Abstract

FEAR OF AQUATIC PREDATORS CAUSE PREY TO ALTER THEIR PHENOTYPE AT MULTIPLE LIFE STAGES

by

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Many organisms can alter their behavior, life history, and morphology in response to their environment. This ability is known as phenotypic plasticity. One of the major environmental cues that triggers a phenotypic plastic response in some organisms is the threat of predation. Predator-induced phenotypic plasticity is finely tuned and organisms can respond differently to different types of predators and responses to a predator can carry over across life stages. These carry over effects may represent trade-offs associated with responding to predators because an adaptive response in one life stage may not be adaptive in a later life stage. In addition, many organisms show phenotypic plasticity in response to competitors and competitor-induced responses are often opposite of predator-induced responses. Since responses to predators are finely tuned and competitors also affect phenotypic plasticity, it remains to be seen as to what extent competitor identity (intra- and interspecific), age, and relative abundance alter the plastic phenotypic response to predators. We sought to determine the extent to which anti-predator responses in an early life stage persist into a later life stage in a natural setting, how competitor age and identity affect the response to a predator, and how the relative strength of intra- and interspecific competition affect the response of two co-occurring prey species to a predator. We used artificial ponds and larval frogs and toads to address these effects. Southern

toads altered their life history and morphology in response to predators but the particular response depended on predator identity. Morphological differences that developed in response to aquatic predators during their larval stage carried over into their terrestrial juvenile stage, but differences only persisted for approximately one month after metamorphosis. Competitors alone had little effect on morphology, but they did strongly affect survival and life history. Predators on the other hand had strong effects on morphology, but competitors altered the way that pinewoods treefrogs responded to the predators. In particular, older intraspecific competitors caused pinewoods treefrogs to develop the most extreme defenses to predators. We also found that the relative strength of intra- and interspecific competition depends on the identity of the species involved. Pinewoods treefrogs seem to be poorer competitors than Cope's gray treefrogs and southern leopard frogs, and both pinewoods treefrogs and leopard frogs survived and grew better when there were more pinewoods present than leopard frogs. Predator identity, competitor age, competitor identity, and the relative strength of intra- and interspecific competition all affected the plastic phenotypic response of frogs and toads to a predator. Responses to a predator in one life stage also carry over into later life stages. These results highlight the importance of adding back the complexity of natural systems into experiments to gain a better understanding of how organisms can persist with predators.

FEAR OF AQUATIC PREDATORS CAUSE PREY TO ALTER THEIR PHENOTYPE AT MULTIPLE LIFE STAGES

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Fear of Aquatic Predators Cause Prey to Alter Their Phenotype at Multiple Life Stages

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Introduction:

Phenotypic plasticity is a change in the expression of a phenotype (including behavior) mediated by the environment (Bradshaw 1965). This phenotypic plasticity is a widespread phenomenon found in plants and animals (Aronson et al. 1992, Via et al. 1995, Robinson and Wilson 1996, Wikelski and Thom 2000, Relyea 2002a, Yeh and Price 2004, Nussey et al. 2005, Pelletier et al. 2007, Brede et al. 2007, Gilbert 2011, Mooney and West 2012). Prey can develop a different morphology or behavior to reduce their vulnerability in the presence of a predator (Relyea 2002b, Gilbert 2011). For example, predators induce *Daphnia pulex* to develop "neck teeth" that makes it harder for predators to swallow them (Tollrian 1995), and predators induce amphibian tadpoles to develop longer tails and shorter bodies as a predator strike to the tail is less likely to be fatal than a strike to the body (Tollrian 1995, Van Buskirk and Mccollum 2000, Relyea 2001a, Stamper et al. 2009). There are consequences, however, of altering phenotype in response to predators. For example, *D. pulex* with neck teeth take longer to reach maturity than *D. pulex* without neck teeth (Tollrian 1995) and wood frog (*Lithobates sylvaticus*) tadpoles raised with predators exhibit shorter bodies and taller tails fins but take longer to grow and develop than wood frog tadpoles raised without predators (Relyea 2001b). Similarly, tadpoles of the common frog (*Rana temporaria*) raised with predators exhibit wider bodies and taller tail fins than tadpoles reared without predators, but tadpoles reared with predators develop into juveniles that swim slower and not as far as common frogs raised without predators (Stamper et al. 2009). We define consequences here as an anti-predator response in morphology or life-history that

differs from the morphology and life-history exhibited by organisms in the absence of a predator or in response to a different stressor such as competition.

One consequence of altering phenotype to respond to a predator is that a response to one predator may not be effective against another predator. Different types of predators often have different impacts on their prey's behavior and morphology. Relyea (2001a) found that invertebrate predators (*Anax* dragonfly larvae) reduced activity of several species of anurans more than fish (*Umbra* sp.), but the fish predators still reduced prey activity relative to the control. One prey species from Relyea's experiment was the wood frog (*Lithobates sylvaticus*), which developed deeper tail fins in the presence of both predators relative to the control, but fish had a larger effect on tail fin depth than dragonfly larvae (Relyea 2001b). In another study, tadpoles of the southern leopard frog (*Lithobates sphenocephalus*) were less visible to an observer with two different fish predators (*Aphredoderus sayanus* and *Lepomis macrochirus*) than with crayfish (*Procambarus acutus*) or salamander (*Notophthalmus viridescens*) predators (Albecker and Vance-Chalcraft 2015). The same study also found that the crayfish and salamander predators consumed more tadpoles, but the tadpoles did not alter their activity in response to these predators, suggesting that these mismatched responses could lead to higher mortality in nature (Albecker and Vance-Chalcraft 2015). In tadpoles of the frog *Rana pirica*, a dragonfly larvae predator induced a taller tail fin whereas a salamander predator induced a wider body (Kishida and Nishimura 2005). In predation trials, salamanders were better at catching the prey morphotype induced by the dragonfly larvae, and dragonfly larvae were better at catching the prey morphotype induced by the salamander (Kishida and Nishimura 2005). This could also carry over to later life stages, as a response to one predator may be adaptive for the juvenile environment, but a response to another predator may be maladaptive.

There is evidence from multiple taxa that responding to a predator via phenotypic plasticity in an early life stage can carry over to later life stages. Larvae of the dragonfly *Leucorrhinia intacta* develop longer tail spines when raised with predators (McCauley et al. 2008) but experience a higher rate of failure to complete metamorphosis (McCauley et al. 2011). The mayfly *Drunella coloradensis* develops longer caudal filaments and a heavier exoskeleton in the presence of brook trout (*Salvelinus fontinalis*), but female mayflies are smaller and have lower fecundity when they transform into adults (Dahl and Peckarsky 2002). Metamorphosed anurans reared as larvae with predators have shorter bodies, longer legs, slower swimming speeds, and shorter swim distances than those reared without predators (Relyea 2001b, Stamper et al. 2009). These results suggest that changes in phenotype that increase survival in an earlier life stage can reduce an individual's fitness and/or chances of surviving in a later life stage, so it is important to understand these lasting impacts better to see just how much early life stages are impacting later ones. It is also unclear how long these changes may persist in juveniles or the adult life stage after larval predators are no longer a threat.

Consequences of responding to a predator as a larvae have been documented in organisms with complex life histories, but they have generally been studied in a laboratory setting and involve a single predator (Relyea 2001b, Relyea and Hoverman 2003, Stamper et al. 2009). Many organisms face other stressors in the wild that could cause predator-induced phenotypes to further diverge from or converge on non-induced phenotypes. For example, food limitation after metamorphosis could result in convergence of phenotypes in the juvenile stage because there are just not enough resources available to maintain a plastic response and undergo normal development. Alternatively, juvenile stage predators could cause differences between treatments to further diverge if they select for or induce altered traits such as longer legs. To

assess how responding to a predator might carry over across life stages under natural conditions, we examined how larval amphibians responded to different predator species and evaluated the extent to which these responses had consequences for individuals following metamorphosis.

Phenotypic plasticity is easily observable in larval amphibians and can involve changes in behavior, life history traits (e.g., length of the larval period), and morphological characters, such as tail and body length (Relyea 2001a, 2001b). The effects of responding to one type of predator as larvae can carry over at least three months after leaving the larval environment (Relyea 2001b, Stamper et al. 2009). Amphibians respond differently to different types of predators (Relyea 2001a, Kishida and Nishimura 2005), but it has not been determined whether different predators have different lasting effects on juvenile morphology and performance. The consequences of amphibians responding to larval predators after metamorphosis have only been tested in the laboratory (Relyea 2001b, Relyea and Hoverman 2003, Stamper et al. 2009), but metamorphosed individuals may differ in predator susceptibility or foraging ability as a function of their larval environment, differences that may not be readily evident in a captive setting.

We were interested in evaluating three hypotheses to confirm that tadpoles exhibit alternative plastic responses to different types of predators in the larval environment and to assess if these plastic responses persisted across life stages. 1) Tadpoles alter their morphology in response to predators but the particular response depends on predator type. 2) Predators impact morphology, performance, and/or life history traits of animals at metamorphosis, but the particular response depends on predator type. 3) Differences in morphology and performance at metamorphosis persist and further diverge with time.

Methods:

To determine the extent to which aquatic predators affect the performance and morphology of prey during their larval (aquatic) and juvenile (terrestrial) life history stages, we 1) raised tadpoles in artificial ponds that differed in the kind of aquatic predator present, 2) measured the morphology of larval individuals, 3) assessed morphology and performance of prey individuals at metamorphosis as individuals transitioned to a terrestrial environment, and 4) measured the morphology and performance of metamorphosed prey individuals raised in outdoor enclosures for several weeks after metamorphosis.

We chose the southern toad (*Anaxyrus terrestris*) as our focal prey species because 1) they breed in ephemeral habitats that can be simulated with tanks; 2) juvenile toads have a very limited ability to escape terrestrial enclosures compared to other anuran species in our study area; and 3) little is known of their morphological response to predators. Tadpoles of the American toad (*A. americanus*), a species closely related to southern toads, has been found to exhibit a different behavior and develop a different morphology in the presence of predators than tadpoles not raised with predators (Relyea 2001a). We used mudminnows (*Umbra* sp.) and darner dragonfly larvae (*Anax* sp.) as predators because members of both of these genera have been shown to induce plastic responses in tadpoles, including the American toad (Relyea 2001a), and they co-occur with southern toads. *Anax* and *Umbra* also have different effects on toad tadpoles. *Anax* reduces tadpole activity more than mudminnows whereas mudminnows induce longer tail growth (Relyea 2001a).

We filled 15 1100 L artificial ponds with well water treated with chlorine on April 1, 2012 and allowed them to sit for four days for dechlorination. We used artificial ponds because they are similar size to many natural temporary ponds and processes that have been found to be important in artificial ponds have also been found to be important in natural ponds, and artificial

ponds allow greater control and replication than natural ponds (Wilbur 1987, Morin 1998, Chalcraft et al. 2005). Experimental tanks were arranged in five spatial blocks. Each block contained three tanks, corresponding to the three treatments (control, dragonfly predator, and fish predator). This resulted in a total of 15 experimental tanks, with each of the three treatments randomly assigned to one tank within each of the five blocks. One kilogram of pine straw was added to each tank 3 days after tanks were filled with water to provide cover for tadpoles, a medium on which algae could grow, and serve as a nutrient source for the pond food web that develops in the tank. Tanks were inoculated with a pint aliquot of pond water 4 days after tanks were filled with water to introduce plankton and algae to the system, better simulating a natural pond and providing food for the tadpoles in the form of the algae. All tanks were covered with mesh window screen to keep metamorphosed toads in the tanks and to prevent wild animals from entering the tanks. Water, litter, and zooplankton inoculations were added to the tanks on a block by block basis.

We collected five southern toad egg masses from Pitt County, North Carolina between 1-3 April 2012, but all of the clutches were likely laid on the night of March 31 as the clutches were at similar stages of development. The eggs were brought back to the laboratory to hatch, allowed to develop for approximately a week, and were added to artificial ponds on April 12, 2012. Each artificial pond received 100 tadpoles, representing an equal mix of individuals from the five clutches. We placed a subset of the remaining tadpoles in separate holding tanks on April 12, 2012, and used them to feed the predators used in the experiment.

Each tank contained two PVC predator cages with window screen fastened on both ends of an 18.5 cm diameter by 30 cm long PVC pipe to allow water and chemical cue exchange between the inside of the cage and the rest of the tank without allowing the predators to escape.

For the dragonfly treatment, each cage contained a single *Anax* larva and for the fish treatment, each cage contained a single mudminnow. We placed two empty predator cages in the control tanks. We caged predators so that tadpoles could detect the presence of predators via water borne cues while preventing predators from killing prey. If predators could kill prey, any difference in phenotype among treatments could be attributed to differential selection on particular phenotypes by predators instead of a change in the way in which phenotypes develop (i.e., phenotypic plasticity) in different treatments. We fed each predator seven tadpoles that derived from separate holding tanks for each week of the experiment.

At two weeks after hatching, a subset of 10 tadpoles was collected from each tank, anesthetized with Orajel (benzocaine), weighed, and photographed. We photographed tadpoles in a water-filled acrylic box placed on a stage with multiple mirrors that allowed us to simultaneously photograph the lateral and ventral views of the tadpole. The acrylic box had a scale etched on it. Tadpoles were then allowed to recover before being returned to their respective tanks. We repeated this process four weeks after the tadpoles hatched. We used these photographs in the program ImageJ (Schneider et al. 2012) to measure five lateral traits: tadpole body length, body depth, tail length, height of the tail fin at the tallest point, height of the tail muscle at the base of the tail, and two ventral traits: body width and tail muscle width (see Relyea 2001a). These traits have all been found to undergo change in response to predators (Relyea 2001a, Relyea 2004, Kishida and Nishimura 2005).

At metamorphosis (determined by the emergence of at least one forelimb), toads were removed from the tanks by hand or net and returned to the lab to allow complete absorption of their tails. Metamorphosis occurred from five to seven weeks post hatching. Once tail absorption was complete, metamorphs were weighed. We measured jumping ability by placing metamorphs

on a sheet of paper, marking their starting location, prodding them to jump, marking their landing location and then measuring the distance between the starting and landing points with a ruler (modified from John-Alder and Morin 1990). We repeated this process three times for each metamorph and calculated the average jump distance. We also measured the cranial width between the eyes (CW), the length from the metamorphs's snout to its urostyle (SUL), the length of its femur (FL), and the length of its tibio-fibula (TFL). These measurements were chosen because previous work has shown that amphibian head size, body size, and limb lengths can change in response to predators (Relyea 2001b, Takatsu and Kishida 2013).

We transferred 45 randomly selected 8 week old, metamorphosed toads from each tank to a corresponding 3 m X 3 m X 0.76 m enclosure made of silt fencing placed in a sandy scrub habitat at the edge of a forest and near several wetlands in Pitt County, North Carolina. Silt fencing was buried 0.15 m into the ground to keep it upright. Silt-fence enclosures have been used to effectively house amphibians (Patrick et al. 2008) and amphibians have been studied for 8-12 months in enclosures with high rates of survival (Boone 2005). Three enclosures were set up within each of five spatial blocks. Enclosures in the same spatial block received metamorphs from the same spatial block of tanks but each enclosure only received metamorphs from one tank. The enclosure to which a tank was assigned within a particular block was randomly assigned. Metamorphs not added to the enclosures were released back into the wild where the eggs were collected.

We weighed, measured the same traits as at metamorphosis, and tested the jumping ability of a subset of up to 10 animals in each of the 15 enclosures at three different times (two, four, and seven weeks after they were placed into the terrestrial enclosures). Sometimes we could not find 10 animals in an enclosure, so we measured as many individuals as could be captured.

Although it is possible that we did not find all of the individuals in an enclosure when we took our measurements, our searches were thorough and we did not find any more remaining individuals at the end of the experiment after removal. Based on this, we feel it is accurate to refer to the number of individuals that we found within an enclosure as a measure of survival. We chose to end the experiment after the third set of enclosure measurements because we only found, on average, two toads remaining in each enclosure, which is too few individuals to draw meaningful conclusions. The surviving toads were returned to the wild where they were collected as eggs.

Statistical Methods:

All analyses were conducted in SAS software, version 9.4 of the SAS System for Windows. Copyright © 2013 SAS Institute Inc, Cary, NC, USA. We averaged values for each response variable across individuals within a tank except for survival, which had a single value for each tank, so we had a sample size of 15 for each response variable. Proportion of individuals surviving was analyzed with a generalized linear mixed model with an expected binomial distribution and logit link function since survival often follows a binomial distribution. Treatment was included as a main effect and block as a random effect in the survival model. All other measurements were analyzed with a linear mixed model with treatment included as a main effect and block as a random effect.

Mean mass of tadpoles and the interaction between mass and treatment were included as fixed effects for the analysis of morphological data and jumping ability. Morphological traits typically scale with body size, so we included body mass in the statistical model to account for this relationship. We also included the interaction between mass and treatment to determine if

the slope of the relationship (allometry) between a morphological trait and mass differed between treatments (i.e. a similar increase in body size causes tadpoles raised with dragonflies get bigger tail fins than tadpoles raised without predators). If there was very weak evidence to suggest that allometric relationships differed among treatments (visual inspection of slopes and *p* for test of equality of slopes >0.3) we excluded the interaction between treatment and mass from the model to enhance statistical power for a test of a treatment effect and a general allometric relationship between the trait and body size. Subsequently, we also removed mass from the model if it appeared that there was no general allometric relationship between the trait and body mass (visual inspection of slope and *p* for test of slope is >0.3). Mass was almost always retained as a covariate. Stronger evidence ($p < 0.3$) for an interaction between mass and treatment for a morphological trait meant that the slope of the allometric relationship between the morphological trait and body size differed among treatments. These differences in allometry often take the form of treatments showing no differences at one body size, but differing at other body sizes (e.g. tail length for small tadpoles does not differ among treatments but tail length of larger tadpoles does differ among treatments). To account for differences in allometry, we compared traits among the three treatments at three body sizes: small sized animals (1 standard deviation below the average mass), average sized animals (at the average mass), and large sized animals (1 standard deviation above the average mass). If the allometry did not vary among the treatments, the treatment differences would be consistent across different body sizes because the allometric relationships across the different treatments are parallel to each other.

We analyzed each response variable for each measurement period (i.e., each time traits were assessed in tanks and enclosures) separately and adjusted *p*-values for each hypothesis test to control the False Discovery Rate for running many tests (Verhoeven et al. 2005). We used our

statistical models to test three hypotheses: 1) caged fish had little effect on response variables, 2) caged dragonflies had little effect on response variables, and 3) caged fish and caged dragonflies did not differ in their effect on response variables. We also calculated the percent difference between the least square means for each hypothesis above and determined that a % difference ≥ 10% implied a biologically meaningful result even in the absence of statistical significance, but only if the *p*-value was less than 0.3. We chose a 10% threshold as this is the level of difference in tail length and depth between tadpoles reared with and without predators in a previous study (Van Buskirk and McCollum 2000), and another study found that tadpoles often exhibit maximum differences in shape of around 20% between those reared with and without predators (Van Buskirk et al. 2003), so 10% is within the expected levels of phenotype change. We chose a *p*-value cutoff of 0.3 as this means that there is a greater than 70% chance that the results were not due to random chance. Some of our small percent differences were found to be statistically significant, we retained these differences in our results. Despite the small magnitude of the difference (as low as 2%), our results are similar to those of previous studies (Van Buskirk and McCollum 2000, Relyea 2001a), suggesting that even small differences are meaningful.

Results:

Tadpoles at two weeks after hatching

The average mass of tadpoles did not differ among treatments (% difference ≤ 7.6%, $F_{2, 8} = 0.55$, $p = 0.595$, adjusted contrast $p \geq 0.597$, Supplemental Table S1, Table 1). All morphological traits increased with increasing mass ($p \leq 0.2872$, Supplemental Table S1), but there were only differences in allometry for body depth and body width ($p \leq 0.259$, Supplemental Table S1).

Predators did not have a large impact (% difference ≤ 5.9% and adjusted contrast $p \geq 0.2868$, Table 1) on the height of the tail fin at the tallest point, body length, tail length, or tail muscle width ($F_{2, 7} \geq 0.48$, $p \geq 0.2173$, Supplemental Table S1, Table 1, Fig. 1, 2). Predators did affect body depth and body width, but the effect varied with tadpole size ($F_{2, 5} \geq 2.12$, $p \leq 0.2152$, Supplemental Table S1, Fig. 1, 2).

Body depth of large and average sized tadpoles did not vary appreciably among treatments (% difference ≤ 3.6%, adjusted contrast $p \geq 0.2937$, Table 1, Fig. 1, 3). Body depth of small tadpoles also did not statistically differ among treatments, but dragonflies appeared to reduce the body depth of small tadpoles by approximately 10% relative to the control (adjusted contrast $p = 0.2405$, Table 1, Fig. 1, 3). Predators did not affect the body width of average sized tadpoles (% difference ≤4.9%, adjusted contrast $p \geq 0.3707$), but predators induced small tadpoles to have a body width that was at least 21% wider (adjusted contrast $p = 0.0623$ for both predators) and both predators induced large tadpoles to have a body width that was at least 10% narrower (adjusted contrast $p \geq 0.135$, Table 1, Fig. 2, 3). The effect of different predator species on tadpole body width was similar across body size (% difference ≤ 3.8%, adjusted contrast $p \geq 0.3064$, Table 1, Fig. 2, 3).

Tadpoles at four weeks after hatching

The average mass of tadpoles did not differ appreciably among treatments (% difference ≤ 6.7%, $F_{2, 8} = 1.05$, $p = 0.3951$, adjusted contrast $p \geq 0.4701$, Table 2, Supplemental Table S2). All measured traits were larger in larger tadpoles, except for body width, which showed no relationship to mass ($p > 0.3$ for body width, $p \leq 0.004$ for others, Supplemental Table S2). Body

length, tail length, and tail muscle depth also showed differences in allometry among the treatments ($p \leq 0.0901$, Supplemental Table S2).

Predators did not have a large impact on body depth or the height of the tail fin at the tallest point (% difference ≤ 1.5%, $F_{2, 7} \geq 0.21$, $p \geq 0.4568$, adjusted contrast $p \geq 0.59$, Supplemental Table S2, Table 2, Fig. 5). Predators impacted body width and tail muscle width irrespective of body size (for body width $F_{2, 8} = 8.24$, $p = 0.0114$, for tail muscle width $F_{2, 7} = 34.39$, $p = 0.0002$, Supplemental Table S2, Fig. 6), but the effects of predators on tadpole body length, tail length, and tail muscle depth varied with body size ($F_{2, 5} \geq 5.39$, $p \leq 0.0565$, Supplemental Table S2, Fig. 5).

Dragonflies induced 6.7% wider bodies than fish and 9.2% wider bodies than the control (adjusted $p \leq 0.0482$), but fish did not have a large effect on body width (% difference = 2.5%, adjusted contrast $p = 0.3587$, Supplemental Table S2, Table 2, Fig. 6, 7). Dragonflies induced 16.0% wider tail muscles in tadpoles than the control and 8.6% wider tail muscles than fish-reared tadpoles, and fish induced 7.4% wider tail muscles than the control ($F_{2, 7} = 34.39$, $p = 0.0002$, adjusted contrast $p \leq 0.0059$ for all contrasts, Supplemental Table S2, Table 2, Fig. 6, 8).

Predators did not alter tadpole tail muscle depth at the small or average size (% difference ≤ 7.2%, adjusted contrast $p \geq 0.0645$, Table 2, Fig. 5, 9). In large tadpoles, dragonflies induced 12.6% deeper tail muscles than no predators and 10.6% deeper tail muscles than fish (adjusted contrast $p = 0.0063$ for both comparisons, Table 2, Fig. 5, 9). Fish did not appreciably alter large tadpole tail muscle depth (% difference = 2.0%, adjusted contrast $p = 0.4135$, Table 2, Fig. 5, 9). Both fish and dragonflies induced at least 5.0% shorter tails in small tadpoles than the control, but fish induced 4.2% shorter tails than dragonflies (adjusted contrast $p \leq 0.0457$, Table 2, Fig. 5, 10). Fish also reduced the tail length of average sized tadpoles by 5.1% relative to no predators

and 3.4% relative to dragonflies (adjusted $p \leq 0.0464$), but dragonflies had no appreciable effect on tail length (% difference = 1.7%, adjusted $p = 0.1914$, Table 2, Fig. 5, 10). Predators did not affect tail length of large tadpoles (% difference ≤ 2.6%, adjusted $p \geq 0.4449$, Table 2, Fig. 5, 10). In small tadpoles, predators induced 3.5-6.5% shorter bodies than the control, and dragonflies induced 3.0% shorter bodies than fish (adjusted $p \leq 0.0332$, Table 2, Fig. 5, 11). At the average mass, predators induced 2.1-2.4% shorter bodies in tadpoles than the control (adjusted contrast $p = 0.0374$ for both), but the two predators did not differ appreciably from each other (% difference < 1%, adjusted contrast $p = 0.6627$, Table 2, Fig. 5, 11). At the large mass, predators had a small effect on tadpole body length (% difference ≤ 2.1%, adjusted $p \geq 0.2238$, Table 2, Fig. 5, 11).

Metamorphosis

Predators did not alter average time to metamorphosis (% difference ≤ 3.0%, $F_{2, 7} = 0.88$, $p = 0.4568$, adjusted contrast $p \geq 0.3858$, Table 3, Supplemental Table S3) or the mass of metamorphs (% difference ≤ 8.3%, $F_{2, 8} = 0.2931$, $p = 0.2931$, adjusted contrast $p \geq 0.5189$, Table 3, Supplemental Table S3). Survival to metamorphosis was 94.89% with caged dragonflies, 84.87% with caged fish, and 79.80% in the control treatment. Dragonflies increased tadpole survival to metamorphosis (adjusted contrast $p \leq 0.018$, % difference ≥ 11.1%), but fish had little effect on survival (% difference = 6.2%, $F_{2, 12} = 7.81$, $p = 0.0067$, adjusted contrast $p = 0.3599$, Table 3, Supplemental Table S3).

Larger metamorphs had wider craniums and longer tibio-fibula lengths ($p < 0.0001$ for both, Supplemental Table S3), but allometry did not differ across the treatments ($p \geq 0.3$), and

predators had little effect on cranial width or tibio-fibula length (% difference ≤ 1.0%, $F_{2, 7} \leq 3.67$, unadjusted $p \leq 0.4166$, adjusted contrast $p \geq 0.0992$, Table 3, Supplemental Table S3).

In general, larger metamorphs had longer snout-urostyle lengths and femurs, and jumped farther ($p \leq 0.0352$, Supplemental Table S3), but the effect of increasing mass depended on treatment ($p \leq 0.2442$, Supplemental Table S3). Despite these differences in allometry, femur length and snout-urostyle length did not differ among the treatments at any body size (% difference ≤ 3.0%, adjusted contrast $p \geq 0.1596$, Table 3). Predators had little effect on jumping ability at the small body size (% difference ≤ 4.3%, adjusted $p = 0.7411$ for all), but predators increased metamorph jump distances at the average and large sizes relative to the control (% difference ≥ 17.2% adjusted $p \leq 0.0081$) and predator effects did not differ (% difference ≤ 6.9%, adjusted $p \geq 0.1168$, Fig. 12, Table 3).

First Enclosure Measurements

We captured at least 5 individuals, and a maximum of 10, in 11 of the replicates. We caught 1-2 individuals in one replicate for each of the three treatments, and 0 individuals from one replicate of the fish treatment.

Mass did not appreciably differ among the treatments (% difference ≤ 17.7%, $F_{2, 7} = 0.66$, $p = 0.5455$, adjusted contrast $p \geq 0.6492$, Supplemental Table S4). Larger juveniles had wider craniums, longer snout-urostyle lengths and tibio-fibulas, and jumped farther (unadjusted $p \leq 0.013$, Supplemental Table S4), but none of these traits differed in allometry ($p \geq 0.3$, Supplemental Table S4). Predator rearing environment had little effect on cranial width, snout-urostyle length, and tibio-fibula length (% difference ≤ 1.2%, $F_{2, 6} \leq 1.60$, $p \geq 0.4049$, adjusted contrast $p \geq 0.3645$, Supplemental Table S4, Table 4). Fish-reared juvenile toads jumped 14%

farther than juvenile toads not raised with predators, but this difference was not statistically different (adjusted contrast $p = 0.3645$), and the dragonfly rearing environment had a small effect on jumping ability (% difference ≤ 8%, adjusted contrast $p = 0.4314$ versus fish and versus control, Table 4).

Larger juvenile toads had longer femurs ($F_{2, 4} = 0.66$, $p = 0.5455$, Supplemental Table S4) and femur length showed differences in allometry ($p = 0.0485$). Predator rearing environments had no appreciable effect on femur length at the small or average mass (% difference ≤ 3.8%, adjusted contrast $p \geq 0.2649$, Table 4, Fig. 13). At the large mass, fish-reared toads had 4.4% longer femurs than control-reared toads (adjusted contrast $p = 0.0465$) and dragonfly-reared toads had 3.3% longer femurs than control-reared toads, but the effects of the dragonfly rearing environment were not statistically significant (adjusted contrast $p = 0.0896$, Table 4, Fig. 13). The effects of dragonfly and fish rearing environments on femur length did not appreciably differ from each other (% difference = 1.1%, adjusted contrast $p = 0.4334$, Table 4, Fig. 13).

Second Enclosure Measurements

We captured an average of 4.07 individuals per enclosure. We captured at least 3 individuals in most of the replicates, but one fish replicate produced 0, one fish replicate and one dragonfly replicate produced 2, and two control replicates produced only a single individual.

At this stage, survival was 13.22% for dragonfly-reared, 5.62% for the control, and 6.72% for fish-reared ($F_{2, 12} = 1.97$, unadjusted $p = 0.1827$, Supplemental Table S5). Dragonflies increased survival of juveniles by at least 65% relative to the other two treatments and fish increased survival by 18% relative to the control, but these effects were not statistically different

(adjusted contrast $p \geq 0.2445$, Table 5). Predators had different effects on mass, with fish increasing it by 10% and dragonflies decreasing mass by 10%, but the effects of predators on mass were not statistically different ($F_{2, 7} = 1.55$, $p = 0.2762$, adjusted contrast $p \geq 0.3666$, Table 5, Supplemental Table S5).

Larger juveniles had wider craniums, longer snout-urostyle lengths and tibio-fibulas, but the allometry of this relationship did not differ across the treatments ($F_{2, 6} \geq 0.60$, $p \leq 0.5802$, mass $p \leq 0.0016$, Supplemental Table S5). Cranial width and snout-urostyle length did not differ across the treatments (% difference ≤ 2.7%, adjusted contrast $p \geq 0.5648$, Supplemental Table S5, Table 5). Tibio-fibula length was 4.5-5.0% longer in juvenile toads raised in the two predator environments than juveniles raised in the control, but this difference was not statistically different and the two predator environments were less than 1% different from each other ($F_{2, 6} = 1.40$, $p = 0.3164$, adjusted contrast $p = 0.3713$ for both, Supplemental Table S5, Table 5).

Larger juveniles generally had longer femurs and jumped farther ($p \leq 0.1183$) and the allometry of this relationship differed across the treatments ($p \leq 0.2772$, Fig. 14, 15 respectively, Supplemental Table S5). Predators had little effect on tadpole femur length (% difference ≤ 8%, $F_{2, 4} = 0.94$, $p = 0.4637$, adjusted contrast $p \geq 0.2211$, Supplemental Table S5, Table 5). Toads exhibited some large differences (% difference ≥ 13.6 %) in jumping ability based on their rearing environment at the small and average sizes, but none of these effects differed significantly from zero ($F_{2, 4} = 1.87$, $p = 0.2673$, adjusted contrast $p \geq 0.6422$, Supplemental Table S5, Table 5, Fig. 15). At the large size, toads from the dragonfly and fish rearing environments both jumped approximately 30% farther than toads reared without predators, but the effect was not statistically significant (adjusted contrast $p \leq 0.1973$, Table 5, Fig. 15). At the

large size, juvenile toads from different predator environments did not differ in their jumping ability (% difference = 1.0%, adjusted contrast $p = 0.9453$, Table 5, Fig. 15).

Third Enclosure Measurements

The average number of individuals we were able to capture in each enclosure at this time was 1.93. No enclosures produced more than 4 toads and four enclosures now contained 0 toads (1 fish replicate, 1 dragonfly replicate, and 2 control replicates).

Juvenile toads reared as larvae with predators survived 10% better than toads reared without predators but survival was only around 4% in all of the treatments, and the rearing environment effects on juvenile survival did not significantly differ from zero ($F_{2, 12} = 0.03$, $p = 0.9688$, contrast adjusted $p = 0.9903$ for all three contrasts, Table 6, Supplemental Table S6). Juvenile toads raised as larvae with fish weighed 26% more than control reared toads and toads reared as larvae with dragonflies weighed 7% less than control reared toads, but none of these effects were statistically different from zero ($F_{2, 5} = 1.48$, $p = 0.3130$, adjusted contrast $p \geq 0.4685$ for all contrasts, Table 6, Supplemental Table S6).

Larger juveniles had longer snout-urostyle lengths and wider craniums, but the allometry did not differ across the treatments ($F_{2, 4} \geq 0.84$, $p \leq 0.4957$, mass $p \leq 0.2469$, interaction $p \geq 0.3$, Supplemental Table S6). Cranial width and snout-urostyle length also did not differ across the treatments (% difference ≤ 4%, adjusted contrast $p \geq 0.3462$, Table 6).

Larger juveniles had longer tibio-fibulas and femurs, and jumped farther, but the allometry of this relationship differed across the three treatments ($F_{2, 2} \geq 2.42$, $p \leq 0.2921$, mass $p \leq 0.0573$, interaction $p \leq 0.2036$, Supplemental Table S6, Fig. 16-17). Predator rearing environment had little effect on tibio-fibula length of juvenile toads at the small body size (%

difference ≤ 4%, adjusted contrast $p \geq 0.1913$, Table 6, Fig. 16). At the average and large body sizes, toads reared as larvae with predators had 4.56-8.90% longer tibio-fibulas, but the effects of the two predator rearing environments differed by 4% or less and none of these differences were significantly different from zero (adjusted contrast $p \leq 0.0785$ for the control versus predator comparisons and adjusted contrast $p \geq 0.1593$ between predator rearing environments, Table 6, Fig. 16). Toads reared as larvae with predators had at least 4.9% longer femurs at all three body sizes and dragonfly-reared toads had the longest femurs (at least 8.3% difference) at small and average body sizes and fish-reared toads had the longest femurs (7.4% difference) at the large body size, but none of these effects were significantly different from zero (adjusted $p \geq 0.0714$, Table 6, Fig. 17). Predator effects also did not differ much between fish and dragonflies for femur length at any body size (% difference ≤ 7.0%, adjusted contrast $p \geq 0.1292$, Fig. 17). Jumping ability of juvenile toads did not differ strongly among toads raised in the different rearing environments at any body size except that dragonfly-reared juveniles jumped 18-22% shorter distances than control or fish-reared juveniles at the large mass (adjusted contrast p = 0.3189 vs. fish, 0.4119 vs. control, % difference ≤ 12.3% for all others, adjusted contrast $p \geq$ 0.5306 for all others, Table 6).

Discussion

Our first hypothesis, that tadpoles would alter their phenotype in response to predators and exhibit different phenotypes to different predators, was supported by predators inducing deeper and wider bodies than the control at 2 weeks after hatching and deeper and wider tail muscles at 4 weeks after hatching. Dragonflies generally had a stronger effect than fish.

Many tadpoles have been found to develop longer and deeper tails with predators because tadpoles are more likely to survive if a predator attacks their tail than their head or body (Van Buskirk et al. 2003). Compared to many other species, toad tadpoles have relatively small tail fins, so they may prioritize developing deeper and wider tail muscles to increase escape speed because they are unable to generate tail fins tall enough to reduce mortality from predator strikes. With similar predator species as those used in this study, American toads developed longer and deeper bodies and shallower tail fins (Relyea 2001a). Although plasticity in American toads differs from southern toads, American toad tadpoles reared with predators had smaller tail fins and deeper and wider tail muscles (Relyea 2001a), which could improve swimming performance. There is some evidence that tadpoles with larger tail musculature are more efficient swimmers because toad tadpoles have half the muscle mass, lower propeller efficiency, and higher tail beat frequencies than ranid frogs of similar size (Wassersug and Hoff 1985).

We found support for our second hypothesis, that predators would impact phenotype at metamorphosis and the phenotype would differ depending on predator identity, because metamorphs reared as tadpoles with predators were capable of longer jumps and dragonflies increased survival of metamorphs relative to fish and the control.

Support for the effects of predators on the time to metamorphosis in the literature is mixed (Relyea 2001b, Relyea and Hoverman 2003, Stamper et al. 2009), but we did not find a delay in time to metamorphosis.

We also found support for our hypothesis that tadpoles raised with different predators would develop different morphologies that would persist after metamorphosis and have important consequences for the performance of individuals during the terrestrial phase of their life. At 3-5 weeks after metamorphosis, dragonfly-reared toads and fish-reared toads had longer

femurs than toads raised as larvae without predators, but the effects were only statistically significant for fish. At 5-8 weeks after metamorphosis, toads raised with either predator had longer jumps than control-reared toads, but dragonfly-reared toads weighed less and had higher survival than toads reared in the other two treatments. At 8-11 weeks after metamorphosis, predator-reared toads had longer legs than control-reared toads, but dragonfly-reared toads had longer femurs at the small and average masses and fish-reared toads had longer femurs and jumps at the large mass.

Differences in morphology and performance between the treatments in our study were still present by 8-11 weeks after metamorphosis, supporting hypothesis three, but the differences were not statistically different. Despite the lack of statistical significance, many of these differences were greater than or equal to 10%, suggesting that they may be biologically significant. Stamper et al. (2009) found that morphological differences were not present at the onset of metamorphosis or at 4-8 weeks after metamorphosis but did appear at 12 weeks after metamorphosis. That study and ours detected performance differences at the onset of metamorphosis (swimming performance for Stamper et al. (2009) and jumping ability in this study) and 4 weeks into the juvenile stage. In Stamper and colleagues' study, swimming differences disappeared at 8-12 weeks after metamorphosis, but we still saw evidence of differences in jumping ability of up to 22% at 8-11 weeks after metamorphosis, though these differences were not statistically different. The appearance of morphological differences at 12 weeks after metamorphosis in the Stamper et al. (2009) study could stem from responding to the juvenile environment. Even though it was not statistically different, we found that toads reared with dragonflies were poorer jumpers than fish and control reared toads at 8-11 weeks after metamorphosis, but were better jumpers than control reared toads at metamorphosis and up to 5-

8 weeks after metamorphosis, suggesting the juvenile environment may be altering jumping ability.

Surprisingly, survival to metamorphosis was higher when individuals were reared with dragonflies than when they were reared with fish or without predators. This could be a function of dragonflies reducing the activity of the tadpoles more than the fish or control treatments, as predators are known to reduce tadpole activity (Relyea 2001a), and this could reduce the stress due to competition as there are fewer competitive interactions or could allow algal food resources time to recover or grow better due to less foraging pressure. Mortality could then be reduced as a direct function of less stress or an increase in the resources needed to maintain growth and survival. Predators have mediating effects on the impacts of competition and removing competitors has more positive impacts on growth and mass than removing predators (reviewed in Gurevitch et al. 2000). Removing predators increases survival more than removing competitors (reviewed in Gurevitch et al. 2000). Support for the increase in algal food resources in the presence of a predator comes from studies on snails and mayflies, which both eat algae. Fish predators (*Galaxia vulgaris* and *Salmo trutta*) reduce mayfly (*Deleatidium sp*.) activity, and this results in higher algal biomass in natural streams, with similar effects in a laboratory experiment (McIntosh and Townsend 1996). Crayfish (*Orconectes rusticus*) cause snails (*Physella gyrina*) to forage at or above the surface of the water, and fish (*Lepomis gibbosus*) cause snails to forage under benthic cover, resulting in 92% higher benthic algae levels in the presence of crayfish and 61% higher surface algae levels in the presence of fish (Turner et al. 2000). We did not assess behavior or algal resources, so it is unclear what mechanism is driving this increase in survival.

