for an effect of predator identity (Table 2.2; Figure 2.2). On average, metamorphs from the No Fish site resorbed their tail more quickly than individuals from the Fish site. In particular, No Fish metamorphs that experienced predator cues as tadpoles resorbed their tail faster than metamorphs that did not have predator cues, with No Fish metamorphs exposed to dragonfly cues resorbing their tails the fastest. In contrast, metamorphs from the Fish site that experienced predator cues resorbed their tails more slowly than Fish control metamorphs, with Fish metamorphs exposed to fish cues resorbing their tails the slowest.

Discussion

I found that site history and lifetime exposure to predation risk influenced most aspects of metamorphosis, but not all. Site of origin and tadpole predation risk treatment influenced tadpole age and size at metamorphosis, but did not affect forearm emergence or rate of weight loss. This study is the first providing evidence for site history having an impact on tail resorption rate in tadpoles and exposure to predation risk resulting in faster tail resorption and therefore duration of metamorphosis. The influence of the tadpole environment and site of origin on metamorphic dynamics highlights an important link between aquatic and terrestrial habitats and the localized spatial heterogeneity or amphibian plasticity.

Tadpoles that came from the Fish site and those from the No Fish site who experienced cues of predation risk during development metamorphosed younger than control tadpoles from the No Fish site. This is in contrast to previous work by Gordon et al. (2016), who found that both pond drying and dragonfly predation risk had no effect on the timing of metamorphosis in Blanchard's cricket frogs. My findings support the idea that a reduced larval period may be an adaptation to predation risk (Werner 1986; Wilbur and Collins 1973). In addition, site of origin had a much stronger effect on tadpole age at metamorphosis than tadpole predation treatments (15 vs 2-0.2

days, respectively; Table 2.2). The two sites used in this study differ in a number of biotic and abiotic factors, including factors like hydroperiod and average water temperature that are known to alter the timing of metamorphosis (e.g., Walsh et al. 2008b). However, even small differences in the age at metamorphosis can have important consequences for survival post-metamorphosis such that a metamorph's odds of surviving decrease by factors of 0.91-0.89 with each day's delay in metamorphosis (Chelgren et al. 2006). Given the contrasting results on the effect of predators on metamorphosis in Blanchard's cricket frog, additional studies are needed using individuals from a variety of populations to determine the biotic and abiotic factors that influence age at metamorphosis in this species.

In addition to the effect of site of origin and tadpole treatment effect on timing of metamorphosis, individuals from different sites and treatments differed in size at metamorphosis. This finding contrasts with previous work on the factors influencing metamorphosis in Blanchard's cricket frog, which found no effect of tadpole predation treatment on mass at metamorphosis (Gordon et al. 2016). Accelerated larval period is assumed to come at the cost of reduced size at metamorphosis (Wilbur and Collins 1973), and my data generally support this – tadpole from the Fish site and those from the No Fish site that experienced fish cues during development were smaller at metamorphosis than control individuals. However, I also found that tadpoles that experienced dragonfly cues during development metamorphosed slightly earlier and at a larger size than controls. The opposing effect of predators on size at metamorphosis observed in my study may reflect predator-specific defenses by cricket frog tadpoles to predators with different hunting strategies. For example, in response to an active predator like a sunfish, it may be advantageous for small-bodied cricket frog tadpoles to reduce activity and foraging, resulting in a smaller size at metamorphosis. For a sit-and-wait predator, like dragonflies, they may rely on alternative defenses. Previous evidence suggests that cricket frog tadpoles employ morphological defenses in the face of predation risk from dragonflies and not fish (Carfagno et al. 2011). Thus, cricket frog tadpoles may not reduce their foraging as much with dragonflies as with fish

predators which could result in a larger body size at metamorphosis. Given that differences in size as small as a millimeter can significantly influence a metamorphs odds of survival (Chelgren et al. 2006), future studies should examine behavioral and morphological differences between cricket frogs exposed to sit-and-wait vs. active pursuit predators to determine the relative importance and cost of behavioral and morphological adaptations in determining size at metamorphosis.

I found that site and the presence of predation risk during tadpole development influenced tadpole tail resorption rate during metamorphosis. In general, metamorphs from the Fish site resorbed their tails more slowly than metamorphs from the No Fish site. Further, metamorphs from the Fish site that experienced predation risk during tadpole development resorbed their tails more slowly while metamorphs from the No Fish site resorbed their tail more quickly in response to tadpole predation risk. This interaction between site and exposure to predation risk could be responsible for conflicting reports of the effects of tadpole predation risk on the duration of metamorphosis (Touchon et al. 2015; Van Buskirk and Saxer 2001; Walsh et al. 2008b). Future work should examine how other aspects of site history may effect metamorphic duration and what population-level and fitness the consequences are for between-population differences in tail resorption rate.

Most tadpoles, when discovered with a single forelimb erupted, had their right forelimb erupt first, but there was no site of origin or tadpole treatment effect. Previous work has found that most species examined did not differ in symmetry of forearm emergence, but if a forearm emerged first, it was the left forearm (but results are conflicted; Malashichev 2002; Zechini et al. 2016). Asymmetry in forelimb emergence may relate to lateralization of behavior later in life (Malashichev 2002). Given that only 42% of metamorphs were detected with one forearm emerged, future studies on the symmetry of forelimb emergence should monitor forelimb emergence more frequently than twice a day (e.g. Zechini et al. 2015).

My study demonstrates that site history and tadpole exposure to predation risk can alter many aspects of metamorphosis, including the rate at which metamorphosing frogs resorb their tail. My findings highlight that there are multiple, potentially plastic aspects to anuran metamorphosis that can vary through both plastic responses and local adaptation (Touchon et al. 2013). Given that anuran metamorphosis is an important link between aquatic and terrestrial ecosystems, we need to better understand the causes and patterns of spatial heterogeneity in - and plasticity of - metamorphic dynamics if we are to predict the effect of both wide-spread (e.g. climate change) and localized (e.g. pollution) environmental perturbations.

Acknowledgements

I would like to thank B. Mausbach and A. Simpson for their help with metamorph maintenance. In addition, I would like to thank the Graduate College at Oklahoma State University (to LEB) and the Animal Behavior Society (to LEB) for financial support. All research was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (protocol: AS-13-1).

Table 2.1. Summary statistics for metamorph morphology and age at metamorphosis for tadpoles originating from different sites treated with different cues of predation risk during the tadpole stage. Weight, snout-vent-length (SVL), and tibiofibula are presented as means ± SD and age at metamorphosis is presented as median ± SD. Forearm emergence ratio is given as the number of Gosner stage 42 individuals with both (B): right (R): left (L) forearms erupted from the skin at first detection.

Site	Treatment	Age at metamorphosis (days)	Tail length (mm)	Weight (g)	SVL (mm)	Tibiofibula (mm)	Detected Forearm Emergence Ratio (B:R:L)
No Fish	Control	51 ± 12	24.5 ± 2.2	0.19 ± 0.04	12.2 ± 1.0	6.6 ± 0.6	29:20:1
	Dragonfly	48 ± 8	24.5 ± 3.3	0.19 ± 0.05	12.4 ± 1.0	6.6 ± 0.8	33:16:2
	Fish	49 ± 13	23.1 ± 2.7	0.16 ± 0.04	11.6 ± 1.1	6.3 ± 0.7	30:15:4
Fish	Control	36 ± 6	24.2 ± 2.6	0.17 ± 0.04	11.8 ± 0.86	6.5 ± 0.6	38:27:2
	Dragonfly	35 ± 7	24.3 ± 2.7	0.18 ± 0.04	11.8 ± 0.88	6.6 ± 0.6	42:22:3
	Fish	36 ± 7	24.0 ± 2.9	0.17 ± 0.03	11.9 ± 0.84	6.4 ± 0.6	30:30:2

Table 2.2. AIC table of alternative models and their parameter estimates for how site of origin and tadpole predation risk treatments affected metamorph age, forearm emergence, initial tail length, weight, snout-vent length (SVL), tibiofibula length, residual leg length, the rate of weight change, and tail resorption rate. The "Predator Presence" term reflects the comparison of control vs. predator treatments (i.e. predator absence and presence, respectively) and the "Predator Identity" term reflects the comparison of control, dragonfly, and fish treatments. Parameter estimates are displayed as mean ± SE. Parameter estimates that contain the effect of site (i.e. "Site", "Site * Predator Identity", and "Site * Predator Presence") represent the effect of the Fish site (i.e. Sanborn Lake) relative to the No Fish site (i.e. Experimental Ponds) alone and in interaction with tadpole predator treatments. Model shown were supported by the data given that they did not contain pretending variables and had ΔAIC < 7. The most supported models are indicated in bold.

Response Variable	**Model**	**df**	**ΔAIC**	**Parameter Estimate**					
				Intercept	**Site**	**Predator Identity**	**Predator Presence**	**Site * Predator Identity**	**Site * Predator Presence**
Age at metamorphosis	**Site + Predator Identity**	**6**	**0.0**	**52.46 ± 1.59**	**-14.11 ± 1.97**	***Dragonfly*: -2.02 ±1.09** ***Fish*: -0.23 ±1.11**	**-**	**-**	**-**
	Site	4	0.1	51.69 ± 1.45	-14.10 ± 1.96	-	-	-	-
Forearm emergence	**Null**	**2**	**0.0**	**2.8 ± 0.62**	**-**	**-**	**-**	**-**	**-**
Initial tail length	**Predator Identity**	**5**	**0.0**	**24.32 0.36**	**-**	***Dragonfly*: 0.04 0.33** ***Fish*: -0.75 0.34**	**-**	**-**	**-**

	Null	3	0.0	24.09 ± 0.31	-	-	-	-	-
Weight	**Site * Predator Identity**	**8**	**0.0**	**0.19 ± 0.008**	**-0.02 ± 0.01**	***Dragonfly*: -0.004 ± 0.008** ***Fish*: -0.03 ± 0.008**	-	***Dragonfly*: 0.01 ± 0.01** ***Fish*: 0.03 ± 0.01**	-
	Predator Identity	5	1.6	0.18 ± 0.005	-	*Dragonfly*: 0.002 ± 0.005 *Fish*: -0.01 ± 0.005	-	-	-
	Site + Predator Identity	6	2.9	0.19 ± 0.007	-0.008 ± 0.009	*Dragonfly*: 0.002 ± 0.005 *Fish*: -0.01 ± 0.005	-	-	-
Metamorph size (SVL)	**Site + Predator Identity**	**6**	**0.0**	**12.2 ± 0.19**	**-0.43 ± 0.24**	***Dragonfly*: 0.08 ± 0.1** ***Fish:* -0.22 ± 0.11**	-	-	-
	Predator Identity	5	0.9	11.97 ± 0.15	-	*Dragonfly*: 0.08 ± 0.1 *Fish:* -0.22 ± 0.11	-	-	-
	Site	4	3.5	12.16 ± 0.18	-0.43 ± 0.24	-	-	-	-
	Null	3	4.3	11.93 ± 0.13	-	-	-	-	-
Tibiofibula length	**Predator Identity**	**5**	**0.0**	**6.52 ± 0.09**	-	***Dragonfly*: 0.05 ± 0.08** ***Fish:* -0.17 ± 0.08**	-	-	-

	Null	3	4.7	6.49 ± 0.07	-	-	-	-	-
Residual leg length	**Site**	**4**	**0.0**	**-0.10 ± 0.07**	**0.17 ± 0.10**	**-**	**-**	**-**	**-**
	Null	3	0.7	-0.006 ± 0.05	-	-	-	-	-
Rate of weight change	**Null**	**3**	**0.0**	**-2 x 10^{-14} ± 0.0005**	-	-	-	-	-
Rate of tail resorption	**Site * Predator Presence**	**6**	**0.0**	**-0.06 ± 0.02**	**0.08 ± 0.03**	-	***Predator*: 0.08 ± 0.03**	-	***Predator*: -0.11 ± 0.04**
	Site * Predator Identity	8	2.6	-0.06 ± 0.02	0.08 ± 0.03	*Dragonfly*: 0.10 ± 0.03 *Fish:* 0.06 ± 0.03	-	*Dragonfly*: -0.12 ± 0.04 *Fish:* -0.09 ± 0.04	-
	Null	3	3.0	-8 x 10^{-13} ± 0.009	-	-	-	-	-

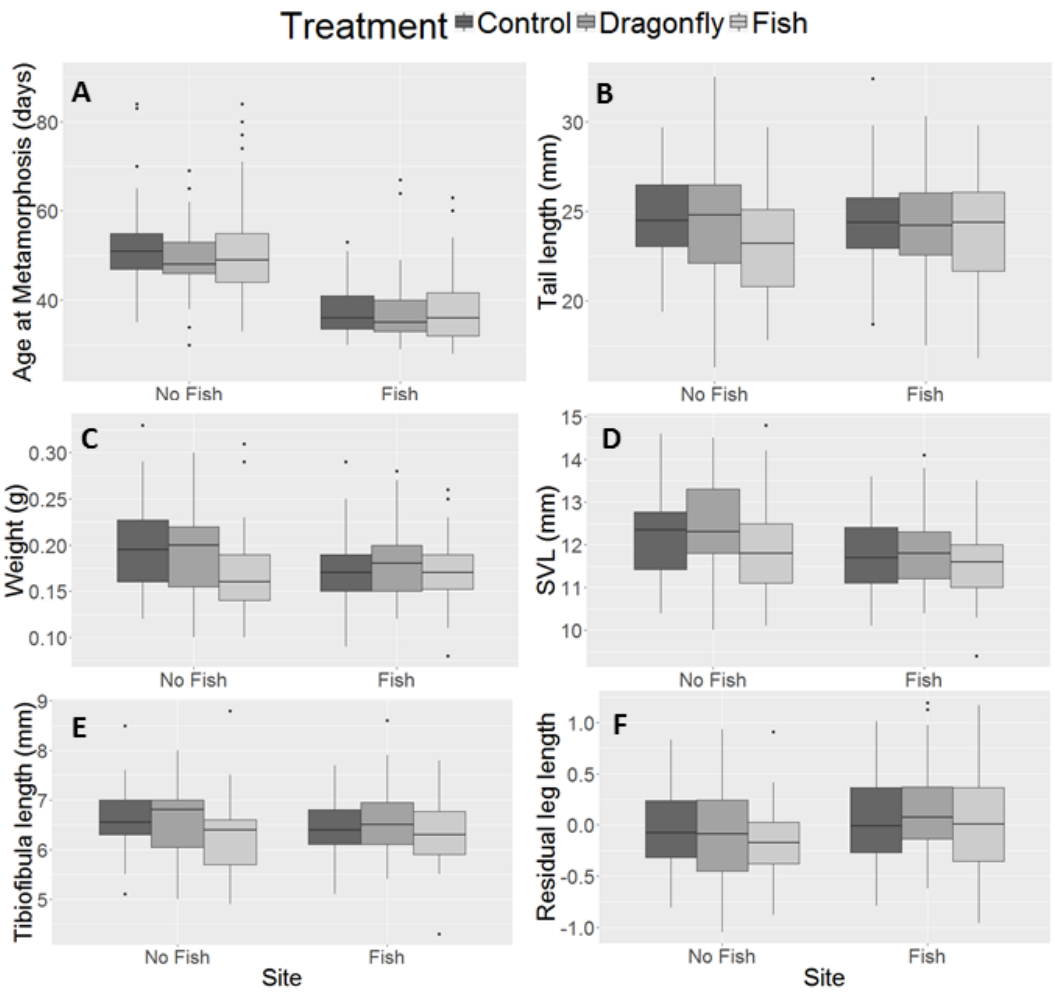

Figure 2.1. The influence of site of origin and tadpole predation risk treatment on a tadpoles' age at metamorphosis (A), tail length at the onset of metamorphosis (B), final weight at the end of metamorphosis (C), final snout-vent length (SVL; D), leg length (E), and residual leg length (F). Outliers are indicated by black circles.