In several traits, allometry differed among the treatments at one time point but not at another time point. For example, body width differed in allometry in tadpoles two weeks after hatching but not at four weeks after hatching (Supplemental Tables S1, S2). At this stage in development, the appearance and disappearance of these treatment specific differences in allometry is likely due to developmental constraints keeping traits on a similar trajectory. For example, body width may not have shown treatment differences in allometry at four weeks after hatching because tadpoles have to maintain a certain body width as they approach metamorphosis, but did differ at two weeks after hatching because body width can vary for younger tadpoles that are further from metamorphosis. Tadpoles at two weeks after hatching showed little difference across the treatments, while many of the response variables differed among the treatments at four weeks after hatching. Previous work has shown that larval treefrogs require two weeks of exposure to predators before differences in tail shape are apparent (McCollum and Van Buskirk 1996). This also occurred in the enclosures and could represent a delayed response to the juvenile environment. It is also possible that there is a delayed cost to responding to predators that may appear in the juvenile stage. Benard and Fordyce (2003) observed no differences in morphology, growth, or development rates between western toad (*Anaxyrus boreas*) tadpoles reared with and without predators. Metamorphs reared as tadpoles with predators were swallowed faster by a recently metmorphosed salamander predator (*Ambystoma tigrinum*) than metamorphs reared without predators (Benard and Fordyce 2003). The fact that dragonfly-reared juveniles jumped shorter distances than juvenile toads reared with fish and without predators in the final set of enclosure measurements supports the idea of delayed constraints (Fig. 14). Dragonfly-reared tadpoles and metamorphs differed from toads

reared in the other two environments in a variety of ways, so it is not clear what might be driving such a cost.

Future work should focus on following amphibians to reproductive maturity to determine if differences in morphology due to larval rearing environment can persist for longer than 12 weeks and if morphologies and performance may converge or diverge over time in the juvenile stage due to new stressors in the juvenile stage or delayed costs from the larval stage as suggested by this study. This will require long-term laboratory and field experiments, and possibly mark-recapture studies to assess how phenotypic plasticity can affect survival in the wild. Increased survival with a caged predator compared to a control environment is also an interesting result, and more work should be undertaken to determine if this is due to a reduction in prey activity and if that in turn results in differences in algal production or reduced stress levels.

Tadpoles responded to predators and responded differently to different predators, and these effects carried over into and after metamorphosis. Differences persisted for 8-11 weeks after metamorphosis, but survival was very low and the differences were largely not statistically different, but were relatively large percent differences in the traits. These results fit with previous studies that examined the long-term effects of predator-induced phenotypic plasticity in amphibians in the laboratory, showing that the effects of different predators can persist across different life stages, and that differences persist under field conditions as well as in the laboratory. Our results suggest that the larval environment still has an impact on the juvenile environment even well after the cues from the larval environment are no longer present, and that these long term impacts of the larval environment can alter the trajectory of responses to the juvenile environment as toads reared with dragonfly predators altered their responses more at the

end of the experiment than toads reared with fish predators. Future work should continue to attempt to determine how long the effects of responding to a larval predator may persist after metamorphosis both in the laboratory and in the field and determine to what extent earlier life stages shape the response to novel cues in later life stages.

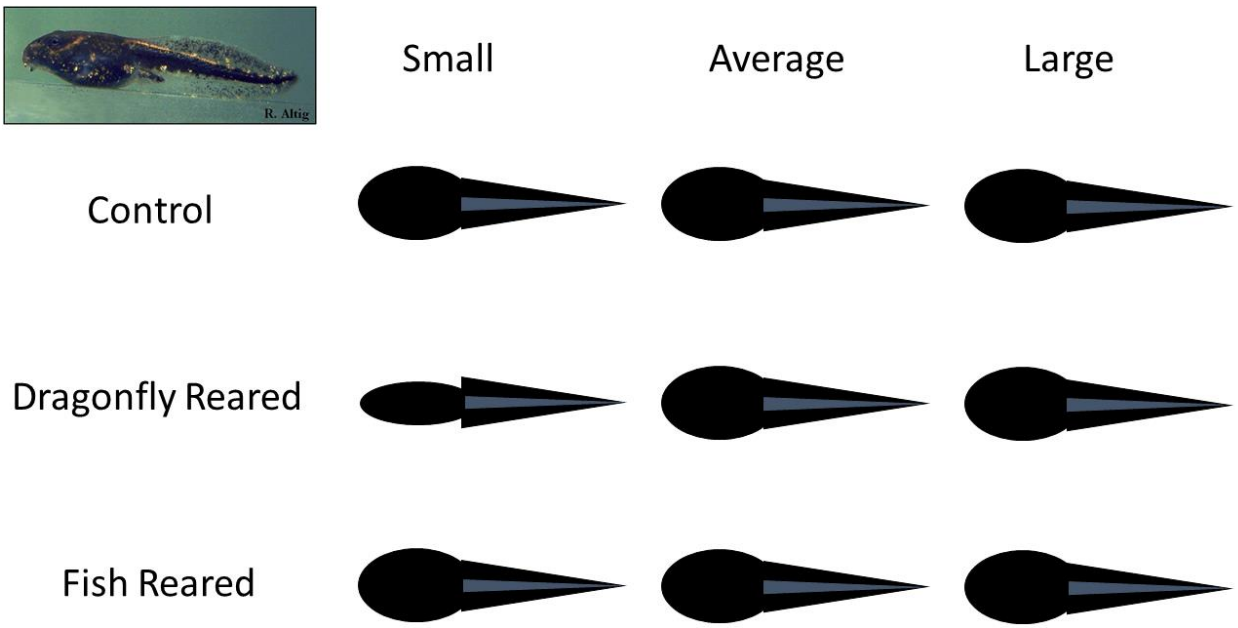

Figure 1. Summary of changes in morphology of tadpoles at two weeks after hatching from the lateral view. Treatments are listed along the y-axis and body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Black ovals represent the body, black triangles represent the tail fin, and gray triangles represent the tail musculature.

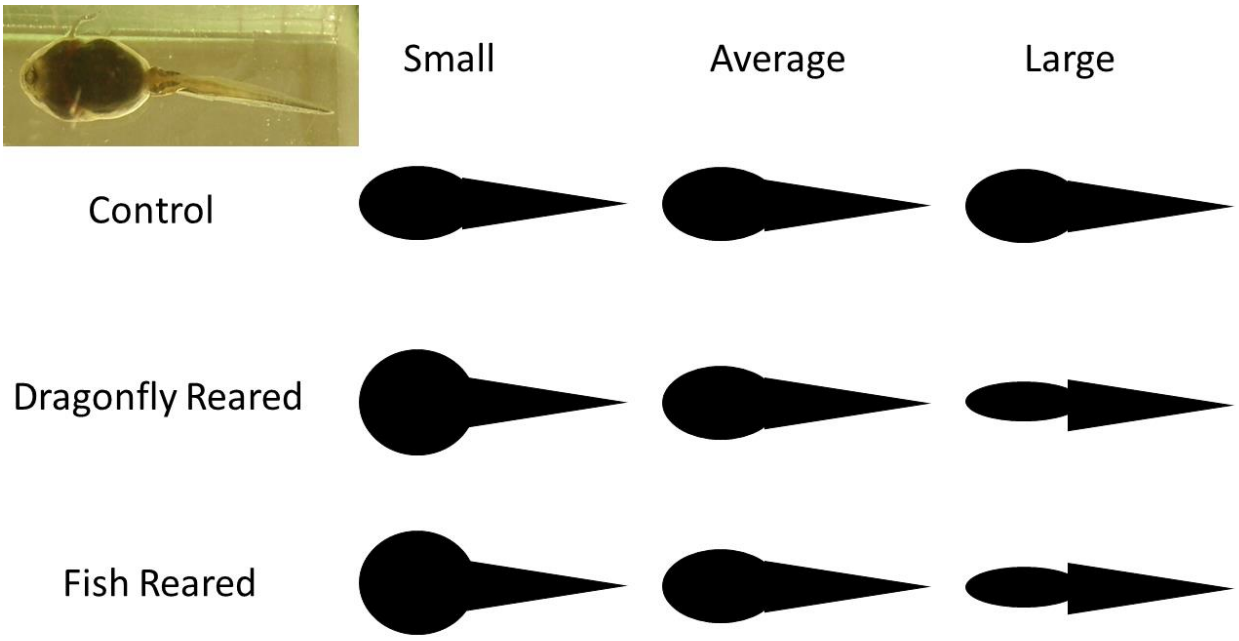

Figure 2. Summary of changes in morphology of tadpoles at two weeks after hatching from the ventral view. Treatments are listed along the y-axis and body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Black ovals represent the body and black triangles represent the tail.

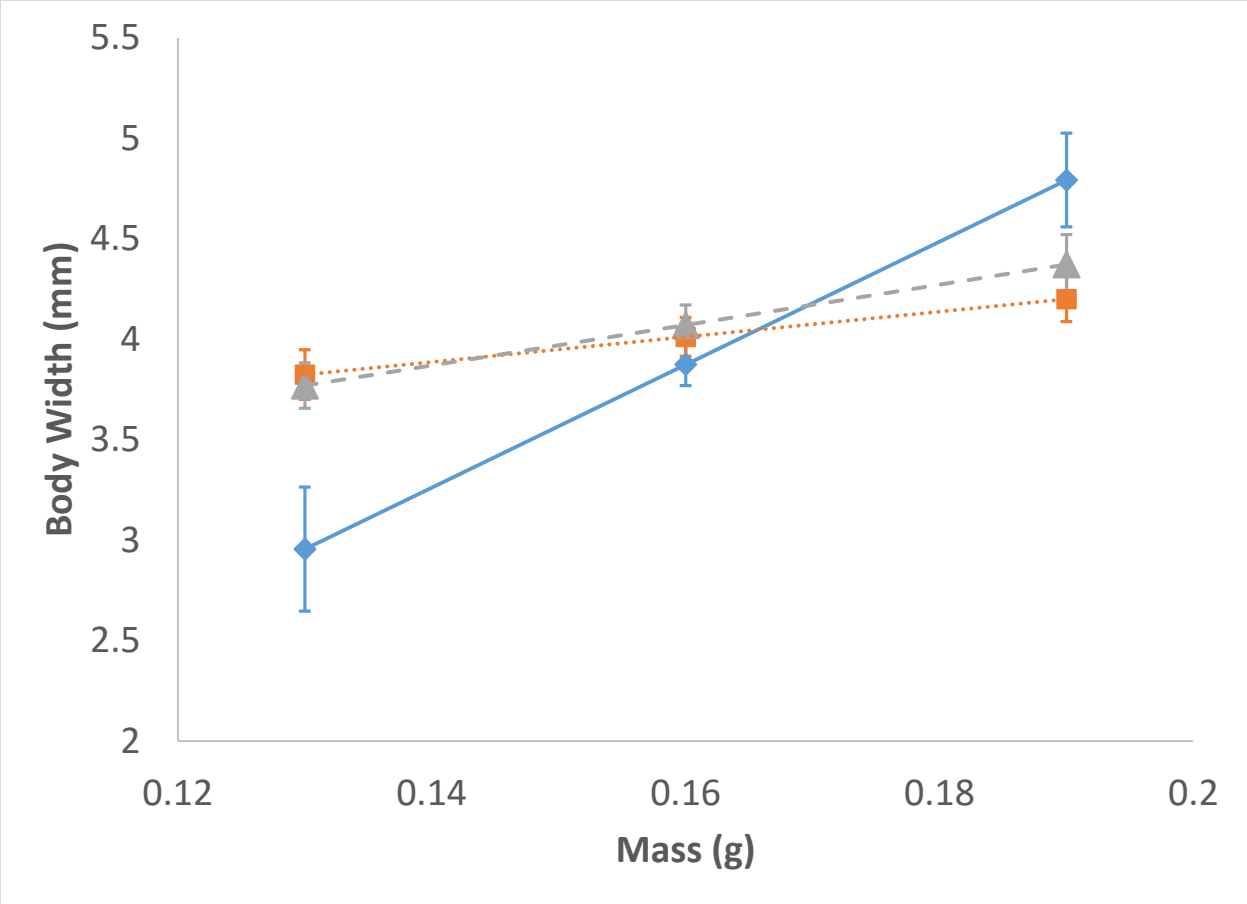

Figure 3. Mass compared to body width of tadpoles two weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars represent one standard error of the mean.

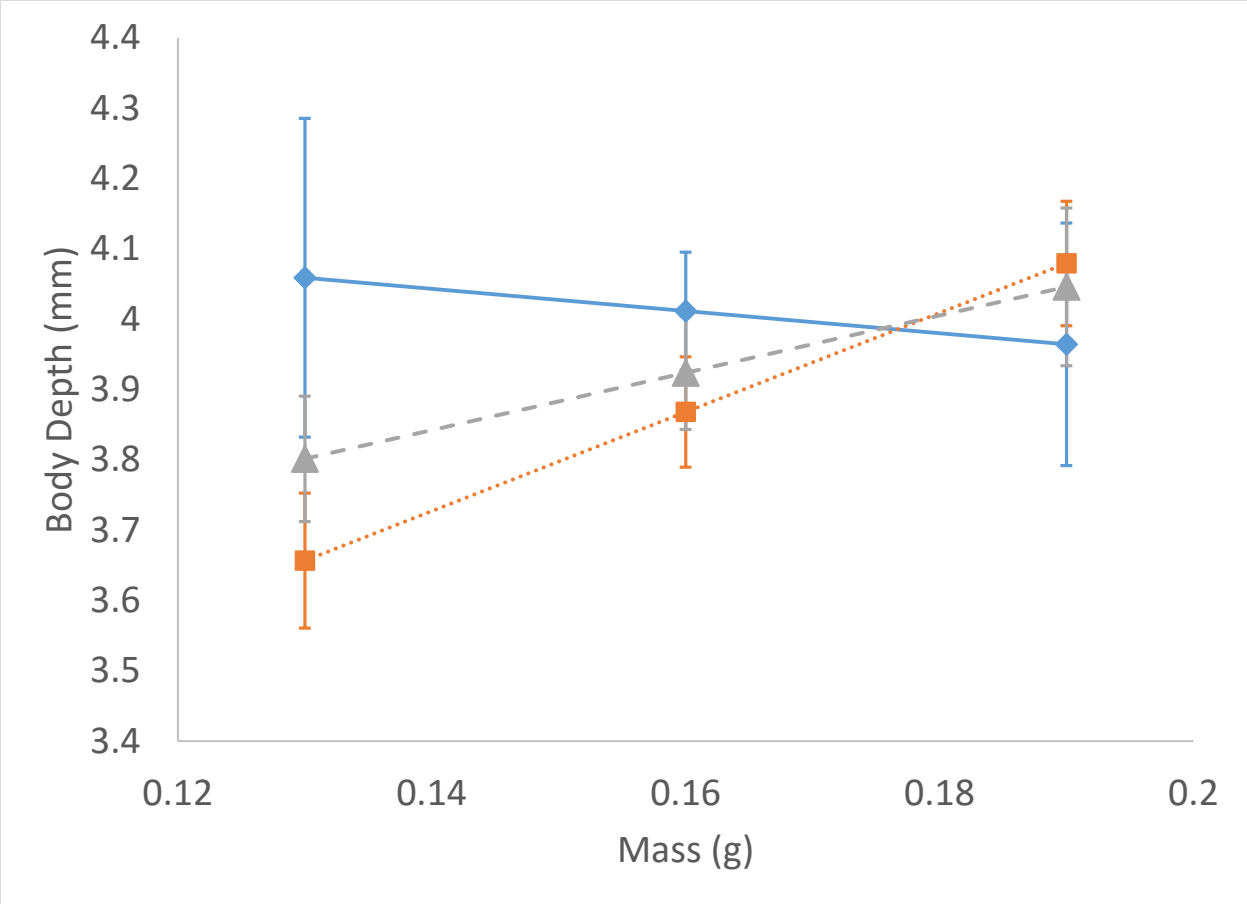

Figure 4. Mass compared to body depth of tadpoles two weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars represent one standard error of the mean.

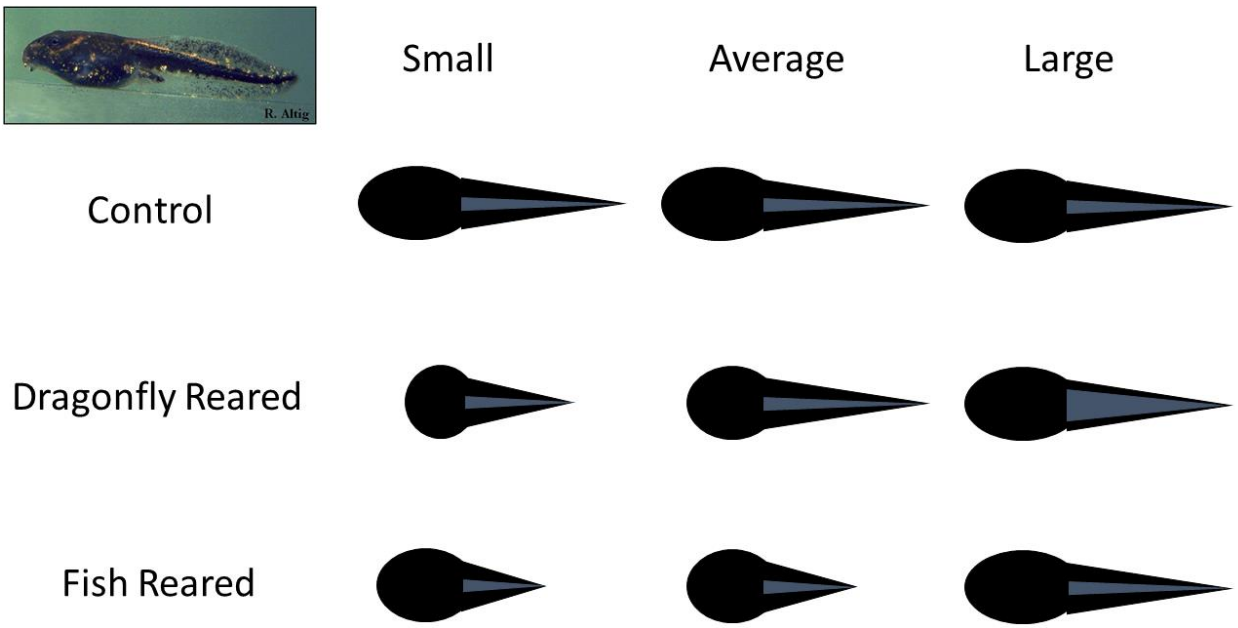

Figure 5. Summary of changes in morphology of tadpoles at four weeks after hatching from the lateral view. Treatments are listed along the y-axis and body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Black ovals represent the body, black triangles represent the tail fin, and gray triangles represent the tail musculature.

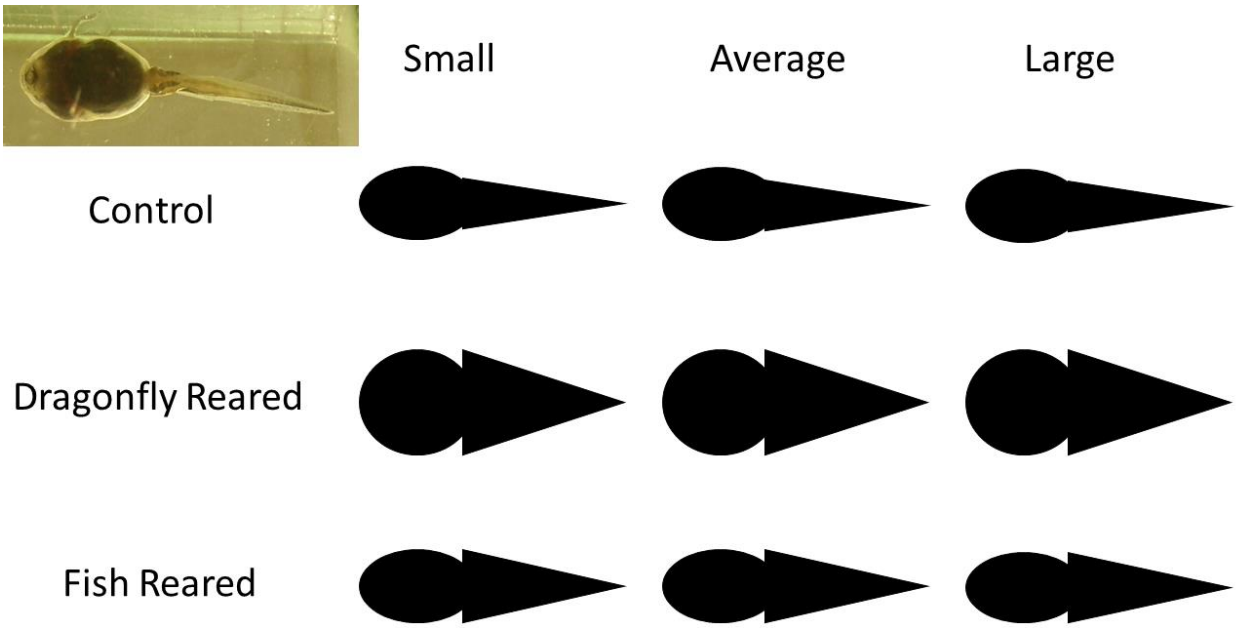

Figure 6. Summary of changes in morphology of tadpoles at four weeks after hatching from the ventral view. Treatments are listed along the y-axis and body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Black ovals represent the body and black triangles represent the tail.

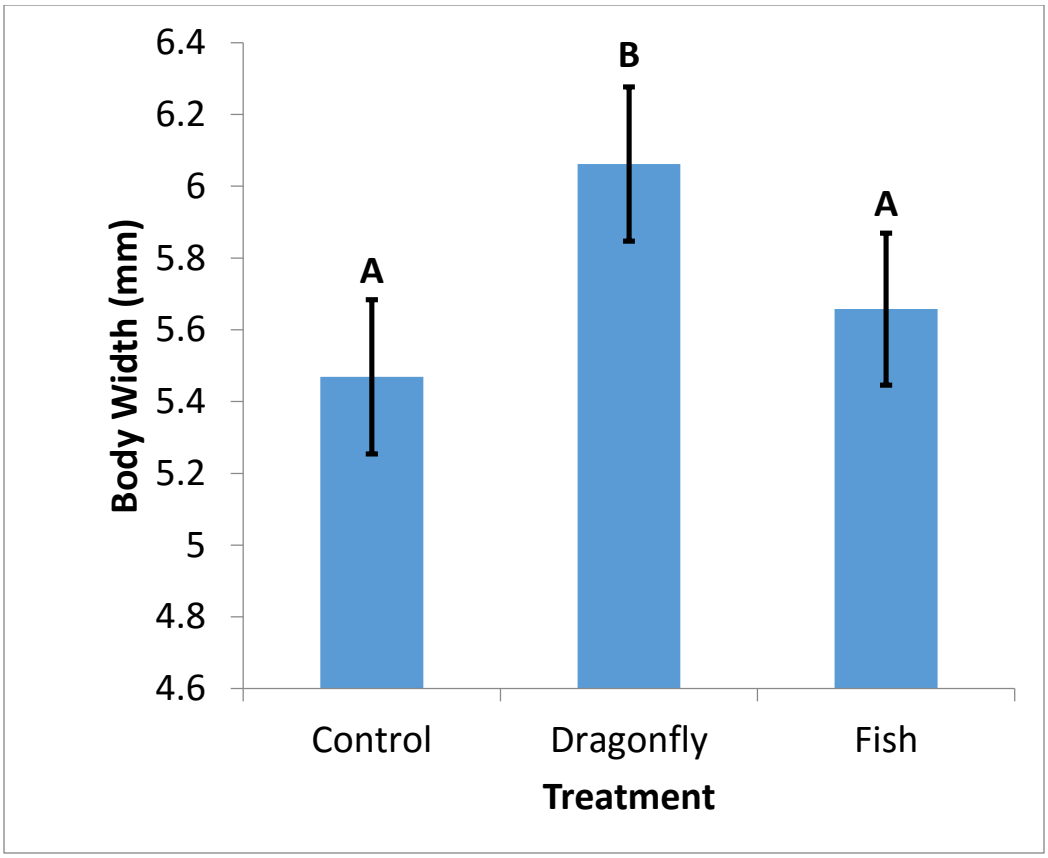

Figure 7. Tadpole body width at four weeks post hatching. Error bars represent one standard error of the mean. Different letters above the error bars indicate significant differences at the p = 0.05 level.

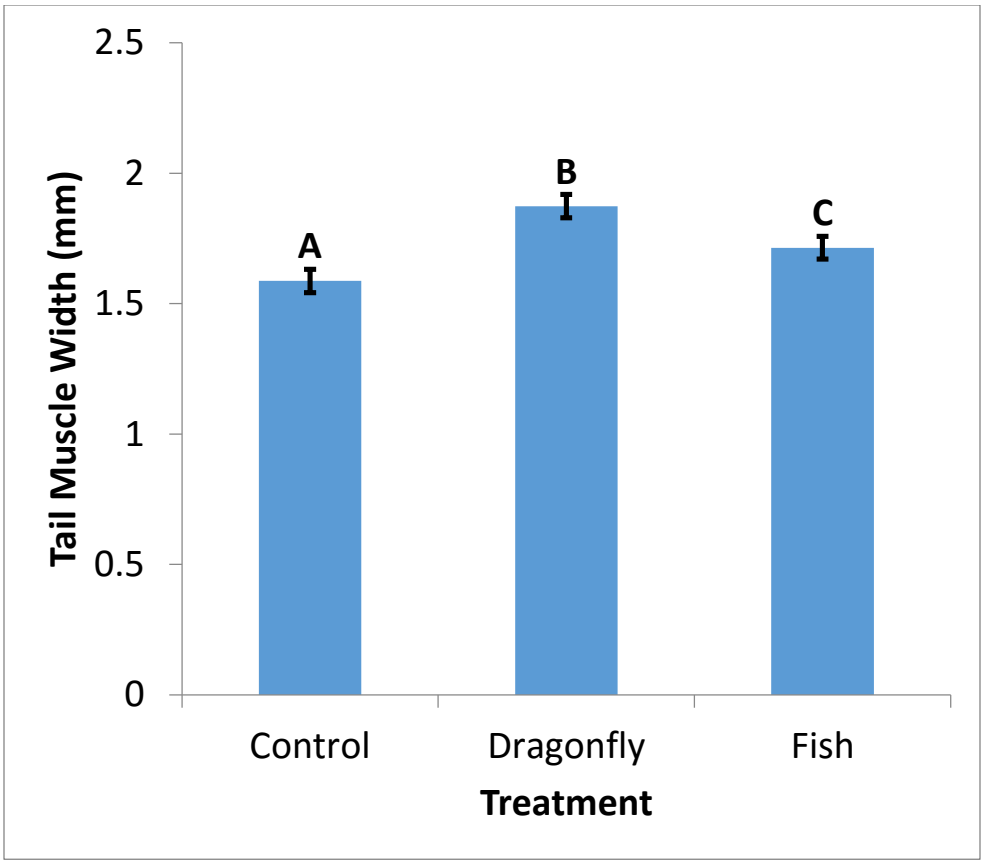

Figure 8. Tadpole tail muscle width at four weeks post hatching. Error bars represent one standard error of the mean. Different letters above the error bars indicate significant differences at the $p = 0.05$ level.

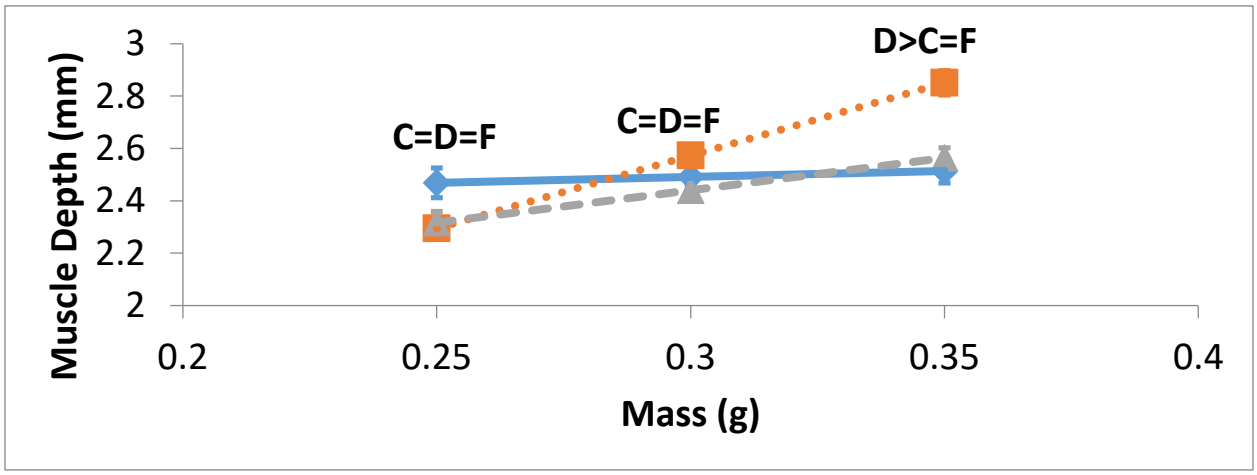

Figure 9. Mass compared to tail muscle depth of tadpoles four weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Letter C above the points indicates the control treatment, D indicates the dragonfly treatment, and F indicates the fish treatment. The < or > symbols between the letters indicate significant differences at the p = 0.05 level. Error bars indicate one standard error of the mean.

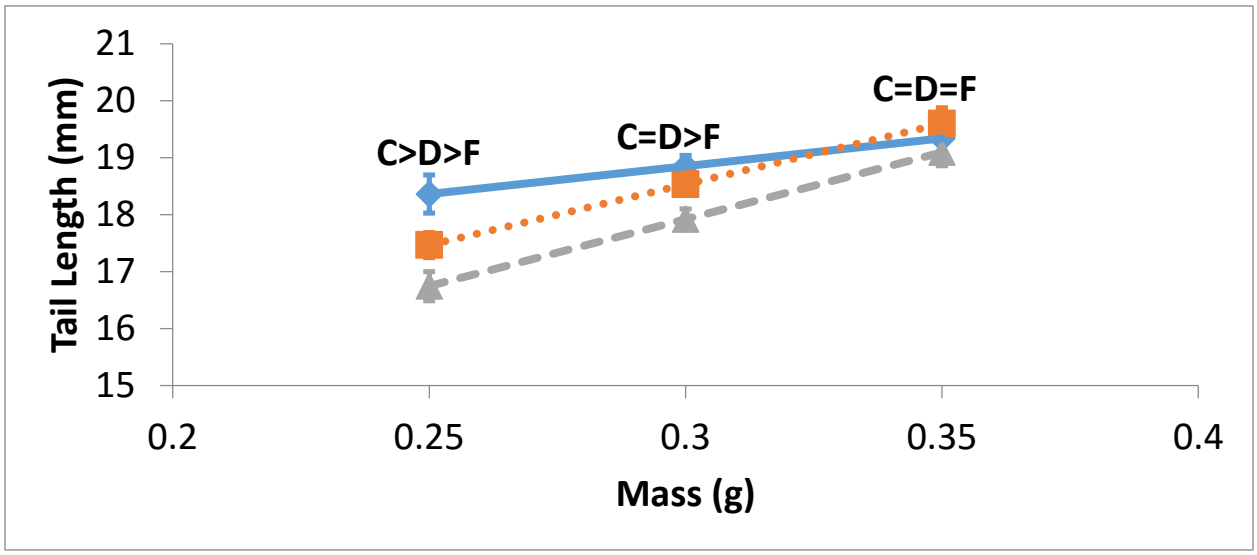

Figure 10. Mass compared to tail length of tadpoles four weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Letter C above the points indicates the control treatment, D indicates the dragonfly treatment, and F indicates the fish treatment. The < or > symbols between the letters indicate significant differences at the p = 0.05 level. Error bars indicate one standard error of the mean.

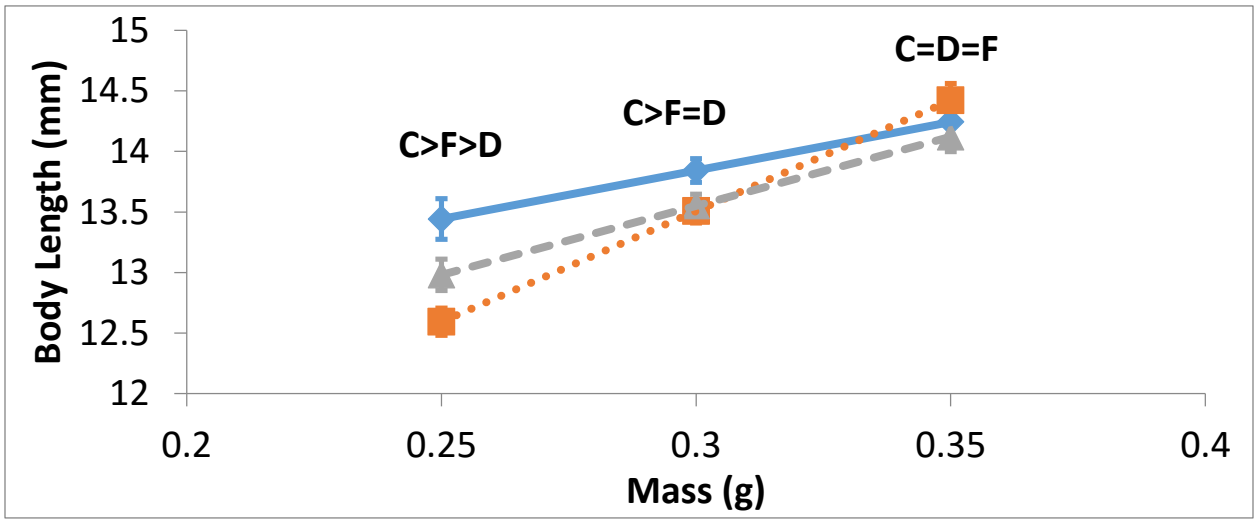

Figure 11. Mass compared to body length of tadpoles four weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Letter C above the points indicates the control treatment, D indicates the dragonfly treatment, and F indicates the fish treatment. The < or > symbols between the letters indicate significant differences at the p = 0.05 level. Error bars indicate one standard error of the mean.

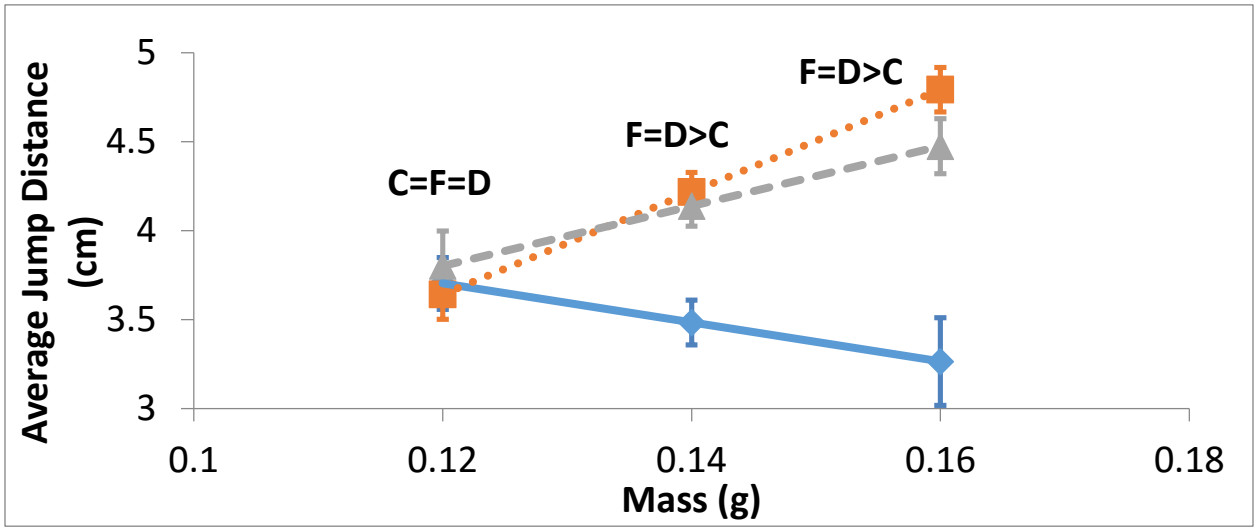

Figure 12. Mass compared to average jump distance of metamorphs 5-7 weeks post hatching. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Letter C above the points indicates the control treatment, D indicates the dragonfly treatment, and F indicates the fish treatment. The < or > symbols between the letters indicate significant differences at the p = 0.05 level. Error bars indicate one standard error of the mean.

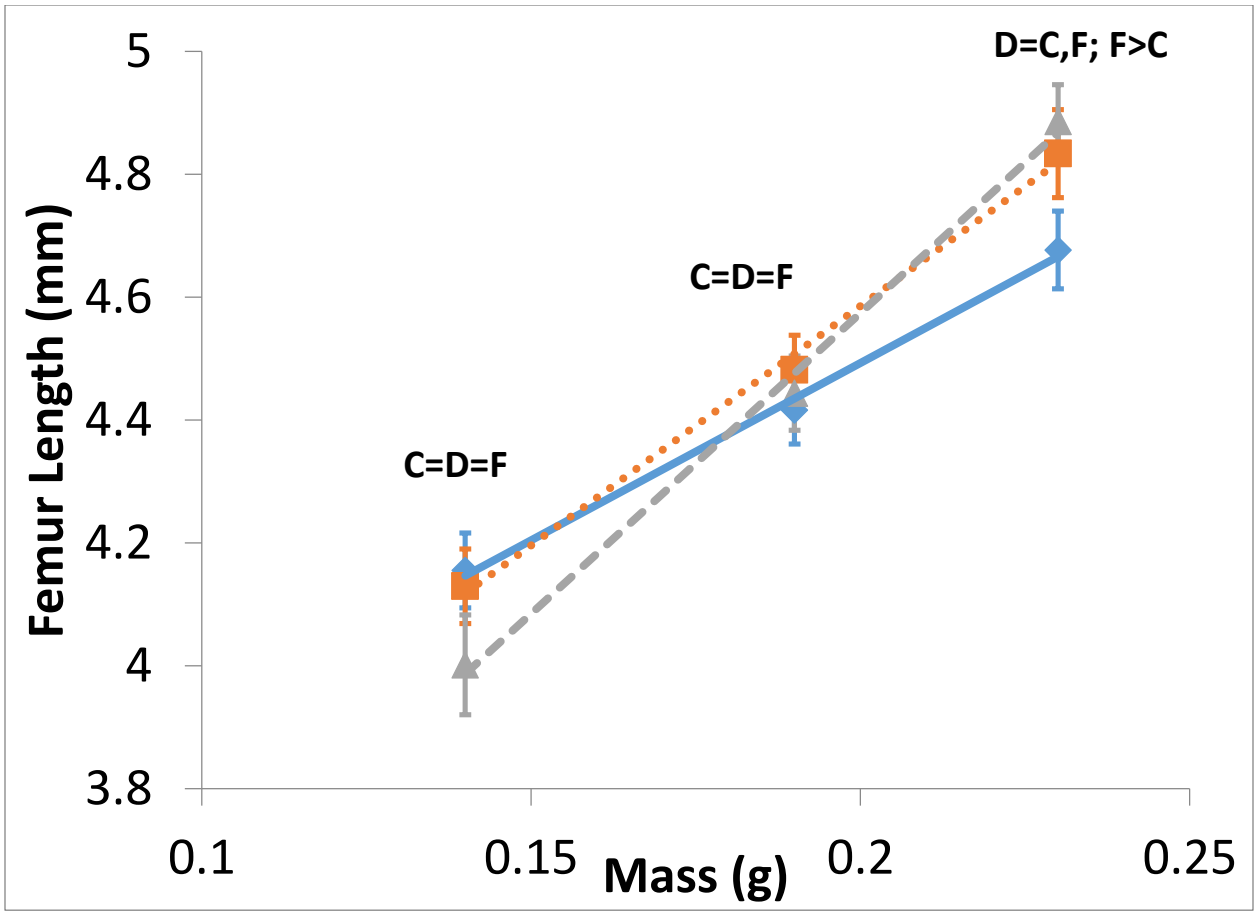

Figure 13. Mass compared to femur length of toads 8-10 weeks post hatching, 3-5 weeks post metamorphosis. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Letter C above the points indicates the control treatment, D indicates the dragonfly treatment, and F indicates the fish treatment. The < or > symbols between the letters indicate significant differences at the p = 0.05 level. Error bars indicate one standard error of the mean.