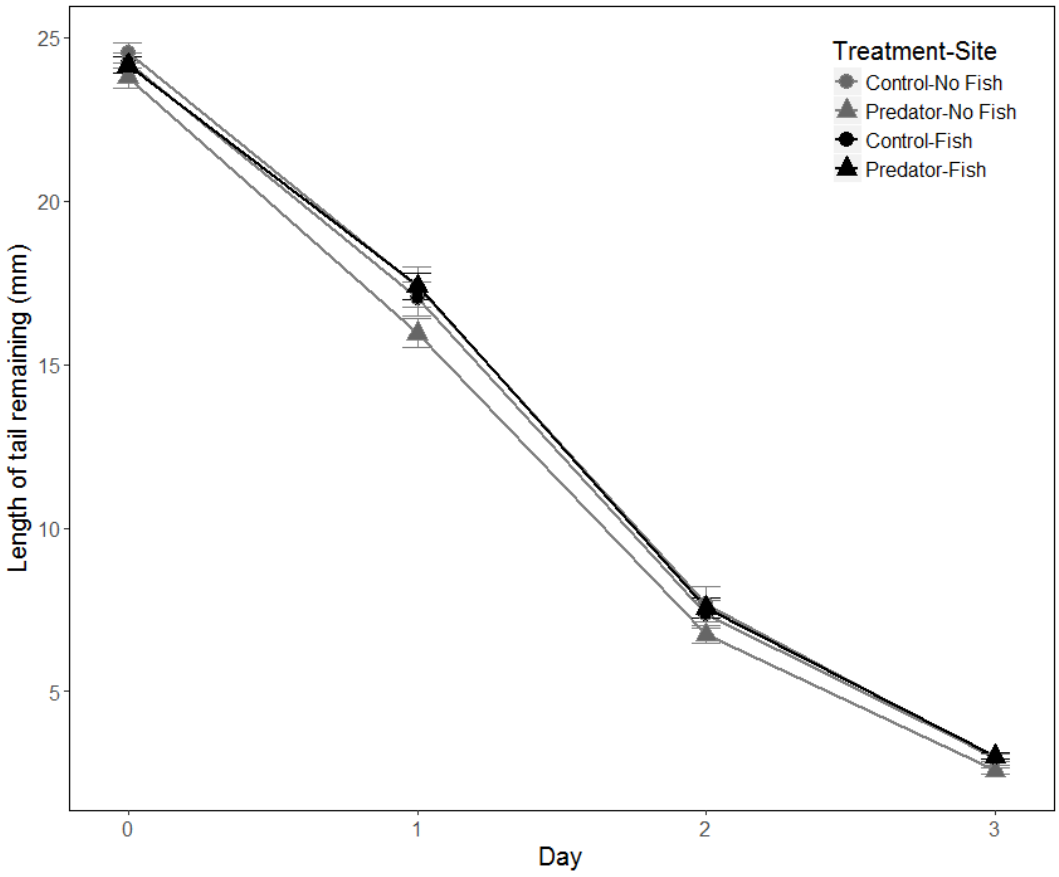

Figure 2.2. The influence of site of origin and tadpole predation risk treatment on tail resorption rate during metamorphosis. “Predator” treatment refers to metamorphs that received cues of either dragonfly or fish predators during development.

CHAPTER III

BEHAVIORAL CARRYOVERS ACROSS ANURAN METAMORPHOSIS: THE IMPACT OF TADPOLE PREDATION RISK ON JUVENILE FROG PHENOTYPE

Abstract: Predation risk influences prey phenotype. Predator-induced changes in prey behavior and morphology are hypothesized to be adaptive, as long as predation risk is fairly constant. For organisms with complex life histories, like frogs, each life stage may experience different predators that may require conflicting changes in phenotype. Further, metamorphosis is not necessarily a new beginning and early life experiences can continue to influence the phenotype of subsequent life stages. To better understand how tadpole predation risk influences frog phenotype throughout development I collected naïve Blanchard's cricket frog (*Acris blanchardi*) tadpoles from two sites around Stillwater, Oklahoma and exposed them to cues from fish or dragonfly predators throughout tadpole development. After repeatedly recording tadpole activity, anti-predator behavior, and size for four weeks, I quantified the activity, anti-predator behavior, and size of juvenile frogs for two months after metamorphosis and determined the effects of site and tadpole predation risk treatment on tadpole and juvenile size and behavior as well as the relationship between an individual's average tadpole and juvenile behavior. While all metrics were affected by trial number, tadpole behavior was also influenced by the interaction of site and tadpole predation risk such that predation risk treatments had contrasting effects on tadpole activity and anti-predator behavior depending on site of origin. Tadpole predation risk treatment

and site also influenced the activity of juvenile frogs with the strongest effect observed in frogs from the site with shorter hydroperiod and absence of fish predators that were exposed to fish cues during development. Indeed, frogs from the site without a history of fish predation risk that experienced fish cues as tadpoles were the only individuals to exhibit a significant positive relationship between their average tadpole and juvenile activity levels. Site of origin also influenced juvenile frog antipredator behavior with juveniles from the site without a history of fish predation exhibited decreased avoidance of predator cues. My study is the first to document the effects of multiple tadpole predators on the behavior of individual frogs from different sites across metamorphosis. This finding highlights the potential for yet un-seen and under-studied linkages between aquatic and terrestrial ecosystems.

Prior experience influences phenotype (West-Eberhard 2003). Predators, in particular, have profound effects on prey, altering prey behavior and morphology simply by being present (Lima and Dill 1990; Sih 1987). Predator-induced changes in phenotype may prove adaptive if predation pressure is constant over time (Walsh et al. 2015) as inducible defenses often come at the cost of altered time and energy allocation (e.g., Van Buskirk 2000), but this requirement does not hold for species with complex life histories whose separate life stages experience distinct predation threats (Benard 2004). Indeed, given the high degree of dissimilarity between adult and larval habitats for many species with complex life histories, it is unclear whether the behavior of different life stages should be related at all (Sih et al. 2004) and for how much and how long we should expect predation risk experienced in early life stages of complex-lived organisms to affect the phenotype of subsequent life stages.

Frogs, whose dramatic ontogenetic niche shift was once thought to preclude them from the influence of natal experience, display the latent effects of predation risk on their morphology and life history transitions. The presence of predation risk is known to influence the time to metamorphosis (Benard 2004; Relyea 2007), with theory predicting that tadpoles exposed to

predation risk should metamorphose earlier, with the cost of a smaller body size (Werner 1986; Werner and Gilliam 1984; Wilbur and Collins 1973), though empirical evidence suggests predator-induced changes in metamorphic size may be both species- and predator-dependent (Chivers et al. 1999; Davenport et al. 2014). Further, predator-induced changes in juvenile frog size persist after metamorphosis with exposure to tadpole predation risk resulting in smaller, more vigorous juveniles with both longer limbs and narrower bodies (Relyea 2001) or shorter, more muscular limbs (Van Buskirk and Saxer 2001) depending on the species. These morphological modifications, however, are not without long-term fitness consequences (e.g. Altwegg & Reyer, 2003) and often give rise to differential survival and growth (Berven 1990; Chelgren et al. 2006).

To date, only a few studies have examined the relationship between the behavior of tadpole and juvenile frogs (Barbasch and Benard 2011; Brodin et al. 2013; Wilson and Krause 2012). One study by Wilson and Krause (2012) showed that consistent individual differences in activity and exploration were retained through metamorphosis in lake frogs (*Rana ribundiula*). While the proximate mechanisms underlying consistent individual differences in behavior (i.e., personalities or behavioral syndromes; Sih et al. 2004) are largely unknown, it is possible that these differences are the result of latent effects from differential early life experiences (Pechenik 2006; Frost et al. 2007). Further, Brodin et al. (2013), found that the boldness and exploratory behavior of tadpole and juvenile common frogs (*Rana temporaria*) differed between island and mainland populations. While these behaviors were not repeatable across life stages, they do highlight the potential for local adaptation in tadpole and juvenile frog behavior. Additional work by Barbasch and Benard (2011) demonstrated that tadpoles exposed to predation risk developed into more active juvenile wood frogs (*Rana sylvatica*) than tadpoles not exposed to predation risk, even though predator cues often result in decreased tadpole activity (e.g. Lawler 1989; Skelly 1994). While these studies examined the relationship between tadpole and juvenile frog behavior, a longitudinal study of the effect of tadpole predation risk on the behavior of individual frogs throughout their life is lacking.

My research aims to fill this gap by generating a more complete picture of the effect of tadpole predation risk on frog phenotype throughout frog development. To do this, I reared naïve Blanchard's cricket frog (*Acris blanchardi*) tadpoles from two sites and quantifying the effect of tadpole experience with two predators: fish and dragonfly nymphs on the size, activity, and anti-predator behavior of individual tadpole and juvenile cricket frogs. In response to cues indicating predation risk, tadpoles often reduce activity (e.g. Relyea 2001) while juvenile frogs exhibit avoidance behaviors (e.g. Flowers and Graves 1997; Belden et al. 2000), but prior experience with predation risk may play an important role in shaping juvenile frog responses to predators (Murray et al. 2004). Specifically, I sought to address how site and tadpole experience with fish and dragonfly predators influence 1) tadpole size, activity, and antipredator behavior; 2) juvenile frog size, activity, and antipredator behavior; and 3) the relationship between an individual frog's tadpole and juvenile behavior.

Methods

Study Species

Blanchard's cricket frogs (*Acris blanchardi*, formerly *A. crepitans blanchardi*; Gamble et al. 2008) are small (1.6-3.8 cm), terrestrial and semi-aquatic hylids that occur around a variety of waterbodies– from lentic, permanent lakes to semi-permanent wetlands –from north of the Ohio River to west of the Mississippi River in the southern United States (Caldwell 1982; Gray et al. 2005; Lehtinen and Skinner 2006). In Oklahoma, Blanchard's cricket frogs breed from April to July and produce tadpoles that are prey for a number of predators including dragonfly nymphs and fish (Caldwell 1982; Carfagno et al. 2011). Tadpoles metamorphose in ~35-90 days into small ~1-1.5 cm juveniles that can reach a sexually-mature body size (~2 cm) in a couple of months (Bayless 1969; Gray et al. 2005).

Site Description

I collected Blanchard's cricket frog eggs from two sites that differ in predator regime: Sanborn Lake and Oklahoma State University's Aquatic Ecology Research Area. Sanborn Lake, Stillwater, OK (Latitude: 36.155, Longitude: -97.078), is a permanent waterbody surrounded by a small tract of woodland. The lake contains a number of vertebrate and invertebrate predators (e.g., largemouth bass - *Micropterus salmoides*, sunfish – *Lepomis* spp., crappie – *Pomoxis* spp., dragonfly nymphs – *Anax* spp., water scorpions - Nepidae, and giant water bugs - Belostomatidae). In contrast, the Aquatic Ecology Research Area, Stillwater, OK (Latitude: 36.134, Longitude: -97.190) is a series of small, temporary to semi-permanent water bodies that contain a diversity of invertebrate predators (e.g. dragonfly nymphs –*Anax* spp., predaceous diving beetles - Dytiscidae, and giant water bugs - Belostomatidae), but no fish. These two sites differ in a number of biotic and abiotic factors, but, because the main difference in tadpole predation risk between my two sites is the presence/absence of fish predators, I will hereafter refer to Sanborn Lake and the Aquatic Ecology Research Area as the "Fish" and "No Fish" sites, respectively.

The maximum reported dispersal by a cricket frog is 1.3 km (Burkett 1984). Given that our Fish and No Fish sites are > 11.3 km apart and have few connecting waterways, there is likely little gene flow between the populations of Blanchard's cricket frog at each of my sites. Thus, my two populations reflect distinct entities to examine for any effect of local adaptation/site history on our measures of metamorphic dynamics.

Animal Collection, Treatment, and Housing

To acquire the tadpoles used in this experiment, I collected amplexed couples of Blanchard's cricket frog at night at the Fish and No Fish sites between 8 May and 14 June 2016 and placed them in 5.7 L plastic bins filled with 1.5 L of dechlorinated tap water overnight to deposit their eggs. The following morning, I released the adults at their site of capture, and brought the eggs

back to a laboratory facility on Oklahoma State University's main campus. This was done to ensure that all eggs were naïve to chemical cues of predation prior to the start of the experiment and to allow me to account for clutch differences in my analyses (Fish site: 7 clutches, No Fish site: 6 clutches, Total = 13 clutches).

Shortly after hatching, tadpoles were placed in 5.7 L clutch-specific replicates of either control, dragonfly, or fish treatments with ten tadpoles per replicate. All tadpoles were kept at a 14:10 light:dark cycle at 25.7 ± 0.7 °C and 52 ± 39 % humidity (mean ± SD). I did complete water changes and fed the tadpoles a mixture of equal parts ground algae wafers (Hikari Inc., Hayward, CA, USA), shrimp pellets (Aqueon, Franklin, WI, USA), and fish flakes (TetraCichlid cichlid flakes; Tetra, Blacksburg, VA, USA) three times a week throughout the experiment. The amount of food each tank of tadpoles was fed depended on tadpole age and density such that each tank was given 0.01g food/week old/individual. For each of the predator cue treatments, 2 mL (dragonfly cue) or 10 mL (fish cue) were added 3 times a week – every 48-72 hrs – immediately after a water change. The volume of predator cue added was lower for the dragonfly cue than the fish cue because the dragonfly predator cues were more concentrated than the fish predator cues (see below). Adding 2 mL and 10 mL of dragonfly and fish predator cues resulted in approximately the same final concentration of predator cue in each tadpole treatment tank. I added 6 mL of dechlorinated water to each control treatment tank to imitate the mechanical disturbance caused by the addition of predator cues in the other treatments.