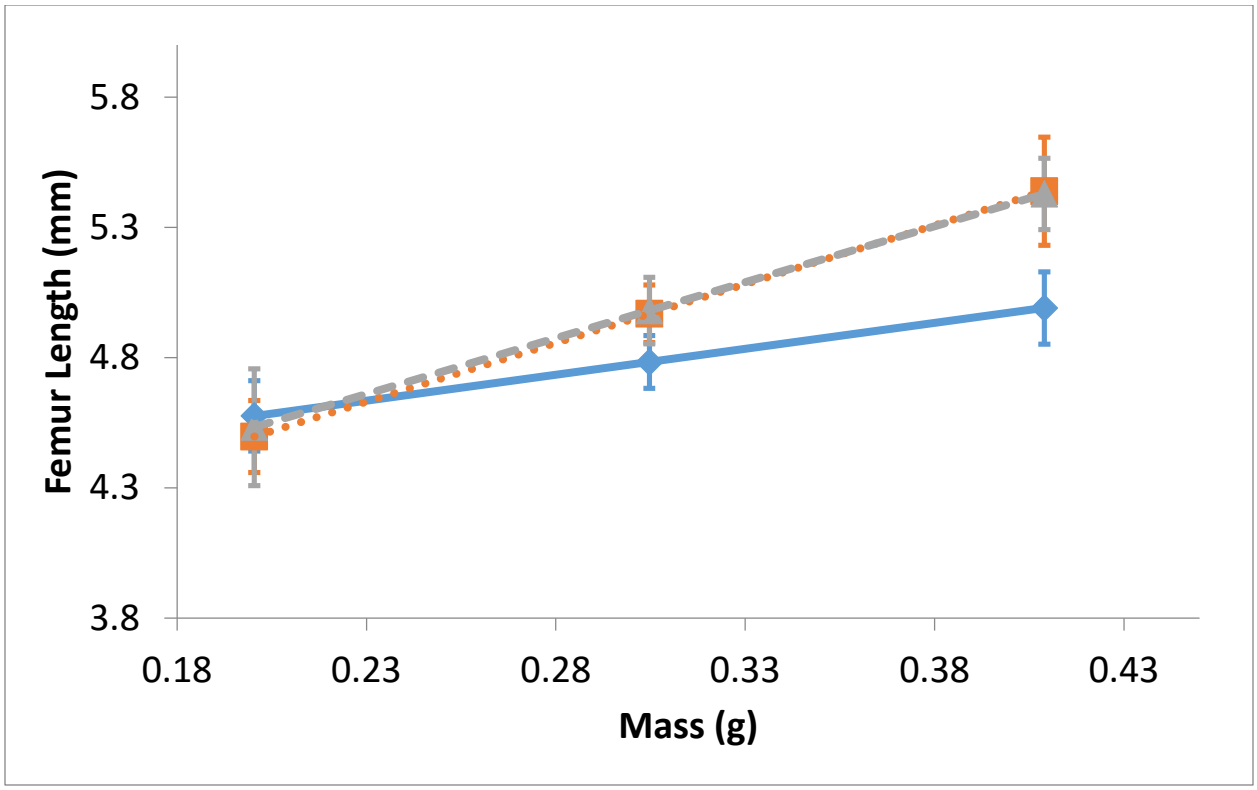

Figure 14. Mass compared to femur length of toads 10-12 weeks post hatching, 5-8 weeks post metamorphosis. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars indicate one standard error of the mean.

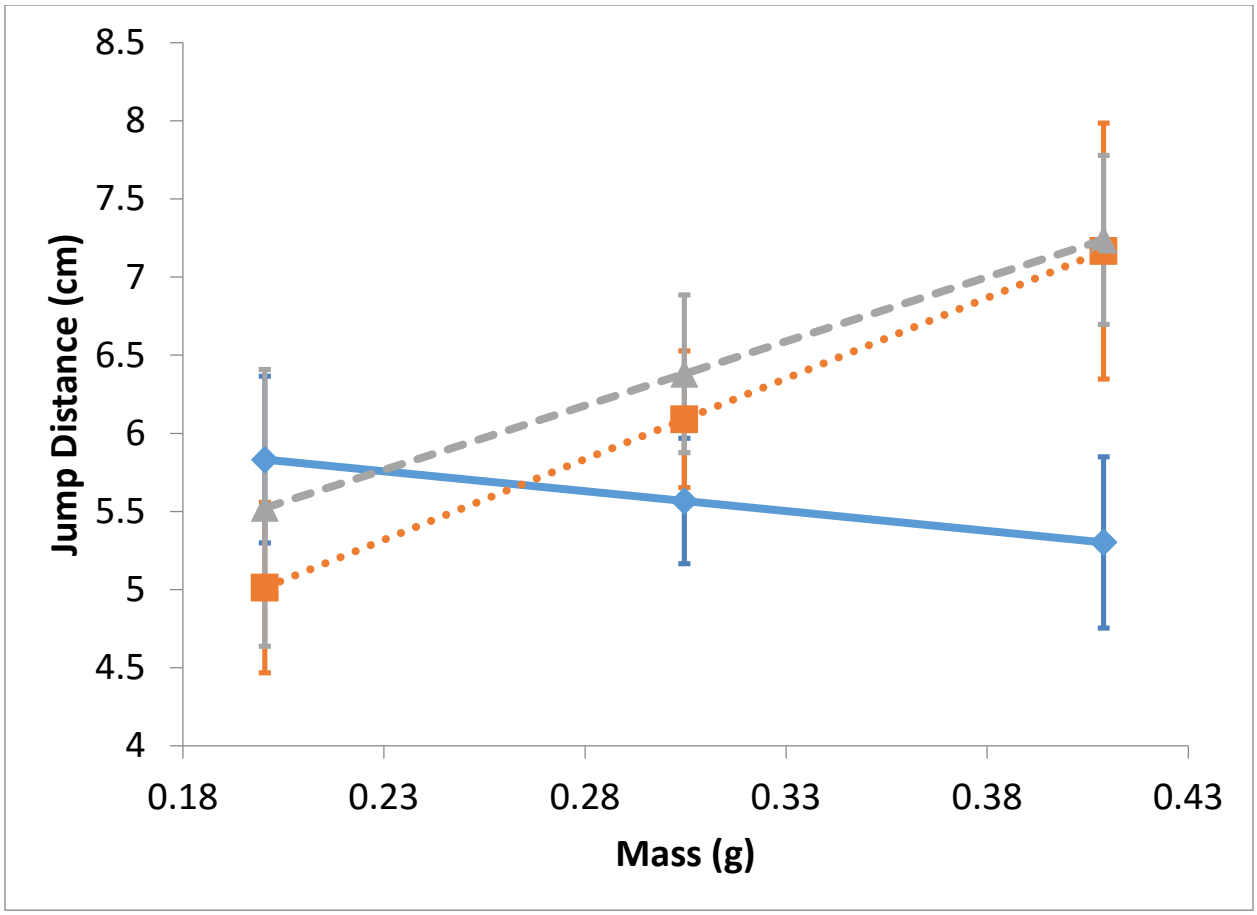

Figure 15. Mass compared to average jump distance of toads 10-12 weeks post hatching, 5-8 weeks post metamorphosis. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars indicate one standard error of the mean.

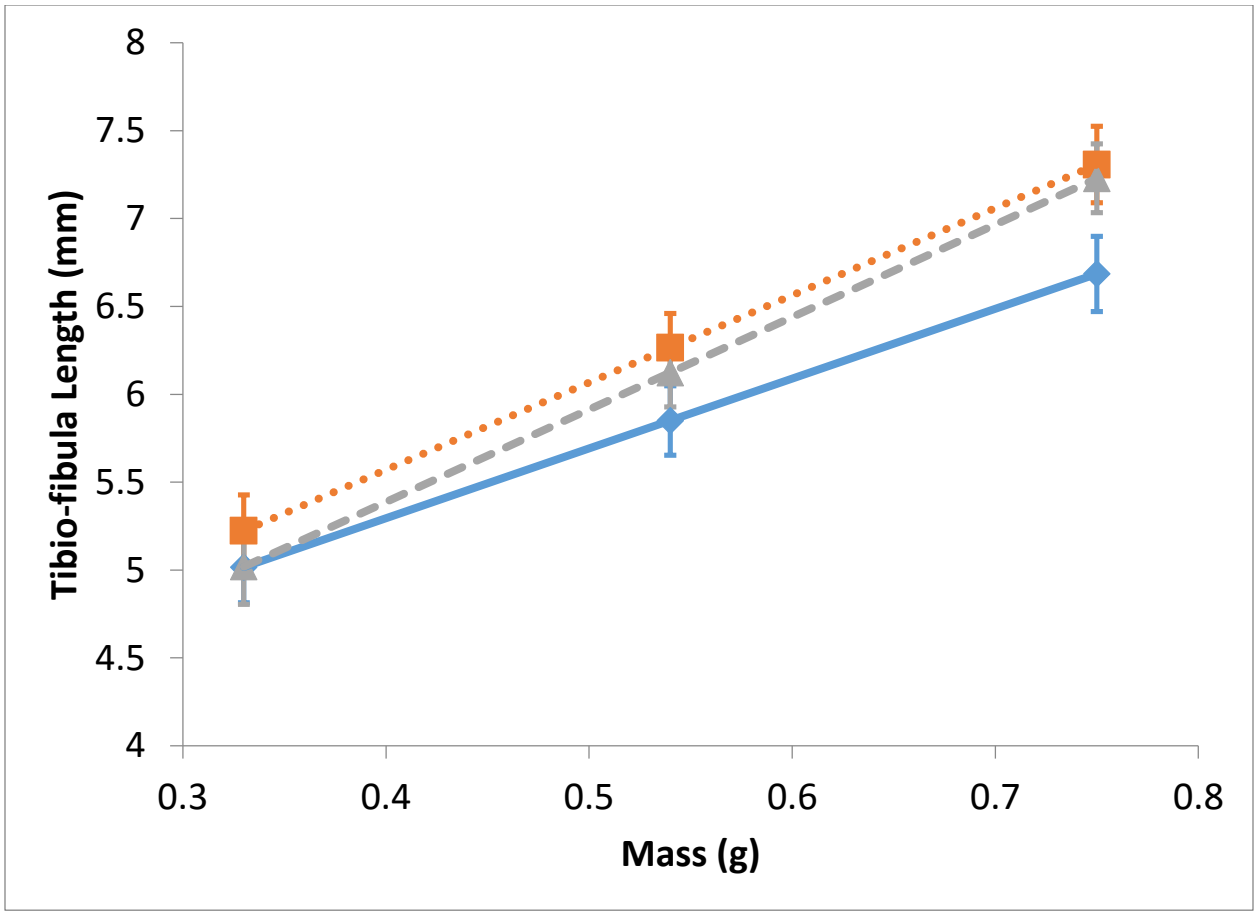

Figure 16. Mass compared to tibio-fibula length of toads 12-14 weeks post hatching, 8-11 weeks post metamorphosis. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars indicate one standard error of the mean.

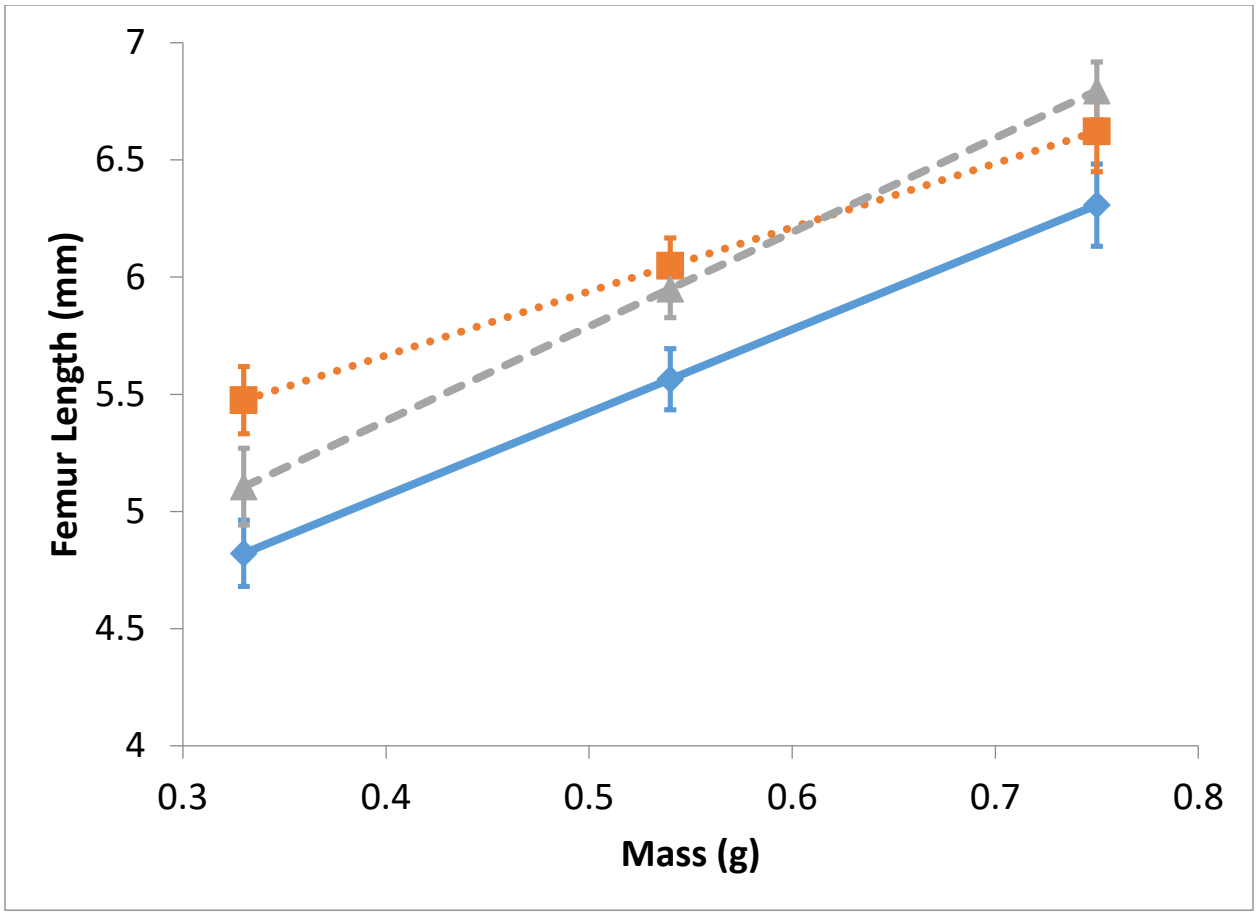

Figure 17. Mass compared to femur length of toads 12-14 weeks post hatching, 8-11 weeks post metamorphosis. The middle point represents the average mass, left point is minus 1 standard deviation, and the right point is plus 1 standard deviation. Blue line and points indicates the control treatment, red line and points indicates the dragonfly treatment, and the gray line and points indicates the fish treatment. Treatment effects were not statistically significant. Error bars indicate one standard error of the mean.

Table 1. Raw and adjusted p-values of planned contrasts for tadpoles at two weeks after hatching. Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.9713	0.3797	0.3980	0.9713	0.597	0.597
Tail Length	0.5467	0.3666	0.7359	0.7359	0.7359	0.7359
Body Length	0.3841	0.3917	0.9929	0.5876	0.5876	0.9929
Body Depth at 0.13 g	0.108	0.2758	0.1603	0.2405	0.2758	0.2405
Body Depth at 0.16 g	0.0979	0.3043	0.4549	0.2937	0.4549	0.4549
Body Depth at 0.19 g	0.4916	0.6265	0.7567	0.7567	0.7567	0.7567
Tail Muscle Depth	0.247	0.705	0.1492	0.3705	0.705	0.3705
Tail Fin Depth	0.3849	0.9376	0.4462	0.6693	0.9376	0.6693
Body Width at 0.13 g	0.0318	0.0415	0.6938	0.0623	0.0623	0.6938
Body Width at 0.16 g	0.2471	0.1401	0.5912	0.3707	0.3707	0.5912
Body Width at 0.19 g	0.045	0.1284	0.3064	0.135	0.1926	0.3064
Tail Muscle Width	0.1055	0.1912	0.7188	0.2868	0.2868	0.7188

Table 2. Raw and adjusted p-values of planned contrasts for tadpoles at four weeks after hatching. Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.2066	0.7728	0.3134	0.4701	0.7728	0.4701
Tail Length at 0.25 g	0.0457	0.0055	0.0449	0.0457	0.0165	0.0457
Tail Length at 0.30 g	0.1914	0.006	0.0309	0.1914	0.018	0.0464
Tail Length at 0.35 g	0.4468	0.4374	0.1483	0.4468	0.4468	0.4449
Body Length at 0.25 g	0.0029	0.0332	0.0256	0.0087	0.0332	0.0332
Body Length at 0.30 g	0.0179	0.0249	0.6627	0.0374	0.0374	0.6627
Body Length at 0.35 g	0.2685	0.3911	0.0746	0.3911	0.3911	0.2238
Body Depth	0.2347	0.6846	0.3933	0.59	0.6846	0.59
Tail Muscle Depth at 0.25 g	0.0433	0.0739	0.6976	0.1109	0.1109	0.6976
Tail Muscle Depth at 0.30 g	0.1015	0.2615	0.0215	0.1523	0.2615	0.0645
Tail Muscle Depth at 0.35 g	0.0027	0.4135	0.0042	0.0063	0.4135	0.0063
Tail Fin Depth	0.5595	0.6438	0.8887	0.8887	0.8887	0.8887
Body Width	0.0045	0.3357	0.0202	0.0135	0.3357	0.0303
Tail Muscle Width	<0.0001	0.0059	0.002	<0.0001	0.0059	0.003

Table 3. Raw and adjusted p-values of planned contrasts for Toads at metamorphosis (5-7 weeks after hatching). Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.3459	0.3391	0.9883	0.5189	0.5189	0.9883
Survival	.0023	0.3599	0.012	0.0069	0.3599	0.018
Survival with end of experiment tadpoles	0.0054	0.4909	0.019	0.0162	0.4909	0.0285
Average Emergence Time	0.1286	0.426	0.4169	0.3858	0.426	0.426
Cranial Width	0.0661	0.8614	0.0436	0.0992	0.8614	0.0992
Snout-Urostyle Length at 0.12 g	0.0204	0.8991	0.0333	0.05	0.8991	0.05
Snout-Urostyle Length at 0.14 g	0.0075	0.1327	0.0421	0.0225	0.1327	0.0632
Snout-Urostyle Length at 0.16 g	0.0583	0.1047	0.6211	0.1571	0.1571	0.6211
Femur Length at 0.12 g	0.0532	0.2943	0.3827	0.1596	0.3827	0.3827
Femur Length at 0.14 g	0.4839	0.493	0.1514	0.493	0.493	0.4542
Femur Length at 0.16 g	0.409	0.1529	0.292	0.409	0.409	0.409
Tibio-Fibula Length	0.7220	0.3711	0.2128	0.722	0.5567	0.5567
Average Jump Distance at 0.12 g	0.7411	0.6637	0.4894	0.7411	0.7411	0.7411
Average Jump Distance at 0.14 g	0.0029	0.0053	0.5459	0.008	0.008	0.5459
Average Jump Distance at 0.16 g	0.002	0.0054	0.1168	0.006	0.0081	0.1168

Table 4. Raw and adjusted p-values of planned contrasts for toads at 3-5 weeks after metamorphosis (8-10 weeks after hatching). Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.7668	0.4328	0.2985	0.7668	0.6492	0.6492
Cranial Width	0.5081	0.2201	0.5027	0.5081	0.5081	0.5081
Snout-Urostyle Length	0.6954	0.2014	0.3392	0.6954	0.5088	0.5088
Femur Length at 0.14 g	0.6065	0.1156	0.1766	0.6065	0.2649	0.2649
Femur Length at 0.19 g	0.124	0.5308	0.3881	0.372	0.5308	0.5308
Femur Length at 0.23 g	0.0597	0.0155	0.4334	0.0896	0.0465	0.4334
Tibio-Fibula Length	0.665	0.3745	0.6131	0.665	0.665	0.665
Average Jump Distance	0.346	0.1215	0.4314	0.4314	0.3645	0.4314

Table 5. Raw and adjusted p-values of planned contrasts for toads at 5-8 weeks after metamorphosis (10-12 weeks after hatching). Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.3802	0.4004	0.1222	0.4004	0.4004	0.3666
Survival	0.0934	0.7314	0.163	0.2445	0.7314	0.2445
Cranial Width	0.3827	0.4026	0.9804	0.6039	0.6039	0.9804
Snout-Urostyle Length	0.2171	0.7741	0.3765	0.5648	0.7741	0.5648
Femur Length at 0.20 g	0.7041	0.8782	0.8977	0.8977	0.8977	0.8977
Femur Length at 0.30 g	0.2871	0.2934	0.942	0.4401	0.4401	0.942
Femur Length at 0.41 g	0.1474	0.088	0.9702	0.2211	0.2211	0.9702
Tibio-Fibula Length	0.1781	0.2475	0.8976	0.3713	0.3713	0.8976
Average Jump Distance at 0.20 g	0.3436	0.7798	0.6499	0.7798	0.7798	0.7798
Average Jump Distance at 0.30 g	0.4281	0.276	0.6858	0.6422	0.6422	0.6858
Average Jump Distance at 0.41 g	0.1315	0.0656	0.9453	0.1973	0.1968	0.9453

Table 6. Raw and adjusted p-values of planned contrasts for toads at 8-11 weeks after metamorphosis (12-14 weeks after hatching). Treatment abbreviations are as follows: C = control, DF = dragonfly-reared, F = fish-reared.

Trait	C vs. DF Raw P	C vs. F Raw P	DF vs. F Raw P	C vs. DF Adjusted P	C vs. F Adjusted P	DF vs. F Adjusted P
Mass	0.7208	0.3123	0.1562	0.7208	0.4685	0.4685
Survival	0.8346	0.8252	0.9903	0.9903	0.9903	0.9903
Cranial Width	0.4939	0.265	0.5568	0.5568	0.5568	0.5568
Snout-Urostyle Length	0.1154	0.3957	0.3774	0.3462	0.3957	0.3957
Femur Length at 0.33 g	0.0248	0.1474	0.0861	0.0744	0.1474	0.1292
Femur Length at 0.54 g	0.0375	0.0476	0.3795	0.0714	0.0714	0.3795
Femur Length at 0.75 g	0.2554	0.0866	0.3061	0.3061	0.2598	0.3061
Tibio-Fibula Length at 0.33 g	0.1053	0.9789	0.1275	0.1913	0.9789	0.1913
Tibio-Fibula Length at 0.54 g	0.0278	0.0458	0.1593	0.0687	0.0687	0.1593
Tibio-Fibula Length at 0.75 g	0.0523	0.0377	0.4907	0.0785	0.0785	0.4907
Average Jump Distance at 0.33 g	0.7522	0.5052	0.3634	0.7522	0.7522	0.7522
Average Jump Distance at 0.54 g	0.3286	0.8524	0.3537	0.5306	0.8524	0.5306
Average Jump Distance at 0.75 g	0.2746	0.7259	0.1063	0.4119	0.7259	0.3189

Chapter 2: Fear, Competition, and Time: The interaction of predation, competition, and phenology on treefrog morphology and life-history.

Introduction:

Predators often cause prey to develop a different phenotype in nature than they would otherwise display if the predators were absent (Relyea 2002c, 2004, Benard 2004, Miner et al. 2005). *Daphnia pulex,* for example, develop neck teeth that make it harder for fish or invertebrate predators to swallow them (Tollrian 1995). In the presence of pike (*Esox lucius*) predators, crucian carp (*Carassius carassius*) develop deeper and more muscular bodies that both make it harder for the predator to swallow the carp and allow the carp to swim faster to better escape the predator (Domenici et al. 2008). Competitors can alter phenotypes as well.

Competitors have many effects on phenotype, such as tadpoles exhibiting longer digestive tracts that increase digestive efficiency at higher competitor densities (Relyea and Auld 2004). Competitor identity may also affect phenotypic plasticity, since interspecific competitors reduce the percentage of brook charr (*Salvelinus fontinalis*) that are benthic specialist feeders and increase the percentage of brook charr that are pelagic specialist or generalist feeders (Bourke et al. 1999). Competitors could also have different effects depending on their age because older competitors may be better able to monopolize shared resources through larger size or higher efficiency than younger competitors. One study on stocked salmon found that older intraspecific competitors reduced growth and survival (Kennedy and Strange 1986). Responding to competitors may alter how organisms respond to predators.

There may be trade-offs to responding to both competitors and predators. Wood frog tadpoles (*Lithobates sylvaticus*) develop longer bodies and shorter tails with competitors but shorter bodies and longer tails with predators (Relyea 2002c, 2004). Predator and competitor effects may also be interactive. Wood frog tadpoles developed shorter oral discs when competitor density was low and predator density was high, and larger oral discs when competitor density was high but predator density was low (Relyea and Auld 2005). Since prey respond to both competitors and predators, different ages and types of competitors may change how prey respond to a predator.

Since different species of competitors can alter the phenotype of a focal species differently and different aged competitors may differ in their traits, the combination of these two types of competition could alter the effects of a predator on prey. We sought to address this combination of factors since natural systems often contain multiple prey species that compete to some extent, multiple age classes of these prey species, and a predator. Phenotypic plasticity has been found to allow organisms to tailor their response to various combinations of predators and intraspecific competitors (Relyea 2004), so it is also likely to allow organisms to tailor their response to different species and age classes of competitors in conjunction with predators.

We chose to use larval amphibians to examine these phenomena as previous work has shown that they respond differently to different competitors (Relyea 2002c) and that this alters their response to predators (Relyea 2004). Anurans also breed multiple times over a breeding season and many species co-occur and this could result in different combinations of competitors that could alter the response to a predator. We were specifically interested in determining if the interaction between age and identity of competitors alters the response of anurans to a predator as this has not been tested.

We hypothesized 1) that predators would induce shorter, shallower, and wider bodies and longer and deeper tails in focal tadpoles, and smaller metamorphs with longer legs as these are typical anti-predator responses in anurans (Relyea 2001b, 2004), 2) competitors would induce opposite changes from those of predators with longer, deeper, and narrower bodies in tadpoles and shorter and narrower tails, and larger metamorphs with shorter legs (Relyea 2002c), 3) competitor age and identity would alter the response of tadpoles and metamorphs to the predator because competitors would use up the resources needed to respond to the predator. We expected that older intraspecific competitors would alter the response of focal tadpoles and metamorphs to predators more than older interspecific competitors or younger competitors of the same or another species because older intraspecific competitors would be the most similar in their resource use and have a competitive advantage, reducing the resources the focal tadpoles have to respond to the predator. Younger intraspecific competitors would have the second largest effect on the response to the predator as they would still have a very similar resource use, but lack the age and size competitive advantage. Interspecific competitors would have a weaker impact on focal tadpoles because their resource use would overlap less, but older interspecific competitors would have a stronger impact than younger interspecific competitors because the older interspecific competitors would have an age and size competitive advantage.

Methods:

To address our hypotheses, we conducted an experiment in artificial ponds consisting of 1100 L Rubbermaid cattle watering tanks. We used artificial ponds for our experiment because they allow more control than field enclosures but more realism than a laboratory experiment and the processes that are important in natural ponds are also important in artificial ponds (Wilbur

1987, Morin 1998, Chalcraft et al. 2005). We used the pinewoods treefrog (*Hyla femoralis*) as our focal species in this experiment to measure its response to predators, competitors, and the interaction of competitors and predators. We used this species as it is known to change its morphology and life history in response to predators (LaFiandra and Babbitt 2004), and competitors alter the response to predators, such as tadpoles raised at a low density of intraspecific competitors developing longer tails than tadpoles raised with predators and a higher density of intraspecific competitors (McCoy 2007). This species also breeds multiple times over the summer, so there can be multiple age classes of this species within a pond (Lannoo 2005). We used Cope's gray treefrog (*Hyla chrysoscelis)* as our interspecific competitor because this species often co-occurs with pinewoods treefrogs (Lannoo 2005) and they have a protracted breeding season which allows for individuals to breed at different times (Lannoo 2005), producing different aged cohorts. Our predators were larval aeshnid dragonflies (*Anax* sp.) which co-occur with both pinewoods and gray treefrogs and are known to induce anti-predator behaviors and responses in treefrog species (Relyea 2001a, Van Buskirk et al. 2003).

To assess how the presence, age, and identity of competitors alter the impacts of predators on the phenotype of their prey, we raised 50 pinewoods treefrogs in the presence/absence of a caged larval dragonfly predator in five different environments that varied in the kind of competitor present: 1) no additional competitors (i.e., low density of pinewoods treefrogs) to serve as a control, 2) 50 additional pinewoods tadpoles (i.e., high density of pinewoods treefrogs) of the same age to examine the effects of increasing competitor density, 3) adding 50 gray treefrogs that are the same age as the pinewoods treefrogs to examine if competitor identity matters, 4) adding 50 older pinewoods to examine the effects of competitor age, and 5) adding 50 older grays to see if competitor age and identity interact. The resulting ten

treatments and their corresponding abbreviations are listed in Table 7. We used caged predators because we wanted to examine morphological and life-history changes due to the presence of the predator without the predator being able to alter density and thereby the strength of competition. Experimental tanks were arranged in five spatial blocks. Each block contained ten tanks, corresponding to the ten treatments. This resulted in a total of 50 experimental tanks, with each of the ten treatments randomly assigned to one tank within each of the five blocks.

All manipulations to artificial ponds were carried out on a block by block basis. Artificial ponds were filled with well water on May 13, 2014 and a kilogram of pine litter was added to each tank on May 13-14 to provide cover for tadpoles and a medium for algal growth. On May 16, we inoculated each tank with one pint of pond water with concentrated zooplankton and phytoplankton to provide more realism and the basis for the pond food web within the tanks. Artificial ponds were covered with fiberglass screens to prevent organisms from entering or leaving the tanks. We collected pairs of pinewoods treefrogs (*Hyla femoralis*) and Cope's gray treefrogs (*Hyla chrysoscelis*) in amplexus from a wetland in Pitt County, North Carolina on June 12, 2014 and again on June 27, 2014. Frogs were taken back to the laboratory at East Carolina University, allowed to finish laying eggs, and then were returned to their place of capture within 36 hours. Tadpoles were counted 1 week after hatching. Tadpoles collected on June 12 were used for the older cohort and were counted on 6/19/2014 and added to holding tanks at ECU's West Research Campus. The older tadpoles were held in the holding tanks for 2 weeks until the younger cohort of tadpoles was ready to be counted on July 4, 2014. For all experimental tadpoles, we used 15 clutches for the focal pinewoods and 4 clutches for the gray treefrogs. Each experimental tank received an equal mix of tadpoles from the 15 clutches for pinewoods. For the four treatments that required gray treefrogs, we used an equal mix of tadpoles from four gray

treefrog clutches. For brevity, we will refer to pinewoods treefrogs as pinewoods and Cope's gray treefrogs as grays throughout the rest of the paper. Focal pinewoods, same age grays, and older grays were all added directly to their corresponding tanks after being counted. Older pinewoods were marked with visible implant elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, WA) in the tail musculature at the base of the tail to differentiate them from the younger cohort of pinewoods, then housed in the lab for at least 12 hours to assess mortality and mark retention before they were added to the tanks for the two older pinewoods treatments. Elastomer tags have been found to be retained well in amphibians, with up to 79.9% retention among individuals even after metamorphosis (Grant 2008, Johnson et al. 2009). All tadpoles were added to the tanks on the same day. Dragonflies were collected from the wild from the Croatan National Forest in Craven County, NC and were weighed and added to the appropriate tanks two days after tadpoles were added. Predators were fed younger pinewoods tadpoles that were collected on June 27, but these feeder tadpoles were housed in separate tanks. The dragonflies were fed 4 tadpoles every 3 days.

We checked the tanks daily after tadpoles were added. We weighed and photographed the focal tadpoles three weeks after they were added to the tanks. We checked pinewoods tadpoles for elastomer marks to determine their cohort. Tadpoles were anesthetized with MS-222 to reduce stress and ensure that tadpoles did not move during photography. We photographed tadpoles in water with a photochamber that used three mirrors to allow us to get a picture of the lateral and ventral view at the same time. We had scales for both the lateral and ventral views to allow for image analysis in the computer program ImageJ (Schneider et al. 2012). Tadpoles were returned to their corresponding experimental tank after being photographed.

Photographs of tadpoles were used to perform image analysis and measure several aspects of tadpole morphology in the program ImageJ. We measured 15 traits: eye width, body length, body depth, length from tail origin to the end of pigment on the tail, tail length from the lateral view, maximum tail fin height, tail muscle height at base of the tail, tail stripe height at the base of the tail, mouth width, distance between the eyes, body width in front of the spiracle, body width behind the spiracle, gut coil length, tail width at the base of the tail, and tail length from the ventral view. We included eye width because it is possible that tadpoles may develop wider eyes to better see predators.

We removed metamorphs (individuals with at least one forelimb) and took them back to the lab where they completed tail resorption. We terminated the experiment when no tadpoles metamorphosed from any tank for two consecutive days. At the end of the experiment, we collected, identified, weighed, and counted the remaining tadpoles. All tadpoles were then returned to their site of capture. Metamorphs were weighed once they completed tail resorption. We calculated the average time of emergence in days for each tank. We also calculated survival as the proportion of individuals surviving within a cohort individually (i.e. focal pinewoods in the same age interspecific competition tanks) and for all animals within a tank (i.e. focal pinewoods and grays combined for the same age interspecific competition tanks). Metamorphs from the older pinewoods treatments were checked with a UV light upon return to the lab after capture and again after metamorphosis was complete to look for elastomer marks to determine if an individual was from an early (focal individuals) or later cohort. Retention of elastomer marks is not 100%, so metamorphs that emerged from the older pinewoods treatments before the other treatments produced focal metamorphs were counted as older metamorphs even if the mark was not visible. We measured the cranial width, snout-urostyle length, femur length, and tibio-fibula

length of focal individuals and tested their jumping ability. We tested jumping ability by inducing frogs to jump, recording where they landed, and taking the average of three jumps (John-Alder and Morin 1990). After being measured, metamorphs were returned to where their parents were captured. Remaining tadpoles and metamorphs from the holding tanks were also returned to where their parents were captured.

Statistical Methods:

All analyses were performed in SAS Enterprise Guide 6.1, for the SAS software, version 9.4 of the SAS System for Windows. Copyright © 2013 SAS Institute Inc, Cary, NC, USA. We used linear mixed models for all of the traits except for survival. Survival was analyzed with a generalized linear mixed model using an expected binomial distribution. We used an expected binomial distribution as this is a common pattern for survival data. All models included block as a random effect and treatment as a main effect. Morphological responses of tadpoles and metamorphs and the jumping ability of metamorphs were analyzed with linear mixed models that also included the effect of mass and the interaction between mass and treatment as covariates. We included mass in the model as morphological traits and jumping ability are known to increase with mass. We included the interaction between mass and treatment in the model to determine whether the allometric relationship between trait values and body mass varied among treatments. We used the geometric mean to summarize the average size of individuals within a tank because mass of individuals within a tank often follow a lognormal distribution and the geometric mean fits a lognormal distribution better than the arithmetic mean. Mass and the interaction between mass and treatment were retained in the model if the *p*-value of that component was less than or equal to 0.3. A *p*-value threshold of 0.3 ensured that we did not

exclude a term from the model that was still having a large effect on our model even though it was not statistically significant. We chose a high threshold to be conservative in our assessment that allometric relationships varied among treatments because visual inspection of scatterplots suggested that the allometric relationship differed substantially among treatments. When we retained the interaction term, it meant that allometries differed, so we looked at the response variables at 3 sizes: the average size, one standard deviation above, and one standard deviation below the average size of the tadpoles to get an idea of how the response differed with mass. If the mass by treatment interaction was above 0.3, the model was rerun without the interaction but with mass retained as a covariate. In all cases mass was retained in the model when the interaction was excluded because mass was still significant ($p < 0.3$).

We analyzed data for each cohort separately because we expected species specific differences in mass and time to metamorphosis for grays and for older pinewoods and grays to respond differently than the younger cohorts since they had more time to develop and were housed at a higher density before being added to the experimental tanks. Each response variable was analyzed with planned contrasts to evaluate specific hypotheses. As some treatment comparisons were not meaningful, we chose to conserve statistical power by only using the contrasts that made comparisons we felt were biologically meaningful. We ended up with 25 contrasts (Table 8). Contrasts 1, 2, 3, and 4 compared the various competitor only environments to the control and contrasts 5, 6, 7, 8 compared the effects of the different competitors to each other (Table 8). Contrasts 9, 10, 11, 12, and 13 looked at how the addition of a predator affected focal tadpoles in each competitive environment relative to environments without predators (Table 8). Contrasts 14, 15, 16, 17, 18, 19, 20, and 21 compared the impacts of the predator across the different competitor environments (Table 8). Contrasts 22, 23, 24, and 25 examined if

the effects of predators and competitors were additive for the four competitor regimes where there were 100 tadpoles present (Table 8). Effects were additive and followed the expected pattern if $p > 0.05$, but if p was less 0.05 then the effects were not additive and deviated from the expected pattern, and we discuss these effects. When the mass by treatment interaction was retained in a model, we examined treatment differences with planned contrasts at all three tested masses. For non-focal metamorph analyses, we used only the contrasts pertaining to the tanks that contained that group of metamorphs (i.e. only contrasts comparing tanks with older pinewoods with and without a predator).

Wild *Pantala* sp. (a dragonfly) and squirrel treefrogs (*Hyla squirella*) colonized some of our experimental replicates. We excluded these tanks from analyses because the density of tadpoles in these tanks was not comparable to uncolonized tanks due to dragonfly larvae eating experimental tadpoles or the increase in density due to the addition of squirrel treefrog tadpoles. This resulted in our initial five replicates being reduced to four in most cases. Both the same age and older gray treefrog treatments without a predator were reduced to 3 replicates. The same age grays with a predator treatment was not contaminated.

Results:

Tadpoles: Tadpole mass was not statistically different across the treatments ($F_{9, 25} = 0.54$, $p = 0.8337$, contrast $p \geq 0.1297$, Supplemental Table S7, Table 9). Despite this, adding competitors reduced focal tadpole mass, but the particular effect depended on the age and identity of the competitor added - older pinewoods had the weakest effect (28% reduction), followed by same age pinewoods (48%), same age grays (60%), and older grays had the strongest effect on mass (111%, Fig. 18). Adding a predator decreased tadpole mass by 50% at the control density, but

competitors altered the predator effect. Species identity and age mattered since same age pinewoods and older grays caused predators to increase mass by at least 39% (pinewoods had a stronger effect, 45%), older pinewoods had little effect (15%), and same age grays decreased mass by 25%.

In general, larger tadpoles had larger morphological traits (i.e., a bigger tadpole had a longer tail than a smaller tadpole) ($p \leq 0.0021$) and the allometry of this relationship varied across treatments for all traits ($p \leq 0.1738$, Supplemental Table S7). Competitors alone had weak effects on tadpole morphology in general, but same age grays had size dependent effects on body depth, tail muscle depth, tail length, mouth width, and gut coil length (Fig. 19, 20). Adding more same age pinewoods, older pinewoods, or older grays increased mouth width for at least one body size (Fig. 20). Adding more same age pinewoods also increased gut coil length at the large mass (Fig. 20). Adding predators generally decreased tail muscle width at the small mass, increased tail length at the average and large mass, and increased tail muscle depth and width at the large mass (Fig. 21). All competitor types altered the effects of predators, and these effects were size dependent (Fig. 22). Older pinewoods had the strongest interacting effects with predators, inducing very large traits in small tadpoles and to a lesser degree average sized tadpoles. In contrast, older pinewoods interacted with predators to induce small traits in large tadpoles (Fig. 22).

Competitor only treatments had weak effects on eye width at any mass (% difference ≤ 11.0%, contrast $p \geq 0.4298$, Table 9, Fig. 24). Adding a predator had a weak effect on eye width at the control density at any mass (% difference ≤ 10.0%, contrast $p \geq 0.4467$, Table 9, Fig. 25). Adding a predator with older pinewoods induced approximately 26% wider eyes at the small mass (contrast $p = 0.0241$), but 11% narrower eyes at the average mass (contrast $p = 0.0734$),

and had little effect (2%) at the large mass (contrast $p = 0.7753$, Table 9, Fig. 25). Adding a predator with same age gray treefrogs did not affect eye width at the small mass (2% difference, contrast $p = 0.9424$), but increased eye width by at least 10% at the average and large mass, but these effects did not significantly differ from zero (contrast $p = 0.1334$ average, 0.1958 large, Table 9, Fig. 25). Adding a predator with same age pinewoods or older grays had little effect at any mass (% difference ≤ 7%, contrast $p \geq 0.4795$, Table 9, Fig. 25). Older competitors altered the impact of the predator on eye width more than same age competitors at the small mass (% difference ≥ 13%), but older competitor effects on the predator got weaker with increasing mass (18% down to 2% difference) and same age competitor effects got stronger as mass increased (≤5% to ≥13%, Fig. 26).

Body length showed significant differences across the treatments ($F_{9, 15} = 3.91$, $p = 0.0099$, Supplemental Table S7). In the absence of predators, the only competitor that had an important effect (% difference ≥ 10%) on body length of tadpoles were gray treefrogs of the same age as the focal prey, and the magnitude of their effect appeared to depend on the body size of focal prey (Fig. 27). Same age gray treefrogs had a very weak effect on body length of the average sized focal prey but induced small focal prey to develop 10-11% longer bodies and induced large focal prey to have 11% shorter bodies (Fig. 27). None of the effects of same age gray treefrogs were significantly different from zero ($p \geq 0.508$, Table 9). Predators had a weak effect on body length (% difference <10%) when no additional competitors of focal prey were present, when older grays were present, or same age pinewoods were present ($p \geq 0.4888$, Fig. 28, Table 9). Adding a predator with older pinewoods and same aged grays had size-dependent effects on the body length of focal prey, but the effects of older pinewoods and same age grays were not the same (Fig. 28, 29). Adding a predator with older pinewoods induced 44% longer

bodies in focal tadpoles at the small mass ($p = 0.001$), had little effect at the average mass (7%, $p = 0.2863$), and induced 27% shorter bodies at the large mass ($p = 0.0033$, Fig. 28, Table 9). Adding a predator with same age grays in contrast, induced 11% shorter bodies in focal tadpoles at the small mass, had little effect at the average mass (1%), and induced 10% longer bodies at the large mass, but none of these effects significantly differed from zero ($p \geq 0.4954$, Fig. 28, 29, Table 9). The effects of older pinewoods competitors and predators were not additive at the small mass ($p = 0.0269$) or large mass ($p = 0.0345$), as the effects were larger than expected (Table 9, Fig. 28, 29).

Body depth responded differently in the different treatments, but followed a similar pattern to body length ($F_{9, 15} = 4.72$, $p = 0.0042$, Supplemental Table S7, Fig. 27-29 for body length). As above in the absence of predators, only same age gray competitors had much of an impact on body depth (≥ 10% difference), but none of these effects were statistically significant ($p \geq 0.0544$, Table 9). Same age gray competitors induced at least 24% deeper bodies at the small mass, had little effect at the average mass, and induced at least 31% shallower bodies in focal prey at the large mass. One difference from body length was that at the small mass in the absence of a predator, same age pinewoods induced 11% shallower bodies in focal prey, but the effect did not significantly differ from zero and there were no other effects of same age pinewoods on body depth ($p \geq 0.6014$, Table 9). Adding a predator had little effect on body depth of focal prey at the control density, with older grays, or with same age pinewoods (% difference ≤ 9%, $p \geq 0.4818$, Table 9). At the small mass, adding predators induced 48% deeper bodies with older pinewoods competitors ($p = 0.0005$) and 28% shallower bodies with same age gray competitors, but the effect of the grays was not statistically different ($p = 0.2383$, Table 9). At the average mass, predators induced approximately 10% deeper bodies with older pinewoods

competitors, but the effect was not statistically different ($p = 0.1561$), and same age grays had little effect on body depth (% difference <1%, $p = 0.9344$, Table 9). At the large mass, predators induced 27% shallower bodies in tadpoles reared with older pinewoods ($p = 0.0047$) and 26% deeper bodies in tadpoles reared with same age grays, but the effect of adding a predator with grays on focal animals did not differ from zero ($p = 0.141$, Table 9). Older pinewoods and older grays both impacted the effect of the predator, but older grays only did so at the large mass by increasing body depth by approximately 10%, and this effect did not differ from zero ($p = 0.3448$). Older pinewoods induced significantly deeper bodies at the small size ($p \leq 0.0098$, $\geq$ 39% difference), converged at the average size (% difference $\leq$ 9%, $p \geq 0.1693$), and induced shallower bodies at the large size ($p \leq 0.0549$, % difference $\geq$ 21%, Table 9). Older pinewoods had non-additive effects with predators at the small mass, inducing deeper bodies than if competitor and predator effects were additive ($p = 0.0185$, Table 9).

The length of the tail from the origin on the head to the end of the pigment near the tip of the tail responded differently in the different treatments ($F_{9, 15} = 3.91$, $p = 0.0099$, Supplemental Table S7). None of the effects of competitors without predators were significantly different from zero ($p \geq 0.4021$, Table 9). Same age pinewoods and older pinewoods in the absence of a predator induced at least 11% shorter tails in small focal tadpoles, small differences (<10%) at the average mass, and same age pinewoods induced 11% longer tails at the large mass (Fig. 30). Adding predators had little effect at the control density at small and average masses (% difference $\leq$ 7%, $p \geq 0.4466$), but predators induced 15% longer tails at the large body size at the control density (Fig. 31, Table 9). The effect of predators on focal prey at the control density was not statistically different ($p = 0.1573$, Fig. 31, Table 9). Adding a predator also induced longer tails in same age and older grays at the average and large masses (% difference $\geq$ 11%), but not

at the small mass (% difference ≤ 9%), but none of the effects significantly differed from zero ($p \geq 0.1386$, Fig. 31, Table 9). Pinewoods competitors induced different patterns with the predator than the other two treatments and also differed from each other, with older pinewoods inducing 55% longer tails at the small body size, same age pinewoods inducing 13% shorter tails at the small body size, older pinewoods inducing approximately 14% longer tails at the average mass while same age pinewoods had little effect (3% difference), and older pinewoods inducing 19% shorter tails and same age pinewoods inducing 18% longer tails at the large mass, but only the effect of older pinewoods at the small size significantly differed from zero ($p = 0.003$ for older pinewoods at small size, $p \geq 0.079$ for others, Fig. 31, Table 9). Only the effects of older pinewoods and predators were not additive, inducing longer tails than expected at the small mass and shorter tails than expected at the large body size ($p \leq 0.0331$, Fig. 31, 32 Table 9). When the full length of the tail was included, adding the tip of the tail or flagellum, the pattern was exactly the same with very similar p – values (Table 9), though differences were approximately 5% weaker with overall tail length.

The height of the tail fin at the tallest point responded differently in different treatments, but generally followed the same pattern as tail length ($F_{9, 15} = 3.76$, $p = 0.0117$, Supplemental Table S7, Fig. 9-11 for tail length). None of the competitor effects in the absence of the predator were significantly different from zero ($p \geq 0.5251$, Table 9). Same age grays and same age pinewoods induced at least 11% shorter tail fins than the other 3 treatments at the small mass, had little effect at the average mass (% difference ≤ 7%), but the only effect at the large mass was that both grays induced approximately 10% shorter tail fins than the pinewoods treatments. Adding a predator had no real effect on tail fin height at any mass for the control density (% difference ≤ 9%, $p \geq 0.4166$), adding a predator with competitors generally increased tail fin

height by at least 16% at any body size, but only the effects of older pinewoods at the small mass and older pinewoods and same age grays at the average mass significantly differed from zero ($p \leq 0.0558$ versus $p \geq 0.2459$ for others, Table 9). With a predator and at any body size, same age pinewoods did not differ from the control density ($p \geq 0.5208$, % difference ≤ 8%), but older pinewoods induced 19% shorter tail fins at the large size, but this effect did not significantly differ from zero ($p = 0.0823$, Table 9). Same age pinewoods also induced at least 10% shorter tail fins than same age grays at the small and average mass, but these effects did not significantly differ from zero ($p \geq 0.1932$, Table 9). The effects of older pinewoods and predators were not additive at the small body size ($p = 0.0341$, Table 9), when they were larger than expected.

Treatments affected tail muscle height differently ($F_{9, 15} = 4.58$, $p = 0.0048$, Supplemental Table S7). Competitors had no significant effects on tail muscle height at any mass ($p \geq 0.1746$, Table 9). Despite this lack of significance, adding same age pinewoods competitors induced 13% shorter tail muscles at the small mass, but had little effect at the average and large masses (≤ 4% difference, Fig. 33). In the absence of predators, same age grays induced at least 28% taller tail muscles in focal pinewoods tadpoles at the small mass, had little effect at the average mass (≤ 4% difference), and induced at least 30% shorter tail muscles at the large mass (Fig. 33). Adding predators at the control density only had an effect on tail muscle height at the large mass, when it induced 21% taller tail muscles, but the effect was not statistically different ($p = 0.5332$, other body size effects ≤ 8%, $p \geq 0.4822$, Fig. 34, Table 9). Older grays followed the same pattern as the control density when predators were added, but tail muscles were at least 12% taller at the average and large body size and the effects did not significantly differ from zero ($p \geq 0.4951$, Fig. 34, Table 9). Adding predators with same age grays induced 27% shorter tail muscles in focal tadpoles at the small mass, and at least 14% taller tail muscles at the average and large

masses, but only the effects at the large mass significantly differed from zero ($p = 0.0549$ at large, ≥ 0.2013 at small and average, Fig. 34, Table 9). The different response of focal tadpoles to same age grays and a predator was probably more a function of the competitor identity than adding a predator, because the predator effects did not differ when same age grays were the competitor from those of the control or older grays at any body size (% difference $\leq 6\%$, Fig. 35, Table 9). Adding a predator with same age pinewoods induced 24% shorter tail muscles at the small mass, had little effect at average mass ($\leq 6\%$ difference), and increased tail muscle height by 20% at the large mass though none of these effects significantly differed from zero ($p \geq 0.1239$, Fig. 34). Adding a predator with older pinewoods induced at least 13% taller tail muscles at the small and average masses ($p \leq 0.00597$), and at least 20% shorter tail muscles at the large mass ($p \geq 0.001$, Fig. 34, 13, Table 9).

The response of tail stripe height to treatment was very similar to the response of tail muscle height ($F_{9, 15} = 2.28$, $p = 0.0766$, Supplemental Table S7, Fig. 33-35 for tail muscle height). For competitors in the absence of predators, tail stripe height differed from tail muscle height in that it was same age pinewoods and not same age grays that induced at least 10% taller tail stripes at the small and average masses ($p \geq 0.3455$, Table 9). Older grays also induced 16% taller tail stripes in focal prey than the control density, but none of the effects of competitors in the absence of predators significantly differed from zero ($p \geq 0.2796$, Table 9). Adding a predator at the control density induced 21% shorter tail stripes at the small mass, had little effect at the average mass (3% difference), and induced 18% taller tail stripes at the large mass. The effects of predators on the control density did not significantly differ from zero ($p \geq 0.2312$, Table 9). Only same age pinewoods competitors showed the same pattern as the control density when a predator was added, but even though they did not significantly differ from zero ($p \geq$

0.1241, Table 9), the effect of adding a predator with same age pinewoods competitors was stronger at the small and large masses than the effect of adding a predator at the control density (57% and 27% difference respectively). As with most other larval morphological traits, adding a predator with older pinewoods greatly increased tail stripe height at the small mass (58%), increased it at the average mass (21%), and decreased it at the large mass (14%). Only the effect of adding a predator with older pinewoods at the small size significantly differed from zero (p = 0.0062 at small mass, $p \geq 0.0735$ for average and large mass, Table 9). Adding a predator with older gray competitors consistently increased tail stripe height by approximately 12%, but the effect was not statistically different at any body size ($p \geq 0.4454$, Table 9). Only the effects of older pinewoods and predators at the small mass were not additive effects, with tail stripe height increasing more than expected (p = 0.0232, Table 9).

Focal tadpole mouth width responded differently in different treatments ($F_{9, 15} = 5.91$, p = 0.0014, Supplemental Table S7). In the absence of predators at the small mass, adding older pinewoods increased mouth width by approximately 10%, while adding same age grays decreased mouth width by approximately 17%, but adding same age pinewoods and older grays did not affect mouth width of focal tadpoles (% difference ≤ 7%, Fig. 36). At the average size, older gray competitors induced at least 10% wider mouths in focal tadpoles, but none of the other competitors altered mouth width (% difference ≤ 7%, Fig. 36). Only the effect of older grays at the average size compared to the control significantly differed from zero (p = 0.0383, others $p \geq 0.099$, Table 9). At the large size, the same age competitors and older grays increased mouth width by at least 15%, with older grays having the strongest effect (at least 22% difference, Fig. 36). Older pinewoods had little effect at the large mass (4% difference) and only the effects of older grays and same age pinewoods compared to the control and older grays

compared to older pinewoods significantly differed from zero ($p \leq 0.0517$, $p \geq 0.1513$ for others, Table 9). Adding a predator had little effect at the control density or with older pinewoods ($p \geq 0.3089$, % difference ≤ 6%), and also had little effect on older grays at the small mass (% difference = 3%, $p = 0.7139$, Fig. 37, 38, Table 9). Adding a predator did increase mouth width by at least 20% with same age grays or same age pinewoods at the small mass, but these effects did not significantly differ from zero ($p \geq 0.0884$, Fig. 37, 38, Table 9). At the average mass, adding a predator only decreased mouth width by 14% with older grays, but this effect did not significantly differ from zero ($p = 0.077$, Fig. 37, 38, Table 9). At the large mass, adding a predator induced at least 20% narrower mouths with same age pinewoods and older gray competitors, but only the effect with same age pinewoods significantly differed from zero ($p = 0.0005$ with grays, 0.0715 with pinewoods, Fig. 37, 38, Table 9). Only predators and same age pinewoods had non-additive effects, and only at the large body size when mouth width decreased more than was expected ($p = 0.0102$, Fig. 37, Table 9).

Body width in front of the spiracle differed among the treatments ($F_{9, 15} = 3.59$, $p = 0.0142$, Supplemental Table S7). Competitors alone only had an impact on body width in front of the spiracle at the small mass, with young pinewoods decreasing body width by 10% relative to the control density and by 10% relative to the older pinewoods, but neither of these effects was significantly different from zero ($p \geq 0.5431$, Fig. 39, Table 9). Adding a predator had little effect on body width in front of the spiracle at the control density (% difference < 10%), but for older pinewoods it did increase body width at the small mass by 30% and decreased body width by 15% at the large mass ($p \leq 0.0198$, Fig. 40, Table 9). Older pinewoods with a predator also induced at least 34% wider bodies than the other competitor and predator environments at the small mass ($p \leq 0.0033$), and at approximately 10% wider bodies than competitors and predators

at the control density and with same age pinewoods (Fig. 41, Table 9). The effects of older pinewoods and a predator on focal prey body width at the average size did not significantly differ from zero ($p \geq 0.0672$, Table 9). At the large size, older pinewoods and a predator induced at least 13% narrower bodies in front of the spiracle than the other competitor and predator environments ($p \leq 0.0521$, Fig. 41, Table 9). None of the other competitor and predator environments differed from each other at any body size ($p \geq 0.1405$, % difference ≤ 8%, Fig. 41, Table 9). Predator and competitor effects were non-additive only for older pinewoods and only at the small mass when body width increased more than expected ($p = 0.0133$, Fig. 40, Table 9).

Body width behind the spiracle and inter-eye distance followed a very similar pattern to body width in front of the spiracle ($F_{9, 15} = 3.62$, $p = 0.0137$ for body width behind the spiracle, $F_{9, 14} = 2.52$, $p = 0.0585$ for inter-eye distance, Supplemental Table S7). In the absence of predators, same age grays did induce 10% narrower bodies behind the spiracle at the small mass, but this effect did not significantly differ from zero ($p = 0.7495$, Table 9). Adding a predator with same age grays also induced 10% wider bodies behind the spiracle at the small mass, but this effect did not significantly differ from zero ($p = 0.7141$), and same age grays did not affect body width at any other mass ($p \geq 0.5626$, % difference ≤ 5%, Table 9). Only older pinewoods with a predator had an effect on body width behind the spiracle compared to the other predator and competitor environments, and this followed the same pattern as with body width in front of the spiracle (≥ 46% increase at small mass, $p \leq 0.0043$, ≥ 10% increase at average mass, $p \geq 0.0607$, ≥ 23% decrease at large mass, $p \leq 0.0236$, Table 9). Like body width in front of the spiracle, competitor and predator effects were only non-additive for older pinewoods, but body width behind the spiracle showed non-additive effects at the large mass (smaller than expected) as well as the small mass (larger than expected, $p \leq 0.0477$, Table 9). Competitors alone did not have

any effects that significantly differed from zero at any mass for inter-eye distance ($p \geq 0.368$, Table 9), but older gray treefrogs at the large mass did induce at least 10% narrower inter-eye distances than older pinewoods and same age grays. Adding predators did induce 13% wider inter-eye distances in focal tadpoles at the large mass, while body width in front of the spiracle did not differ, but this effect was not statistically different for inter-eye distance ($p = 0.341$, Table 9).

The length of the gut coil (distance from the most anterior point of the head/gut interface to the midpoint of the tail base) showed differences among the treatments ($F_{9, 15} = 4.48$, $p = 0.0053$, Supplemental Table S7). The effects of competitors alone on length of the gut coil were not statistically different ($p \geq 0.2326$, Table 9). In the absence of predators, only same age grays differed from the control by inducing 23% shorter gut coil lengths at the small mass and 15% longer gut coils at the large mass, but same age grays did not differ from the control at the average mass (Fig. 42). Same age pinewoods induced 16% longer gut coils than same age grays at the small mass and 12% longer gut coils than older pinewoods at the large mass, but same age pinewoods did not have any other effects (% difference ≤ 8%, Fig. 42). Adding a predator decreased gut coil length at the small mass in the control density by approximately 17%, but this effect was not statistically different ($p = 0.365$), and the predators did not have an effect on gut coil length at the average or large mass (% difference ≤ 8%, $p \geq 0.2996$, Fig. 43, Table 9). Adding a predator had little effect on older grays at any size ($p \geq 0.6223$, % difference ≤ 6%) and the effects of adding a predator at the average size with any competitor were small ($p \geq 0.495$, % difference ≤ 5%), but both ages of pinewoods with a predator induced at least 15% longer gut coils at the small size and approximately 19% shorter gut coils at the large mass (Fig. 43, Table 9). The effects of a predator on same age pinewoods at the small mass were not statistically

different ($p = 0.4833$), but the other effects of predators on same age and older pinewoods were significant ($p \leq 0.059$, Table 9). Adding a predator with same age grays only had an effect at the small mass, when it induced 22% longer gut coils, but there was no other effect on same age grays and the effect at the small size did not significantly differ from zero ($p \geq 0.4546$ for all, % difference for other sizes ≤ 7%, Fig. 43, Table 9). With a predator, all of the competitors increased gut length by at least 11% relative to the control density at the small mass, but only the effect of older pinewoods significantly differed from zero ($p = 0.0066$ versus $p \geq 0.1772$, Fig. 44, Table 9). No competitor and predator effects differed at the average mass ($p \geq 0.234$, % difference ≤ 8%), but both ages of pinewoods with a predator induced at least 20% shorter guts than the other three predator and competitor environments ($p \leq 0.0282$, Fig. 44, Table 9). Same age pinewoods with a predator also induced 12% longer gut coils than older pinewoods with a predator at the large mass, but this effect did not significantly differ from zero ($p = 0.1481$, Fig. 44, Table 9). At the small mass, older pinewoods and a predator had a positive non-additive effect on gut coil length ($p = 0.0233$, Fig. 44, Table 9). Older pinewoods and same age pinewoods and a predator had non-additive effects on gut coil length at the large mass, decreasing gut coil length more than expected ($p \leq 0.0401$, Fig. 44, Table 9).

Different treatments impacted focal tadpole tail width in different ways ($F_{9, 15} = 3.82$, $p = 0.0109$, Supplemental Table S7). Competitor environments without predators did not have any impacts on tail width that differed significantly from zero ($p \geq 0.4783$, Table 9). At the small mass, both age pinewoods induced at least 15% narrower tails, but adding same age pinewoods had the strongest effect on tail width (16% narrower tails than older pinewoods, Fig. 45). Tail width did not differ across the competitor only environments at the average mass (≤ 7% difference, Fig. 45). At the large mass, only same age grays altered tail width by inducing at least

11% narrower tails (Fig. 45). Adding predators at the control density decreased tail width by 40% at the small mass, had little effect at the average mass, and increased tail width by 19% at the large mass, but none of these effects differed significantly from zero ($p \geq 0.1569$, Fig. 46, Table 9). None of the other competitor regimes followed the same pattern as the control density when predators were added (Fig. 46). Adding predators with older grays had little effect on tail width ($p \geq 0.7424$, % difference $\leq 8\%$), induced at least 23% wider tails with older pinewoods at small and average masses and 22% narrower tails at the large mass, 12% wider tails with same age pinewoods at the large mass, and at least 25% wider tails with same age grays at the average and large masses (Fig. 46, Table 9). Only the effects of predators on older pinewoods at the small mass and older pinewoods and same age grays at the average mass significantly differed from zero ($p \leq 0.0524$, $p \geq 0.1099$ for others, Table 9). Older pinewoods and same age grays generally had the strongest effects in the presence of the predator (up to 45% differences for same age grays and 91% differences for older pinewoods), but only the effects of older pinewoods significantly differed from zero and only at the small and average masses ($p \leq 0.0233$ for older pinewoods except at large mass compared to older grays, there $p = 0.0769$, $p \geq 0.073$ for all others, Fig. 47, Table 9). Only the effects of older pinewoods and predators at the small and large masses were non-additive, with a larger increase than expected at the small mass and a larger decrease than expected at the large mass ($p \leq 0.0418$ for older pinewoods, $p \geq 0.133$ for others, Table 9).

Metamorphs:

Average emergence time (the time it took for tadpoles to turn into frogs) was impacted by several of the treatments ($F_{9, 25} = 1.24$, $p = 0.3143$, Supplemental Table S8). Older gray treefrogs

and same age gray treefrogs increased average emergence time of focal tadpoles by 29% and 30% respectively relative to the control density ($p \leq 0.0441$) and same age pinewoods increased emergence time by 11%, but the effect of same age pinewoods did not significantly differ from zero ($p = 0.4197$, Fig. 48, Table 10). Older grays caused tadpoles to take 25% longer to emerge than older pinewoods and same age grays caused tadpoles to take 19% longer to emerge than same age pinewoods, but neither of these effects significantly differed from zero ($p \geq 0.0738$, Fig. 48, Table 10). Adding predators had little effect on emergence time at the control density ($p = 0.5569$), but it did decrease emergence time by 14% with older gray competitors, but this effect did not significantly differ from zero ($p = 0.2815$, Fig. 48, Table 10). Only same age grays altered the impacts of predators on focal tadpoles, increasing emergence time by 15% relative to the control density with a predator and increasing emergence time by 16% relative to same age pinewoods, but neither of these effects significantly differed from zero ($p \geq 0.1841$, Fig. 48, Table 10).

The effects of treatment on the geometric mean of mass of metamorphs did not significantly differ from zero ($F_{9, 25} = 0.85$, $p = 0.5764$, Supplemental Table S8, contrast $p \geq 0.0785$, Table 10). In the absence of predators, older gray competitors decreased focal metamorph mass by 16%, same age grays decreased mass by 30%, and same age pinewoods decreased mass by 29%. Older grays reduced mass by 25% and same age pinewoods reduced mass by 39% relative to older pinewoods and same age grays reduced mass by 14% relative to older age grays. Adding a predator had no impact on the control density or older pinewoods competitors, but increased mass by 10% with older grays, by 24% with same age pinewoods, and by 33% with same age grays. Only older pinewoods altered the response of mass to the predator by increasing focal metamorph mass by 16% relative to the control density metamorphs. Older

pinewoods with a predator also induced 17% larger masses in metamorphs than older grays with a predator and 16% larger masses than same age pinewoods with a predator.

As with mass, the effect of treatment on the percentage of individuals surviving to metamorphosis was not statistically different ($F_{9, 29} = 0.86$, $p = 0.5686$, Supplemental Table S8, contrast $p \geq 0.0698$, Table 10). Survival at the control density without predators was 46%. The addition of competitors relative to the control density reduced survival by 29% with older grays, increased it by 32% with same age grays, increased it by 10% with same age pinewoods, and had little effect with older pinewoods. Gray treefrogs of either age had at least 23% stronger effects on survival than pinewoods. Survival at the control density with a predator was 61%. Adding predators increased survival to metamorphosis by 28% at the control density, increased it by 63% with older grays, had little effect on older pinewoods, increased it by 15% with same age pinewoods, and decreased it by 47% with same age grays. Older pinewoods and younger grays with a predator increased survival by at least 25% relative to the control, but the other treatments did not differ from the control density with a predator (% difference ≤ 8%). Adding tadpoles in to the percentage of individuals surviving to metamorphosis by the end of the experiment gave an estimate of overall survival, but the results did not differ much from those for the percentage of individuals surviving to the end of the experiment other than increasing the percent differences to some extent. New effects were that older pinewoods exhibited 15% higher survival than the control without a predator and that adding a predator with older pinewoods decreased survival by 10%, but neither of these effects differed significantly from zero ($p \geq 0.5914$).

Metamorph femur length, tibio-fibula length, and average jump distance all increased with increasing mass ($p < 0.0001$ for all) and the allometry of these three traits did not vary with treatment ($p \geq 0.3$, Supplemental Table S8). Tibio-fibula length did not vary across the

treatments ($F_{9, 24} = 0.68$, $p = 0.7226$, Supplemental Table S8, contrast $p \geq 0.0776$, Table 10). Femur length did not differ across the treatments ($F_{9, 24} = 2.70$, $p = 0.0249$, Supplemental Table S8, contrast $p \geq 0.0672$, Table 10) except that in the presence of a predator same age pinewoods induced 6% longer legs than same age gray competitors in focal metamorphs ($p = 0.0021$) and older pinewoods with a predator induced 4% longer legs in focal metamorphs than same age pinewoods with a predator ($p = 0.0362$, Table 10). These effects are small enough that despite the statistical significance, they are not likely biologically significant. Despite this lack of differences in leg lengths, there were some effects of treatment on jumping ability ($F_{9, 24} = 1.44$, $p = 0.2271$, Supplemental Table S8). With competitors alone, only older pinewoods had an effect on jump distance compared to the control, increasing average jump distance of focal metamorphs by 10% and older pinewoods also increased focal metamorph jump distance by 14% relative to older grays without a predator (Fig. 49). Only the difference between older pinewoods and older grays on jump distance was significant ($p = 0.0411$, $p = 0.0919$ between older pinewoods and control, Table 10). The predator environments did not differ from each other in the jumping ability of metamorphs ($p \geq 0.3555$), but adding predators increased metamorph jumping ability by 11% at the control density and by 14% when older grays were the competitors, but only the effect of older grays significantly differed from zero ($p = 0.0462$ for older grays, 0.0795 for control density, Fig. 49, Table 10).

Metamorph snout-urostyle length and cranial width both increased with increasing mass ($p < 0.0001$), and both of these traits showed different allometries in different treatments ($p \leq 0.1691$, Supplemental Table S8). Although there were differences in snout-urostyle length and cranial width across the treatments in terms of their statistical significance ($F_{9, 15} \leq 1.83$, $p \geq 0.1441$, Supplemental Table S8), none of these differences was at or above 10%. This suggests

that there were no biologically significant effects of treatment on snout-urostyle length or cranial width.

Same Age and Older Gray Treefrog and Older Pinewoods Competitor Results:

Older pinewoods competitors did not differ in their mass or average emergence time whether or not a predator was present ($F_{1, 3} = 0.21$, $p = 0.6812$ for mass, $F_{1, 3} = 0.36$, $p = 0.5932$ for emergence). Older pinewoods metamorphs showed 16% higher survival when a predator was present than when a predator was absent, but this effect did not significantly differ from zero ($F_{1, 6} = 1.18$, $p = 0.3187$). Average emergence time of gray treefrogs differed across the treatments ($F_{3, 7} = 2.19$, $p = 0.1771$). The addition of a predator had little effect on emergence time for same age or older gray treefrogs ($p \geq 0.6437$, Table 11). Same age grays took 26% longer to emerge than older grays in the absence of a predator and 27% longer to emerge in the presence of a predator, but neither effect significantly differed from zero ($p \geq 0.0876$, Table 11). Mass of gray treefrogs varied with treatment ($F_{3, 7} = 2.05$, $p = 0.1951$). Adding predators increased mass of gray treefrog metamorphs by approximately 28% when they were older gray treefrogs and 30% when younger gray treefrogs, but the effect was not statistically different ($p \geq 0.1865$, Table 11). Older gray treefrog metamorphs were 23% larger than same age gray treefrog metamorphs when a predator was present and 25% larger when a predator was absent, but these effects also did not significantly differ from zero ($p \geq 0.21$, Table 11). There was an effect of treatment on survival to metamorphosis of gray treefrogs ($F_{3, 11} = 1.91$, $p = 0.1859$). The addition of a predator did not alter the survival to metamorphosis of older gray treefrogs ($p = 0.4009$, 5% difference, Table 11), but adding a predator decreased the survival of same age gray treefrogs by approximately 12%. The effect of adding a predator on same age gray treefrog survival did not significantly differ

from zero (p = 0.0938, Table 11). In the absence of predators, gray treefrog age also did not affect survival (5% difference, p = 0.4275), but with predators same age gray survival was approximately 12% lower than that of older grays, though the effect was not statistically different (p = 0.164).

Discussion:

Competitors alone had little impact on tadpole morphology. Older pinewoods had the smallest impact of competitors alone, gray treefrog competitors had more of an impact than pinewoods competitors, and younger competitors had more of an impact than older competitors. Adding a caged predator typically had little impact on focal tadpole morphology at the control density, but adding a caged predator did impact tadpole morphology when density was increased, particularly when same age gray treefrog or older pinewoods treefrog competitors were present. Competitors often altered the impacts of predators, particularly same age grays and older pinewoods, and these effects on predator impacts often varied with different competitors. The effects of older pinewoods competitors and predators were also often additive. The interaction between mass and the other morphological traits was usually positive, but older pinewoods consistently induced a negative relationship between mass and morphology, with smaller tadpoles exhibiting larger traits than large tadpoles. Larval aeshnid dragonflies such as the *Anax* sp. that we used as predators can kill prey close to their own size (Relyea and Yurewicz 2002), so it is unlikely that tadpole morphology became smaller with increasing mass because defensive morphologies were no longer needed. Emergence time did not differ between older pinewoods competitors with a predator and the other competitor/predator environments, but older pinewoods with predators were around 16% larger than metamorphs from the other

predator/competitor environments. Tadpoles that metamorphose at a larger mass, are often larger frogs or at least grow faster (Altwegg and Reyer 2003), so perhaps there is a trade-off or cost to maintaining altered morphologies at larger tadpole masses in the presence of a predator. Focal tadpoles raised with older pinewoods competitors in the absence of a predator did not show a negative relationship between morphology and mass, but still became relatively large metamorphs and metamorphosed around the same time as tadpoles reared with older pinewoods competitors and a predator, suggesting that this pattern stems from the interaction of the competitor and predator environment. It is also unclear as to where the biomass is going, considering that all morphological traits measured except for mouth width decreased with increasing mass when older competitors and a predator were present (Fig. 38). It is possible that the biomass may be incorporated into hind legs, but femur length at metamorphosis did not differ among the treatments and tibio-fibula length for older pinewoods metamorphs with a predator was only longer compared to same age pinewoods with a predator. We did not measure leg length in the tadpoles, so it could be an effect only at this stage. Perhaps the gut coil is longer or denser in this treatment, but the space that the coil occupies follows the same pattern of decreasing with increasing body mass (Fig. 44). It is also possible that bone density may have increased in this treatment as there is evidence that other species of frogs (*Agalychnis callidryas* and *Xenopus laevis*) responded to higher temperatures and lower food availability by increasing the ossification of their bodies (Gomez-Mestre and Buchholz 2006). This pattern disappeared after metamorphosis, with cranial width and snout-urostyle length both increasing with increasing mass for pinewoods metamorphs reared as tadpoles with older pinewoods competitors and a caged dragonfly larvae (not pictured).

Competitor effects in the absence of predators may have been relatively minor because this species has evolved to change its morphology more in response to predators. Pinewoods Treefrogs exhibit exaggerated tail fins and also change the color of the tail fin to red in the presence of predators to make the tail a bigger target (Van Buskirk and Mccollum 2000, Van Buskirk et al. 2003), and this may preclude them from developing competitor induced morphologies such as shorter, shallower tails and longer, deeper bodies that have been documented in Wood Frogs (*Lithobates sylvaticus*) (Relyea 2002c). Same age gray treefrogs did reduce body depth relative to the other treatments at the large mass, but actually increased body depth at the small mass and the same pattern was evident with tail muscle height (Fig. 33). Same age pinewoods increased tail stripe height at the small mass and decreased it at the large mass. There were other cases where all competitors induced similar slopes in the trait, but one competitor induced different changes in the trait relative to the other treatments depending on the mass. An example of this is gut coil length, where same age grays induced the smallest gut coil lengths relative to the other treatments at the small mass, but the longest gut coil lengths at the large mass. This suggests that there are size-dependent strategies in these tadpoles. The data suggest that they are not time lags, because small size pinewoods tadpoles raised with older pinewoods competitors and a predator often exhibited the largest traits (i.e. gut length, Fig. 44).

Both ages of gray treefrogs may have used up more resources than pinewoods competitors as they decreased tadpole mass and increased emergence time of focal pinewoods metamorphs, but oddly same age grays increased focal pinewoods survival while older grays decreased pinewoods survival (Fig. 18, 48). Older grays may have eaten more algal resources than same age grays due to their large size and greater developmental stage, but could have decreased the survival of the focal pinewoods tadpoles through predation. Many species of frogs,

including other hylid treefrogs, have been documented to eat the eggs and occasionally tadpoles of their own or other species of frogs (reviewed in Petranka & Kennedy, 1999). One species of frog, the Wood Frog (*Lithobates sylvaticus*) consumed most of the freshly laid eggs or hatchlings of the American Toad (*Anaxyrus terrestris*) within as little as 45 minutes (Petranka et al. 1994). In this same study, the older the toad tadpoles were, the greater their chance of surviving (Petranka et al. 1994). This suggests that tadpoles are better at catching and eating smaller tadpoles, which could explain why survival decreased with older gray treefrog competitors but not same age gray treefrog competitors. Older pinewoods did not alter survival compared to the control, but we collected the older and same age pinewoods from the same area, so individuals may have shared at least a single parent. In that case eating a conspecific could potentially reduce inclusive fitness which might discourage cannibalism. Pinewoods Treefrog tadpoles also do not get as large as Gray Treefrog tadpoles before metamorphosis since our gray treefrog metamorphs were around a tenth of a gram larger than pinewoods metamorphs, so the same age pinewoods tadpoles may also have been a bit too large for older pinewoods to consume.

Adding a predator at the control density largely just exaggerated the tail by making it wider, taller, and longer, but had little effect on the body (Fig. 29, 32, 35, 41, 47). Adding predators in the other competitor environments had similar effects to adding predators at the control density, exaggerating tails but having little effect on the body. Competitors also altered the response of tadpoles to the predator, producing more exaggerated traits than those produced by adding a predator at the control density. Sometimes competitors altered the impact of the predator by inducing opposite changes from the predator at the control density. At the small mass for example, same age pinewoods competitors with a predator induced narrower eyes than same age pinewoods without a predator, but the predator at the control density induced wider eyes than

the control density without a predator (Fig. 25). This suggests that responding to competitors alone or predators alone is less important than being able to respond to both. Since competitor effects on predators varied with competitor identity, intra- and interspecific competition also place very different stresses on tadpoles. The age of competitors also mattered, since older gray treefrogs reducd survival of focal tadpoles to metamorphosis, but younger gray treefrogs increased or at least did not lower survival to metamorphosis. Some of these differences in the impacts of different aged competitors could stem from the fact that the older competitors metamorphosed earlier than the younger competitors, reducing competitive effects. A good example of this is body width behind the spiracle, where with and without a predator older gray treefrogs induced very similar body widths to the control density in the focal pinewoods tadpoles but same age grays altered body width relative to the control density.

The interaction of predators and competitors increased eye width when older pinewoods treefrogs were present and younger gray treefrogs were present, and to a lesser extent when same age pinewoods were present (Fig. 26). Perhaps these competitors force pinewoods treefrogs to forage more actively and wider eyes allow them to spot predators earlier. Same age grays and same age pinewoods competitors alone did increase eye width by around 10% at the small mass, so there is some impact of the competitors alone, but it was much more exaggerated with predators present (Fig. 24-26).

Not only does the species identify of competitors, even closely related individuals matter, but their age also matters. We found that gray treefrogs often had stronger effects on tadpoles than pinewoods treefrogs, but that these effects were often idiosyncratic to a particular trait. Competitor age also strongly mattered, with different age competitors often inducing weaker trait changes or trait changes in the opposite direction from younger competitors. Older gray treefrogs

also decreased focal pinewoods tadpole survival, suggesting that some competitors may directly affect mortality. Competitors also alter how tadpoles respond to predators. In particular, the older intraspecific competitor induced a different response than the other competitor types in most larval traits. This study adds to our understanding of natural systems by showing that individuals can respond to a complex gauntlet of different types and ages of competitors and predators, and that despite this range of different cues, the individuals that survive to the next life history stage may be relatively similar. This suggests that at least for our focal species, the Pinewoods Treefrog, there may not be much of a lasting trade-off in responding to different types of competitors versus a predator. However, since our predator was caged, it could be that the lasting non-lethal effects of the predator are similar across different competitor regimes, but individuals may be more susceptible to predation depending on the competitor environment. Future work should test to see if different types of competitor induced morphologies do increase susceptibility of tadpoles to their predators.

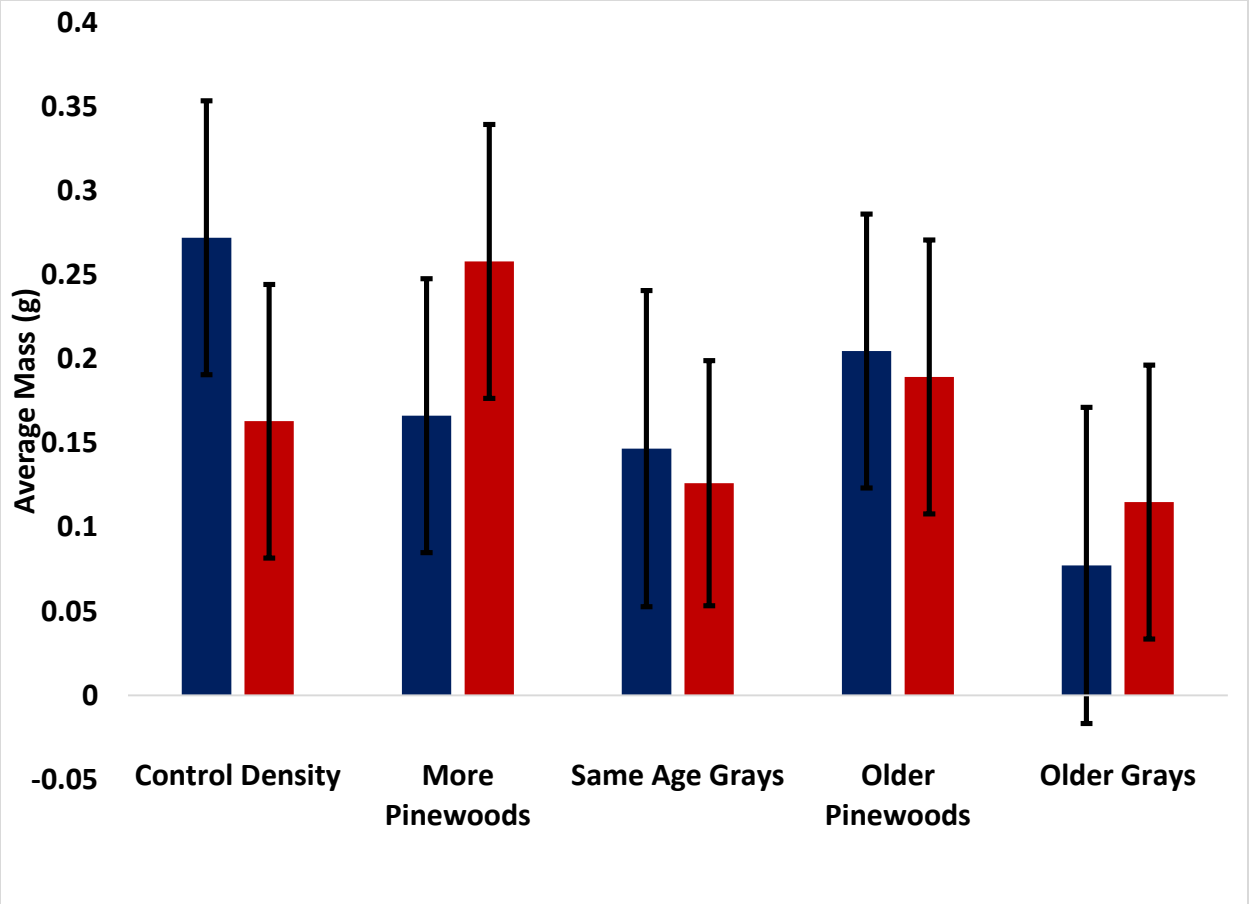

Figure 18. Geometric mean of tadpole mass. Blue bars indicate predator absence and red bars indicate presence of a caged predator. Values are least square means and error bars are one standard error of the mean. Letters above the bars indicate significant differences at the $p = 0.05$ level.