To make dragonfly predation cues, I collected four green darner (*Anax junius*) nymphs by dip net from Teal Ridge Wetland (Latitude: 36.100, Longitude: -97.080). While green darner nymphs are present at both the Fish and No Fish sites, they are relatively more abundant and easier to catch at Teal Ridge. Each nymph was housed in an individual 0.5 L plastic container filled with dechlorinated tap water and plastic vegetation. To generate predator cues, each nymph was starved for 48 hrs, placed in an individual 0.25 L plastic container filled with dechlorinated tap water and a piece of plastic vegetation from which to stalk prey. Each nymph was fed 0.06 -

0.15g (1-2 individuals) tadpoles and allowed to feed for 1 hour. Any tadpoles that were not consumed within that hour were removed and weighed to determine the resulting predator cue concentration (0.26 ± 0.07 mg/mL). After the dragonfly nymph was returned to its home container, the water in which each nymph fed was mixed together, passed through a coffee filter, and immediately frozen so that it could be later thawed for predator cue application.

To make fish predation cues, I collected four juvenile bluegill sunfish (*Lepomis macrochirus*) by seine from Sanborn Lake in May 2016 (15 ± 5 cm total length). Each fish was housed individually in a 10 gal tank, and fed fish flakes *ad libidum* (TetraCichlid cichlid flakes; Tetra, Blacksburg, VA, USA). For cue generation, one of the fish was starved for 48 hrs, placed in a covered and visually-isolated 9.5 L tank filled with 7.6 L of dechlorinated water. After an hour of acclimation, I allowed the fish to feed on 0.15 – 1.2 g (2-11 individuals) tadpoles for an hour. Any tadpoles that were not consumed during that time were removed and weighed to determine the resulting concentration of the predator cue (0.08 ± 0.07 mg/mL). The fish was then returned to its home tank and the water was immediately filtered and frozen so it could be thawed later for predator cue application.

Once a tadpole started to metamorphose (i.e. erupted a forearm and reached Stage 42; Gosner 1960), it was removed from the tadpole tank, identified from its unique pattern of dorsal saddle spots, and placed in individual housing to complete metamorphosis. While resorbing their tail, metamorphs were housed in 16 oz deli cups filled with 75 mL of dechlorinated water. These cups were placed at a 30° angle so that metamorphs could easily come out of the water when metamorphosis was complete. I changed metamorph water and monitored metamorphosing individuals daily so that they could be transferred to terrestrial housing once tail resorption was complete. After an individual had resorbed its tail, I placed it in a 16 oz deli cup filled with 2 cm of dampened coconut fiber (Zoo Med Laboratories, California) and sphagnum moss (Zoo Med Laboratories, California), a 35x10 mm petri dish filled with dechlorinated water, and covered with a 20x20 cm piece of unbleached cheesecloth (Pure Acres Farm, Colorado) to allow for

sufficient ventilation. Juvenile frogs were housed individually for the remainder of the experiment and fed 20-30 flightless *Drosophila melanogaster* and *D. hydei* dusted with calcium and multivitamin powder every other day.

Tadpole Phenotype

Once a week beginning one week after tadpoles were initially put into treatments I recorded the weight, developmental stage (Gosner 1960), activity, and anti-predator responses of five focal tadpoles from each of the predator treatments and all control treatment tadpoles for each clutch. The control tadpoles were divided in half so that five individuals served as controls for the dragonfly treatment, while the remaining five served as controls for the fish treatment. I kept track of individual tadpoles by taking photographs of their unique, black saddle patterns on the dorsal portion of their tail prior to measurement. These markings only shifted slightly during development and varied sufficiently between individuals to be able to reliably identify one individual out of a tank of ten siblings.

To quantify tadpole activity and anti-predator behavior, I placed individual focal tadpoles in labeled, opaque 200 mL cups filled with 150 mL of dechlorinated water and allowed them to acclimate for at least 15 minutes. I then placed tadpole cups under a video camera and recorded tadpole activity for 15 minutes. After 15 minutes, I slowly added treatment-specific predator cue to each cup and recorded tadpole activity for an additional 15 minutes. For the tadpoles in the dragonfly treatment and their control counterparts I added 1 mL of dragonfly cue and, for the fish-treated tadpoles and their paired controls, I added 2 mL of fish cue. Tadpole activity was then later scored from the video recordings in three two-minute increments both before and after the addition of predator cue (before cue between minutes: 2-4, 6-8, and 10-12, after cue addition between minutes: 1-3, 5-7, and 9-11). I then defined tadpole activity as the average time the tadpole spent actively swimming out of two minutes before predator cue was added. Tadpole anti-predator behavior, on the other hand, was defined as the average amount of time the tadpole

spent swimming out of two minutes after the predator cue was added. Tadpole trials continued for four consecutive weeks, which is when the first tadpoles started metamorphosing.

Juvenile Phenotype

I recorded juvenile frog weight, snout-vent-length (SVL), activity, and anti-predator behavior once a month for two months beginning one month after an individual had completed metamorphosis. The month interval was chosen to allow juveniles to complete tail resorption and adjust to life on land. I conducted juvenile frog activity trials in visually isolated, 38 L aquaria in which the floor was been divided into a 5 x 13 grid. The floor of this arena was then lined with a paper towel dampened with dechlorinated water to prevent desiccation and was replaced after every individual. To quantify juvenile frog anti-predator behavior, I lined one end of the floor of a visually isolated 38 L glass aquaria with a paper towel (20 cm x 35 cm) that had been dampened with 15 mL of predator cue matching the predator that each individual experienced as a tadpole, the other end of the same aquaria with a matching paper towel that had been dampened with 15 mL of dechlorinated water, and left the remaining, central third of the arena empty, as a neutral zone. These paper towels were refreshed and the arena wiped clean with dechlorinated water after each individual. For each of these trials, an individual frog was placed under an opaque lid in the center of the arena and allowed to acclimate for 15 minutes. At the end of the acclimation period, I slowly removed the lid and began scoring behavior five minutes after the lid had been removed. Each trial lasted 50 minutes during which I recorded juvenile frog position (i.e. which grid cell or whether or not it was on the predator-scented paper towel) every 5 minutes. Anti-predator behavior was defined as the number of position checks during which a frog was on or touching the paper towel dampened with the scent of predator cues. Given that juvenile frogs typically avoid the scent of predators (e.g. Murray 2004), individuals that were in contact with the predator-scented paper towel less were considered to be exhibiting a greater degree of anti-

predator behavior. Frogs that did not complete two juvenile behavioral observations were excluded from analysis, leaving me with 180 individuals

Statistical Analysis

I analyzed how tadpole weight, activity, and anti-predator behavior were affected by site history, tadpole experience with predation, and the individual's age. Tadpole weight and Gosner stage were highly correlated (Pearson's $r = 0.94$, $n = 720$, $p < 0.0001$), as were juvenile frog weight and SVL (Pearson's $r = 0.93$, $n = 720$, $p < 0.0001$). Thus, because weight was measured across both life stages, I used it as my measure of individual size in my analyses.

I used model selection approaches to analyze my data, comparing the Akaike's Information Criterion (AIC) values of alternative linear (weight) or generalized linear (activity and anti-predator behavior) mixed effect models (LMM and GLMM, respectively) with individual nested within clutch as random effects and tadpole treatment, site of origin, trial number, and their interactions as fixed effects. All behavioral metrics were treated as proportions (e.g., for the tadpole, the number of seconds moving out of 120, and, for the juvenile, the number of times an individual moved grid cells out of 10 location checks) and were fit using a binomial GLMM. Given that weight may be influenced by tadpole treatments and increases as tadpoles develop, which may, either independently or in concert with tadpole treatments, influence behavior I also included weight as a fixed effect in all behavioral analyses for both tadpoles and juveniles. In all analyses, tadpole treatments were coded in two ways: 1) predator presence with both predator treatments pooled (i.e., control vs. predator treatments), and 2) expanded to include predator identity (i.e., control vs. dragonfly vs. fish treatments). No model contained both the predator presence and predator identity treatment effects. Models receive support from the data when $\Delta AIC \leq 7.0$. I also excluded models with ΔAIC greater than a simpler version of the model, because this indicates that the model included a pretending variable, i.e. a variable that does not

explain much of the variation and has a parameter estimate near zero (Anderson 2008; Richards 2008). All model selection analyses were run using the "nlme" (Pinheiro et al. 2016) and "lme4" (Bates et al. 2014) packages in R Statistical Software (R Core Team 2016).

In addition, to determine the relationship between an individual's tadpole and juvenile behaviors, I calculated individual average activity and anti-predator behavior scores for each life stage and ran separate ANCOVAs for each site with tadpole predator treatments as explanatory factors. This analysis was also run in R Statistical Software (R Core Team 2016).

Results

Tadpole Phenotype

Tadpole weight was influenced by trial number, site of origin, and their interaction (Table 3.1; Figure 3.1). As trial numbers increased (i.e. as tadpoles developed), they weighed more. Tadpoles reared from eggs collected from the Fish site weighed more during each trial and gained weight more quickly as they developed. I found no evidence that tadpole predator treatments influenced tadpole weight.

The most-supported model for tadpole activity contained weight and a three-way interaction between site of origin, tadpole predator identity, and trial number (Table 3.1, Figure 3.2). On average, heavier tadpoles were more active than lighter tadpoles. Initially tadpoles from the Fish site that were reared with cues of fish predation were less active than other Fish site tadpoles, while Fish-treated tadpoles from the No Fish site were more active. However, as time passed tadpoles from the Fish site that developed in fish cues became more active, while fish-treated tadpoles from the No Fish site became less active. Tadpoles reared in dragonfly cues from the No Fish site became less active as trials progressed and becoming less active, on average, compared to other No Fish tadpoles.

Similar to tadpole activity, the most-supported model for tadpole anti-predator behavior contained tadpole weight and a three-way interaction between site of origin, tadpole predator

identity, and trial number (Table 3.1; Figure 3.3). After predator cues were added, heavier tadpoles were more active than lighter tadpoles. On average, tadpoles from the No Fish site were more active than tadpoles from the Fish site. Developing in cues of fish predation had conflicting effects on activity in the presence of fish predator cues in tadpoles from Fish and No Fish sites such that fish-treated tadpoles from the Fish site were the least active after predator cue addition, while No Fish tadpoles exposed to fish cues were, on average, more active than other No Fish tadpoles. In contrast, dragonfly-treated Fish tadpoles were more active after dragonfly predator cues were added than other Fish tadpoles, while dragonfly-treated No Fish tadpoles were less active, on average, than other No Fish tadpoles. With the exception of fish-exposed Fish tadpoles, most tadpoles were less active after predator cues were added as trials progressed.

Juvenile Phenotype

Juvenile frog weight was affected by trial number (Table 3.2; Figure 3.1). As trials progressed, frogs got heavier.

For the activity of juvenile frogs the most supported model contained the interaction between trial number, site of origin, and the presence of predators in the tadpole's treatment (Table 3.2; Figure 3.2). A slightly less supported model was the equivalent model, but with predator identity replacing predator presence. As more time passed after metamorphosis, juvenile frogs became more active. This increase in activity, however, was not as pronounced in juveniles from the No Fish site. According to the second-most supported model, Juvenile frogs from the No Fish site that were exposed to cues of fish predation while tadpoles were, on average, more active than other No Fish juveniles. In contrast, juvenile frogs from the Fish site that were exposed to dragonfly cues during tadpole development were more active than juvenile frogs from the same site that experienced either control or fish treatments as tadpoles.

Juvenile frog anti-predator behavior was primarily influenced by individual weight and the interaction between trial number and site (Table 3.2; Figure 3.3). In general, heavier frogs spent

less time on predator-scented paper towel, even though, over time, frogs spent more time on predator-scented paper towel. On average, initially frogs from the No Fish site spent more time on the predator-scented paper towel, but over trials the amount of time spent on the predator paper towel increased more for Fish juveniles than No Fish juveniles.

Tadpole and juvenile frog activity levels were only significantly related in individuals from the No Fish site ($F(5,61) = 2.448$, $p = 0.04$; Figure 3.4). This was driven largely by the significant interaction of average tadpole activity and exposure to fish cues during tadpole development (average tadpole activity * fish treatment: $p = 0.006$). Anti-predator behavior of tadpoles and juveniles from the No Fish site was not significantly related ($F(5,61) = 1.329$, $p = 0.26$; Figure 3.5), nor were the tadpole and juvenile behaviors of individuals from the Fish site (Activity: $F(5,107) = 1.762$, $p = 0.13$, Anti-predator behavior: : $F(5,107) = 0.3861$, $p = 0.86$; Figures 3.4,3.5).

Discussion

I found that site and tadpole exposure to predation risk influenced frog phenotype during the tadpole stage and for months after metamorphosis. Overall, site of origin had a stronger effect than tadpole experience - influencing more aspects of frog phenotype and modifying the direction and magnitude of predation risk-induced changes. Where tadpole predation risk treatments had an effect, however, cues from dragonfly and fish predators had different effects on tadpole and juvenile frog behavior with fish cues having the largest impact. This longitudinal study is the first to document the interactive effects of multiple tadpole predators and site history on the phenotype of individual frogs throughout tadpole development and across metamorphosis.

Tadpoles from the Fish site weighed more and grew faster than tadpoles from the No Fish site. Exposure to predation risk, both through population history and lifetime experiences, has been shown to alter tadpole development rate (Benard 2004; Relyea 2007). While predation risk generally elicits a reduction in the rate of tadpole development (Davenport et al. 2014), tadpole

development is a balance between size and safety such that accelerated growth and development comes at the cost of smaller body size at metamorphosis and increased risk of predation (Wilbur and Collins 1973). Thus, the accelerated growth exhibited by tadpoles from the Fish site may be a consequence of generations of experience with fish predators shaping the size-time trade-off that determines tadpole growth rate. It is important to note, however, that my two sites differ in a variety biotic and abiotic factors known to influence tadpole development (e.g. hydroperiod - Rowe and Dunson 1995) other than tadpole predator regime, so my observed site effects may represent local adaptation to a variety or combination of site-specific factors.

For both tadpole activity and anti-predator behavior, site of origin and exposure to predation risk interacted to yield differences in behavior. In general, tadpoles from the Fish site that were exposed to fish cues during development were more active, both before and after the addition of predator cues, while tadpoles from the No Fish site that were exposed to fish cues were less active. This interactive effect of site and predator treatment occurred for the dragonfly treatment too, but, in contrast, dragonfly-treated tadpoles from the No Fish site were less active before and after the addition of predator cues while dragonfly-treated tadpoles from the Fish site were more active in both behavioral assays. These results align with previous studies that have documented local adaptation and differences in responsiveness to predators in amphibians (Berven and Grudzien 1990; Relyea 2002; Storfer and Sih 1998) and highlight the importance of multiple source populations when examining predator-induced behavioral plasticity in amphibians.