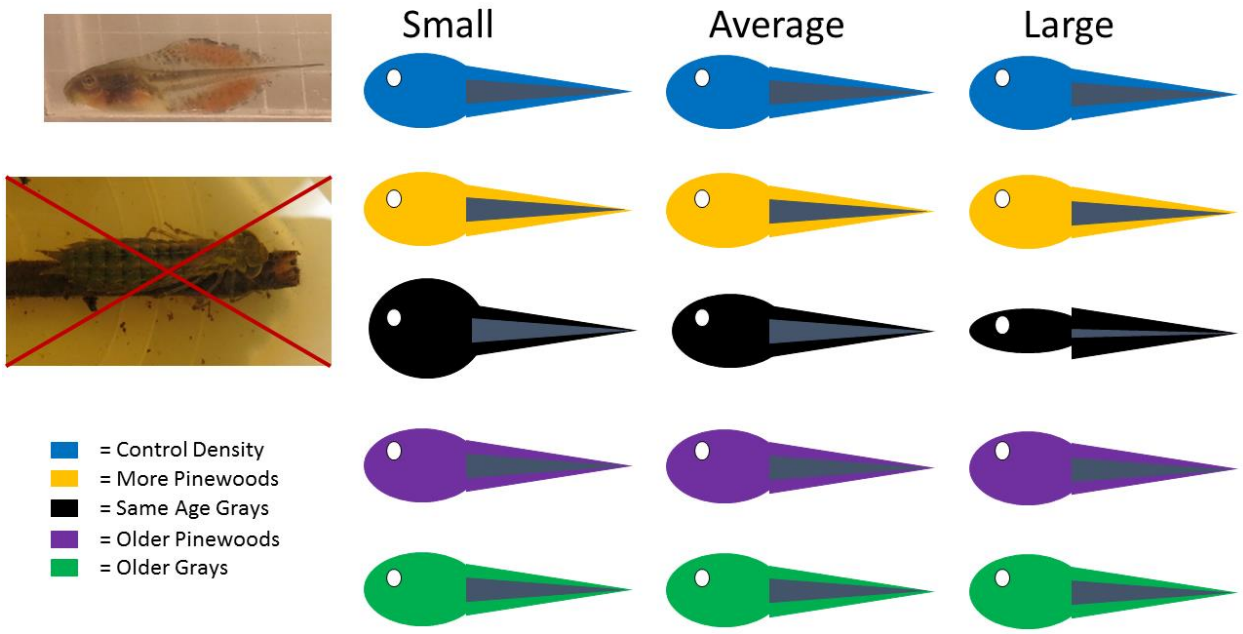

Figure 19. Summary of changes in morphology of tadpoles from the lateral view. The dragonfly picture with the red X corresponds to the fact that predators were absent from these comparisons. The legend lists the treatments by color based on what competitors were added. Body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Large ovals represent the body, small white ovals represent the eye, large triangles represent the tail fin, and gray triangles represent the tail musculature.

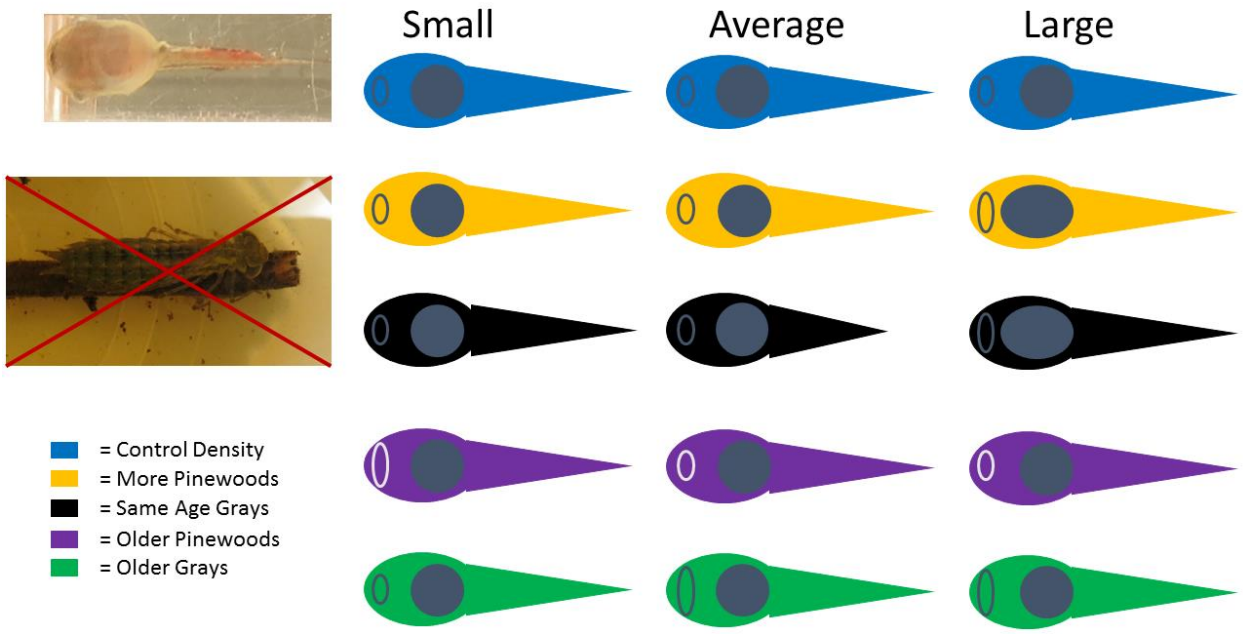

Figure 20. Summary of changes in morphology of tadpoles from the ventral view. The dragonfly picture with the red X corresponds to the fact that predators were absent from these comparisons. The legend lists the treatments by color based on what competitors were added. Body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Large ovals represent the body, small gray ovals that are open represent the mouth, large gray ovals that are filled represent the gut coil, and large triangles represent the tail.

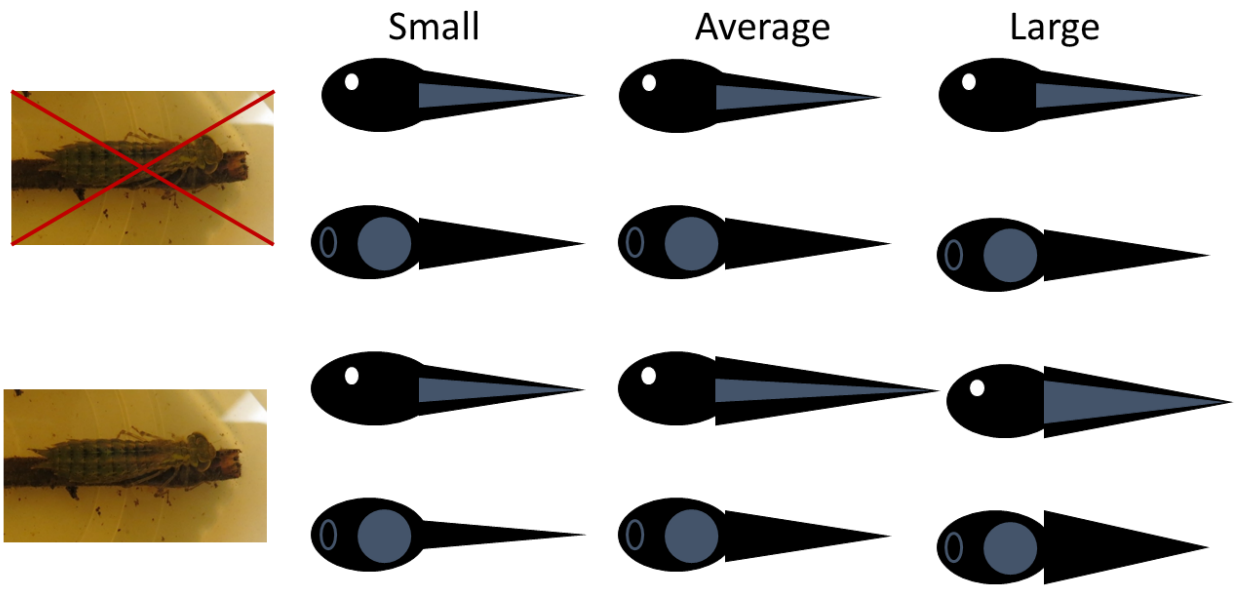

Figure 21. Summary of changes in morphology of tadpoles from the lateral and ventral views based on predator addition. The dragonfly picture with the red X corresponds to when predators were absent and the dragonfly picture without the X corresponds to when caged dragonfly predators were present. Body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). For the top row of tadpole views large ovals represent the body, small white ovals represent the eye, large triangles represent the tail fin, and gray triangles represent the tail musculature. For the bottom row of tadpoles views large ovals represent the body, small gray ovals that are open represent the mouth, large gray ovals that are filled represent the gut coil, and large triangles represent the tail.

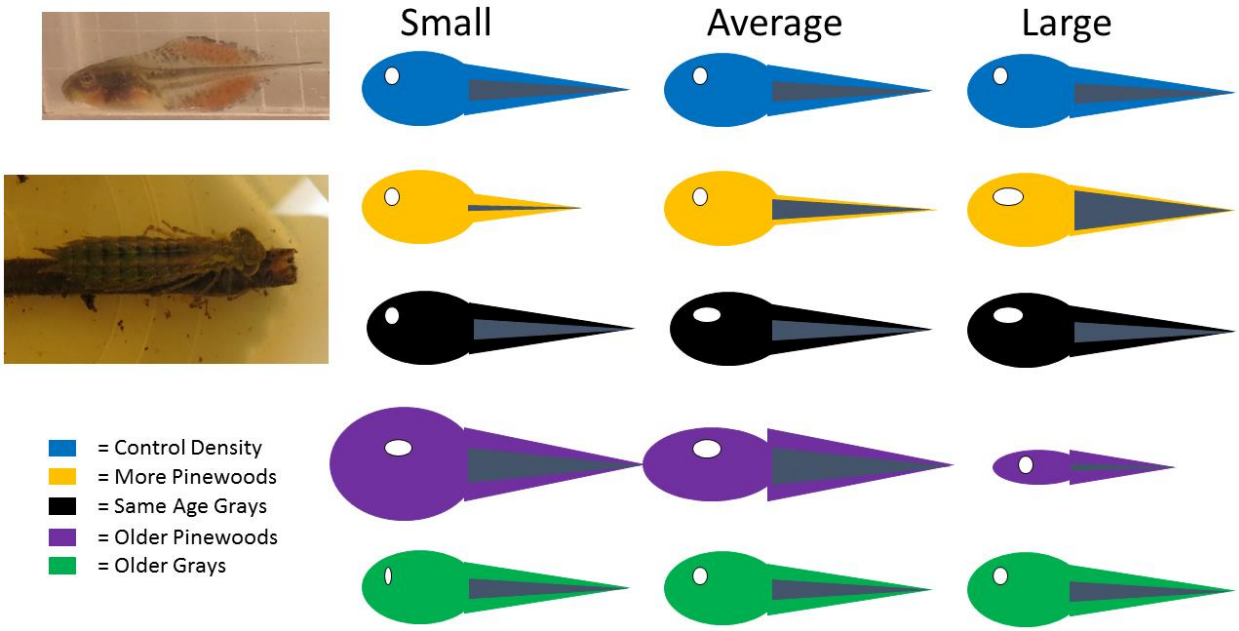

Figure 22. Summary of changes in morphology of tadpoles from the lateral view. The dragonfly picture to the fact that caged predators were present for these comparisons. The legend lists the treatments by color based on what competitors were added. Body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Large ovals represent the body, small white ovals represent the eye, large triangles represent the tail fin, and gray triangles represent the tail musculature.

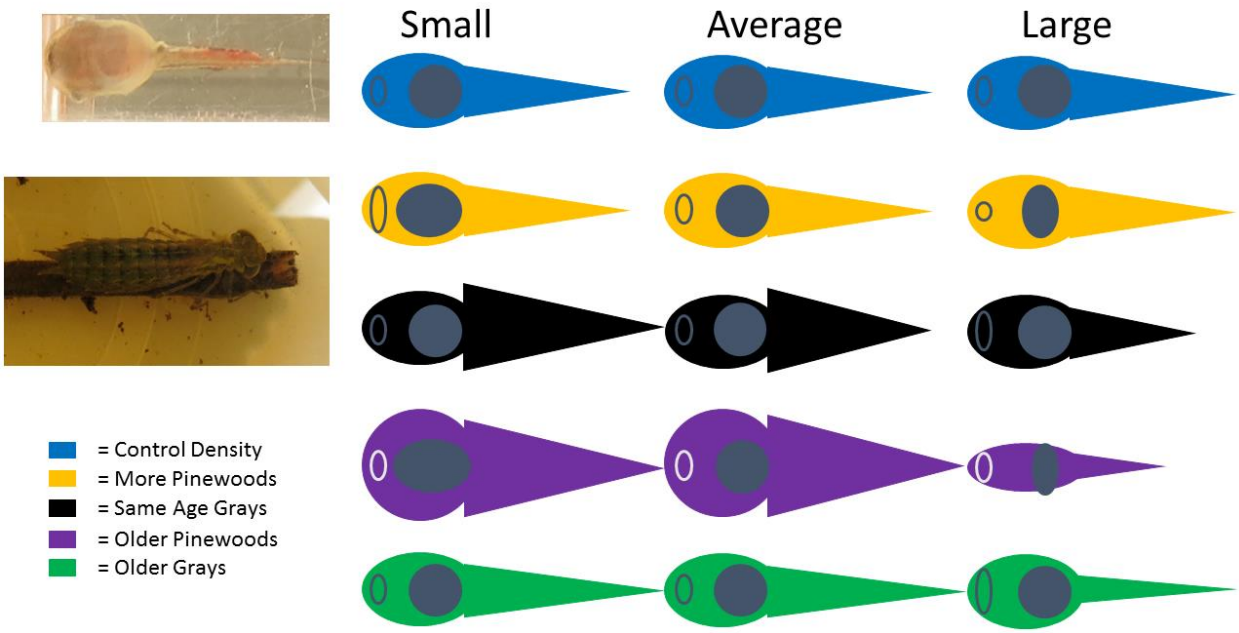

Figure 23. Summary of changes in morphology of tadpoles from the ventral view. The dragonfly picture corresponds to the fact that caged predators were present for these comparisons. The legend lists the treatments by color based on what competitors were added. Body size of the tadpole is listed along the x-axis. Body sizes correspond to one standard deviation below the average mass (small), the average mass (average), and one standard deviation above the average mass (large). Large ovals represent the body, small gray ovals that are open represent the mouth, large gray ovals that are filled represent the gut coil, and large triangles represent the tail.

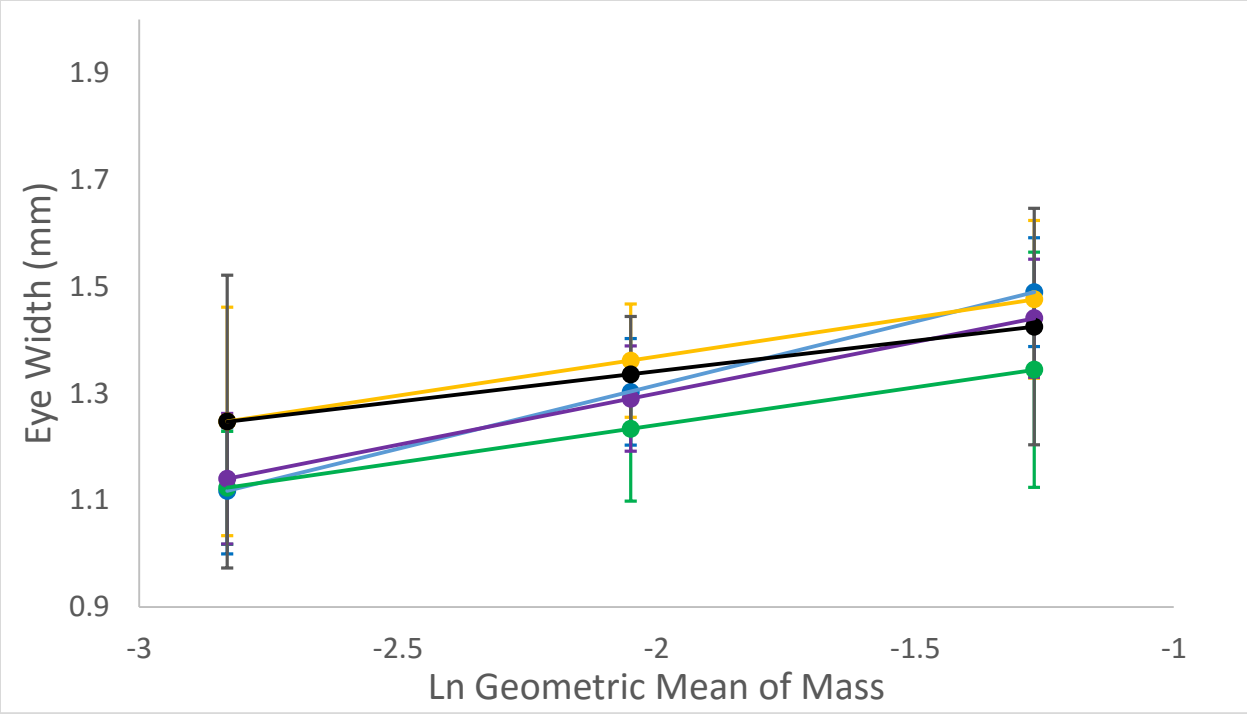

Figure 24. Tadpole eye width versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

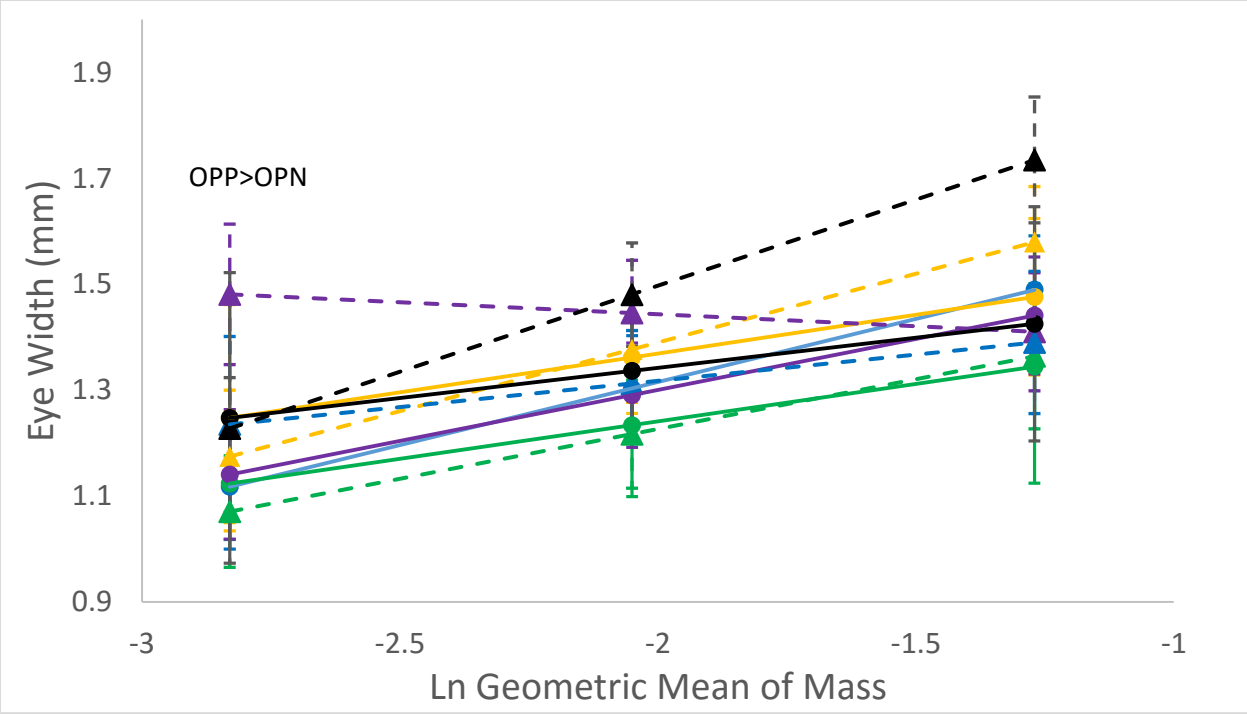

Figure 25. Tadpole eye width versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

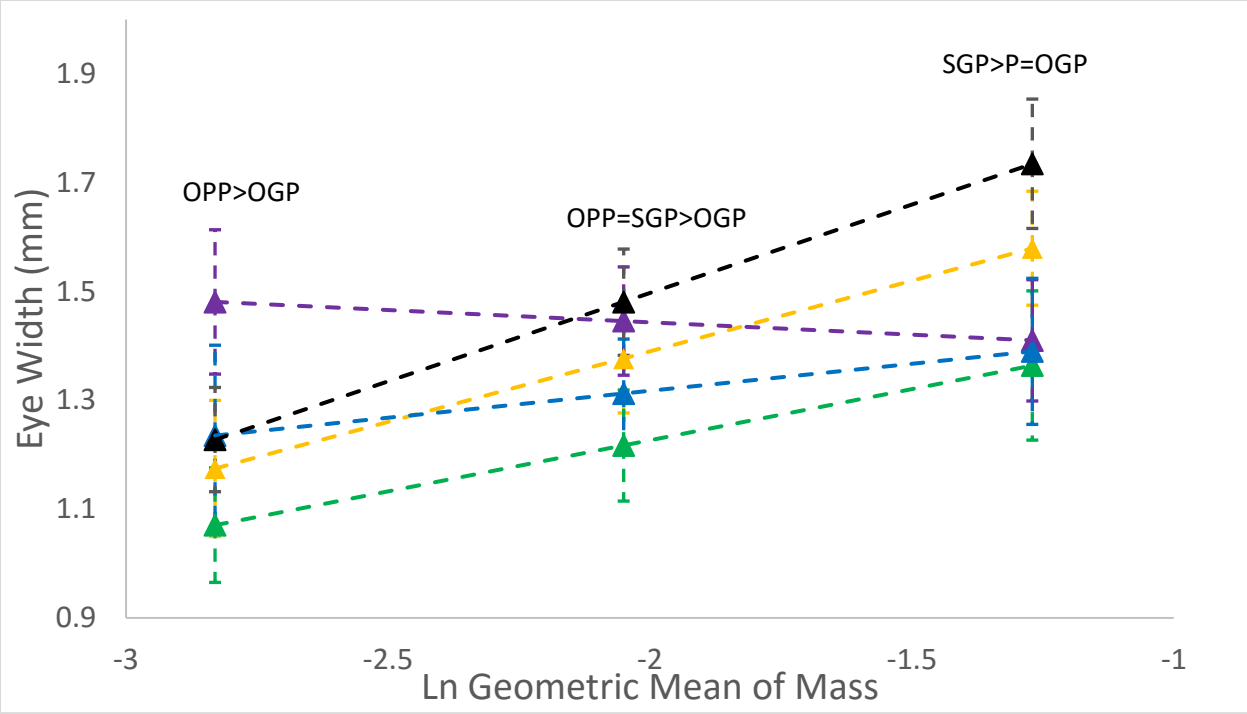

Figure 26. Tadpole eye width versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

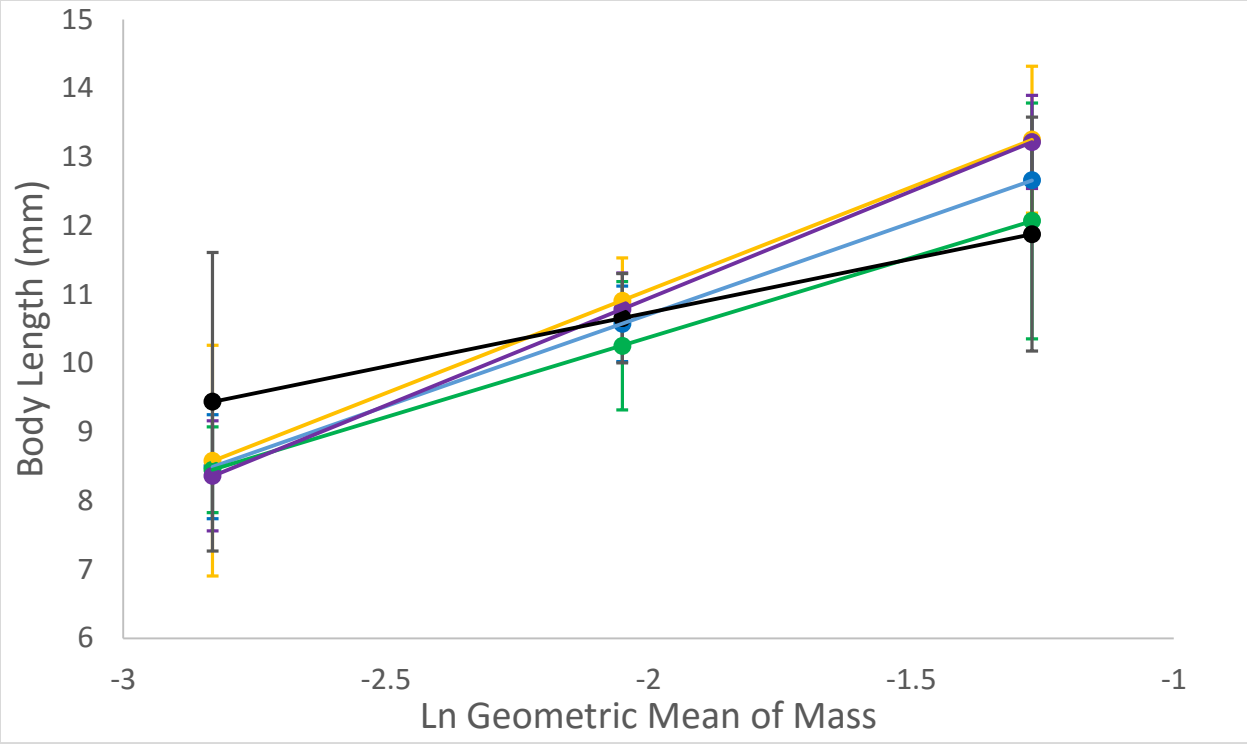

Figure 27. Tadpole body length versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

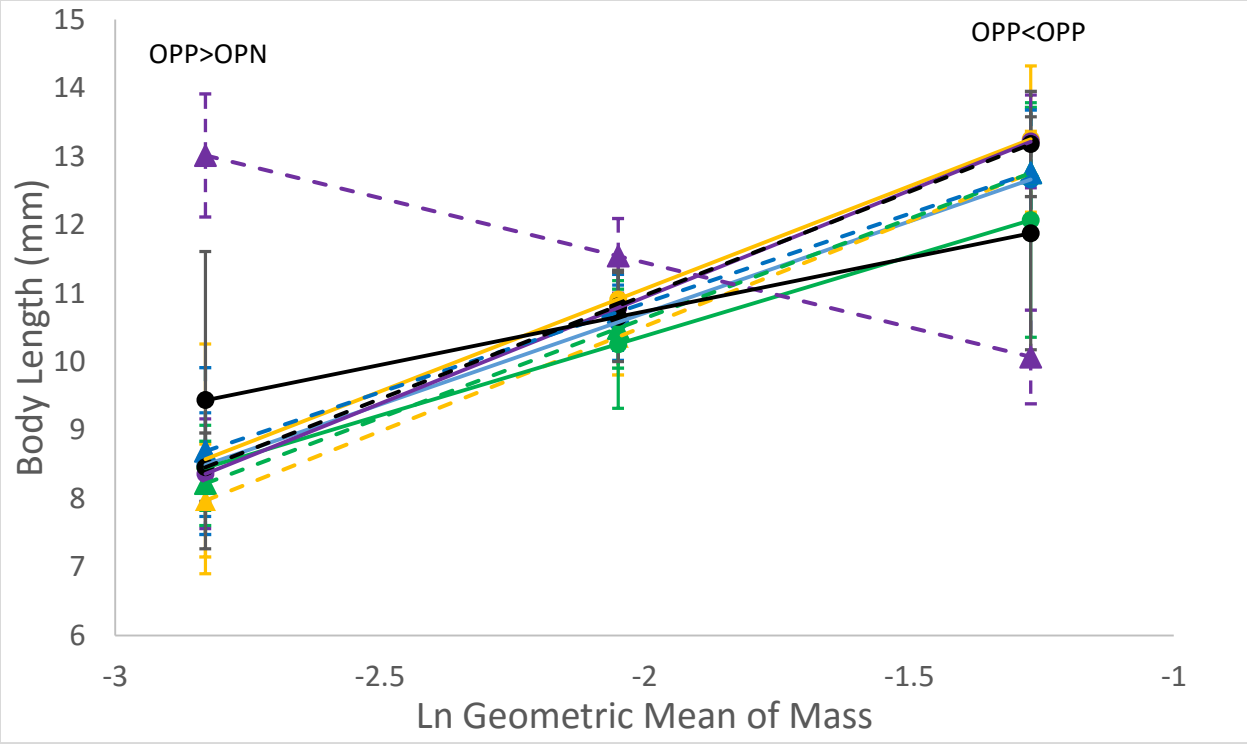

Figure 28. Tadpole body length versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

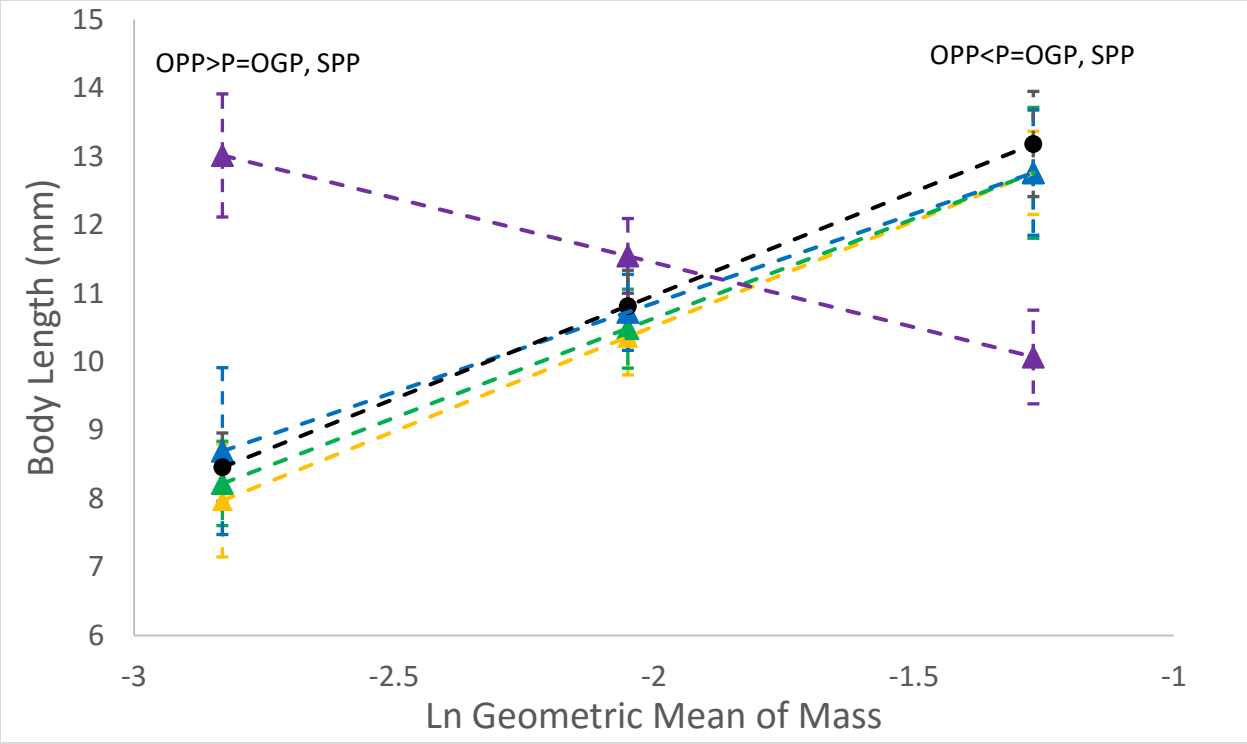

Figure 29. Tadpole eye width versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

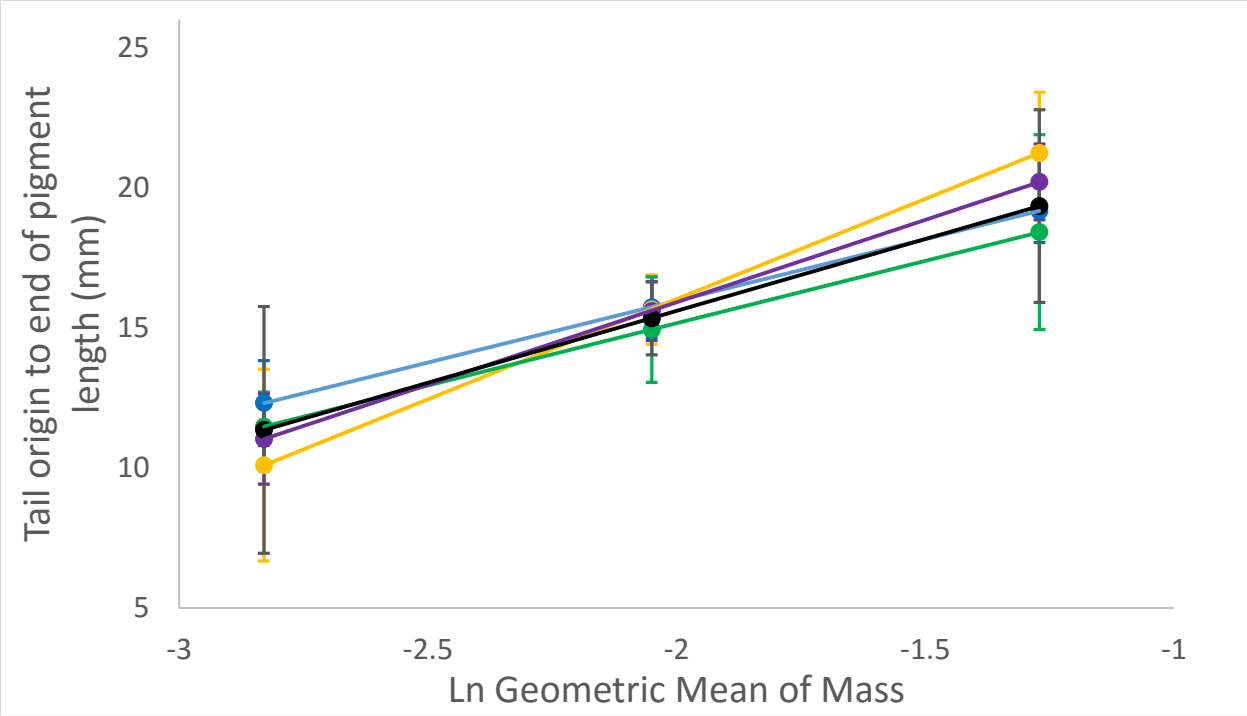

Figure 30. Tadpole tail length from the tail origin to the end of pigment on the tail versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

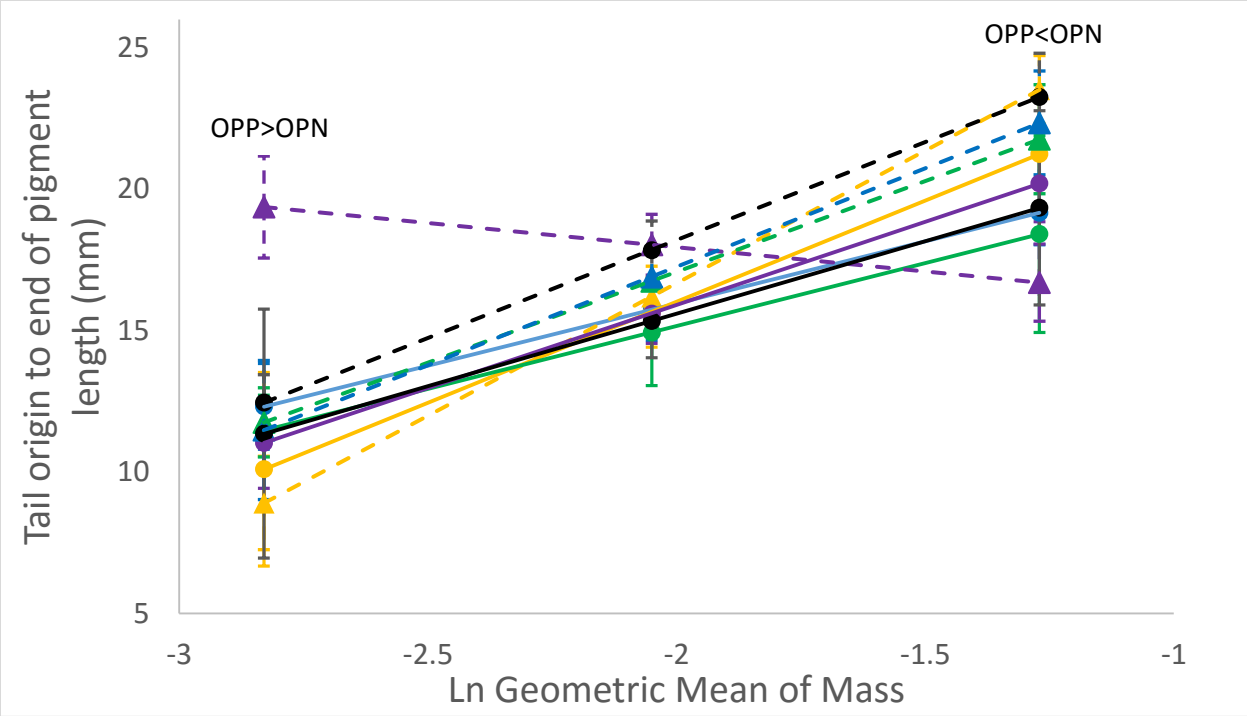

Figure 31. Tadpole tail length from the tail origin to the end of pigment on the tail versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

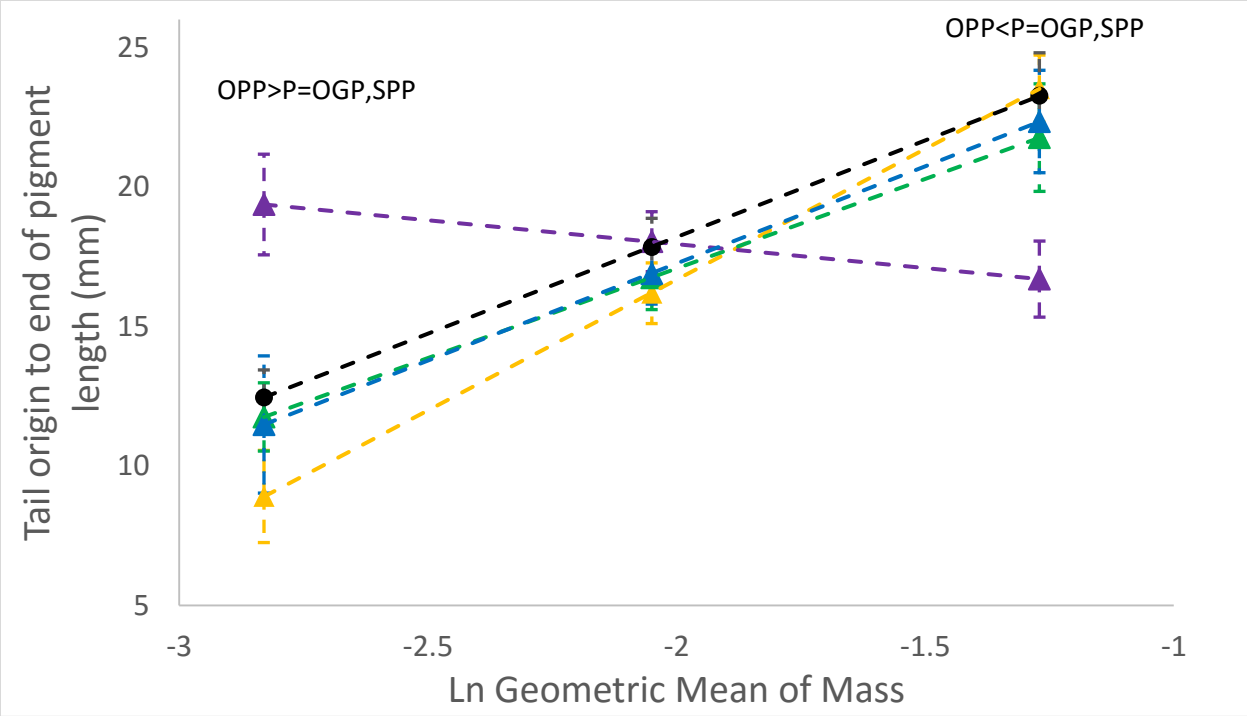

Figure 32. Tadpole tail length from the tail origin to the end of pigment on the tail versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