Heavier tadpoles were more active both before and after the addition of predator cues. This finding is in agreement with other studies that have found that bigger tadpoles tend to be more active (Nicieza 1999). Furthermore, given that predation rates decrease with increasing tadpole size (e.g. Eklöv and Werner 2000), potentially due to a size threshold imposed by gape-limited predators, larger tadpoles may use alternative anti-predator strategies (e.g. spatial avoidance) in lieu of reducing activity in response to predator cues.

Most tadpoles exhibited reduced activity after the addition of predator cues (i.e. a stronger anti-predator response), and over time, tadpoles became less active both before and after the addition of predator cues. These changes in tadpole behavior could be state-dependent (Laurila et al. 2004) and are in agreement with the asset protection principle (Clark 1994). For example, a tadpole that is closer to metamorphosis has more to protect than an individual that is recently hatched and should respond more strongly to the threat of predation.

Tadpole experience with predation risk, interacting with site of origin, influenced the activity level of juvenile frogs. While all juvenile frogs got more active over time, my second-most supported model indicated that juveniles from the No Fish site that were exposed to cues of fish predation while tadpoles were the most active juvenile frogs, particularly during the first month after metamorphosis. In contrast to the findings of Barbasch and Benard (2011), not all predator treatments, including exposure to chemical cues of dragonfly nymphs – which was the predator used in their study – resulted in more active juvenile frogs. However, predator-exposed individuals from the Fish site did rapidly increase in activity as time passed after metamorphosis. Given that tadpole experience with predation risk continues to influence frog behavior months after metamorphosis, when some individuals were approaching the size threshold for sexual maturity, future studies should continue to examine if tadpole experiences persist after the hormonal changes involved with puberty.

While tadpole treatment did not affect juvenile anti-predator behavior, site of origin did. This finding aligns with Brodin et al. (2013) who found differences in juvenile frog boldness between populations. In addition, the absence of an effect of tadpole predation risk treatment on juvenile anti-predator behavior confirms the results of Barbasch and Benard (2011), who also found that tadpole experiences with predation risk did not influence juvenile responses to predators. Even though frogs from both sites exhibited less avoidance of predator scent over time, frogs from the No Fish site exhibited less predator avoidance overall. This difference in anti-predator behavior between individuals from Fish and No Fish sites could reflect local adaptation to a number of

site-specific differences. For example, the observed site effect could represent differences in terrestrial predator threats or the perceived relative risk of aquatic vs. terrestrial predators. If individuals from the No Fish site perceive terrestrial predators to be the greater threat, then they may exhibit reduced spatial avoidance of the scent of an aquatic predator. Indeed, given that cricket frogs often respond to terrestrial predation threats by jumping into water (Gray 1978), individuals may favor close proximity to water regardless of predator scent.

The tadpole and juvenile behavior of individual frogs was only significantly related in individuals from the No Fish site, and only in individuals who experienced cues of fish predation as tadpoles. The relationship between tadpole and juvenile behavior in fish-exposed No Fish individuals was positive – meaning that individuals that were more active as tadpoles were also more active as adults. While this finding aligns with previous work that has found that significant positive relationships between tadpole and juvenile frog behavior (Wilson and Krause 2012) and tadpole exposure to predation risk can result in increased behavioral consistency (Urszán et al. 2015), I found that other predator-stressed individuals did not exhibit a similar degree of behavioral consistency across life stages. This finding is in support of theories that suggest that selection should decouple behavior through ontogeny when environmental conditions experienced by one life stage are substantially different from the subsequent life stage (Sih et al. 2004). Indeed, the environments experienced by tadpole and juvenile Blanchard's cricket frog meet this criterion. Proximately, the lack of a relationship between tadpole and juvenile behavior could be due to the myriad physiological and morphological changes that occur during and immediately after metamorphosis (Pough and Kamel 1984).

One possible mechanism underlying the presence of a behavioral carryover lasting months after anuran metamorphosis is predator- and stress-induced changes in physiology. Chronic stress can have profound, prolonged effects on individual phenotype (Lupien et al. 2009; Romero et al. 2009). Furthermore, exposure to persistent stressors as tadpoles have been shown to have carryover effects on juvenile frog physiology (Crespi and Warne 2013; Denver 2009). Future

studies should examine the relationship between behavior and stress physiology throughout tadpole development and into adulthood.

Altogether, the presence of fish predation, either historically or within a tadpole's lifetime had a large impact on tadpole and post-metamorphic frog behavior. Fish have been shown to have large effects on frog populations, usually through direct, lethal reductions in frog abundance (Bronmark and Edenhamn 1994; Hecnar and M'Closkey 1997; Kats et al. 1988). My studies show that fish can have indirect and lasting impacts on frog populations by altering frog behavior before and after metamorphosis and emphasizes potential under-studied links between aquatic and terrestrial ecosystems.

The totality of my research shows that local adaptation and tadpole predation risk, as well as the resulting alterations that tadpoles make to their behavior and morphology, impact current and future life stages. In my study system, metamorphosis is not a new beginning and is, instead, one of many aspects of a frog's life that can be influenced by early experience with predator stress. My research highlights the importance and utility of longitudinal studies in organisms with complex life histories in order to understand the total effects of early life experience and tadpole plasticity.

Acknowledgements

I would like to thank B. Atherton, K. Alvarez, A. Simpson, M. Ambardar, S. Beaty, and B. Mausbach for their assistance with animal maintenance and M. Byrne, M. Naylor, and J. Agan for their help locating adult frogs in amplexus. In addition, I would like to thank the Graduate College at Oklahoma State University (to LEB) and the Animal Behavior Society (to LEB) for financial support. All research was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (protocol: AS-13-1).

Table 3.1. AIC table of alternative models for how site of origin, tadpole predation risk treatments, and trial number affected tadpole weight, activity and anti-predator behavior. Tadpole weight is also included as a possible fixed factor in the analysis of tadpole activity and anti-predator behavior. The "Predator Presence" term reflects the comparison of control vs. predator treatments (i.e. predator absence and presence, respectively) and the "Predator Identity" term reflects the comparison of control, dragonfly, and fish treatments. I show the models that had $\Delta AIC < 7$ and did not contain pretending variables.

Model	df	ΔAIC	*w*
Weight			
Trial Number * Site	7	0.0	0.98
Activity			
Trial Number * Predator Identity * Site + Weight	15	0.0	1.0
Anti-predator Behavior			
Trial Number * Predator Identity * Site + Weight	15	0.0	0.58
Trial Number * Predator Identity * Site	14	0.8	0.39
Trial Number * Predator Presence * Site + Weight	11	6.9	0.02

Table 3.2. AIC table of alternative models for how site of origin, tadpole predation risk treatments, and trial number affected juvenile weight, activity, and anti-predator behavior. Juvenile weight is also included as a possible fixed factor in the analysis of juvenile activity and anti-predator behavior. The "Predator Presence" term reflects the comparison of control vs. predator treatments (i.e. predator absence and presence, respectively) and the "Predator Identity" term reflects the comparison of control, dragonfly, and fish treatments. Models in bold were supported by the data given that they did not contain pretending variables and had $\Delta AIC < 7$.

Model	df	ΔAIC	*w*
Weight			
Trial Number	**5**	**0.0**	**0.21**
Trial Number + Predator Identity	7	0.4	0.18
Trial Number + Predator Presence	6	0.8	0.14
Trial Number + Site	6	1.7	0.09
Trial Number + Predator Identity + Site	8	1.9	0.08
Trial Number + Predator Presence + Site	7	2.5	0.06
Trial Number * Predator Identity	9	2.8	0.05
Trial Number * Predator Presence	7	2.8	0.05
Trial Number * Site	7	3.7	0.03
Trial Number * Predator Identity + Site	10	4.3	0.02
Trial Number * Predator Presence + Site	8	4.5	0.02
Trial Number + Predator Presence * Site	8	4.5	0.02
Trial Number + Predator Identity * Site	10	4.6	0.02
Activity			
Trial Number * Predator Presence * Site	**10**	**0.0**	**0.22**
Trial Number * Predator Identity * Site	**14**	**0.1**	**0.21**
Trial Number * Predator Presence * Site + Weight	11	1.5	0.10
Trial Number * Predator Identity * Site + Weight	15	1.8	0.09

Trial Number	4	2.9	0.05
Trial Number + Predator Presence	5	3.4	0.04
Trial Number * Predator Presence	5	3.6	0.04
Trial Number + Site	5	4.0	0.03
Trial Number + Weight	5	4.1	0.03
Trial Number * Site	6	4.4	0.02
Trial Number + Predator Presence + Site	6	4.5	0.02
Trial Number * Predator Presence + Site	7	4.7	0.02
Trial Number + Weight + Site	6	5.0	0.02
Trial Number * Predator Identity + Site	9	5.2	0.02
Trial Number + Predator Identity	6	5.4	0.01
Trial Number * Site + Weight	7	5.5	0.01
Trial Number + Predator Presence * Site	7	6.2	0.01
Trial Number + Predator Identity + Site	7	6.5	0.01
Anti-predator Behavior			
Trial Number * Site + Weight	**7**	**0.0**	**0.81**
Trial Number * Predator Presence * Site + Weight	11	3.4	0.15
Trial Number * Predator Identity * Site + Weight	15	6.3	0.03

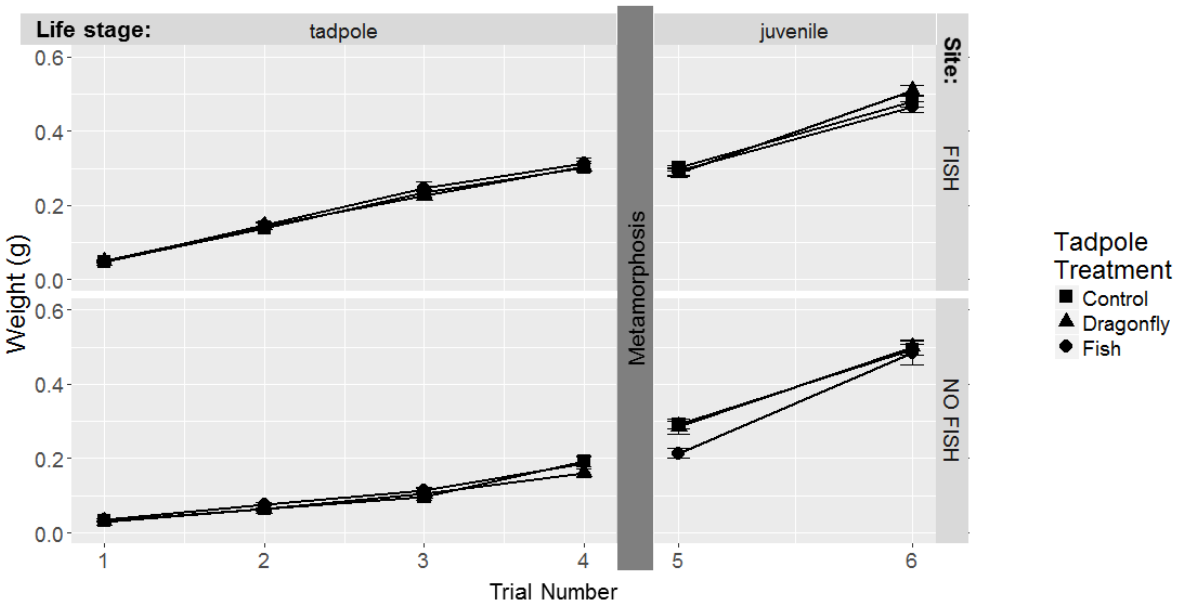

Figure 3.1. Weight of tadpoles and juvenile frogs from Fish and No Fish sites during behavioral trials after exposure to chemical cues of dragonfly predators, fish predators, and water (control) during tadpole development.

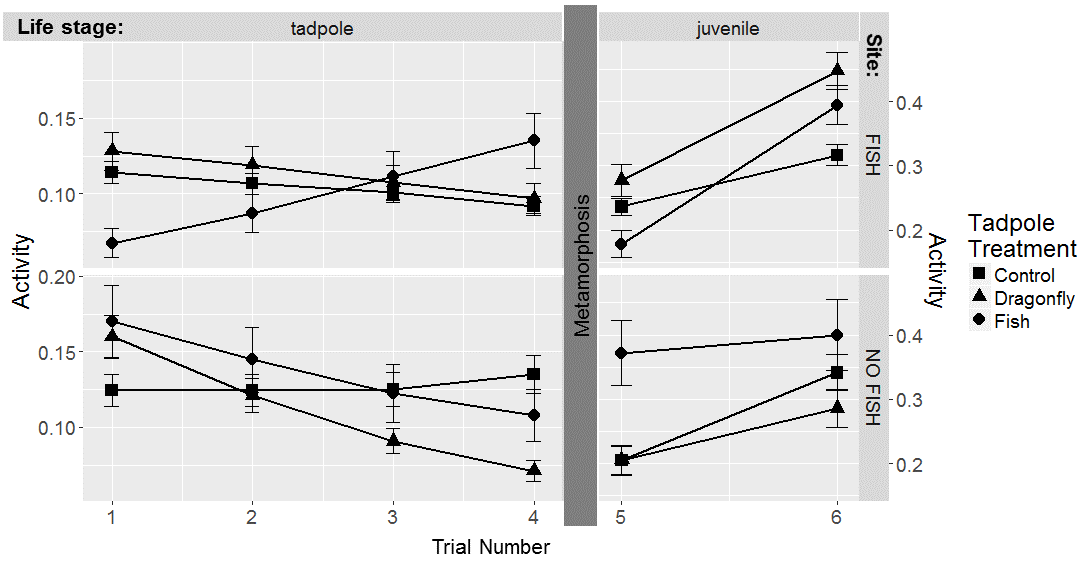

Figure 3.2. Activity levels of tadpoles and juvenile frogs from Fish and No Fish sites after exposure to chemical cues of dragonfly predators, fish predators, and water (control) during tadpole development as predicted by the most supported model for tadpole activity and the second-most supported model for juvenile activity. Tadpole activity is the predicted average number of seconds that a tadpole was actively swimming out of three two-minute intervals before predator cues were added. Juvenile frog activity is the predicted number of times that a frog moved cells in a gridded behavioral arena out of ten position observations.

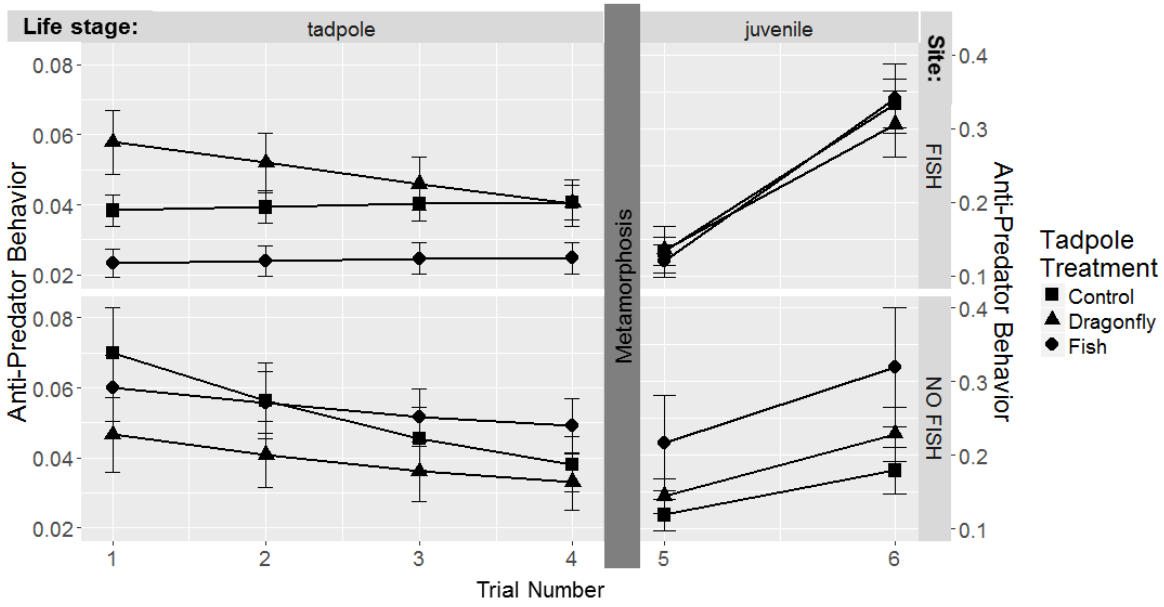

Figure 3.3. Anti-predator behavior of tadpoles and juvenile frogs from Fish and No Fish sites after exposure to chemical cues of dragonfly predators, fish predators, and water (control) during tadpole development as predicted by the most supported models for tadpole and juvenile anti-predator behavior. Tadpole anti-predator behavior is the predicted average number of second moving of three two-minute intervals after predator cues were added to water. Juvenile frog anti-predator behavior is the predicted number of position observations, out of ten, during which the frog was on a paper towel dampened with predator cues. For both tadpole and juvenile frog behaviors, larger values represent reduced anti-predator behavior as increased movement and less spatial avoidance increase an individual's likelihood of being detected by a predator.

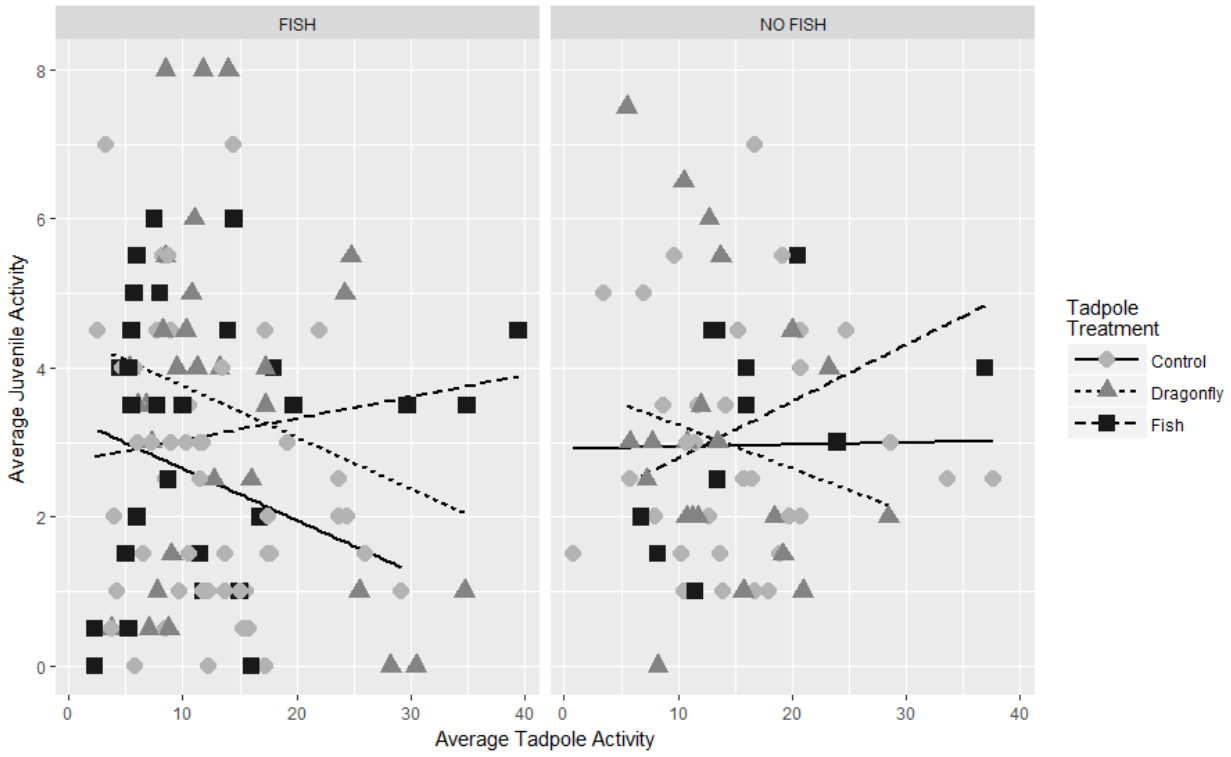

Figure 3.4. Relationship between an individual's activity level as a tadpole and juvenile frog from a Fish or No Fish site after exposure to chemical cues of dragonfly predators, fish predators, and water (control) during tadpole development. Tadpole activity is the average number of seconds that a tadpole was actively swimming out of three two-minute intervals before predator cues were added. Juvenile frog activity is the number of times that a frog moved cells in a gridded behavioral arena out of ten position observations.

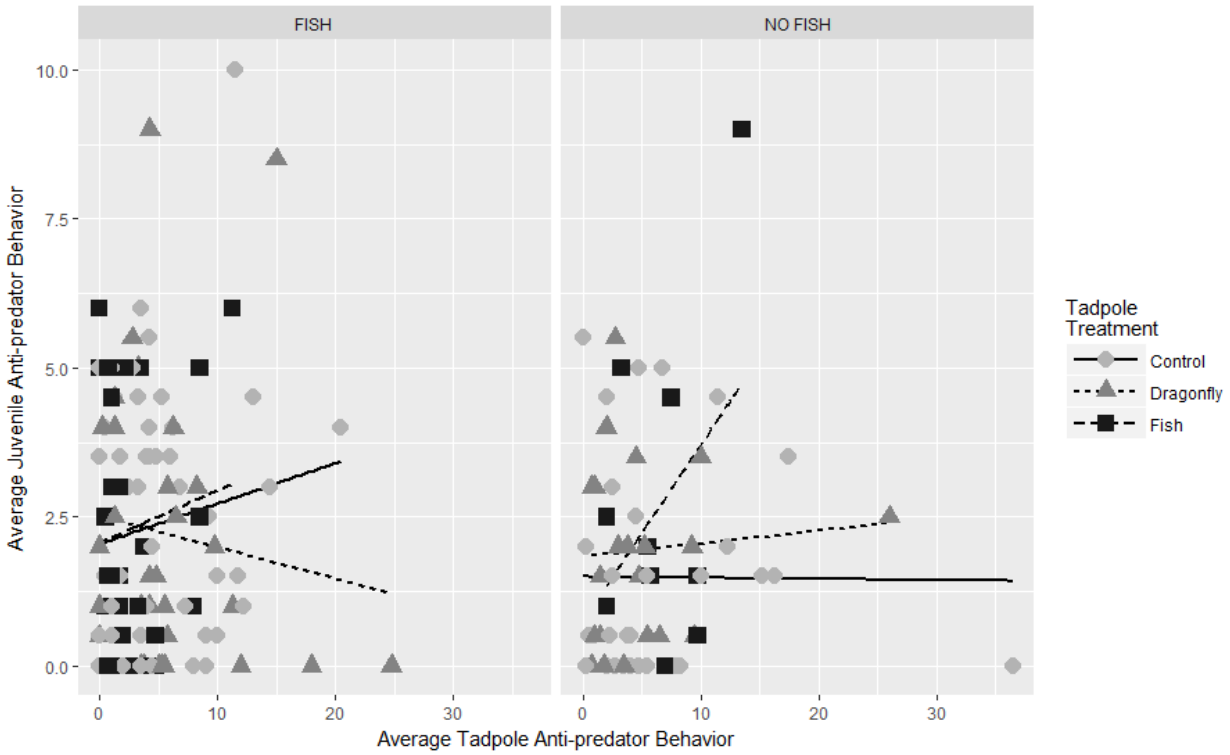

Figure 3.5. Relationship between anti-predator behavior of individuals as a tadpoles and juvenile frogs from Fish and No Fish sites after exposure to chemical cues of dragonfly predators, fish predators, and water (control) during tadpole development. Tadpole anti-predator behavior is the average number of second moving of three two-minute intervals after predator cues were added to water. Juvenile frog anti-predator behavior is the number of position observations, out of ten, during which the frog was on a paper towel dampened with predator cues.

CHAPTER IV

VALIDATING A NON-INVASIVE CORTICOSTERONE ASSAY FOR AMPHIBIAN TADPOLES

Abstract: Individual differences in stress physiology is one of the proposed mechanisms underlying individual variation in behavior. Repeatedly quantifying the stress physiology of individual organisms, however, is difficult, particularly for small-bodied and sensitive vertebrates like frogs. This has prompted the development and validation of non-invasive stress assays, like waterborne corticosterone (CORT) assays. To date, a significant positive correlation has been established between circulating CORT, the primary glucocorticoid produced in the vertebrate stress response, and waterborne CORT release rates in adult frogs, but a similar validation has not been attempted for tadpoles. After exposing Blanchard's cricket frog (*Acris blanchardi*) tadpoles to four different concentrations of exogenous CORT to augment differences between individuals, I collected water and whole body samples from each tadpole and examined their relationship. While collecting tadpole water samples, I simultaneously recorded tadpole activity levels to determine the relationship between tadpole activity and CORT. I found a significant positive relationship between tadpole waterborne CORT release rates and whole body CORT levels. On average, however, tadpole waterborne CORT release rates greatly exceeded that of the whole body CORT concentrations. Furthermore, exposure to increasing concentrations of exogenous CORT increased tadpole waterborne CORT release rates and whole body CORT concentrations,

though the later was not statistically significant. In addition, I found that tadpole average activity levels were not significantly related to either metric of CORT. These results indicate that waterborne CORT can serve as a proxy for whole body CORT levels in cricket frog tadpoles, and future work is needed to determine if waterborne CORT release rates in tadpoles reflect baseline levels or some aspect of the stress response. I discuss the utility of waterborne assays for studies of the mechanisms underlying tadpole plasticity and potential interpretations of differences in tadpole CORT measures.

Since the publication of Tinbergen's four questions (Tinbergen 1963), researchers have sought to understand the proximate mechanisms underlying behavior (e.g., metabolism, hormones, and genes). Recently, however, there has been a surge of interest in identifying the mechanisms responsible for behavioral differences between individuals (Careau et al. 2008; Koolhaas et al. 2010; Stamps and Groothuis 2010). Such lines of inquiry not only involve repeatedly quantifying behavior, but also repeatedly and simultaneously quantifying the potential mechanistic trait of choice (Dingemanse and Dochtermann 2013). In particular, stress physiology is one of the leading traits that has been examined as a mechanism underlying individual differences in behavior (Koolhaas et al. 1999). Repeatedly measuring physiological traits that could be causal mechanisms of behavior, like stress hormones, of individuals is difficult, but advanced technologies and methodologies are increasingly making it more it achievable.

Amphibians add an additional level of difficulty to this endeavor because of their endangered status and relatively small body size, which makes sufficient tissue acquisition difficult. Though amphibians are one of the most threatened taxa worldwide, they are also one of the most understudied groups (Lawler et al. 2006; Stuart et al. 2004), and are extremely sensitive to stressors (Kiesecker et al. 2001). In amphibians, the primary stress response is increased production of glucocorticoids, predominantly corticosterone (CORT), which are produced and

released when the hypothalamus-pituitary-interrenal (HPI) axis is activated. While common stress hormone assays require the use of plasma, urine, or whole body samples to effectively quantify stress hormones (e.g., Kindermann et al., 2012, Bennett et al., 2016, Narayan, 2013), the tadpoles, juveniles, and even adults of many amphibian species are too small to collect a sufficient amount of sample from which to measure CORT without killing the animal (Burraco et al. 2015).

To avoid lethal sampling, techniques have been developed to quantify CORT through non-invasive and non-stressful methods. In aquatic organisms, this has included the use of waterborne CORT assays, which have been widely used in fish (reviewed in Scott and Ellis, 2007) and recently validated for adults of the common midwife toad (*Alytes obstetricans*) and San Marcos salamander (*Eurycea nana*) (Gabor et al. 2013a). While Gabor et al. (2013a; 2013b) assessed the waterborne CORT release rates of *A. obstetricans* tadpoles using CORT EIA kits, a significant positive relationship between tadpole circulating CORT and waterborne CORT release rates has not, to my knowledge, been established. Instead, Gabor et al. (2013a; 2013b) found a significant positive relationship between the circulating CORT in the plasma of adult *A. obstetricans* and their waterborne CORT release rates and assumed that waterborne CORT will adequately serve as a proxy for tadpole circulating CORT as well. Given the dramatic changes that occur in tadpole stress physiology during metamorphosis (Krain and Denver 2004), this is not a safe assumption. I seek to fill this gap in our understanding of the utility of non-invasive waterborne CORT assays for tadpoles by formally examining the relationship tadpole circulating CORT levels and waterborne CORT release rates.

To evaluate the relationship between tadpole circulating CORT concentrations and waterborne CORT release rates, I exposed tadpoles to different concentrations of exogenous CORT and subsequently extracted and measured CORT from water and whole body samples using a CORT enzyme immune assay (EIA) kit. Exposure to exogenous CORT augmented differences between individual tadpoles and allowed for the CORT EIA to be validated over a broader range of CORT concentrations. Because CORT has been linked to stress-related changes

in tadpole phenotype (e.g. Middlemis-Maher et al. 2013) and may be the mechanism underlying changes in tadpole behavior in response to predator stress (Bennett et al. 2016), I concurrently measured tadpole activity level while collecting water samples for CORT analysis. I then examined the relationship between tadpole waterborne CORT release rates and whole body CORT concentrations with activity levels to determine if tadpole CORT and activity were related.

Methods

Species Description

Blanchard's cricket frogs (*Acris blanchardi*, formerly *A. crepitans blanchardi*; Gamble et al. 2008) are wide-spread, small (1.6-3.8 cm), terrestrial and semi-aquatic hylids found from north of the Ohio River to west of the Mississippi River in the southern United States (Caldwell 1982; Gray et al. 2005; Lehtinen and Skinner 2006). These tadpoles are too small (typically 0.1-0.5 g) to repeatedly extract a sufficient volume of plasma to quantify circulating CORT, which would lead investigators to use whole body, or even pooled whole body samples from several individuals (Burraco et al. 2015) and render repeated individual measures of CORT impossible. Thus, this species is an ideal candidate with which to try this assay.