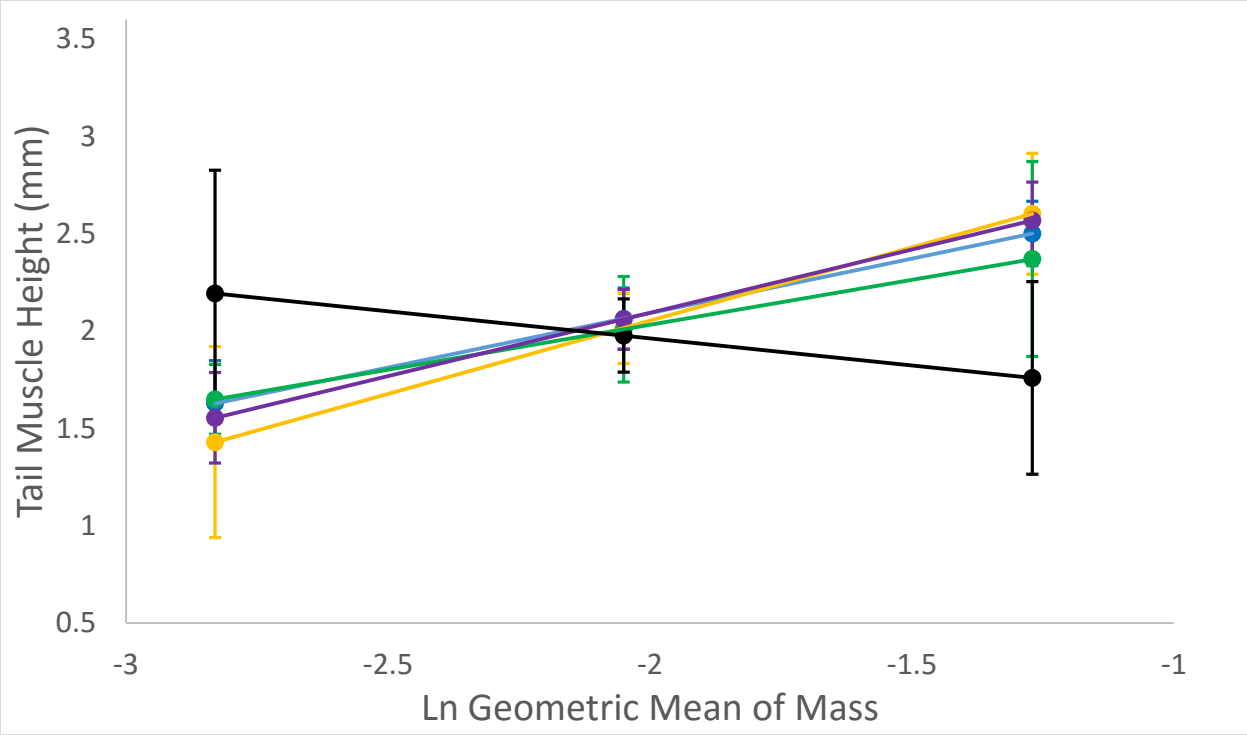

Figure 33. Tadpole tail muscle height at the base of the tail versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

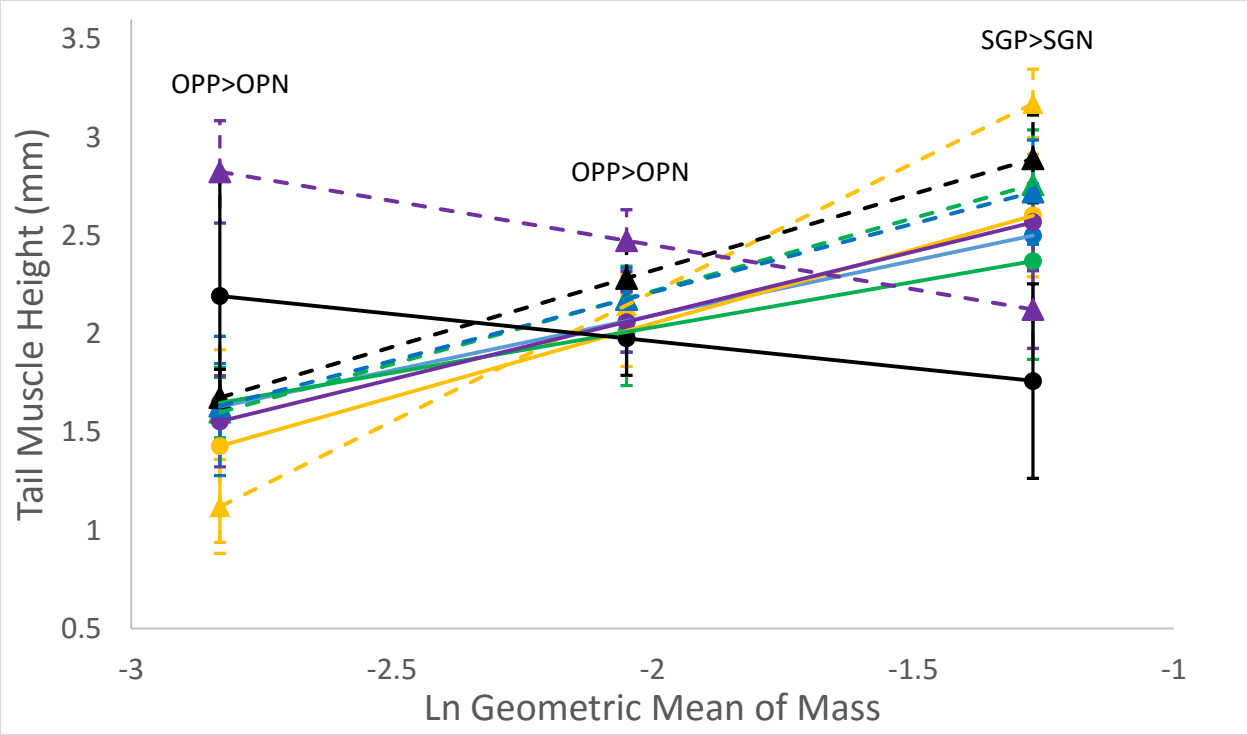

Figure 34. Tadpole tail muscle height at the base of the tail versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

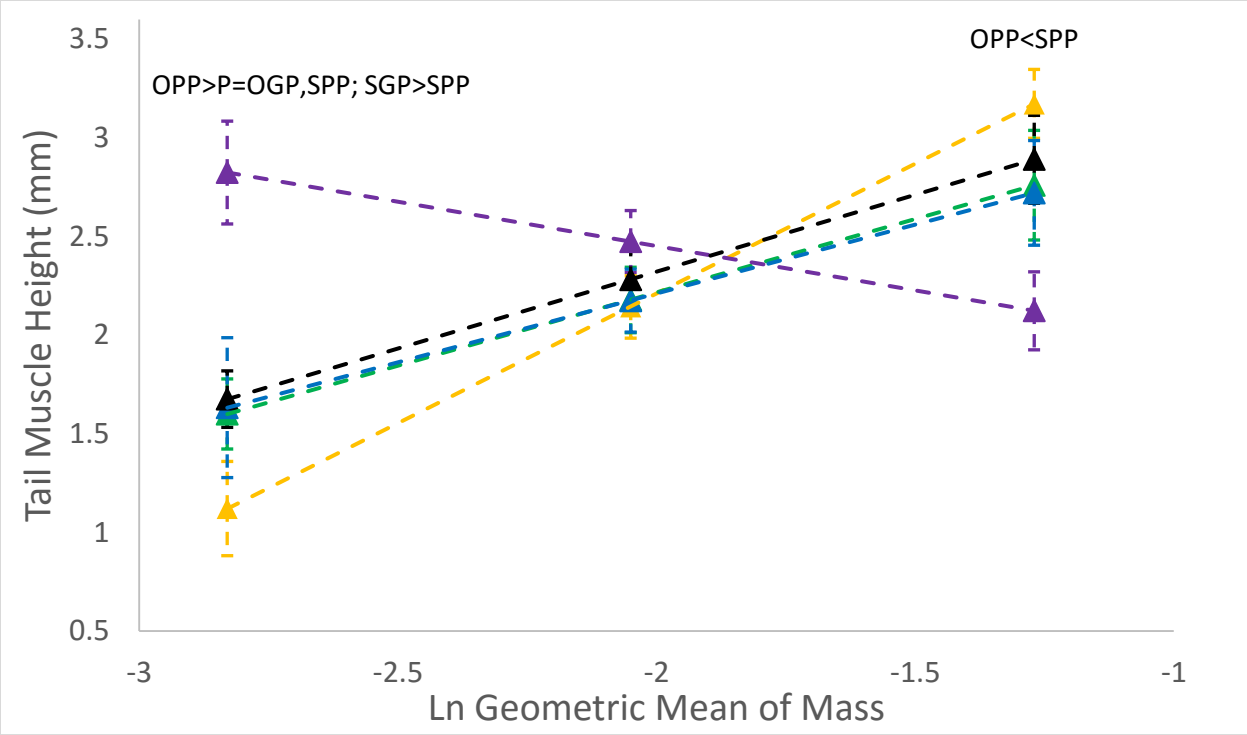

Figure 35. Tadpole tail muscle height at the base of the tail versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

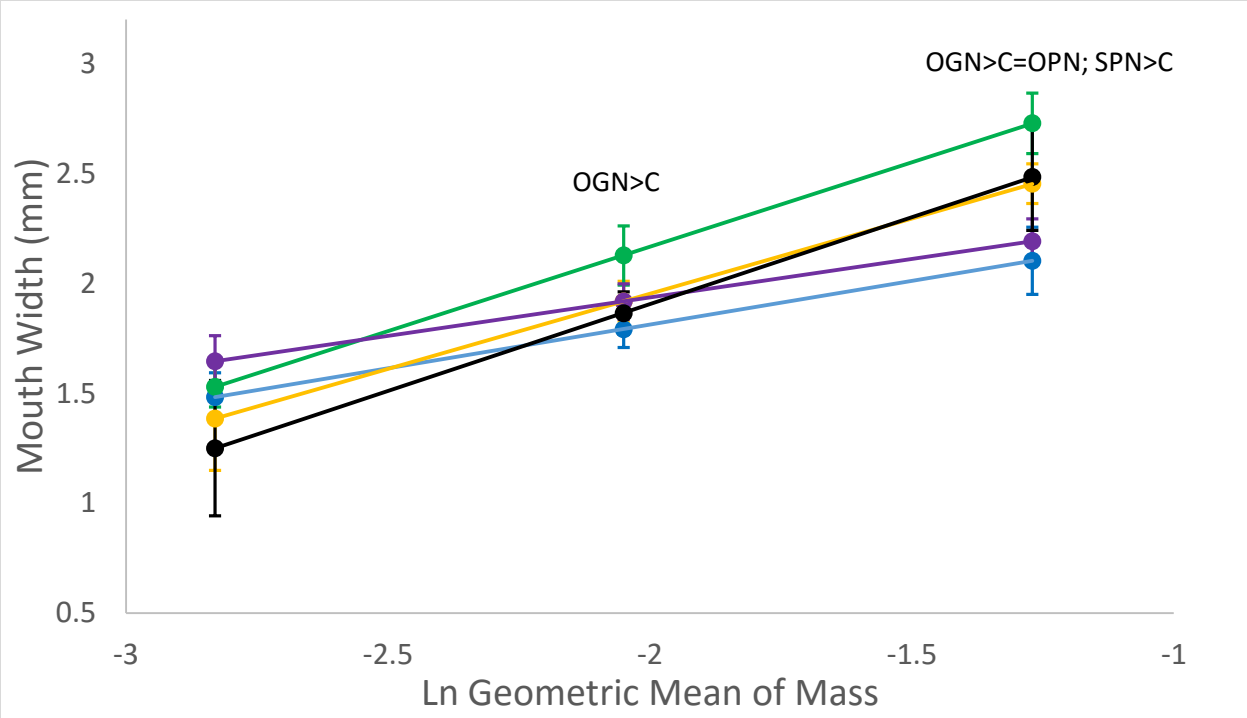

Figure 36. Tadpole mouth width versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

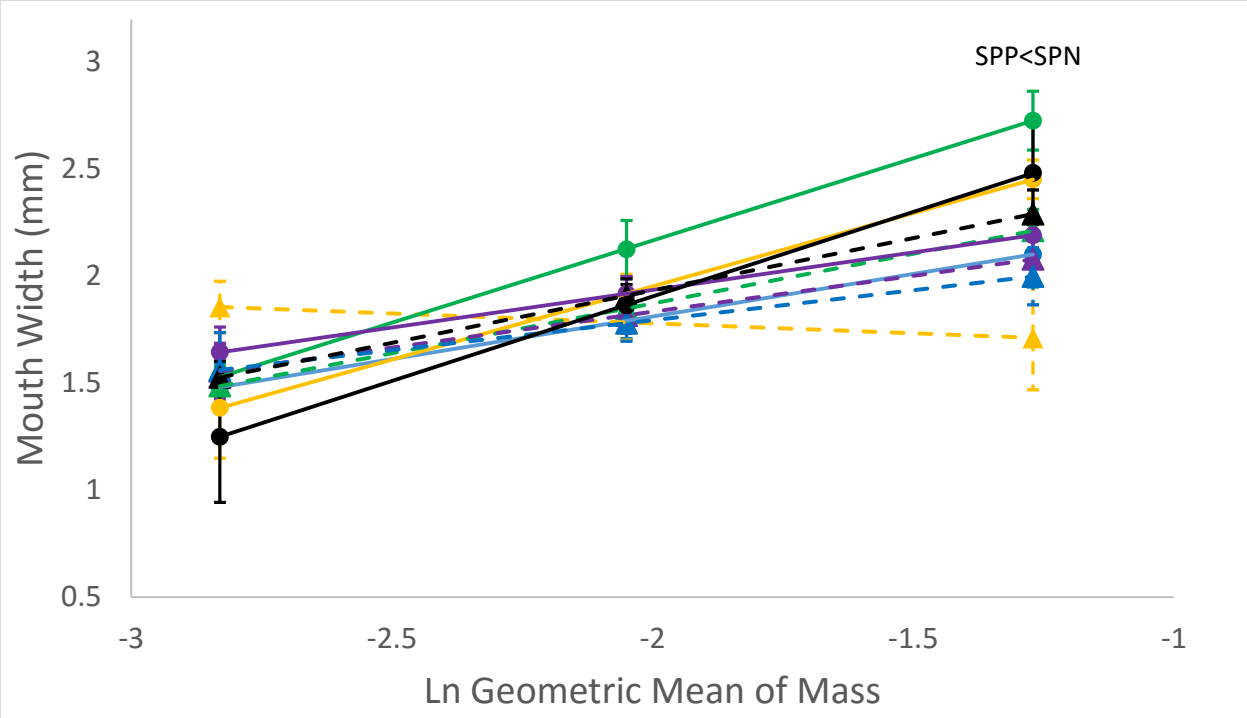

Figure 37. Tadpole mouth width versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

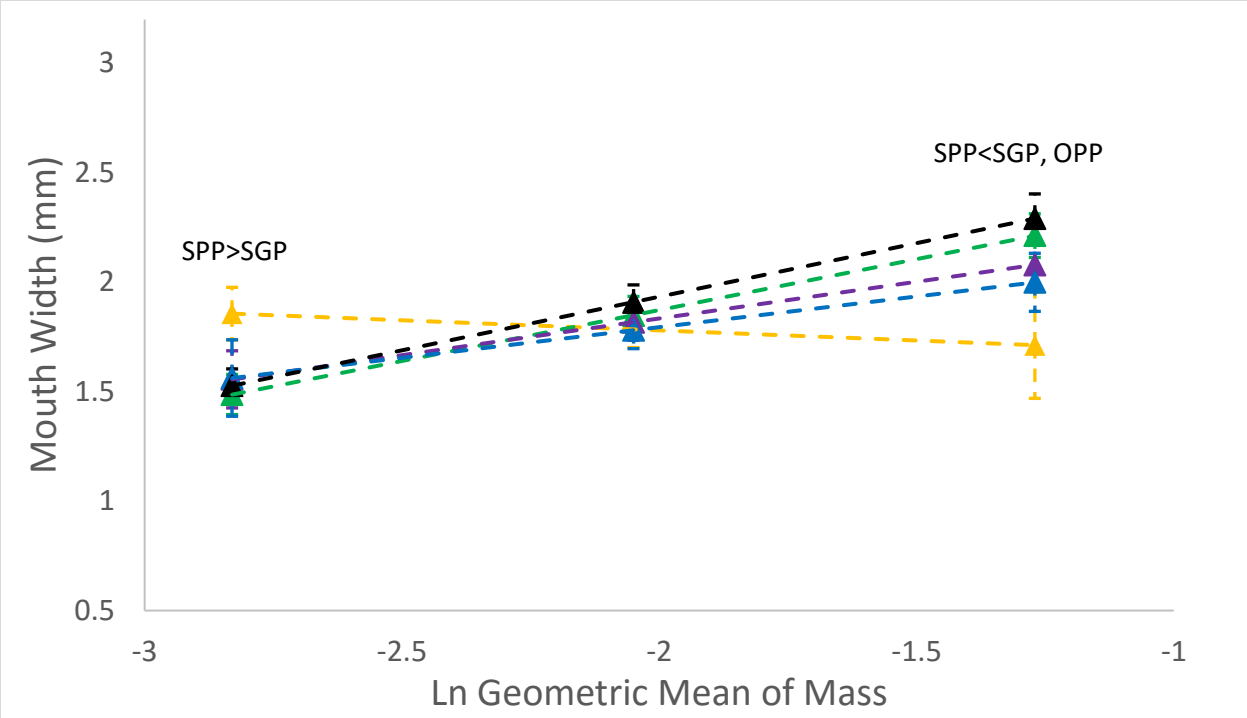

Figure 38. Tadpole mouth width versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

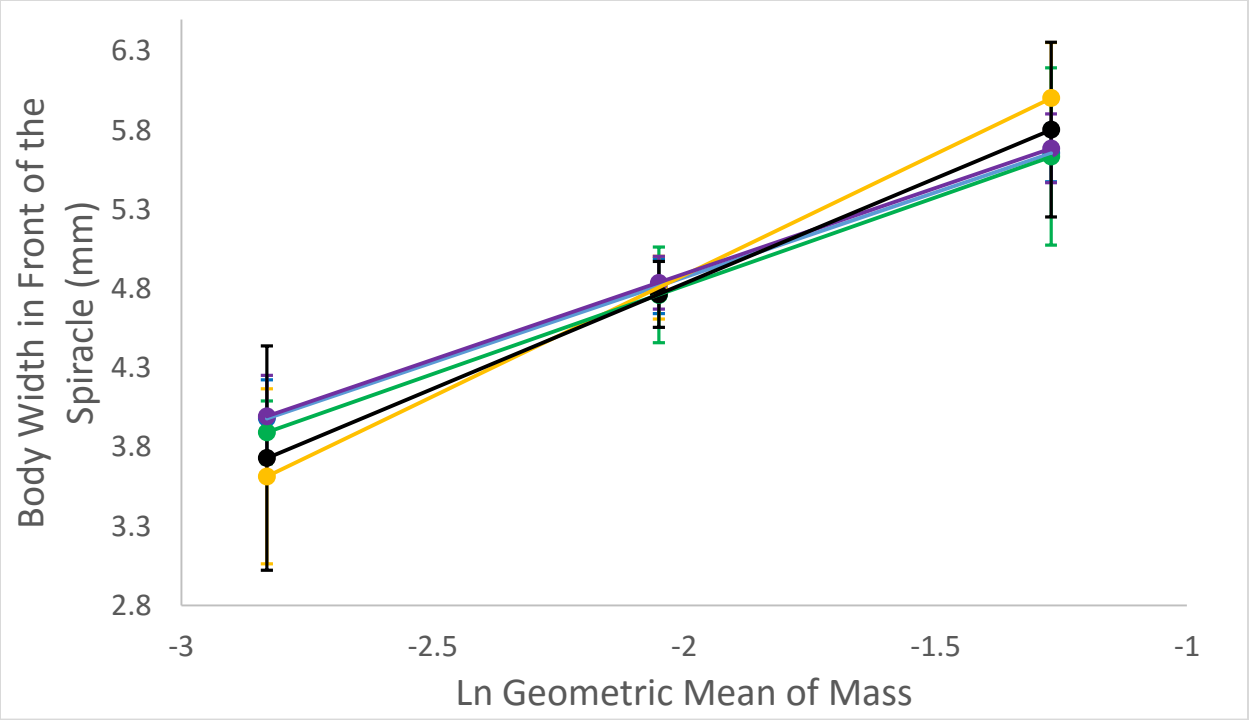

Figure 39. Tadpole body width in front of the spiracle versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

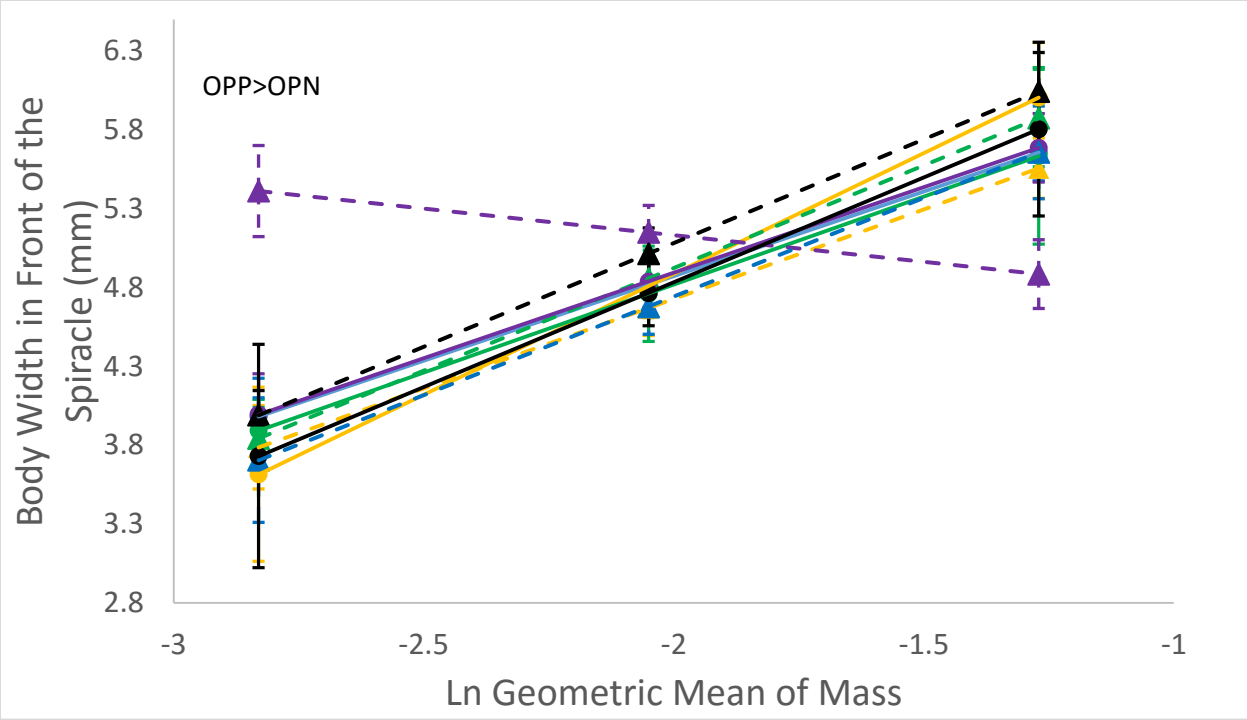

Figure 40. Tadpole body width in front of the spiracle versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

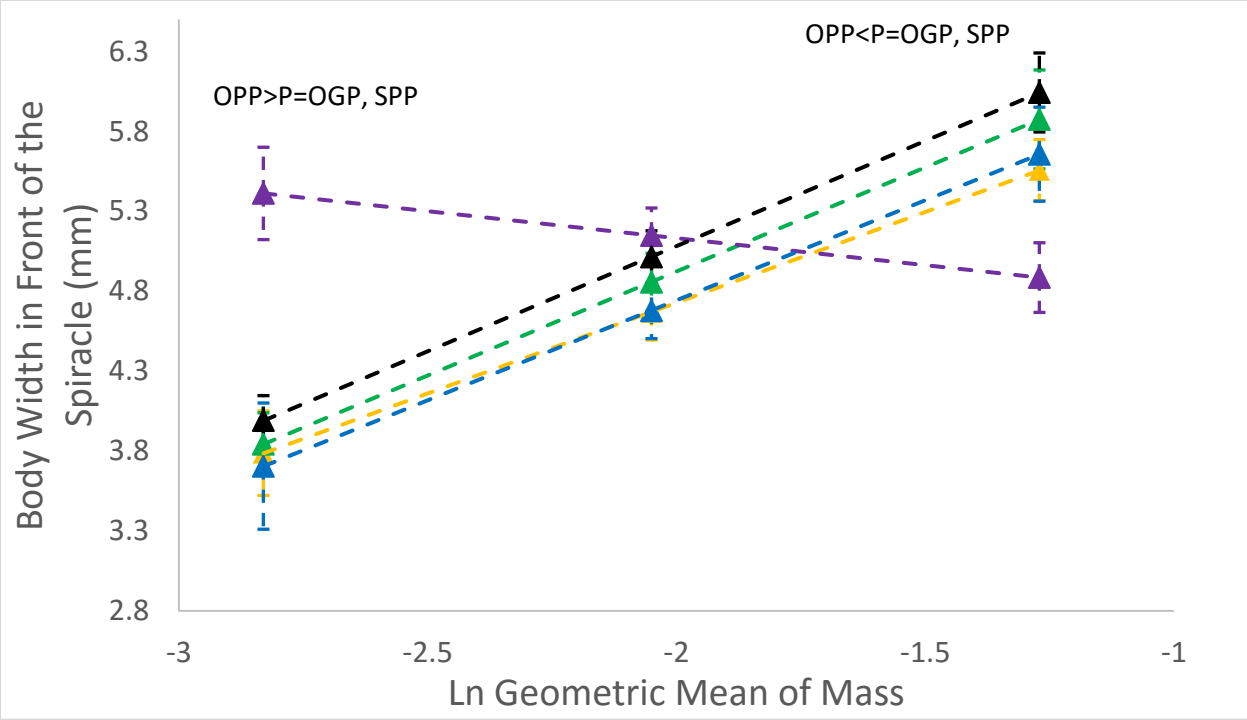

Figure 41. Tadpole body width in front of the spiracle versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

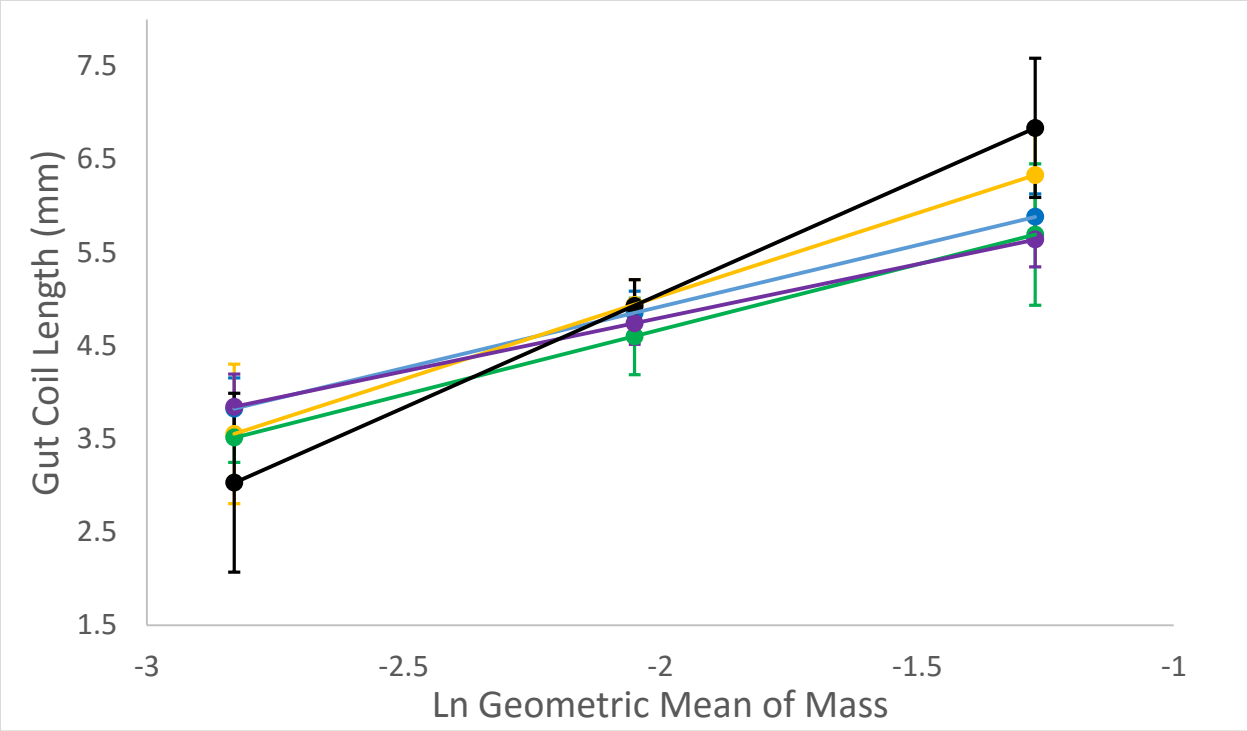

Figure 42. Tadpole gut coil length versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

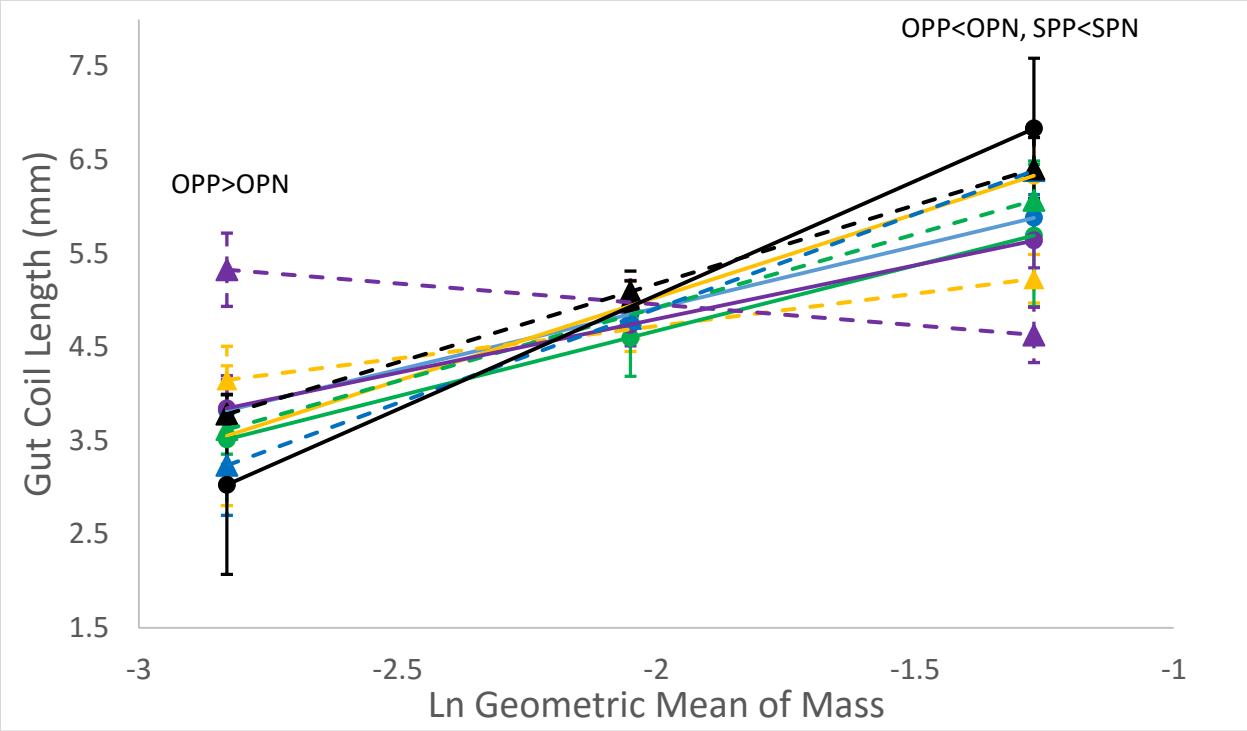

Figure 43. Tadpole gut coil length versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

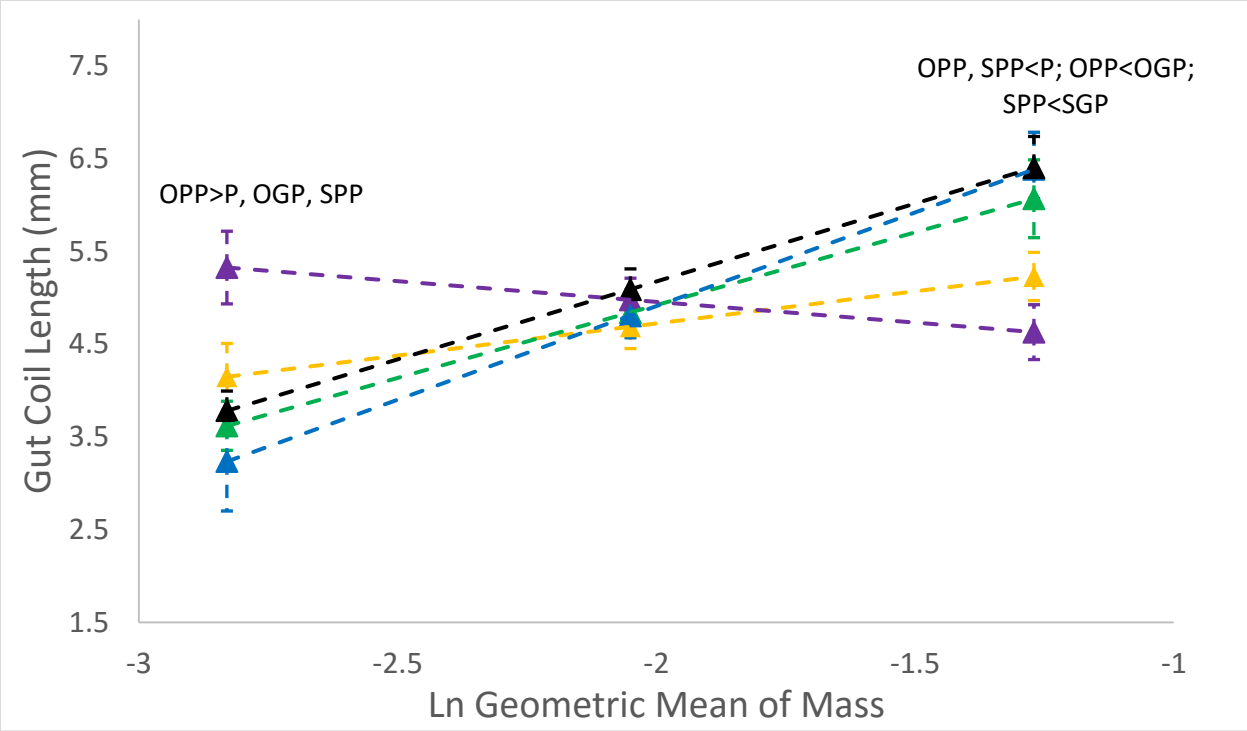

Figure 44. Tadpole gut coil length versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

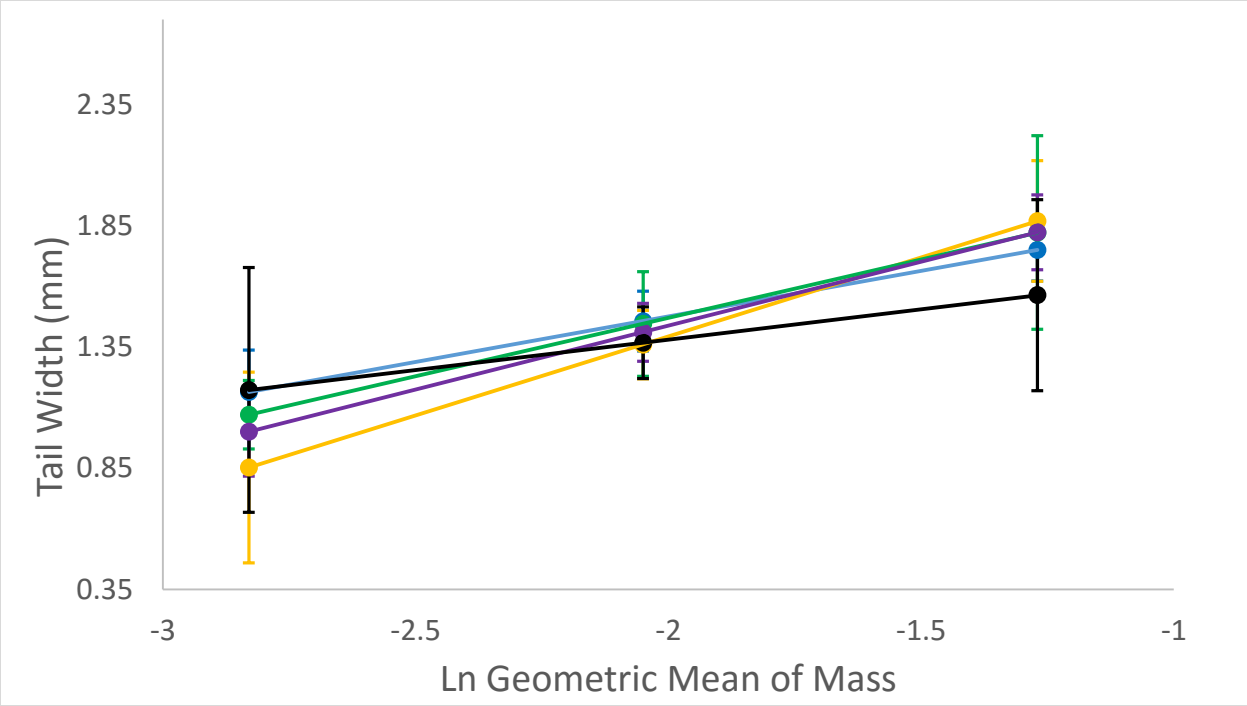

Figure 45. Tadpole tail width versus the natural log of the geometric mean of mass for all no predator treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

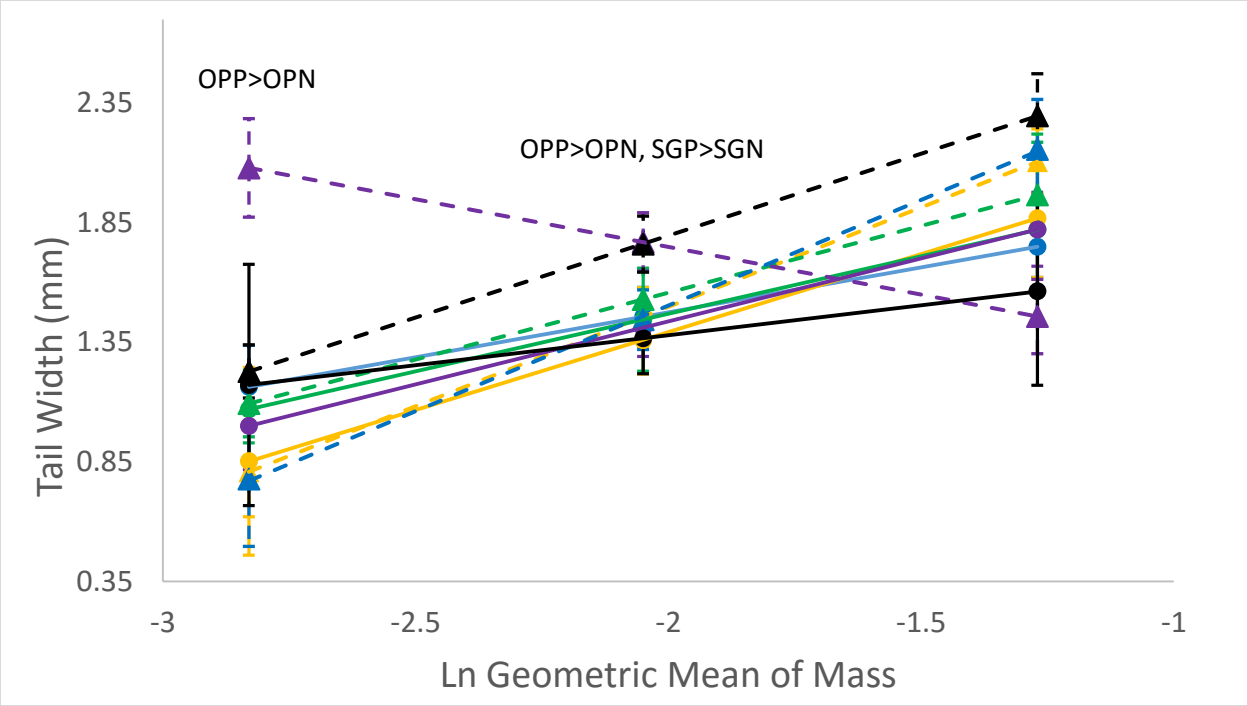

Figure 46. Tadpole tail width versus the natural log of the geometric mean of mass for all treatments. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation.