To acquire the tadpoles used in this experiment, I collected three amplexed couples of Blanchard's cricket frog from Sanborn Lake, Stillwater, OK (Latitude: 36.155, Longitude: -97.078) on 14 June 2016 placed them in 5.7 L plastic bins filled with 1.5 L of dechlorinated tap water overnight to deposit their eggs. The next morning, I released the adult frogs at their site of capture and brought the eggs back to a laboratory facility on Oklahoma State University's main campus. Given that experience with predation risk can alter mean levels of CORT in tadpoles (Middlemis-Maher et al. 2013), eggs were collected in lieu of wild tadpoles to ensure that all individuals were equally naïve to stressors prior to the start of the experiment. All tadpoles were kept at a 14:10 light:dark cycle at 25.7 ± 0.7 °C and 52 ± 39 % humidity (mean ± SD) for 35 days. I did complete water changes and fed the tadpoles a mixture of equal parts ground algae

wafers (Hikari Inc., Hayward, CA, USA), shrimp pellets (Aqueon, Franklin, WI, USA), and fish flakes (TetraCichlid cichlid flakes; Tetra, Blacksburg, VA, USA) three times a week.

Exogenous CORT Treatments

To augment variation in CORT between tadpoles, I placed groups of four tadpoles in treatment-specific replicates of glass bowls containing 1 L of dechlorinated water and four different concentrations of exogenous CORT or control treatments. To make exogenous CORT treatments, I dissolved crystalline CORT (Sigma Aldrich C2505) in ethanol to make the following concentrations: 0.05 µM [0.017 mg of CORT in 0.1 mL of ethanol added to 1 L of water], 0.1 µM [0.035 mg of CORT in 0.1 mL of ethanol added to 1 L of water], 0.25 µM [0.0867 mg of CORT in 0.1 mL of ethanol added to 1 L of water], and 0.5 µM [0.173 mg of CORT in 0.1 mL of ethanol added to 1 L of water]. In addition to these treatments, there were two control treatments: a vehicle control [0.1 mL ethanol added to 1 L of water] and a plain water control [0.1 mL water added to 1 L of water]. Five additional tadpoles were group housed in a separate glass bowl to be later homogenized and to construct a serial dilution curve and validate use of enzyme-immunoassay (EIA) kits.

Tadpoles were exposed to exogenous CORT or control treatments for seven days. Each day, I did a complete water change, added fresh, ground fish flakes, algae wafers, and shrimp pellets *ad libidum*, and spiked each glass bowl with the appropriate CORT treatment or control solution. On the seventh day, individual tadpoles were placed in 100-mL glass beakers filled with 40 mL of dechlorinated water. These beakers were then placed under a video camera for an hour to be able to simultaneously collect waterborne CORT samples and document activity for each tadpole. From the hour-long videos, I scored the amount of time each tadpole was moving for 5 minutes and then skipped ahead 5 minutes. This resulted in six activity scores per tadpole that were then averaged to obtain the average activity level per tadpole during that hour. Videos were scored by an observer who was blind to both tadpole identity and treatment.

After an hour, tadpoles were moved to clean water and immediately euthanized in an overdose of MS-222 [250 mg MS-222 in 1 L of water; Sigma Aldrich]. Water samples were then transferred to sterile, small centrifuge tubes (50 mL) and stored in -20°C until they were thawed for hormone extraction. Freezing of water samples has been shown to not influence steroid hormone concentrations (Ellis et al. 2004). After euthanasia, I measured the weight, SVL, and total length of each tadpole. I then removed the gut of each tadpole via dissection, homogenized the remaining tadpole tissue using a micro tissue homogenizer, centrifuged the sample for 3 mins at 5 x *g*, and preserved the resulting supernatant at -80°C until assayed. The five individuals used for the serial dilution curve were also euthanized with an overdose of MS-222, measured, dissected, and the remaining tissue from all five individuals was homogenized and centrifuged. The resulting supernatant was then stored at -80°C until assayed.

Whole Body CORT Extraction

After removing the gut from each tadpole, I homogenized the four tadpoles from each treatment replicate in 1 mL of Millipore ultrapure water using a tissue homogenizer. The sample was centrifuged for 3 mins at 5 x *g*, and the supernatant was transferred to a 10 mL dram vial. I then performed a two-step liquid-liquid extraction using reagent-grade diethyl ether. In the first step, I extracted hormone from the water sample adding 4 mL of diethyl ether and agitating the sample for 4 mins with a vortex mixer (VWR). I allowed the layers to separate for 2 mins, transferred the organic layer to a borosilicate vial, and added an additional 4 mL of solvent to the dram vial to perform a secondary clean-up extraction. I repeated the extraction, transferred the final 4 mL of solvent to the same borosilicate vial, and evaporated the 8 mL of solvent under a gentle stream of nitrogen. Finally, I re-suspended the hormone pellet in a 5% ethanol and 95% enzyme-immunoassay (EIA) buffer solution for a final resuspension volume of 500 µL. Whole body CORT samples were measured in duplicate with an EIA kit (Cayman Chemicals Inc.) on a SPECTRAmax® microplate spectrophotometer (Molecular Devices, California) set to 405 nm.

Waterborne CORT Extraction

I extracted the hormones from the thawed water sample by passing the entire sample through sterile tubing into C18 solid phase extraction columns under vacuum pressure. Following a modified protocol based on Earley and Hsu (2008), I washed the columns with 5 mL ethyl acetate, then primed the columns with 5 mL of HPLC-grade methanol followed by two 5 mL washes of deionized water. After the entire water sample had passed through the column, I centrifuged each column for 3 mins at 5 x *g*. Next, I eluted the columns with two 4 mL washes of HPLC-grade methanol into borosilicate vials. Following this, I evaporated the eluted solvent under a gentle stream of nitrogen in a 37°C analog heat block (VWR) and re-suspended the resulting hormone pellet in 200 µL of Millipore ultrapure water. I then performed a liquid-liquid extraction using the same protocol as deployed on the whole-body samples.

Following the liquid-liquid extractions, I evaporated the solvent (diethyl ether) under a gentle stream of nitrogen, and re-suspended the hormone pellet in 5% ethanol and 95% enzyme-immunoassay (EIA) buffer for a final resuspension volume of 500 µL. Waterborne CORT samples were measured in duplicate with an EIA kit (Cayman Chemicals Inc.) on a SPECTRAmax® microplate spectrophotometer (Molecular Devices, California) set to 405 nm.

CORT EIA Validation

I validated the use of this CORT EIA kit for *A. blanchardi* tadpoles using pooled whole body samples from the five group-housed individuals described above. These pooled samples were then diluted 1:2 for serial dilutions and quantitative recovery. I included three pooled controls on each plate to determine the intra-assay coefficient of variation (CV) for both the waterborne and whole body assays. For the waterborne assay, the mean intra-assay CV of the pooled controls was 26.18% and the intra-assay CV of the entire plate was 70.75%. For the whole body assay, the mean intra-assay CV was 48.11% and 15.98% for the pooled controls and entire plate, respectively. The inter-assay CV of the standards was 12.7% and the inter-assay CV of the

pooled controls was 37.14%. The assay sensitivities of the waterborne assay and the whole body assay were 12.76 pg/ml and 11.71 pg/ml, respectively. The serial dilution curve was parallel to the serial dilution curve (comparison of slopes: waterborne: $t_6 = -0.0003$, $p = 1.0$; whole body: $t_6 = 0.0007$, $p = 1.0$). I conducted cold spikes using known CORT concentrations to determine quantitative recovery, an estimate of assay performance with waterborne or whole body samples. I mixed either a waterborne or whole body sample diluted 1:2 with an equal volume of a high (5000 pg/ml standard), medium (320 pg/ml standard), and low (20.5 pg/ml standard) CORT spike. The known concentrations of each CORT spike were used to establish expected recovery concentrations. I also included a pooled control with no CORT spike as a reference. The minimum observed recovery for the waterborne and whole body samples was 65% (average = 93%) and 52% (average = 71%), respectively. For the waterborne assay, the regression coefficient for observed vs. expected concentrations of CORT was 0.63 ($F_{1,1} = 652$, $r^2 = 1.0$, $p = 0.02$), while the regression coefficient was 0.84 for the whole body assay ($F_{1,1} = 1513$, $r^2 = 1.0$, $p = 0.001$).

Statistical Analysis

To correct for differences in body size, I divided individual waterborne and whole body CORT estimates by tadpole weight. In *A. blanchardi* tadpoles, total length and weight are positively correlated ($F_{1,22} = 157.6$, $r^2 = 0.87$, $p < 0.0001$). Because CORT is assumed to pass through both the gills and skin of tadpoles (Gabor et al. 2013a), tadpole CORT release rates may be more strongly related to a tadpole's surface area to volume ratio, which is inversely proportional to size, than tadpole mass. To account for the potential scaling of surface area to volume ratio with tadpole size, I fit linear and exponential models to the relationship between mass-corrected measures of waterborne and whole body CORT and compared the fit of these alternative models using Akaike's Information Criterion (AIC). To determine if exogenous CORT treatments had an effect on waterborne or whole body CORT levels, I used ANOVAs to test for differences

between treatments followed by Fisher's LSD comparisons between each treatment. I again used a Pearson's correlation to examine the relationship between waterborne and whole body CORT and average tadpole activity levels. All model selection analyses and statistical tests were run using R statistical software (R Core Team 2016).

Results

There was more support for an exponential model of the relationship between waterborne and whole body CORT in *A. blanchardi* tadpoles (exponential vs. linear ΔAIC = 383.9). According to the exponential model, waterborne CORT was significantly and positively related to whole body CORT (whole body = 0.002 ± 0.0004, $p = 0.0003$; Figure 4.1). Prior treatment with exogenous CORT significantly increased tadpole waterborne CORT release rates, while the effect on whole body CORT concentrations was only marginally significant (Waterborne: $F_{5,18} = 3.80$, $p = 0.02$; Whole body: $F_{5,18} = 2.64$, $p = 0.06$; Figure 4.2). Tadpoles exposed to exogenous CORT at a concentration of 0.1 µM or 0.5 µM had significantly higher waterborne CORT release rates than tadpoles exposed to 0.05 µM, 0.25 µM, or either of the controls (Fisher's LSD: 0.1 µM or 0.5 µM vs. 0.05 µM, 0.1 µM, plain water, or ethanol – all pairwise $p <= 0.02$). Tadpole average activity levels were not related to either waterborne CORT release rates or whole body CORT concentration (Waterborne: Pearson's $r = -0.05$, $n = 24$, $p = 0.82$; Whole body: Pearson's $r = 0.21$, $n = 24$, $p = 0.32$; Figure 4.3).

Discussion

I found a significant positive relationship between waterborne CORT release rates and whole body CORT concentrations for *A. blanchardi* tadpoles. The establishment of a positive relationship between waterborne CORT release rates and whole body circulating CORT represents a potentially powerful tool to study the relationship between the stress physiology and

behavior of individual tadpoles. In addition to this, I also found that treatment with exogenous CORT increased tadpole whole body CORT and waterborne CORT release rates but that neither CORT metric was significantly related to tadpole activity.

While I found a positive relationship between the amount of CORT released by tadpoles in the water and their circulating CORT levels, tadpoles released more CORT in an hour than was contained in their bodies at the end of the assay. One potential reason for this discrepancy is the difference in sample duration. The whole body CORT concentration represents a brief moment in time – i.e. the quantity of CORT circulating in an individual tadpole's body at the moment of death – while the waterborne CORT is an hour of hormone release, allowing for CORT to accumulate in the water over time. It is also possible that the elevated CORT concentrations in the water sample reflect the tadpole's response to confinement stress, as they can in fish (Wong et al. 2008). While housing tadpoles in similarly-sized beakers has resulted in elevated tadpole CORT levels (Belden et al. 2003; Belden et al. 2010; Chambers et al. 2011), I did not disturb the beakers during sample collection, as previous studies have done. If the elevated CORT measured from waterborne samples does reflect a stress response, this measure can still be used as a relative baseline and point of comparison to examine relative differences in stress responses. Further, since tadpole CORT responses appear to be rapid (Bennett et al. 2016) and it is possible that the CORT measured in water samples over an hour may represent the sum of CORT released during that time (i.e. the area under the curve of the CORT response), this value may be used to examine the influence of extended exposure to CORT (McEwen and Wingfield 2010; Romero et al. 2009) on individual behavior and allow for tracking the time course of CORT responses. To determine if waterborne CORT does reflect a stress response (either the max CORT or the total CORT released), water samples should be collected at shorter time intervals and compared to whole body amounts.

Regardless of the cause of the higher CORT levels measured from the water samples, that they can be quantified from water has interesting implications for interactions between tadpoles.

It is possible that this elevated CORT released into the water is a mechanism by which tadpoles can communicate the presence of stressors to other tadpoles. Tadpoles have been demonstrated to learn to recognize novel predator cues by being paired with one or more trained, tutor tadpoles (Ferrari et al. 2007), even when the tutor is a different species of tadpole (Ferrari and Chivers 2008). Increased releases of CORT into water by a trained tadpole and subsequent detection and/or absorption of CORT by naïve tadpoles may facilitate social learning of novel predator threats, particularly in small volumes of water.

In general, exposure to higher concentrations of exogenous CORT increased tadpole waterborne CORT release rates and, nearly significantly, whole body CORT concentrations. The addition of exogenous CORT has been shown to increase whole body CORT levels in tadpoles (e.g., Glennemeier and Denver, 2002; Belden et al., 2005), but a concurrent increase in waterborne CORT is, to my knowledge, a novel finding. In my study, an increase in whole body CORT was likely not significant due to the large amount of variation in whole body CORT concentrations after exposure to 0.5 μM of exogenous CORT.

I found no relationship between tadpole activity levels and either waterborne CORT release rates or whole body CORT concentrations. Given that tadpoles have acute and immediate changes in whole body CORT in response to cues of at least some stressors (e.g., predation risk; Bennett et al. 2016) and tadpoles typically alter their behavior in response to stressors (Fraker 2008; Orizaola et al. 2012), it is possible that an hour was too coarse to detect a relationship between tadpole CORT and activity. Indeed, other studies that have exposed tadpoles to CORT for longer periods of time (e.g. 18 days; Glennemeier and Denver, 2002) found no relationship between tadpole CORT and activity. Regardless of the relationship detected, the relative ease with which behavior and physiology were simultaneously documented highlight the utility of this assay for examining potential physiological mechanisms underlying tadpole behavior.

The ability to quantify individual CORT from water has the potential to be a powerful tool to assess and establish links between individual physiology and behavior in aquatic and semi-

aquatic organisms. This link is especially valuable for tadpoles whose stress physiology is not only the proposed mechanism underlying plastic responses to stressors (e.g. Bennett et al., 2016, Hossie et al., 2010, Middlemis-Maher et al., 2013) and development (Denver 1998; Denver 2009), but also post-metamorphic stress physiology (Crespi and Warne 2013). The use of waterborne CORT assays as a non-invasive method of stress hormone collection for small-bodied, early-stage amphibians will allow investigators to repeatedly assess physiological characteristics of sensitive, plastic animals and further our understanding of the mechanisms underlying differences in individual behavior of a broad range of taxa.

Acknowledgements

I would like to thank J. Grindstaff and J. Belden for use of their laboratory facilities. In addition, I would like to thank the Graduate College at Oklahoma State University (to LEB) and the Animal Behavior Society (to LEB) for financial support. All research was approved by the Institutional Animal Care and Use Committee of Oklahoma State University (protocol: AS-16-4).

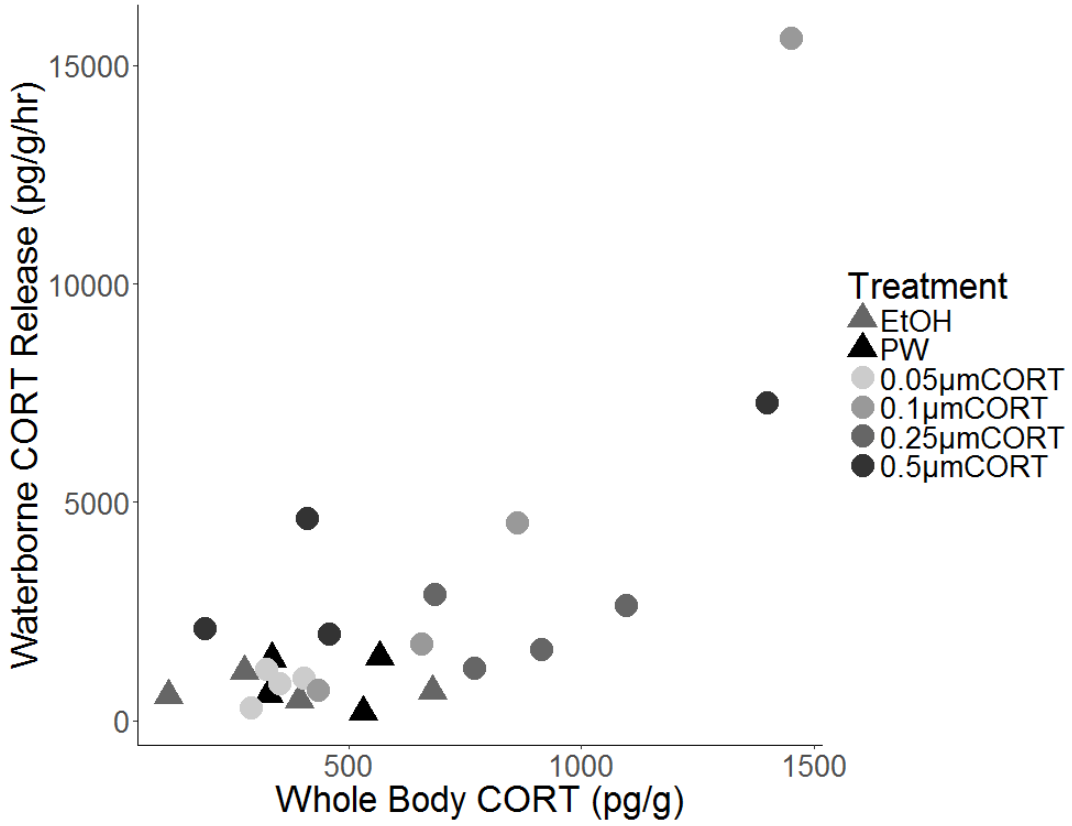

Figure 4.1. Significant positive, exponential relationship between release rates of waterborne corticosterone (CORT) and whole body CORT concentrations in *A. blanchardi* tadpoles. Values represent individual tadpoles exposed to either ethanol ("EtOH") or plain water ("PW") controls or exogenous CORT for seven days. Individual tadpoles from control treatments are represented by triangles while tadpoles from exogenous CORT treatments are represented by circles.

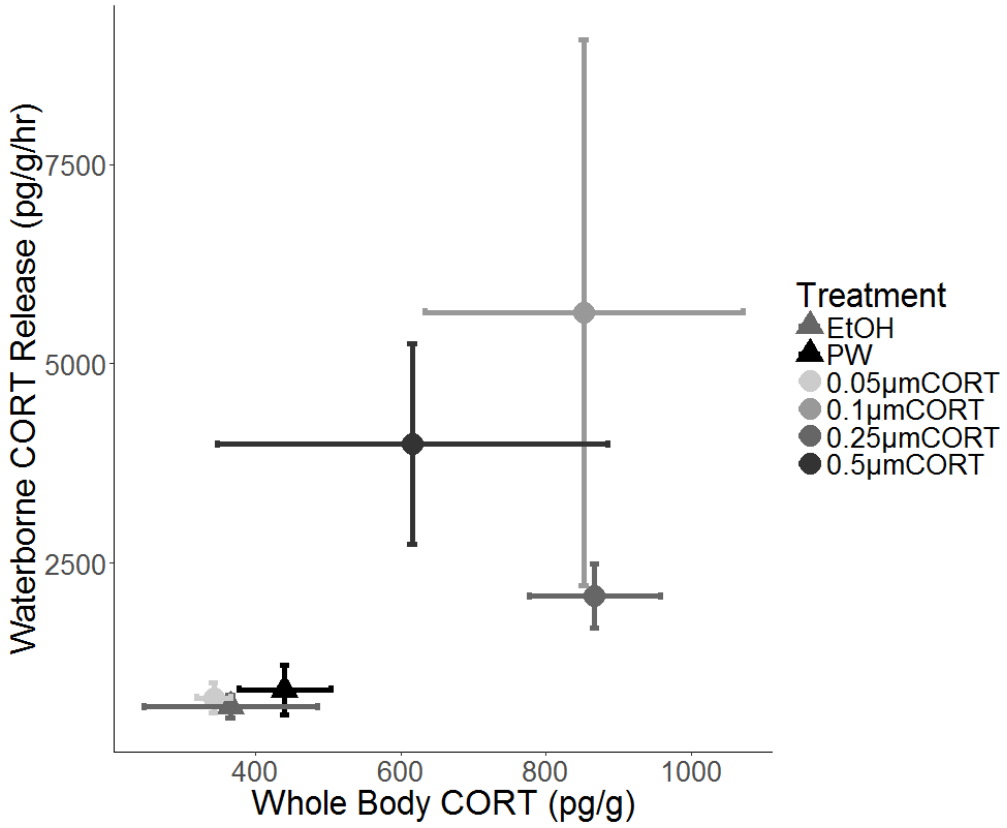

Figure 4.2. Waterborne corticosterone (CORT) release rates and whole body CORT concentrations of tadpoles exposed to different concentrations of exogenous CORT for seven days. Values represent treatment means ± SE of group housed tadpoles (*n* = 4 per treatment). Control treatments are represented by triangles and exogenous CORT treatments are represented by circles. "EtOH" = ethanol, "PW" = plain water.

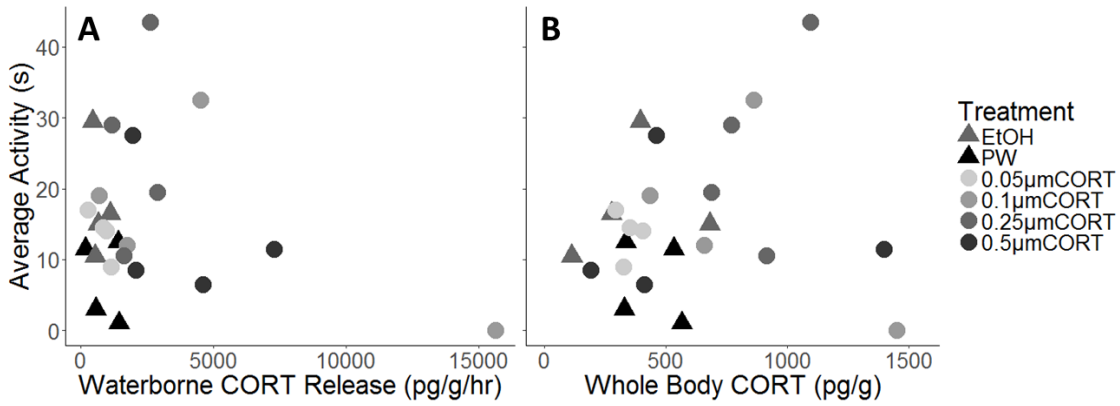

Figure 4.3. Relationship between average tadpole activity level in an hour and (A) waterborne corticosterone (CORT) release rates and (B) whole body CORT concentrations of tadpoles exposed to different concentrations of exogenous CORT for seven days. Values represent individual tadpoles (n = 4 per treatment). Individual tadpoles from control treatments are represented by triangles while tadpoles from exogenous CORT treatments are represented by circles. "EtOH" = ethanol, "PW" = plain water.

REFERENCES

Altwegg, R., and H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872-882.

Anderson, D. R. 2008, Model based inference in the life sciences: A primer on evidence, Springer.

Azevedo-Ramos, C., M. Van Sluys, J.-M. Hero, and W. E. Magnusson. 1992. Influence of tadpole movement on predation by odonate naiads. Journal of Herpetology 26:335-338.

Barbasch, T., and M. F. Benard. 2011. Past and present risk: exposure to predator chemical cues before and after metamorphosis influences juvenile wood frog behavior. Ethology 117:367-373.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4, version 1.1-7.

Bayless, L. E. 1969. Post-metamorphic growth of *Acris crepitans*. American Midland Naturalist 81:590-592.

Belden, L., I. Moore, R. Mason, J. Wingfield, and A. Blaustein. 2003. Survival, the hormonal stress response and UV-B avoidance in Cascades Frog tadpoles (*Rana cascadae*) exposed to UV-B radiation. Functional Ecology 17:409-416.

Belden, L. K., I. T. Moore, J. C. Wingfield, A. R. Blaustein, and S. J. Beaupre. 2005. Corticosterone and growth in Pacific treefrog (*Hyla regilla*) tadpoles. Copeia 2005:424-430.

Belden, L. K., E. L. Wildy, A. C. Hatch, and A. R. Blaustein. 2000. Juvenile western toads, *Bufo boreas*, avoid chemical cues of snakes fed juvenile, but not larval, conspecifics. Animal Behaviour 59:871-875.

Belden, L. K., J. C. Wingfield, and J. M. Kiesecker. 2010. Variation in the hormonal stress response among larvae of three amphibian species. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 313:524-531.

Bell, A., and A. Sih. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters 10:828-834.

Bell, A. M., S. J. Hankison, and K. L. Laskowski. 2009. The repeatability of behaviour: a meta-analysis. Animal Behaviour 77:771-783.

Benard, M. F. 2004. Predator-induced phenotypic plasticity in organisms with complex life histories. Annual Review of Ecology, Evolution, and Systematics 35:651-673.

—. 2006. Survival trade-offs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). Ecology 87:340-346.

Bennett, A. M., J. N. Longhi, E. H. Chin, G. Burness, L. R. Kerr, and D. L. Murray. 2016. Acute changes in whole body corticosterone in response to perceived predation risk: A mechanism for anti-predator behavior in anurans? General and Comparative Endocrinology 229:62-66.

Berven, K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599-1608.

Berven, K. A., and T. A. Grudzien. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. Evolution 44:2047-2056.

Biro, P. A., and B. Adriaenssens. 2013. Predictability as a personality trait: consistent differences in intraindividual behavioral variation. The American Naturalist 182:621-629.

Briffa, M. 2013. Plastic proteans: reduced predictability in the face of predation risk in hermit crabs. Biology Letters 9:20130592.

Brodin, T., M. I. Lind, M. K. Wiberg, and F. Johansson. 2013. Personality trait differences between mainland and island populations in the common frog (*Rana temporaria*). Behavioral Ecology and Sociobiology 67:135-143.

Bronmark, C., and P. Edenhamn. 1994. Does the presence of fish affect the distribution of tree frogs (*Hyla arborea*)? Conservation Biology 8:841-845.

Burkett, R. 1984. An ecological study of the cricket frog, *Acris crepitans*, Pages 89-103 *in* R. Seigel, L. Hunt, J. Knight, L. Malaret, and N. Zuschlag, eds. Vertebrate Ecology and Systematics - A Tribute to Henry S. Fitch. Lawrence, Kansas, University of Kansas Museum of Natural History.

Burraco, P., R. Arribas, S. S. Kulkarni, D. R. Buchholz, and I. Gomez-Mestre. 2015. Comparing techniques for measuring corticosterone in tadpoles. Current Zoology 61:835-845.

Buskirk, J. V., and G. Saxer. 2001. Delayed costs of an induced defense in tadpoles? Morphology, hopping, and development rate at metamorphosis. Evolution 55:821-829.

Caldwell, J. P. 1982. Disruptive selection: a tail color polymorphism in *Acris* tadpoles in response to differential predation. Canadian Journal of Zoology 60:2818-2827.

Careau, V., D. Thomas, M. Humphries, and D. Réale. 2008. Energy metabolism and animal personality. Oikos 117:641-653.

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

Chambers, D. L., J. M. Wojdak, P. Du, and L. K. Belden. 2011. Corticosterone level changes throughout larval development in the amphibians *Rana sylvatica* and *Ambystoma jeffersonianum* reared under laboratory, mesocosm, or free-living conditions. Copeia 2011:530-538.

Chelgren, N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications 16:250-261.

Chivers, D. P., J. M. Kiesecker, A. Marco, E. L. Wildy, and A. R. Blaustein. 1999. Shifts in life history as a response to predation in western toads (*Bufo boreas*). Journal of Chemical Ecology 25:2455-2463.

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

Clark, C. W. 1994. Antipredator behavior and the asset-protection principle. Behavioral Ecology 5:159-170.

Crespi, E. J., and R. W. Warne. 2013. Environmental conditions experienced during the tadpole stage alter post-metamorphic glucocorticoid response to stress in an amphibian. Integrative and Comparative Biology 53:989-1001.

Davenport, J. M., B. R. Hossack, and W. H. Lowe. 2014. Partitioning the non-consumptive effects of predators on prey with complex life histories. Oecologia 176:149-155.

Denver, R. J. 1998. Hormonal correlates of environmentally induced metamorphosis in the Western spadefoot toad, *Scaphiopus hammondii*. General and Comparative Endocrinology 110:326-336.

Denver, R. J. 2009. Stress hormones mediate environment-genotype interactions during amphibian development. General and Comparative Endocrinology 164:20-31.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13:77-81.

Dingemanse, N. J., and N. A. Dochtermann. 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. Journal of Animal Ecology 82:39-54.

Downie, J., R. Bryce, and J. Smith. 2004. Metamorphic duration: an under-studied variable in frog life histories. Biological Journal of the Linnean Society 83:261-272.