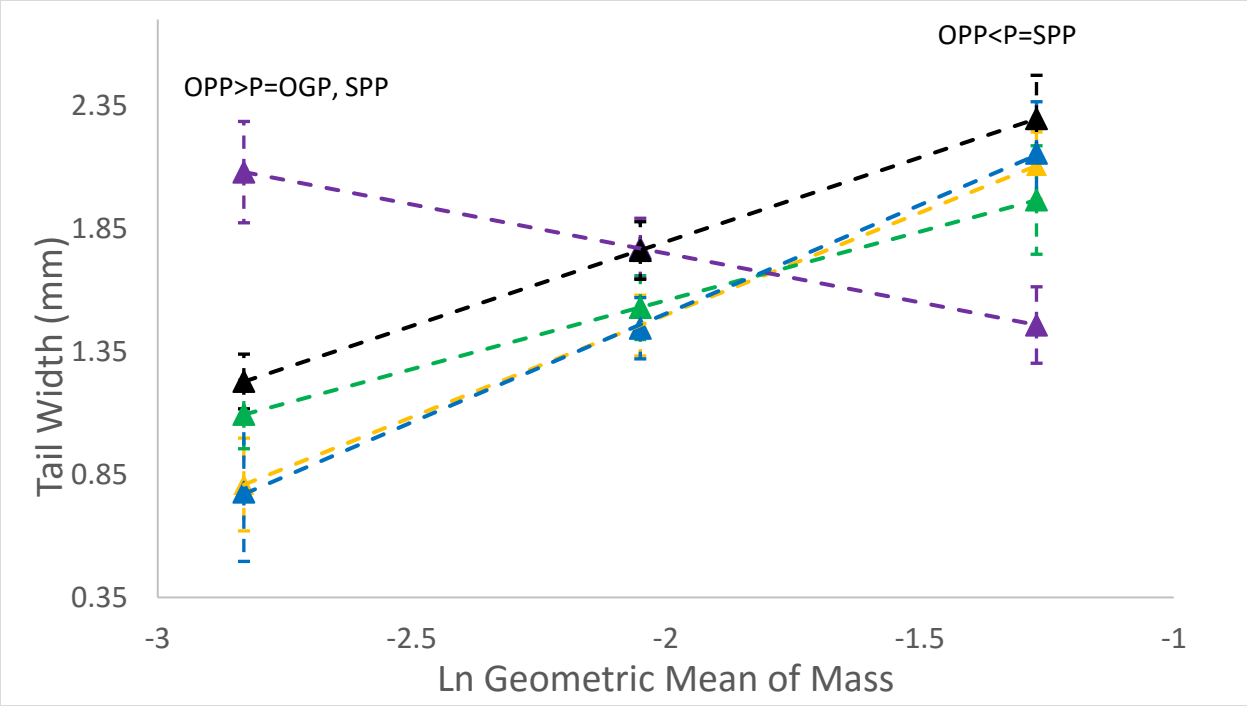

Figure 47. Tadpole tail width versus the natural log of the geometric mean of mass for all treatments containing predators. Mass increases to the right after transformation. Solid lines and circles indicate that no predator was present in that treatment. Dashed lines and triangle symbols indicate the presence of a caged predator in that treatment. Blue lines represent the control density of only 50 pinewoods tadpoles. All other treatments contained 50 pinewoods tadpoles and some more competitors. Yellow lines indicate the presence of an additional 50 same age pinewoods tadpoles. Black lines indicate the addition of 50 same age gray treefrog tadpoles. Purple lines indicate the addition of 50 older pinewoods treefrog tadpoles. Green lines indicate the addition of 50 older gray treefrog tadpoles. Error bars are one standard error of the mean and symbols represent least square means. The three mass values are from left to right: average minus 1 standard deviation, average, average plus 1 standard deviation. See Table 7 for treatment abbreviations. Greater than symbols indicate significant differences at the $p \leq 0.05$ level.

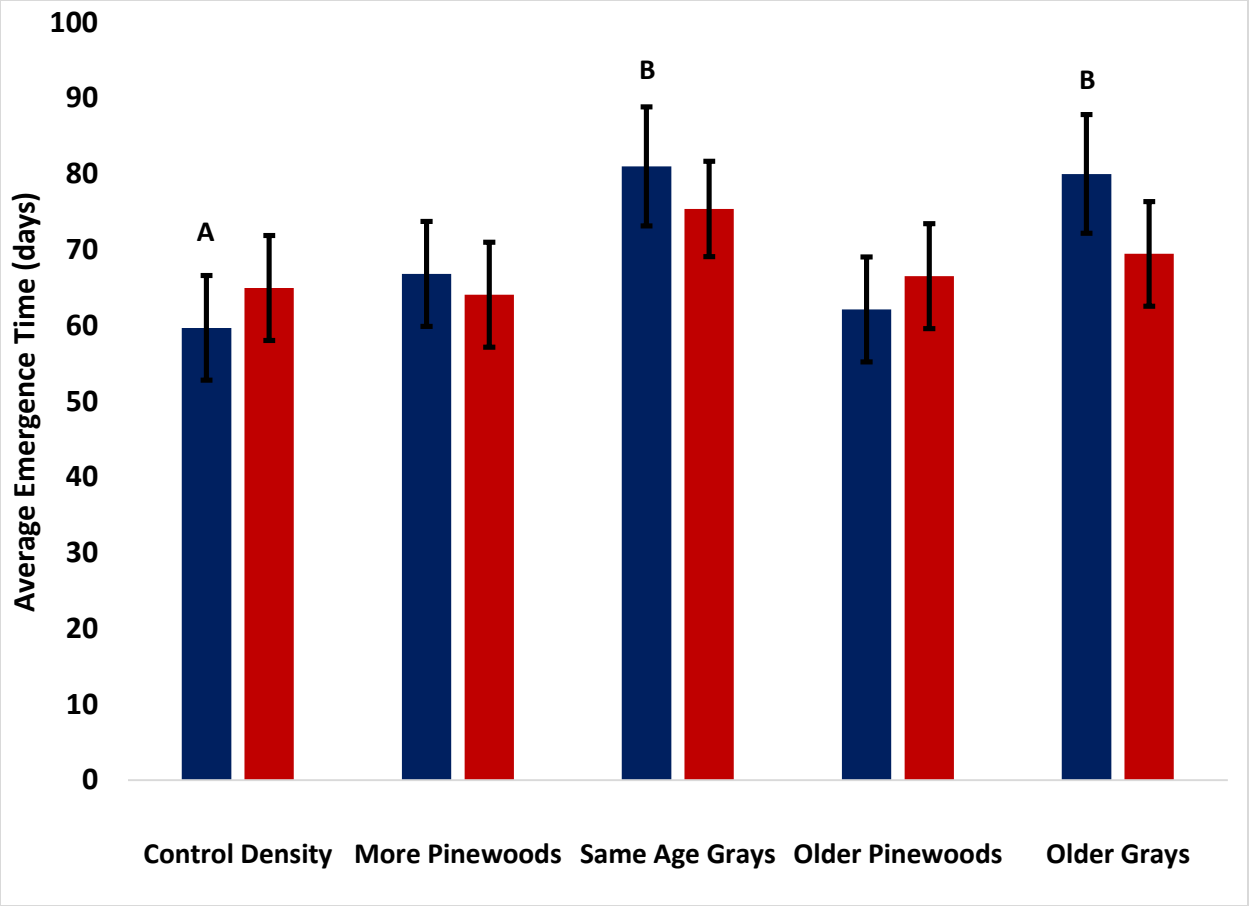

Figure 48. Average emergence time of metamorphs. Blue bars indicate predator absence and red bars indicate presence of a caged predator. Values are least square means and error bars are one standard error of the mean. Letters above the bars indicate significant differences at the $p = 0.05$ level.

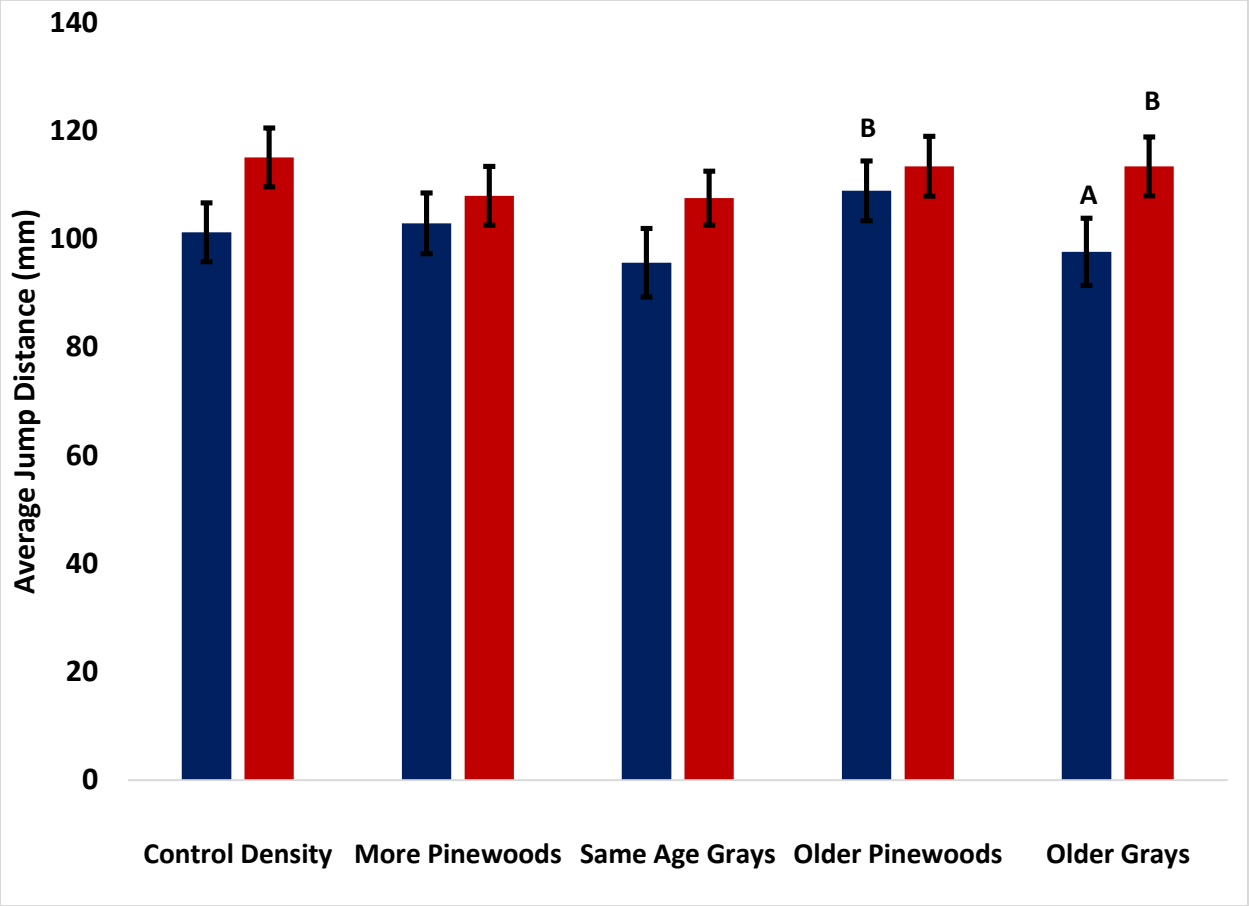

Figure 49. Average jump distance of focal metamorphs. Blue bars indicate predator absence and red bars indicate presence of a caged predator. Values are least square means and error bars are one standard error of the mean. Letters above the bars indicate significant differences at the p = 0.05 level.

Table 7. Treatment names and abbreviations

Treatment	Treatment ID Letters
Control	C
Predator	P
More Pinewoods No Predator	SPN
More Pinewoods and Predator	SPP
Same Age Grays No Predator	SGN
Same Age Grays and Predator	SGP
Older Pinewoods No Predator	OPN
Older Pinewoods and Predator	OPP
Older Grays No Predator	OGN
Older Grays and Predator	OGP

Table 8. Planned Contrasts for the Focal Tadpoles and Metamorphs

Contrast #	Description	Treatment Comparisons
1	Does an older interspecific competitor affect pinewoods?	C vs OGN
2	Does the addition of older pinewoods affect pinewoods?	C vs. OPN
3	Do same age grays affect pinewoods?	C vs. SGN
4	Does higher initial density of pinewoods affect pinewoods?	C vs. SPP
5	Is the addition of older grays the same as adding older pinewoods?	OPN vs OGN
6	Does the identity of the second cohort matter when the second cohort arrives at the same time as the first?	SPN vs. SGN
7	Does the timing of the addition of grays matter?	OGN vs. SGN
8	Does the timing (early vs same time) of more pinewoods arrival matter when pinewoods number constant?	OPN vs. SPN
9	Does a caged predator affect pinewoods?	C vs P
10	Does a caged predator affect pinewoods when an older interspecific competitor is present?	OGN vs. OGP
11	Does non-lethal predator affect focal pinewoods when a second older cohort is present?	OPN vs OPP
12	Does a caged predator affect pinewoods when more pinewoods are present	SPN vs. SPP
13	Does a caged predator affect pinewoods when same age grays are present?	SGN vs. SGP
14	Do older grays affect how pinewoods respond to a predator?	P vs. OGP
15	Do older pinewoods change the response to a predator?	P vs. OPP
16	Do more pinewoods affect how pinewoods respond to non-lethal predator?	P vs. SPP
17	Do same age grays affect how pinewoods respond to non-lethal predator?	P vs. SGP
18	Do focal pinewoods respond to predators the same if they are present with older grays or older pinewoods?	OPP vs. OGP
19	Does the non-lethal effect of predators depend on the identity of the same age cohort?	SPP vs. SGP
20	Does the non-lethal effect of predators differ when grays added early or at same time?	OGP vs. SGP
21	Do non-lethal predators affect pinewoods differently when second cohort of pinewoods enters early or at same time?	OPP vs. SPP
22	Are the effects of predators and older competitors additive?	P and OGN vs. C and OGP
23	Are the effects of older pinewoods and predators additive?	P and OPN vs. C and OPP

Table 8. Continued

24	Are the effects of adding same age grays and predators additive?	P and SGN vs. C and SGP
25	Are the effects of adding more pinewoods and predators additive?	P and SPN vs. C and SPP

Table 9. Part 1. Contrast *p*-values for the geometric mean of mass (mass), eye width (EW), and body length (BL) for tadpoles. Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	Mass	EW (-1SD)	EW (Avg.)	EW (+1SD)	BL (-1SD)	BL (Avg.)	BL (+1SD)
1	C vs P	0.6545	0.4896	0.9144	0.4467	0.8879	0.8384	0.9253
2	C vs OGN	0.1142	0.9573	0.592	0.5041	0.9613	0.7664	0.7423
3	OGN vs. OGP	0.5897	0.5959	0.8939	0.9327	0.7859	0.8298	0.7219
4	P vs. OGP	0.4716	0.3163	0.2841	0.8728	0.7262	0.7501	0.9986
5	P and OGN vs. C and OGP	0.4867	0.3736	0.8673	0.6601	0.7908	0.9513	0.792
6	C vs. OPN	0.6322	0.8599	0.8828	0.6186	0.9018	0.7718	0.508
7	P vs. OPP	0.9357	0.2127	0.1326	0.885	0.0124	0.265	0.0294
8	OPN vs OPP	0.9106	0.0241	0.0734	0.7753	0.001	0.2863	0.0033
9	P and OPN vs. C and OPP	0.6915	0.3248	0.2319	0.6901	0.0269	0.547	0.0345
10	OPN vs OGN	0.2452	0.8839	0.6446	0.6633	0.9286	0.6119	0.5352
11	OPP vs. OGP	0.4246	0.0065	0.0203	0.7305	0.0004	0.1712	0.0291
12	C vs. SGN	0.5932	0.6451	0.7347	0.7745	0.685	0.9193	0.6682
13	C vs. SPP	0.8378	0.5535	0.5238	0.9237	0.9631	0.6662	0.62
14	SPN vs. SPP	0.925	0.7451	0.8725	0.4795	0.7466	0.4888	0.685
15	SGN vs. SGP	0.4181	0.9424	0.1334	0.1958	0.6643	0.8417	0.4954
16	P vs. SPP	0.7345	0.7262	0.4682	0.135	0.6189	0.6246	0.998
17	P vs. SGP	0.3067	0.9621	0.0571	0.0257	0.8573	0.8983	0.7223
18	SPN vs. SGN	0.7275	0.9996	0.8052	0.8406	0.7603	0.7705	0.5084
19	SPP vs. SGP	0.1717	0.6355	0.2282	0.1856	0.6024	0.5269	0.6587
20	P and SGN vs. C and SGP	0.7859	0.6818	0.2998	0.1616	0.6648	0.9942	0.5943
21	P and SPN vs. C and SPP	0.7004	0.5065	0.9649	0.2877	0.7346	0.5201	0.7089
22	OPM vs. SPN	0.7822	0.6378	0.4474	0.8136	0.9078	0.8708	0.9779
23	OGN vs. SGN	0.314	0.6588	0.4298	0.7886	0.6669	0.714	0.9379
24	OGP vs. SGP	0.79	0.0861	0.0064	0.0147	0.7493	0.6506	0.7194
25	OPP vs. SPP	0.7953	0.0634	0.4341	0.132	0.0009	0.1206	0.0079

Table 9. Part 2. Contrast *p*-values for tadpole body depth (BD) and tail length from the origin of the tail to the end of the tail pigment (TPL). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	BD (-1SD)	BD (Avg.)	BD (+1SD)	TPL (-1SD)	TPL (Avg.)	TPL (+1SD)
1	C vs P	0.8005	0.7956	0.4818	0.7733	0.4466	0.1573
2	C vs OGN	0.7975	0.6741	0.7163	0.6644	0.715	0.8372
3	OGN vs. OGP	0.9151	0.6216	0.5541	0.8717	0.4143	0.4046
4	P vs. OGP	0.6085	0.7172	0.3448	0.9217	0.9218	0.8308
5	P and OGN vs. C and OGP	0.7853	0.5814	0.396	0.7384	0.8094	0.9686
6	C vs. OPN	0.6917	0.7708	0.9957	0.5628	0.9304	0.552
7	P vs. OPP	0.0098	0.1727	0.0549	0.0212	0.4513	0.0251
8	OPN vs OPP	0.0005	0.1561	0.0047	0.003	0.111	0.079
9	P and OPN vs. C and OPP	0.0185	0.2376	0.1186	0.026	0.5479	0.0331
10	OPN vs OGN	0.8517	0.823	0.72	0.8253	0.7546	0.6353
11	OPP vs. OGP	0.0002	0.3209	0.0069	0.0031	0.4088	0.0436
12	C vs. SGN	0.3157	0.4968	0.0649	0.8388	0.8135	0.9627
13	C vs. SPP	0.62	0.854	0.6014	0.5587	0.9601	0.4021
14	SPN vs. SPP	0.8039	0.9457	0.6353	0.7565	0.7419	0.3656
15	SGN vs. SGP	0.2383	0.9344	0.141	0.8097	0.1386	0.3152
16	P vs. SPP	0.5788	0.9912	0.4579	0.3903	0.6442	0.5856
17	P vs. SGP	0.5914	0.6773	0.9353	0.715	0.5218	0.7023
18	SPN vs. SGN	0.2483	0.6359	0.0544	0.8257	0.8601	0.6497
19	SPP vs. SGP	0.9067	0.6849	0.4649	0.0776	0.2711	0.8942
20	P and SGN vs. C and SGP	0.2601	0.8132	0.11	0.7243	0.5492	0.8678
21	P and SPN vs. C and SPP	0.9634	0.8993	0.9157	0.9406	0.7794	0.7852
22	OPM vs. SPN	0.794	0.9333	0.6206	0.8089	0.9756	0.6882
23	OGN vs. SGN	0.2615	0.9181	0.2511	0.9794	0.8584	0.8524
24	OGP vs. SGP	0.9918	0.4388	0.3424	0.6472	0.4675	0.5396
25	OPP vs. SPP	0.0006	0.1693	0.0029	0.0007	0.2327	0.0017

Table 9. Part 3. Contrast *p*-values for tadpole tail length from the origin on the head to the tip of the tail (HTL) and tail height at the tallest point of the tail fin (MTH). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	HTL (-1SD)	HTL (Avg.)	HTL (+1SD)	MTH (-1SD)	MTH (Avg.)	MTH (+1SD)
1	C vs P	0.8438	0.7616	0.4928	0.6991	0.3392	0.4166
2	C vs OGN	0.8564	0.6809	0.6934	0.8722	0.7071	0.7156
3	OGN vs. OGP	0.9011	0.5293	0.4545	0.7673	0.2459	0.2468
4	P vs. OGP	0.9984	0.9852	0.9846	0.7392	0.8382	0.5719
5	P and OGN vs. C and OGP	0.9149	0.7295	0.7415	0.8526	0.6725	0.53
6	C vs. OPN	0.6334	0.7084	0.9751	0.9419	0.8798	0.7199
7	P vs. OPP	0.0195	0.4974	0.0185	0.0157	0.1961	0.0628
8	OPN vs OPP	0.0027	0.1759	0.0311	0.0008	0.0386	0.0823
9	P and OPN vs. C and OPP	0.0276	0.4558	0.0547	0.0341	0.4053	0.0881
10	OPN vs OGN	0.7263	0.8729	0.7104	0.9416	0.6219	0.6052
11	OPP vs. OGP	0.0021	0.5216	0.0193	0.0006	0.2905	0.0163
12	C vs. SGN	0.8704	0.5686	0.7538	0.7594	0.5251	0.8567
13	C vs. SPP	0.5991	0.7993	0.6307	0.6487	0.8953	0.5911
14	SPN vs. SPP	0.8605	0.7954	0.5409	0.9528	0.6717	0.5208
15	SGN vs. SGP	0.8296	0.235	0.4391	0.6561	0.0558	0.2571
16	P vs. SPP	0.4829	0.769	0.5761	0.3087	0.5208	0.599
17	P vs. SGP	0.7686	0.7026	0.8872	0.8476	0.6028	0.4032
18	SPN vs. SGN	0.8303	0.7587	0.5764	0.9641	0.6306	0.6404
19	SPP vs. SGP	0.1454	0.4957	0.6559	0.1932	0.2447	0.6395
20	P and SGN vs. C and SGP	0.7765	0.4978	0.7386	0.8655	0.4143	0.5596
21	P and SPN vs. C and SPP	0.9826	0.9868	0.992	0.7823	0.7286	0.9538
22	OPM vs. SPN	0.8083	0.9276	0.6333	0.6878	0.7865	0.7864
23	OGN vs. SGN	0.928	0.9782	0.9523	0.8068	0.9128	0.8949
24	OGP vs. SGP	0.6136	0.7225	0.9055	0.7896	0.7635	0.8387
25	OPP vs. SPP	0.0009	0.3363	0.0011	0.09004	0.00645	0.0061

Table 9. Part 4. Contrast *p*-values for tadpole tail muscle height at the base of the tail (TMH) and tail stripe height at the base of the tail (TSH). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	TMH (-1SD)	TMH (Avg.)	TMH (+1SD)	TSH (-1SD)	TSH (Avg.)	TSH (+1SD)
1	C vs P	0.9928	0.6056	0.4822	0.5332	0.7898	0.2312
2	C vs OGN	0.9404	0.8607	0.8038	0.7299	0.708	0.5309
3	OGN vs. OGP	0.8423	0.5891	0.4951	0.5122	0.4454	0.5744
4	P vs. OGP	0.9351	0.9883	0.9205	0.4119	0.1928	0.4746
5	P and OGN vs. C and OGP	0.9112	0.8796	0.7954	0.3857	0.6323	0.9312
6	C vs. OPN	0.8142	0.9863	0.7832	0.7875	0.8702	0.5372
7	P vs. OPP	0.0167	0.1732	0.0868	0.0107	0.0967	0.1397
8	OPN vs OPP	0.0018	0.0597	0.1137	0.0062	0.0735	0.316
9	P and OPN vs. C and OPP	0.0309	0.318	0.124	0.0232	0.269	0.1259
10	OPN vs OGN	0.7419	0.8663	0.713	0.9693	0.7909	0.7441
11	OPP vs. OGP	0.0013	0.1919	0.0711	0.0085	0.7255	0.0335
12	C vs. SGN	0.4112	0.7188	0.1766	0.8595	0.9066	0.904
13	C vs. SPP	0.7113	0.8285	0.7712	0.3455	0.4354	0.6714
14	SPN vs. SPP	0.5779	0.5786	0.1239	0.1381	0.5554	0.1241
15	SGN vs. SGP	0.4368	0.2013	0.0549	0.7223	0.389	0.7389
16	P vs. SPP	0.2385	0.8833	0.1547	0.8734	0.9522	0.8938
17	P vs. SGP	0.9099	0.6118	0.6227	0.3348	0.5786	0.6943
18	SPN vs. SGN	0.3591	0.8857	0.1746	0.4347	0.4119	0.7176
19	SPP vs. SGP	0.0562	0.5124	0.3213	0.1236	0.5367	0.529
20	P and SGN vs. C and SGP	0.5132	0.5464	0.1757	0.5272	0.648	0.7517
21	P and SPN vs. C and SPP	0.655	0.9552	0.4573	0.4011	0.5406	0.6854
22	OPM vs. SPN	0.8203	0.8407	0.9269	0.2796	0.5207	0.4109
23	OGN vs. SGN	0.4219	0.9217	0.4017	0.9732	0.6583	0.7031
24	OGP vs. SGP	0.7301	0.6295	0.704	0.8394	0.4098	0.2551
25	OPP vs. SPP	0.0002	0.1383	0.001	0.0016	0.0861	0.0499

Table 9. Part 5. Contrast *p*-values for tadpole mouth width (MW) and inter-eye distance (IED). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	MW (-1SD)	MW (Avg.)	MW (+1SD)	IED (-1SD)	IED (Avg.)	IED (+1SD)
1	C vs P	0.6878	0.9103	0.4957	0.892	0.99	0.8405
2	C vs OGN	0.719	0.0383	0.0234	0.7283	0.5157	0.5566
3	OGN vs. OGP	0.7139	0.077	0.0715	0.9021	0.3632	0.341
4	P vs. OGP	0.6903	0.5113	0.2662	0.9787	0.7078	0.6377
5	P and OGN vs. C and OGP	0.5851	0.1601	0.2025	0.8575	0.4572	0.454
6	C vs. OPN	0.2931	0.2237	0.4443	0.8586	0.7157	0.6914
7	P vs. OPP	0.978	0.7149	0.6201	0.0188	0.2053	0.0943
8	OPN vs OPP	0.5838	0.3089	0.3835	0.0091	0.3352	0.0388
9	P and OPN vs. C and OPP	0.5177	0.5288	0.9632	0.06	0.503	0.1173
10	OPN vs OGN	0.4051	0.161	0.0517	0.5982	0.368	0.448
11	OPP vs. OGP	0.61513	0.7636	0.4034	0.0021	0.376	0.0389
12	C vs. SGN	0.4808	0.524	0.1554	0.7997	0.9252	0.6774
13	C vs. SPP	0.706	0.2538	0.0477	0.9885	0.8401	0.7694
14	SPN vs. SPP	0.0884	0.2374	0.0005	0.9894	0.8742	0.9171
15	SGN vs. SGP	0.3887	0.6924	0.4782	0.7355	0.4336	0.7743
16	P vs. SPP	0.1595	0.9642	0.0614	0.9346	0.9847	0.9466
17	P vs. SGP	0.8506	0.2047	0.0923	0.7809	0.3413	0.3743
18	SPN vs. SGN	0.733	0.6647	0.9181	0.84	0.923	0.8459
19	SPP vs. SGP	0.0213	0.2248	0.0005	0.9422	0.4867	0.7191
20	P and SGN vs. C and SGP	0.6093	0.7162	0.7829	0.7207	0.5694	0.8812
21	P and SPN vs. C and SPP	0.251	0.4253	0.0102	0.9407	0.8905	0.8765
22	OPM vs. SPN	0.3341	0.9992	0.1513	0.9062	0.8941	0.9942
23	OGN vs. SGN	0.394	0.099	0.4859	0.912	0.4935	0.4572
24	OGP vs. SGP	0.6929	0.5548	0.629	0.6046	0.5777	0.7162
25	OPP vs. SPP	0.1064	0.7538	0.0095	0.1267	0.3506	0.6321

Table 9. Part 6. Contrast *p*-values for tadpole body width in front of the spiracle (BWBS) and body width behind the spiracle (BWAS). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	BWBS (-1SD)	BWBS (Avg.)	BWBS (+1SD)	BWAS (-1SD)	BWAS (Avg.)	BWAS (+1SD)
1	C vs P	0.5598	0.5804	0.9997	0.8577	0.7596	0.5106
2	C vs OGN	0.7823	0.8759	0.9701	0.6453	0.9259	0.8953
3	OGN vs. OGP	0.8598	0.7923	0.7127	0.6151	0.6833	0.8163
4	P vs. OGP	0.7575	0.4911	0.6179	0.8569	0.8805	0.9885
5	P and OGN vs. C and OGP	0.681	0.5954	0.7459	0.675	0.8715	0.9089
6	C vs. OPN	0.9712	0.9311	0.9188	0.8418	0.7824	0.8255
7	P vs. OPP	0.0033	0.0729	0.0521	0.0043	0.1269	0.0231
8	OPN vs OPP	0.0023	0.2118	0.0198	0.001	0.1097	0.0218
9	P and OPN vs. C and OPP	0.0133	0.2083	0.1034	0.0167	0.3556	0.0477
10	OPN vs OGN	0.7601	0.8279	0.9325	0.5117	0.7729	0.9771
11	OPP vs. OGP	0.0004	0.258	0.0196	0.0004	0.1804	0.0236
12	C vs. SGN	0.7428	0.8495	0.8031	0.7495	0.8815	0.5884
13	C vs. SPP	0.5528	0.9695	0.3893	0.9227	0.753	0.5731
14	SPN vs. SPP	0.7838	0.6092	0.2775	0.8486	0.6737	0.7997
15	SGN vs. SGP	0.7257	0.3614	0.698	0.7141	0.5626	0.9626
16	P vs. SPP	0.8665	0.9707	0.779	0.8442	0.6736	0.7432
17	P vs. SGP	0.5116	0.1816	0.3305	0.7965	0.6172	0.7503
18	SPN vs. SGN	0.8989	0.886	0.7625	0.8411	0.8869	0.8772
19	SPP vs. SGP	0.5161	0.1692	0.1405	0.507	0.3555	0.4557
20	P and SGN vs. C and SGP	0.543	0.2996	0.7361	0.6913	0.8287	0.7838
21	P and SPN vs. C and SPP	0.5687	0.9977	0.4079	0.9645	0.6075	0.5256
22	OPM vs. SPN	0.5431	0.9055	0.4493	0.8345	0.9534	0.6984
23	OGN vs. SGN	0.8283	0.9931	0.8319	0.8929	0.8395	0.7596
24	OGP vs. SGP	0.5654	0.5292	0.6761	0.8984	0.7368	0.737
25	OPP vs. SPP	0.0009	0.0672	0.0352	0.0005	0.0607	0.0125

Table 9. Part 7. Contrast *p*-values for tadpole gut coil length (GTBL) and tail width (TW). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	GTBL (-1SD)	GTBL (Avg.)	GTBL (+1SD)	TW (-1SD)	TW (Avg.)	TW (+1SD)
1	C vs P	0.365	0.8966	0.2996	0.2581	0.9471	0.1569
2	C vs OGN	0.4755	0.6031	0.8142	0.6773	0.9636	0.8666
3	OGN vs. OGP	0.7813	0.6223	0.671	0.9063	0.7424	0.7547
4	P vs. OGP	0.531	0.9222	0.5912	0.3282	0.6436	0.6294
5	P and OGN vs. C and OGP	0.3589	0.6323	0.8993	0.2991	0.7587	0.6745
6	C vs. OPN	0.9653	0.7387	0.5343	0.5238	0.7888	0.7216
7	P vs. OPP	0.0066	0.6087	0.003	0.002	0.0787	0.0233
8	OPN vs OPP	0.0129	0.4707	0.0285	0.0013	0.0494	0.1133
9	P and OPN vs. C and OPP	0.0233	0.5502	0.029	0.0038	0.1449	0.0418
10	OPN vs OGN	0.4631	0.7689	0.9494	0.7646	0.8886	0.9986
11	OPP vs. OGP	0.0026	0.6864	0.0134	0.0012	0.1923	0.0769
12	C vs. SGN	0.4455	0.8369	0.244	0.9882	0.6475	0.6581
13	C vs. SPP	0.7449	0.8087	0.4123	0.4783	0.6081	0.6753
14	SPN vs. SPP	0.4833	0.495	0.059	0.9218	0.6136	0.4154
15	SGN vs. SGP	0.4546	0.6568	0.6047	0.9153	0.0524	0.1099
16	P vs. SPP	0.1772	0.7281	0.0282	0.9275	0.9519	0.9683
17	P vs. SGP	0.3575	0.397	0.9725	0.1565	0.0809	0.5173
18	SPN vs. SGN	0.673	0.9775	0.5764	0.6258	0.9696	0.5224
19	SPP vs. SGP	0.393	0.234	0.0143	0.073	0.0902	0.4029
20	P and SGN vs. C and SGP	0.2682	0.6788	0.337	0.4832	0.133	0.4732
21	P and SPN vs. C and SPP	0.2734	0.6791	0.0401	0.5408	0.6782	0.7344
22	OPM vs. SPN	0.7284	0.581	0.2326	0.7386	0.7856	0.8749
23	OGN vs. SGN	0.6336	0.5204	0.2996	0.8486	0.7681	0.6519
24	OGP vs. SGP	0.6367	0.4645	0.541	0.458	0.1984	0.2541
25	OPP vs. SPP	0.0426	0.3915	0.1481	0.0004	0.0882	0.0067

Table 10. Part 1. Contrast *p*-values for focal metamorph average emergence time (AET), geometric mean of mass (GeoMass), survival to metamorphosis (STM), survival at the end of the experiment including tadpoles (SET), tibio-fibula length (TFL), and femur length (FL).

Contrast #	Treatment Comparisons	AET	GeoMass	STM	SET	TFL	FL
1	C vs P	0.5569	0.7903	0.3496	0.2924	0.9367	0.4029
2	C vs OGN	0.0441	0.5004	0.4825	0.6793	0.1265	0.191
3	OGN vs. OGP	0.2815	0.6838	0.0698	0.0915	0.1314	0.5988
4	P vs. OGP	0.6121	0.979	0.7433	0.7135	0.9154	0.2403
5	P and OGN vs. C and OGP	0.239	0.6334	0.4607	0.5809	0.2854	0.8577
6	C vs. OPN	0.7858	0.6147	0.8795	0.5914	0.7656	0.6972
7	P vs. OPP	0.8582	0.3848	0.3878	0.3837	0.8953	0.5189
8	OPN vs OPP	0.6174	0.9234	0.9388	0.726	0.9307	0.0672
9	P and OPN vs. C and OPP	0.9457	0.7975	0.4737	0.3216	0.9063	0.4638
10	OPN vs OGN	0.0738	0.2603	0.4027	0.3657	0.0776	0.1036
11	OPP vs. OGP	0.7401	0.3762	0.2382	0.2207	0.8142	0.5931
12	C vs. SGN	0.0356	0.2346	0.2929	0.2365	0.716	0.2767
13	C vs. SPP	0.4197	0.1922	0.7608	0.7191	0.6311	0.7162
14	SPN vs. SPP	0.7574	0.3012	0.5776	0.4225	0.6346	0.7028
15	SGN vs. SGP	0.5416	0.1577	0.1366	0.1062	0.8418	0.2506
16	P vs. SPP	0.9224	0.9992	0.9218	0.942	0.9459	0.1183
17	P vs. SGP	0.2191	0.6401	0.1579	0.1272	0.4694	0.09
18	SPN vs. SGN	0.1518	0.9994	0.4295	0.3789	0.4101	0.1468
19	SPP vs. SGP	0.1841	0.6392	0.1842	0.1059	0.5161	0.0021
20	P and SGN vs. C and SGP	0.4028	0.2324	0.0887	0.0616	0.8415	0.7809
21	P and SPN vs. C and SPP	0.524	0.3554	0.776	0.8395	0.6918	0.3904
22	OPM vs. SPN	0.5978	0.0785	0.8813	0.85	0.8507	0.99
23	OGN vs. SGN	0.9224	0.6192	0.1075	0.1394	0.2681	0.8496
24	OGP vs. SGP	0.481	0.6203	0.0837	0.0607	0.5413	0.6279
25	OPP vs. SPP	0.7844	0.3896	0.4384	0.3406	0.8441	0.0362

Table 10. Part 2. Contrast *p*-values for focal metamorph average jump distance (JAVG), snout-urostyle length (SUL), and cranial width (CW). Values in parentheses represent masses at which treatments were compared in the planned contrasts: Avg. = average mass, -1SD = one standard deviation below the average mass, and +1SD = one standard deviation above the average mass.