Earley, R. L., and Y. Hsu. 2008. Reciprocity between endocrine state and contest behavior in the killifish, *Kryptolebias marmoratus*. Hormones and behavior 53:442-451.

Eklöv, P., and E. E. Werner. 2000. Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. Oikos 88:250-258.

Ellis, T., J. James, C. Stewart, and A. Scott. 2004. A non-invasive stress assay based upon measurement of free cortisol released into the water by rainbow trout. Journal of Fish Biology 65:1233-1252.

Ferrari, M. C., and D. P. Chivers. 2008. Cultural learning of predator recognition in mixed-species assemblages of frogs: the effect of tutor-to-observer ratio. Animal Behaviour 75:1921-1925.

Ferrari, M. C., F. Messier, and D. P. Chivers. 2007. First documentation of cultural transmission of predator recognition by larval amphibians. Ethology 113:621-627.

Ferrari, M. C., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus Canadian Journal of Zoology 88:698-724.

Flowers, M. A., and B. M. Graves. 1997. Juvenile toads avoid chemical cues from snake predators. Animal Behaviour 53:641-646.

Fraker, M. E. 2008. The dynamics of predation risk assessment: responses of anuran larvae to chemical cues of predators. Journal of Animal Ecology 77:638-645.

Frost, A. J., A. Winrow-Giffen, P. J. Ashley, and L. U. Sneddon. 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? Proceedings of the Royal Society B-Biological Sciences 274:333-339.

Gabor, C. R., J. Bosch, J. N. Fries, and D. R. Davis. 2013a. A non-invasive water-borne hormone assay for amphibians. Amphibia-Reptilia 34:151-162.

Gabor, C. R., M. C. Fisher, and J. Bosch. 2013b. A non-invasive stress assay shows that tadpole populations infected with *Batrachochytrium dendrobatidis* have elevated corticosterone levels. PloS one 8:e56054.

Gamble, T., P. B. Berendzen, H. B. Shaffer, D. E. Starkey, and A. M. Simons. 2008. Species limits and phylogeography of North American cricket frogs (Acris: Hylidae). Molecular Phylogenetics and Evolution 48:112-125.

Gastwirth, J. L., Y. R. Gel, W. W. Hui, V. Lyubchich, W. Miao, and K. Noguchi. 2017. lawstat: Tools for biostatistics, public policy, and law, version 3.1.

Glennemeier, K. A., and R. J. Denver. 2002. Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components. General and Comparative Endocrinology 127:16-25.

Gordon, A. M., M. B. Youngquist, and M. D. Boone. 2016. The effects of pond drying and predation on Blanchard's cricket frogs (*Acris blanchardi*). Copeia.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.

Gray, R. 1978. Nondifferential predation susceptibility and behavioral selection in three color morphs of Illinois cricket frogs, *Acris crepitans*. Transactions Illinois State Academy of Science 71:356-360.

Gray, R., L. Brown, and L. Blackburn. 2005. *Acris crepitans* Baird 1854(b): Northern Cricket Frog,, Pages 441-443 *in* M. Lannoo, ed. Amphibian Declines: The Conservation Status of United States Species. Berkeley, California, University of California Press.

Hecnar, S. J., and R. T. M'Closkey. 1997. The effects of predatory fish on amphibian species richness and distribution. Biological Conservation 79:123-131.

Hossie, T., K. Landolt, and D. L. Murray. 2016. Determinants and co-expression of anti-predator responses in amphibian tadpoles: a meta-analysis. Oikos.

Hossie, T. J., B. Ferland-Raymond, G. Burness, and D. L. Murray. 2010. Morphological and behavioural responses of frog tadpoles to perceived predation risk: a possible role for corticosterone mediation?

Houston, A. I., and J. M. McNamara. 1992. Phenotypic plasticity as a state-dependent life-history decision. Evolutionary Ecology 6:243-253.

Hugie, D. M. 2003. The waiting game: a “battle of waits” between predator and prey. Behavioral Ecology 14:807-817.

Kats, L. B., J. W. Petranka, and A. Sih. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology 69:1865-1870.

Kiesecker, J. M., A. R. Blaustein, and L. K. Belden. 2001. Complex causes of amphibian population declines. Nature 410:681-684.

Kiesecker, J. M., D. P. Chivers, M. Anderson, and A. R. Blaustein. 2002. Effect of predator diet on life history shifts of red-legged frogs, *Rana aurora*. Journal of Chemical Ecology 28:1007-1015.

Kindermann, C., E. J. Narayan, and J.-M. Hero. 2012. Urinary corticosterone metabolites and chytridiomycosis disease prevalence in a free-living population of male Stony Creek frogs (*Litoria wilcoxii*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 162:171-176.

Koolhaas, J. M., S. F. de Boer, C. M. Coppens, and B. Buwalda. 2010. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Frontiers in Neuroendocrinology 31:307-321.

Koolhaas, J. M., S. M. Korte, S. F. De Boer, B. J. Van Der Vegt, C. G. Van Reenen, H. Hopster, I. C. De Jong et al. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience and Biobehavioral Reviews 23:925-935.

Krain, L., and R. Denver. 2004. Developmental expression and hormonal regulation of glucocorticoid and thyroid hormone receptors during metamorphosis in *Xenopus laevis*. Journal of Endocrinology 181:91-104.

Krause, J., and G. D. Ruxton. 2002, Living in Groups, Oxford University Press.

Laurila, A., M. Järvi-Laturi, S. Pakkasmaa, and J. Merilä. 2004. Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. Oikos 107:90-99.

Laurila, A., B. Lindgren, and A. T. Laugen. 2008. Antipredator defenses along a latitudinal gradient in *Rana temporaria*. Ecology 89:1399-1413.

Laurila, A., S. Pakkasmaa, and J. Merilä. 2006. Population divergence in growth rate and antipredator defences in *Rana arvalis*. Oecologia 147:585-595.

Lawler, J. J., J. E. Aukema, J. B. Grant, B. S. Halpern, P. Kareiva, C. R. Nelson, K. Ohleth et al. 2006. Conservation science: a 20-year report card. Frontiers in Ecology and the Environment 4:473-480.

Lawler, S. P. 1989. Behavioural responses to predators and predation risk in four species of larval anurans. Animal Behaviour 38:1039-1047.

Lehtinen, R. M., and M. C. MacDonald. 2011. Live fast, die young? A six-year field study of longevity and survivorship in Blanchard's cricket frog (*Acris crepitans blanchardi*). Herpetological Review 42:504-507.

Lehtinen, R. M., and A. A. Skinner. 2006. The enigmatic decline of Blanchard's cricket frog (*Acris crepitans blanchardi*): A test of the habitat acidification hypothesis. Copeia 2006:159-167.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. Bioscience 48:25-34.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619-640.

Lupien, S. J., B. S. McEwen, M. R. Gunnar, and C. Heim. 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience 10:434-445.

Malashichev, Y. B. 2002. Asymmetries in amphibians: A review of morphology and behaviour. Laterality: Asymmetries of Body, Brain and Cognition 7:197-217.

McEwen, B. S., and J. C. Wingfield. 2010. What's in a name? Integrating homeostasis, allostasis and stress. Hormones and Behavior 57:105.

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

Murray, D. L., J. D. Roth, and A. J. Wirsing. 2004. Predation risk avoidance by terrestrial amphibians: The role of prey experience and vulnerability to native and exotic predators. Ethology 110:635-647.

Nicieza, A. G. 1999. Context-dependent aggregation in Common Frog *Rana temporaria* tadpoles: influence of developmental stage, predation risk and social environment. Functional Ecology 13:852-858.

Orizaola, G., E. Dahl, and A. Laurila. 2012. Reversibility of predator-induced plasticity and its effect at a life-history switch point. Oikos 121:44-52.

Pechenik, J. A. 2006. Larval experience and latent effects - metamorphosis is not a new beginning. Integrative and Comparative Biology 46:323-333.

Pechenik, J. A., D. E. Wendt, and J. N. Jarrett. 1998. Metamorphosis is not a new beginning. Bioscience 48:901-910.

Pigliucci, M. 2001, Phenotypic plasticity: beyond nature and nurture, JHU Press.

Pinheiro, J., D. Bates, S. DebRoy, and R. C. Team. 2016. nlme: Linear and nonlinear mixed effects models, version R package version 3.1-128.

Pough, F. H., and S. Kamel. 1984. Post-metamorphic change in activity metabolism of anurans in relation to life history. Oecologia 65:138-144.

R Core Team. 2016. R: A language environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Réale, D., and N. J. Dingemanse. 2010. Personality and individual social specialisation, Pages 417-441 *in* T. Székely, A. J. Moore, and J. Komdeur, eds. Social Behaviour: Genes, Ecology and Evolution, Cambridge University Press.

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

—. 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. Ecological Monographs 72:77-93.

—. 2003. Predators come and predators go: the reversibility of predator-induced traits. Ecology 84:1840-1848.

—. 2007. Getting out alive: how predators affect the decision to metamorphose. Oecologia 152:389-400.

Richards, S. A. 2008. Dealing with overdispersed count data in applied ecology. Journal of Applied Ecology 45:218-227.

Romero, L. M., M. J. Dickens, and N. E. Cyr. 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. Hormones and Behavior 55:375-389.

Rosseel, Y. 2012. lavaan: An R package for structural equation modeling. Journal of Statistical Software 48:1-36.

Rowe, C. L., and W. A. Dunson. 1995. Impacts of hydroperiod on growth and survival of larval amphibians in temporary ponds of central Pennsylvania, USA. Oecologia 102:397-403.

Scott, A. P., and T. Ellis. 2007. Measurement of fish steroids in water—a review. General and Comparative Endocrinology 153:392-400.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview, Pages 203-224 *in* W. Kerfoot, and A. Sih, eds. Predation: Direct and Indirect Impacts on Aquatic Communities. Hanover, University Press of New England.

—. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. The American Naturalist 139:1052-1069.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378.

Skelly, D. K. 1994. Activity level and the susceptibility of anuran larvae to predation. Animal Behaviour 47:465-468.

Snell-Rood, E. C. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour 85:1004-1011.

Stamps, J., and T. G. Groothuis. 2010. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85:301-325.

Stamps, J. A. 2015. Individual differences in behavioural plasticities. Biological Reviews of the Cambridge Philosophical Society 2:534-567.

Stamps, J. A., M. Briffa, and P. A. Biro. 2012. Unpredictable animals: Individual differences in intraindividual variability (IIV). Animal Behaviour 83:1325-1334.

Stamps, J. A., and V. Krishnan. 2017. Age-dependent changes in behavioural plasticity: Insights from Bayesian models of development. Animal Behaviour 126:53-67.

Storfer, A., and A. Sih. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. Evolution 52:558-565.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

Tinbergen, N. 1963. On aims and methods of ethology. Ethology 20:410-433.

Touchon, J. C., R. R. Jiménez, S. H. Abinette, J. R. Vonesh, and K. M. Warkentin. 2013. Behavioral plasticity mitigates risk across environments and predators during anuran metamorphosis. Oecologia 173:801-811.

Touchon, J. C., M. W. McCoy, T. Landberg, J. R. Vonesh, and K. M. Warkentin. 2015. Putting μ/g in a new light: plasticity in life history switch points reflects fine-scale adaptive responses. Ecology 96:2192-2202.

Trokovic, N., A. Gonda, G. Herczeg, A. Laurila, and J. Merilä. 2011. Brain plasticity over the metamorphic boundary: carry-over effect of larval environment on froglet brain development. Journal of Evolutionary Biology 24:1380-1385.

Urszán, T. J., L. Z. Garamszegi, G. Nagy, A. Hettyey, J. Török, and G. Herczeg. 2015. No personality without experience? A test on *Rana dalmatina* tadpoles. Ecology and Evolution 5:5847-5856.

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

Van Buskirk, J., and G. Saxer. 2001. Delayed costs of an induced defense in tadpoles? Morphology, hopping, and development rate at metamorphosis. Evolution 55:821-829.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution:505-522.

Walsh, M., F. Cooley, K. Biles, and S. Munch. 2015. Predator-induced phenotypic plasticity within-and across-generations: a challenge for theory? Proceedings of the Royal Society of London B: Biological Sciences 282:20142205.

Walsh, P., J. Downie, and P. Monaghan. 2008a. Plasticity of the duration of metamorphosis in the African clawed toad. Journal of Zoology 274:143-149.

—. 2008b. Predation-induced plasticity in metamorphic duration in *Xenopus laevis*. Functional Ecology 22:699-705.

Walsh, P. T. 2010. Anuran life history plasticity: variable practice in determining the end-point of larval development. Amphibia-Reptilia 31:157-167.

Wassersug, R. J., and D. G. Sperry. 1977. The relationships of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). Ecology 58:830-839.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. The American Naturalist:319-341.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393-425.

West-Eberhard, M. J. 2003, Developmental plasticity and evolution, Oxford University Press.

Westneat, D. F., J. Wright, and N. J. Dingemanse. 2015. The biology hidden inside residual within-individual phenotypic variation. Biological Reviews 90:729-743.

Wilbur, H. M., and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305-1314.

Williams, G. 1966, Adaptation and Natural Selection. Princeton, New Jersey, Princeton University Press.

Wilson, A. D. M., and J. Krause. 2012. Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? Behavioral Ecology 23:1348-1359.

Wong, S. C., M. Dykstra, J. M. Campbell, and R. L. Earley. 2008. Measuring water-borne cortisol in convict cichlids (*Amatitlania nigrofasciata*): is the procedure a stressor? Behaviour 145:1283-1305.

Zechini, L., A. Lilley, J. R. Downie, and P. T. Walsh. 2015. Why do frog and toad forelimbs suddenly (but asynchronously) appear, every time metamorphosis is near? Functional Ecology 29:816-822.

Zechini, L., A. Lilley, E. Waddell, T. J. Burns, J. R. Downie, and P. T. Walsh. 2016. Turning into frogs: Asymmetry in forelimb emergence and escape direction in metamorphosing anurans. Laterality: Asymmetries of Body, Brain and Cognition Advanced Online Publication. doi: 10.1080/1357650X.2016.1235175.

APPENDICES

VITA

Lynne Elizabeth Beaty

Candidate for the Degree of

Doctor of Philosophy

Thesis: LATENT EFFECTS OF LARVAL EXPERIENCE: THE PERVASIVE EFFECTS OF TADPOLE PREDATION RISK ON FROG PHENOTYPE

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Zoology at Oklahoma State University, Stillwater, Oklahoma in May, 2017.

Completed the requirements for the Master of Science in Biology at Texas Tech University, Lubbock, Texas in 2012.

Completed the requirements for the Bachelor of Science in Wildlife Science at the State University of New York College of Environmental Science and Forestry, Syracuse, New York in 2010.

Professional Memberships:

American Society of Naturalists
Animal Behavior Society
Southwestern Association of Naturalists