Contrast #	Treatment Comparisons	JAVG	SUL (-1SD)	SUL (Avg.)	SUL (+1 SD)	CW (-1SD)	CW (Avg.)	CW (+1 SD)
1	C vs P	0.0795	0.6783	0.239	0.3208	0.9674	0.3601	0.5029
2	C vs OGN	0.568	0.2104	0.0102	0.266	0.6425	0.1048	0.2831
3	OGN vs. OGP	0.0462	0.1846	0.3714	0.3452	0.1386	0.0777	0.9834
4	P vs. OGP	0.869	0.5138	0.3943	0.389	0.5024	0.2811	0.8334
5	P and OGN vs. C and OGP	0.756	0.2843	0.1509	0.4712	0.428	0.0616	0.6579
6	C vs. OPN	0.0919	0.8624	0.272	0.1179	0.899	0.4887	0.2141
7	P vs. OPP	0.7744	0.6376	0.107	0.3146	0.2664	0.3256	0.4178
8	OPN vs OPP	0.8314	0.063	0.054	0.09997	0.0206	0.0187	0.2841
9	P and OPN vs. C and OPP	0.1592	0.692	0.5256	0.336	0.306	0.2348	0.7498
10	OPN vs OGN	0.0411	0.2398	0.0708	0.2585	0.7001	0.0362	0.1027
11	OPP vs. OGP	0.9051	0.0753	0.3117	0.2497	0.0086	0.0417	0.1868
12	C vs. SGN	0.9069	0.8944	0.0128	0.2222	0.3203	0.1078	0.2302
13	C vs. SPP	0.327	0.6523	0.2196	0.3241	0.249	0.2455	0.4134
14	SPN vs. SPP	0.4377	0.4149	0.2816	0.3209	0.4995	0.2318	0.2727
15	SGN vs. SGP	0.3312	0.0022	0.5815	0.2137	0.4861	0.2197	0.0782
16	P vs. SPP	0.9982	0.7216	0.3528	0.3175	0.8191	0.3306	0.34
17	P vs. SGP	0.3555	0.4485	0.0162	0.315	0.5565	0.6691	0.2889
18	SPN vs. SGN	0.2945	0.4979	0.5333	0.3695	0.9406	0.9936	0.9889
19	SPP vs. SGP	0.3598	0.0017	0.0006	0.1099	0.2337	0.5011	0.7653
20	P and SGN vs. C and SGP	0.6219	0.4958	0.6989	0.3854	0.8036	0.1339	0.1262
21	P and SPN vs. C and SPP	0.474	0.8318	0.132	0.4537	0.9055	0.1406	0.2164
22	OPM vs. SPN	0.5032	0.4799	0.5197	0.318	0.2646	0.1337	0.2006
23	OGN vs. SGN	0.6713	0.2212	0.8953	0.3198	0.7765	0.9037	0.9553
24	OGP vs. SGP	0.4512	0.0199	0.053	0.2502	0.0337	0.4231	0.1066
25	OPP vs. SPP	0.7805	0.0556	0.0122	0.1088	0.0332	0.0513	0.648

Table 11. Contrast *p*-values for gray treefrog metamorph average emergence time (AET), geometric mean of mass (GeoMass), and survival to metamorphosis (STM).

Contrast #	Treatment Comparisons	AET	GeoMass	STM
1	OGP vs OGN	0.7796	0.1865	0.4009
2	SGP vs SGN	0.6437	0.241	0.1996
3	SGP vs OGP	0.0876	0.21	0.164
4	SGN vs OGN	0.185	0.3931	0.4275

Chapter 3: How does the relative strength of inter- and intraspecific competition impact how a prey species responds to its predator?

Introduction:

Many organisms have been shown to exhibit phenotypic plasticity, the ability to alter their phenotype in response to various stressors in the environment (Via et al. 1995, Miner et al. 2005, Pfennig et al. 2010). One of the major stressors is predation, and it can alter behavior, life history, and even morphology of organisms through phenotypic plasticity (Tollrian 1995, Relyea 2001b, Domenici et al. 2008). Competition may alter the effects of predation because competitors can take away the resources necessary to respond to predators or because surviving with competitors requires different strategies than surviving with predators.

Strategies for dealing with competitors are often different from those for dealing with predators. In wood frog (*Lithobates sylvaticus*) tadpoles, responding to predators results in longer tails and shorter bodies, while responding to competitors results in longer bodies and shorter tails (Relyea 2002c, 2004). The reason for the decrease in body length and increase in tail length with predators is that the tail becomes a bigger target because tadpoles are more likely to survive strikes to the tail than to the body or head (Van Buskirk et al. 2003). The reduction in body size with predators results in a reduction in gut length, while competitors induce longer guts that increase digestive efficiency to give an edge over competitors (Relyea and Auld 2004).

An intraspecific competitor may have very different impacts on phenotype than an interspecific competitor. In Brook Charr (*Salvelinus fontinalis*), intraspecific competitors result in around 40% benthic specialist feeders, 40% generalist feeders, and 20% pelagic specialist

feeders (Bourke et al. 1999). Adding an interspecific competitor alters these ratios by reducing the number of benthic feeders by 20 to 30% (Bourke et al. 1999). Intraspecific and interspecific competitors may also have reciprocal effects on each other.

There is evidence of reciprocal effects of phenotypic plasticity from studies on the *Rana pirica, Hynobius retardatus* system. In this system from Japan, *R. pirica* responds to the predatory *H. retardatus* by developing wider bodies and *H. retardatus* responds by increasing the size of its head (Takatsu and Kishida 2013). Since competitors can also alter phenotypic plasticity it is possible that interspecific competitors may alter the phenotype of a focal species and the new phenotype of the focal species may then alter the phenotype of the interspecific competitor.

We sought to examine how differing levels of intra- and interspecific competition altered the response of both competitor species to a predator. Though phenotypic plasticity has been studied in many species, rarely has it been examined in pairs of competitor species within the same environment. We were also interested in assessing how differing levels of intra- and interspecific competition would alter the response to a predator, since different species differ in their competitive ability and some species might not impact phenotype at low abundance but would at high abundances. Looking at the response in both species is also interesting because the responses could be equal and opposite or they could be completely different. We used amphibians to address this interplay between predation and relative strength of intra- and interspecific competition on phenotypic plasticity. We chose amphibians as our model system because many of them exhibit phenotypic plasticity, they can be raised easily in a controlled environment, and many species co-occur, allowing us to look for reciprocal effects of competitors.

Hypotheses:

We hypothesized that 1) predators will alter the morphology, life history, and resource use of tadpoles and metamorphs, but the effect of predators will be strongest when intraspecific and interspecific competition are equal because when interspecific competition is higher, the predator is eating more interspecific competitors and so is producing less intraspecific alarm cue, and when intraspecific competition is higher competitor feeding strategies should be more similar and it will be harder to get enough resources to respond to the predator; 2) In the absence of predators, tadpoles and metamorphs will alter their morphology, life history, and resource use more in response to higher intraspecific competition because intraspecific competitors share more similar resource use.

Methods:

We used Pinewoods Treefrogs (*Hyla femoralis*) and Southern Leopard Frogs (*Lithobates sphenocephalus*) as our two competitor species. These species co-occur in the wild (Lannoo 2005), exhibit altered phenotypes in response to predators (Babbitt 2001, LaFiandra and Babbitt 2004), and also exhibit altered phenotypes in response to differing levels of competition (Mills and Semlitsch 2004, McCoy 2007). This makes them a good model system for examining the effects of differing levels of intra- and interspecific competition on the plastic response to a predator. We used larval aeshnid dragonflies (*Anax* sp.) as our predator because they co-occur with both of these species and many larval amphibians respond to aeshnid dragonfly predators (Relyea 2001a).

Our experimental units were artifical ponds made from Rubbermaid 1100 L cattle watering tanks. We used artificial ponds because they allow more control than a field

experiment, but offer more realism than a laboratory experiment, and the processes that are important in natural ponds are also important in artificial ponds (Wilbur 1987, Morin 1998, Chalcraft et al. 2005). To address our hypotheses, our experiment consisted of seven treatments: 1) an algae control with no animals, 2) equal levels of Pinewoods Treefrogs and Southern Leopard Frogs (100 tadpoles of each species) with a caged *Anax* larvae, 3) higher intraspecific competition (150 Pinewoods Treefrog tadpoles and 50 Southern Leopard Frog tadpoles with a caged *Anax* larvae predator), 4) higher interspecific competition (50 Pinewoods Treefrog tadpoles and 150 Southern Leopard Frog tadpoles with a caged *Anax* larvae predator), 5) equal levels of competition without a caged predator, 6) higher intraspecific competition without a caged predator, and 7) higher interspecific competition without a caged predator. Since we measured the responses of both species present, for Southern Leopard Frogs treatments 3 and 6 are higher interspecific competition and treatments 4 and 7 are higher intraspecific competition.

Tanks were filled with well water on May 6 and 7, 2015 then allowed to sit for approximately a week to allow any chlorine to evaporate from the tanks. We added 1 kilogram of mixed hardwood and pinestraw litter to all of the experimental tanks and 1300 grams of the same mix of litter to the holding tanks on May 13-14. We also added two pint aliquots containing natural pond water, phytoplankton, zooplankton, and algae to each of the tanks on May 14. We collected these aliquots from a natural pond at the experimental site. We had six replicates of each treatment and tanks were arranged in randomized blocks with one randomly assigned replicate of each treatment, so each block consisted of seven tanks. We also had a seventh block of holding tanks. Tanks were covered with two fiberglass screens to prevent experimental animals from escaping and wild animals from colonizing the tanks.

Both Pinewoods Treefrogs and Southern Leopard Frogs were collected from the Croatan National forest in eastern North Carolina (Craven County). We collected 25 pairs of *H. femoralis* on May 11, 2015 and collected seven *L. sphenocephalus* clutches on May 13. We collected pairs and egg masses in tupperware containers filled with pond water from the site of capture and used these to transport the pairs and eggs back to the lab at East Carolina University. Eggs of both species were then allowed to hatch and develop for approximately one week before they were counted out for the experiment. We used a mix of 8 pinewoods clutches for the experimental pinewoods tadpoles and an even mix of all 7 leopard frog clutches and each tank received an even mixture of these 15 clutches. Tadpoles were counted on 5/18 and 5/19/2015 and were added to the corresponding tanks on 5/20/2015. We also counted feeder *H. femoralis* and *L. sphenocephalus* to feed to the dragonfly predators and added them to separate holding tanks.

We used caged predators because we wanted to test the effects of predators on morphology, life-history, and resource use without allowing the predators to consume experimental individuals and alter the density and so that we could rule out the effects of selective predation in the tadpole responses we measured. We used 8.5 cm in diameter by 30 cm long PVC cages with window screen fastened to either end to allow cues and water to pass freely between the tank and the cage. One end of the cage was shut with a hose clamp that could be opened and closed with a screw driver to allow us to add the predator to the cage and to feed and check on the predator as necessary. We used dragonfly larvae (*Anax* sp.) as our predator collected from the Croatan National Forest in eastern North Carolina on May 21. We added predators to the predator treatment tanks and fed the predators on May 22. Cages were suspended with string from wires that stretched across the top of the tank to prevent screens from sinking into the tank, in order to allow dragonflies space to emerge as adults by leaving several inches of

air at the top of the cage and room for adult dragonflies to cling to the cage cover that was suspended out of the water. Each dragonfly was fed four tadpoles every 3 days for the duration of the experiment. Dragonflies were fed tadpoles of both species in the relative abundance that they were present in the tank, so that if density was equal, dragonflies received two tadpoles of each species and if density was unequal they received 3 tadpoles of the more abundant species and 1 tadpole of the less abundant species.

We added strips of flagging tape to each tank as substrate for algae/periphyton to grow on so that we could collect these strips later and measure algal abundance as a food resource. We collected the strips from all tanks, including the algae only control tanks, on 6/19/2015. We labelled each sample and put it on ice. We also collected phytoplankton samples by collecting one liter of pond water out of the middle of the water column in each tank on the same day. We collected phytoplankton samples because phytoplankton could compete with periphyton or be a direct food resource for tadpoles. These samples were also placed on ice. We cut off a section of the periphyton strip approximately 7 cm long, measured the exact length of the strip and filtered the algae scraped off of the strip with approximately 150 mL of water through a hand operated vacuum pump flask. We recorded the exact volume of water filtered and the length of the periphyton strip from which algae was removed, folded the filter paper in aluminum foil, labelled it and placed it back on ice and in a freezer. We similarly filtered approximately 150 mL of the phytoplankton samples through the vacuum pump apparatus, recorded the volume, placed the filter paper in aluminum foil, labelled it, and placed it back on ice and in a refrigerator. We allowed the phytoplankton and periphyton samples to sit in the refrigerator for approximately four weeks while we finished the rest of the experiment. We removed the samples from the refrigerator, placed them back on ice, carefully ground up the filter papers in acetone, let them sit

in the acetone and extract, centrifuged them, poured a subset of the supernatant into a sample cuvette, and read them using a fluorometer. We recorded this value for both periphyton and phytoplankton. For phytoplankton, this concentration was then analyzed directly, but for periphyton we converted to concentration per area of the flagging tape that we scraped off.

We weighed and photographed a subset of up to 10 tadpoles of each species from each of the six experimental tanks containing tadpoles, so up to 20 tadpoles from each tank. We always tried to photograph 10 tadpoles, but it was not possible to capture tadpoles from some of the tanks, particularly when there were only 50 *H. femoralis* added to a tank initially. We took photographs from June 8 – June 12, approximately three weeks after the eggs hatched. This gave the tadpoles sufficient time to achieve a size where morphological differences were likely to accrue. We anesthetized tadpoles with 0.02 g of MS-222 buffered with 0.04 g of baking soda in one pint of water. We used a fresh dose of anesthetic for each block of six tanks. Anesthetized tadpoles were weighed, then placed into a water filled transparent container on a wooden stage with three mirrors that allowed us to simultaneously photograph the lateral and ventral views of the tadpole. Each tadpole was then placed into recovery water from one of the stock tanks. Tadpoles were allowed to sit in the recovery water for at least 15 minutes. We did not have any mortality from the anesthesia. Once photographs were complete, tadpoles were returned to their experimental tank. We used these photographs to measure morphology with geometric morphometrics. We used the TPSDig2 software (Rohlf 2013) to place points corresponding to various landmarks on the lateral view of the tadpoles after setting a scale bar using the millimeter scale present in each picture (see Supplementary Figures 1 and 2 for Landmarks). The points for *H. femoralis* and *L. sphenocephalus* were different since these two species have slightly different morphology.

We collected metamorphs (individuals with at least one forelimb) from the tanks as we checked them daily. Metamorphs were taken back to the lab and held in containers with a tiny volume of water to prevent dessication. We checked the metamorphs in the lab daily and weighed them once they completed tail resorption (no tail was remaining). We also recorded the day that we collected each metamorph and the day that we weighed them to calculate emergence time and total time to complete metamorphosis for each individual. We then calculated a tank average for each of the following: mass, emergence, and metamorphosis time. Metamorphs were returned to their site of capture after they were weighed.

We terminated the experiment approximately 9 weeks after the eggs were laid and 8 weeks after tadpoles were added to the tanks as many of the *H. femoralis* had metamorphosed by this point, *L. sphenocephalus* had begun metamorphosing, and we were out of feeder tadpoles as most of them had also metamorphosed by this date. When taking down the experiment, we carefully drained all of the tanks into a net, sorted through the litter to catch any remaining tadpoles, and took these individuals back to the lab. Tadpoles were examined for the presence of hind legs and limb buds and were weighed. Metamorphs were held in the lab until tail resorption as described above. All tadpoles were returned to their site of capture after they were measured. For both species we calculated the percent survival of all individuals surviving to the end of the experiment (metamorphs and tadpoles) for each tank and calculated what percentage of tadpoles achieved metamorphosis in each tank. We also looked at total survival to the end of the experiment within a treatment (average of the sum of metamorphs and tadpoles surviving to the end of the experiment for both species in the tanks of that treatment) and compared the overall percent survival of Pinewoods Treefrogs to Southern Leopard Frogs within each treatment.

Statistical Methods:

All analyses were performed in SAS Enterprise Guide 6.1, for the SAS software, version 9.4 of the SAS System for Windows. Copyright © 2013 SAS Institute Inc, Cary, NC, USA. We used the tadpole mass data from when we photographed the tadpoles to calculate the geometric mean of mass for each tank. Mass often follows a lognormal distribution and the geometric mean provides a better estimate than the arithmetic mean when variables are lognormally distributed. We repeated this procedure with tadpole mass at the end of the experiment and metamorph mass. For the environmental variables, plankton data, masses, and measures of metamorph emergence time, and total metamorphosis time, we used a linear mixed model. These linear mixed models included treatment as a fixed effect and block as a random effect. For the percent survival and percent achieving metamorphosis data, we used generalized linear mixed models with treatment as a fixed effect and block as a random effect. The generalized linear mixed models used a binomial expected distribution with a logit link function for the percent survival and percentage of tadpoles reaching metamorphosis, as these measures often follow a binomial distribution.

For tadpole morphology, we loaded the pictures into TPSRelw (Rohlf 2010) to construct a consensus morphology of the tadpoles using Procrustes superimposition, then used the Procrustes coordinates to calculate centroid size for each tadpole, and ran a PCA on the Procrustes coordinates to calculate shape variables (relative warps). We used the average relative warp scores for each tank in SAS as dependent variables to run a MANCOVA with treatment as the main effect to look for differences in tadpole shape. We used the first 12 relative warps in the MANCOVA as they explained over 90% of the variation. We also included centroid size as a covariate and the interaction between centroid size and treatment as a covariate. We used Proc GLM in SAS to run the MANCOVA.

We performed planned contrasts for all of the response variables to compare treatments. We used planned contrasts as we were only interested in a subset of the comparisons and not all of the comparisons. We used planned contrasts that incorporated the algae only control tanks as well as the other six treatments for analysis of algal abundance (Table 12), but excluded the algae control from the planned contrasts for survival, morphology, and life history data for the two frog species (Table 12). For the overall survival comparison between the pinewoods and leopard frogs within each treatment, we used a separate set of contrasts (Table 12). With tadpole morphology we also looked at tadpoles at the average centroid size, and one standard deviation above and below the average centroid size for each of the 11 planned contrasts because allometry differed among the treatments (the interaction between centroid size and treatment $p \leq 0.3$). A *p*-value threshold of 0.3 ensured that we did not exclude a term from the model that was still having a large effect on our model even though it was not statistically significant. We chose a high threshold to be conservative in our assessment that allometric relationships varied among treatments because visual inspection of scatterplots suggested that the allometric relationship differed substantially among treatments. For the tadpole morphology, we ran a principle components (PCA) analysis in SAS on the sum of squares cross-product matrix from the MANCOVA for each planned contrast, and used the eigenvectors from this PCA and the relative warps for each tadpole from the treatments involved in the contrast to calculate a divergence factor (Langerhans 2009). We did this to calculate the greatest divergence in tadpole shape between the treatments(Langerhans 2009). We then used the average of this divergence factor for each treatment in TPSRegr (Rohlf 2016) to visualize the shape of the tadpoles from the treatments being compared. We used Wilk's λ as the test statistic for the MANCOVA (Langerhans and DeWitt 2002, Dayton et al. 2005, Langerhans 2009, Sharpe et al. 2015).

Results:

Periphyton levels did not significantly differ across the treatments ($F_{6, 30} = 0.3$, $p = 0.9331$, contrast $p \geq 0.3309$). Adding leopard frogs did increase periphyton levels by approximately 11% relative to the equal density and by approximately 18% relative to increasing pinewoods density. Adding a predator increased periphyton levels by 18% at the equal density, 23% with more pinewoods, and 13% with more leopard frogs. Relative to the algae control, adding pinewoods and leopard frogs at equal densities reduced algal resources by 23%, adding more pinewoods and fewer leopard frogs reduced algal resources by 30%, and adding more leopard frogs and fewer pinewoods decreased algal resources by approximately 13%. Percent differences between the predator treatments and the algae control were ≤ 7%. For phytoplankton, there were significant treatment effects ($F_{6, 30} = 4.44$, $p = 0.0025$). Changing tadpole density in favor of pinewoods or leopard frogs in the absence of a predator increased phytoplankton levels by 51% and 34% respectively, but only the effect of increasing pinewoods density relative to the equal density treatment was significantly different from zero (contrast $p = 0.0157$ for more pinewoods, 0.1411 for more leopard frogs). The difference between adding more pinewoods and adding more leopard frogs was 18% with pinewoods increasing phytoplankton levels, but this effect did not significantly differ from zero ($p = 0.3024$). Adding predators always decreased phytoplankton abundance (% difference = 31% at equal density, 89% with more pinewoods, 76% with more leopard frogs), but the effect was significant when densities were unequal ($p \leq 0.0075$) but not when densities were equal ($p = 0.3217$). Altering competitor density did not have effects on phytoplankton that were statistically significant ($p \geq 0.7296$). Increasing pinewoods or increasing leopard frog abundance decreased phytoplankton by 11% and 14% respectively, but

the effects of increasing either competitor with a predator did not differ (approximately 3% difference). Competitors alone increased phytoplankton abundance relative to the algae control by 52% when pinewoods were more abundant, 34% when leopard frogs were abundant, and had no effect (<1% difference) when tadpoles were present at equal densities, but only the effect of more pinewoods significantly differed from zero ($p = 0.0151$ for pinewoods, ≥ 0.1369 for others). Adding a predator with the competitors decreased phytoplankton abundance by 31-44% across the competitor densities, but these effects did not significantly differ from zero ($p \geq 0.1903$).

Pinewoods Treefrogs

There were significant effects of treatment on body mass across life stages ($F_{5, 24} = 3.41$, $p = 0.0182$ at 3 weeks, $F_{5, 16} = 1.6$, $p = 0.2165$ at 8 weeks, $F_{5, 19} = 2.12$, $p = 0.1072$ at metamorphosis). Adding more pinewoods in the absence of a predator relative to the equal density without a predator increased body mass by 29% at 3 weeks after hatching when tadpoles were photographed (contrast $p = 0.0396$), but had no effect at 8 weeks after hatching (5% difference, $p = 0.8413$), and increased mass by approximately 14% at metamorphosis but the effect at metamorphosis did not significantly differ from zero ($p = 0.3015$, Fig. 50). Increasing leopard frog abundance relative to the equal density in the absence of a predator had no effect at 3 weeks, but increased mass by 17% at 8 weeks and 23% at metamorphosis but the effects did not significantly differ from zero ($p \geq 0.0946$, Fig. 50). The effect of increasing the relative abundance of either competitor was not statistically significant at any life stage ($p \geq 0.0657$). Increasing leopard frog relative abundance increased mass of pinewoods tadpoles by 26% at three weeks, decreased it by 22% at eight weeks, and increased mass of frogs by 10% at

metamorphosis relative to increasing pinewoods abundance (Fig. 50). Adding a predator had no effect on mass for any of the densities at 3 weeks (% difference ≤ 6%, $p \geq 0.5881$), but at 8 weeks adding a predator increased mass by 18% at the equal density and by 44% when more pinewoods were present and decreased mass by 42% when more leopard frog tadpoles were present (Fig. 50). Only the effect of more pinewoods was significantly different from zero at 8 weeks after hatching ($p = 0.042$ for more pinewoods, 0.4312 for equal, 0.2792 for more leopard frogs). At metamorphosis, only adding a predator with more pinewoods altered mass, inducing 36% larger metamorphs than more pinewoods raised without a predator ($p = 0.0053$, $p \geq 0.4565$ and % difference ≤ 9% for other treatments, Fig. 50). Increasing pinewoods density and adding a predator produced 37% larger tadpoles at 3 weeks after hatching, 22% larger at 8 weeks, and 19% smaller metamorphs than adding a predator with equal densities of tadpoles but only the effect at 3 weeks significantly differed from zero ($p = 0.0083$, $p \geq 0.0831$ for others, Fig. 50). Increasing the relative density of leopard frogs in the presence of a predator resulted in no effects on mass of tadpoles at 3 weeks ($p = 0.9516$, % difference <1%), 43% smaller tadpoles at 8 weeks ($p = 0.2165$), and 19% smaller metamorphs ($p = 0.083$) than pinewoods tadpoles reared at the equal density with a predator (Fig. 50). Increasing the density of pinewoods relative to increasing the density of leopard frogs resulted in larger pinewoods tadpoles at three weeks ($p = 0.0072$, % difference = 37%) and eight weeks ($p = 0.2165$, % difference = 37%), but they were the same size at metamorphosis ($p = 0.9391$, % difference < 1%, Fig. 50). The effects of increasing either species' density and adding a predator on mass were not additive at 3 weeks, but were additive for both species at 8 weeks ($p \leq 0.1866$, % difference ≥ 29%), and at metamorphosis ($p \leq 0.0563$, % difference ≥ 16%, Fig. 50).

Treatment largely had no effect on emergence time ($F_{5, 19} = 1.54$, $p = 0.2251$, contrast $p \geq 0.1854$), but increasing pinewoods density in the absence of a predator reduced average emergence time by 8% or around 4 days from equal density without a predator ($p = 0.0556$). For Pinewoods Treefrogs, total metamorphosis time followed the same pattern as emergence time, with only increasing pinewoods density in the absence of a predator decreasing total metamorphosis time by 9% or around 5 days compared to equal densities without a predator ($F_{5, 19} = 0.66$, $p = 0.6599$, contrast $p = 0.1585$ for more pinewoods and no predator vs equal and no predator, contrast $p \geq 0.2425$ for all others).

The effect of treatment on the percentage of tadpoles metamorphosing did not significantly differ across the treatments and the numbers were consistently low (Mean ≤ 17% metamorphosing, $F_{5, 30} = 1.04$, $p = 0.4141$, contrast $p \geq 0.1322$). Despite this, adding more pinewoods tadpoles without a predator increased metamorphosis rates by 100% compared to equal density without a predator and by 98% compared to increasing leopard frog tadpole density without a predator. Adding a predator at the equal and higher pinewoods densities reduced metamorphosis by 18 and 11% respectively, but adding a predator with higher leopard frog density increased metamorphosis by 46%. Increasing pinewoods density in the presence of a predator also increased metamorphosis by approximately 100% relative to equal density with a predator, and increasing leopard frog density with a predator increased metamorphosis by approximately 68% relative to the equal density with a predator. Adding a predator with more pinewoods increased metamorphosis by 48% compared to adding a predator with more leopard frogs. The effects of adding leopard frogs and predators were also additive, increasing metamorphosis by 22%.

Survival of pinewoods tadpoles and metamorphs to the end of the experiment varied with treatment and was relatively low (16-34%, $F_{5, 30} = 2.35$, $p = 0.0647$). Increasing pinewoods density without a predator increased survival by 36% and increasing leopard frog density decreased survival by 37% relative to the equal density, but neither effect significantly differed from zero ($p \geq 0.1767$). More pinewoods also induced 70% higher survival than more leopard frogs without a predator. Adding a caged predator decreased pinewoods survival by 23% at equal density, had no effect on survival (2% increase) when pinewoods density was higher, and increased survival by 54% when leopard frog density was higher. None of the effects of adding a predator were significantly different from zero, but they came close when leopard frog density was higher ($p = 0.0894$ with more leopard frogs, $p \geq 0.433$ for others). When predators were present, increasing pinewoods or leopard frog density increased survival relative to the equal density (59% increase with pinewoods, $p = 0.0318$, 41% increase with leopard frogs, $p = 0.1696$). When a predator was present, increasing pinewoods density resulted in 20% higher survival than increasing leopard frog density ($p = 0.1696$). The effects of pinewoods and predators on survival were not additive, but the effects of leopard frogs and predators were ($p = 0.5389$ for pinewoods, 0.0774 for leopard frogs).

The overall effect of treatment on shape was significant ($F_{60, 36.557} = 1.62$, $p = 0.0593$), and many of the contrasts showed statistical significance ($p \leq 0.05$). Centroid size and the interaction between treatment and centroid size were retained in the model because there was weak evidence to suggest that the allometric relationship between body shape and body size varied among treatments (i.e., $p = 0.1592$ for interaction between centroid size and treatment). At the small centroid size and in the absence of a predator, increasing pinewoods density or leopard frog density had little effect on pinewoods tadpole shape ($p \geq 0.3532$, Fig. 53). Increasing

leopard frog density in the absence of a predator resulted in slightly longer and deeper bodies, and taller tail fins than increasing pinewoods density without a predator, but this effect was not statistically significant ($p = 0.1084$, Fig. 53). At the average mass, increasing pinewoods density did not alter phenotype relative to the equal density, but increasing leopard frog density resulted in longer and shallower bodies and shallower tail muscles (Fig. 54). The effects of increasing leopard frog density were not statistically significant and there was no difference in morphology between pinewoods tadpoles reared at a higher density of pinewoods or a higher density of leopard frogs ($p \geq 0.0861$). At the large mass, competitors alone had little effect on pinewoods treefrog tadpole morphology ($p \geq 0.3806$).

At the small mass, adding predators at the equal density and when more leopard frogs were present reduced pinewoods tadpole body length and body depth, but increased the length of the tail and height of the tail fin ($p \leq 0.0543$, Fig. 55). Adding a predator when pinewoods density was higher and at the small mass caused pinewoods tadpoles to develop slightly longer, but shallower bodies and slightly taller tails, but the effect was not statistically significant ($p = 0.0805$, Fig. 55). At the average mass, adding a predator at the equal density caused pinewoods tadpoles to develop longer, shallower bodies with longer tails, taller tail fins, and shallower tail muscles ($p = 0.0064$). Morphology followed the same general pattern at the average mass with a predator and a higher density of pinewoods, but the effects were not as strong and not statistically significant ($p = 0.0762$). Adding a predator with more leopard frogs at the average mass had little effect on morphology ($p = 0.3545$). At the large mass, adding a predator at the equal density still increased body length and tail fin height, and reduced body depth and tail muscle height, but had little effect on tail length and the effects were not statistically significant ($p = 0.0798$). Adding a predator with more pinewoods had little effect on morphology at the

large mass (p = 0.4198). Adding a predator with more leopard frogs induced shorter, shallower bodies, and longer tails, but the effects were not statistically significant (p = 0.1784, Fig. 57).

Increasing pinewoods density from equal to higher altered the effect of the predators on pinewoods tadpoles by inducing shorter and shallower bodies and longer tails, but these effects were not statistically significant (p = 0.0873, Fig. 56). Increasing leopard frog density from equal to higher at the small mass significantly increased body length and depth, shortened tail length, and slightly decreased tail fin height (p = 0.0103, Fig. 56). Increasing leopard frog density at the small mass also induced longer and deeper bodies, shorter tails, and slightly shorter tail fins compared to increasing pinewoods density (p = 0.006, Fig. 56). At the small mass, the effects of increasing leopard frog density and adding a predator were not additive (p = 0.0442), but the effects of increasing pinewoods density and adding a predator were additive (p = 0.1371). At the average mass, increasing leopard frog density with a predator had very similar effects on morphology to adding a predator at the equal density (p = 0.166). Increasing pinewoods density with a predator at the average body size resulted in slightly longer and deeper bodies and slightly shorter tails relative to the equal density with a predator, but these effects were not statistically significant (p = 0.2182). Increasing pinewoods density with a predator relative to increasing leopard frog density decreased body length and depth of pinewoods tadpoles and increased tail length, but these effects were not statistically significant (p = 0.191). The effects of increasing pinewoods density and adding predators at the average size were additive (p = 0.3034), but not the effects of increasing leopard frog density and adding predators (p = 0.0587). At the large mass, increasing pinewoods density did not alter the effects of the predator (p = 0.6257). Relative to the equal density and increasing pinewoods density with a predator, increasing leopard frog density induced longer, deeper bodies and shorter tails with shorter tail fins in pinewoods

tadpoles (Fig. 57). The effects of increasing leopard frog density on predators were not statistically significant for either comparison ($p \geq 0.2306$). The effects of increasing competitor density and adding a predator were additive for both species at the large body size ($p \geq 0.1506$).

Leopard Frogs:

As with the Pinewoods Treefrogs, Southern Leopard Frog mass was affected by treatment across life stages ($F_{5, 25} = 3.78$, $p = 0.011$ for tadpoles 3 weeks after hatching, $F_{5,25} = 10.54$, $p < 0.0001$ for tadpoles 8 weeks after hatching, and $F_{5, 18} = 4.08$, $p = 0.0119$ for metamorphs). In the absence of predators, increasing pinewoods density relative to the equal density had no effect on mass of tadpoles at 3 weeks (% difference = 8%, $p = 0.6664$), increased mass of tadpoles by 48% at 8 weeks ($p = 0.0033$), and increased mass of frogs by 32% at metamorphosis ($p = 0.0052$, Fig. 51). Increasing leopard frog density relative to equal density in the absence of a predator decreased mass by 26% in tadpoles at 3 weeks ($p = 0.225$), decreased mass by 20% in tadpoles at 8 weeks ($p = 0.3566$), and had no effect on metamorph mass ($p = 0.9008$, 1% difference, Fig. 51). In the absence of a predator, increasing leopard frog density decreased mass by 17% at three weeks, 67% at eight weeks, and 30% at metamorphosis relative to more pinewoods without a predator (Fig. 51). Only the effect of increasing leopard frog density without a predator at three weeks did not significantly differ from zero ($p = 0.4268$ at three weeks, $p \leq 0.0047$ for the other two, Fig. 51). Adding predators at the equal density increased leopard frog tadpole mass by 10% at 3 weeks, 22% at 8 weeks, and increased metamorph mass by just 5% but none of these effects significantly differed from zero ($p \geq 0.2188$, Fig. 51). Adding predators when there were more pinewoods tadpoles than leopard frog tadpoles present increased tadpole mass by 48% at 3 weeks ($p = 0.0044$) and by 15% at 8 weeks

($p = 0.1983$), but decreased mass of metamorphs by 19% ($p = 0.0531$, Fig. 51). Increasing leopard frog density and adding a predator did not have any effects that were statistically significantly ($p \geq 0.1421$), but at 3 weeks adding a predator at the high leopard frog density increased mass by 16% and metamorphs reared in the high density leopard frog with a predator environment were 29% smaller than leopard frog metamorphs reared at high density without a predator (Fig. 51). Increasing pinewoods density from equal density with a predator increased leopard frog tadpole and metamorph mass by 30% at 3 weeks ($p = 0.0455$), 41% at 8 weeks ($p = 0.0029$), and by only 8% for metamorphs ($p = 0.4803$, Fig. 51). Increasing leopard frog density with a predator relative to equal density with a predator decreased leopard frog tadpole mass by 21% at 3 weeks ($p = 0.2711$), 43% at 8 weeks ($p = 0.0332$), and metamorph mass by 32% ($p = 0.1086$). Leopard frogs always showed higher masses when reared with more pinewoods and a predator than when reared with more leopard frog tadpoles and a predator, and this difference was 50% at 3 weeks ($p = 0.0035$), 81% at 8 weeks ($p < 0.0001$), and 40% in metamorphs ($p = 0.032$, Fig. 51). The effects of increasing competitor density and adding a predator were additive at three weeks for pinewoods competitors ($p = 0.0845$, 21% difference), but were not for leopard frog competitors ($p = 0.9337$, 1 % difference, Fig. 51). Increasing pinewoods density and adding a predator did not have additive effects on leopard frog tadpoles at 8 weeks ($p = 0.9664$, <1% difference), but adding a predator and increasing leopard frog density did have an additive effect at 8 weeks ($p = 0.3613$, 13% difference, Fig. 51). Both increasing pinewoods density and increasing leopard frog density and adding a predator had additive effects on leopard frog metamorphs ($p = 0.1166$, 13% difference for pinewoods, $p = 0.161$, 16% difference for leopard frogs, Fig. 51).

The only treatment effect on emergence time was that adding a predator when more leopard frog tadpoles were present than pinewoods tadpoles reduced emergence time by approximately 6% or 3 days ($F_{5, 20} = 1.58$, $p = 0.2117$, contrast $p = 0.0443$, contrast $p \geq 0.1718$ for all others, %difference $\leq 4\%$). Total time to complete metamorphosis was affected more by treatment ($F_{5,18} = 2.19$, $p = 0.1009$). Relative to equal density without a predator, increasing leopard frog density without a predator increased total metamorphosis time by 5% or around 3 days ($p = 0.1008$). Adding a predator with a higher density of leopard frogs reduced total time to complete metamorphosis by approximately 12% or about 8 days ($p = 0.005$) and also reduced total metamorphosis time by 10% or 6 days compared to the equal density with a predator treatment ($p = 0.0271$). The effects of increasing leopard frog density with a predator and increasing pinewoods density with a predator were not equivalent, with higher densities of leopard frogs inducing 9% shorter metamorphosis times or about 6 days shorter than higher densities of pinewoods with a predator ($p = 0.0331$). The effects of adding a predator and increasing leopard frog tadpole density were also additive ($p = 0.0084$).

Metamorphosis ranged from 4 to 24% on average for leopard frogs across the treatments ($F_{5, 30} = 12.13$, $p < 0.0001$). Metamorphosis was highest by far when pinewoods were more abundant, whether or not a predator was present, increasing by 100% relative to the equal density and by over 100% relative to more leopard frogs ($p \leq 0.0083$). Increasing leopard frog density greatly reduced the percentage of leopard frog tadpoles that metamorphosed relative to the equal density with and without a predator, though it decreased much more when a predator was present (62%, $p = 0.1696$ without predator, 172%, $p = 0.0007$ with predator). Adding a predator decreased metamorphosis by 35% at the equal density ($p = 0.4472$), had no effect at higher pinewoods density ($p = 0.8707$, 6% difference), and decreased metamorphosis by 163% when

leopard frogs were more abundant ($p = 0.0021$). The effects of pinewoods and predators on metamorphosis of leopard frogs were not additive ($p = 0.6586$, 2% difference) but the effects of leopard frogs and predators were ($p = 0.0288$, 10%). Total survival ranged from 36 to 44% ($F_{5, 30} = 0.76$, $p = 0.5824$). Increasing pinewoods density or leopard frog density in the absence of a predator did not alter survival relative to the equal density without a predator and the two increased density treatments did not differ from each other ($p \geq 0.6689$, % difference $\leq 5\%$). Adding a predator at the equal density or when leopard frogs were more abundant also did not affect overall leopard frog survival ($p \geq 0.438$, % difference $\leq 6\%$). Adding a predator when more pinewoods were present did increase leopard frog survival by 15%, but this effect did not significantly differ from zero ($p = 0.4741$). Increasing leopard frog density with a predator also did not differ from the addition of a predator at the equal density, but increasing pinewoods density increased survival by 17% relative to the equal density with a predator and by 13% relative to increasing leopard frog density with a predator but again the effects did not significantly differ from zero ($p \geq 0.1303$). The effects of adding predators and increasing the density of one species did not alter overall percent survival of leopard frogs ($p \geq 0.3301$, % difference $\leq 5\%$).

Morphology of leopard frog tadpoles differed overall among the treatments ($F_{60, 41.239} = 1.71$, $p = 0.0356$). Centroid size and the interaction between centroid size and treatment were retained in the model because the interaction between centroid size and treatment was statistically significant ($p = 0.0410$ respectively). At the small centroid size without predators, increasing pinewoods density caused leopard frog tadpoles to shorten their tails and tail fins, slightly shorten their body length and depth, and develop taller tail muscles ($p = 0.0234$, Fig. 58). Increasing leopard frog density without a predator at the small centroid size resulted in longer

and deeper bodies and longer tails in leopard frog tadpoles, but the effects were not statistically significant (p = 0.1409, Fig. 58). Compared to increasing pinewoods density, increasing leopard frog density without a predator at the small centroid size induced slightly shorter and shallower bodies, and shorter tails in leopard frog tadpoles (p = 0.001, Fig. 58). At the average centroid size without predators, increasing pinewoods density relative to the equal density had little effect on leopard frog tadpole morphology (p = 0.4016). Increasing leopard frog density relative to the equal density resulted in tadpoles with longer but shallower bodies, longer tails, and shorter tail fins (p = 0.0366, Fig. 59). Increasing leopard frog density relative to increasing pinewoods density induced slightly shorter and shallower bodies, taller tail muscles, and slightly shorter tails with shorter tail fins (p = 0.0236, Fig. 59). Increasing pinewoods density without predators at the large size resulted in tadpoles with shorter and shallower bodies, but taller tail fins (p = 0.0381, Fig. 60). Increasing leopard frog density without a predator appeared to have a small impact on leopard frog tadpole morphology at the large centroid size, but tadpoles did show slightly longer bodies and shorter tail muscles relative to the equal density and the difference was not statistically significant (p = 0.0988, Fig. 60). Comparing the two unequal density competitor environments at the large centroid size, more leopard frogs induced slightly shorter and shallower bodies, taller tail muscles and tail fins, and shorter tails (Fig. 60). The effects of the two unequal competitor densities were not statistically significant (p = 0.2474).

Adding a predator at the equal density and small centroid size resulted in leopard frog tadpoles with longer, but shallower bodies and longer tails with deeper tail muscles, and taller tail fins (Fig. 61). The effects of predators at the equal density were not statistically significant (p = 0.1117). Adding a predator with more pinewoods present at the small centroid size induced slightly shorter and shallower bodies in leopard frog tadpoles (p = 0.0558, Fig. 61), but the

effects appear small compared to the other competitor environments at the same centroid size (Fig. 61). Adding a predator with a higher density of pinewoods at the small centroid size induced leopard frog tadpoles to develop shorter and shallower bodies and longer tails with taller tail fins ($p = 0.0008$, Fig. 61). At the average centroid size, adding a predator at the equal density and when leopard frog density was higher induced shorter and shallower bodies, longer tails, and taller tail fins (Fig. 62). Adding a predator at the higher leopard frog density also increased the height of the tail muscles (Fig. 62). The effects of adding the predator on the equal density and when leopard frog density was higher at the average centroid size were not statistically significant ($p = 0.1726$, 0.1398 respectively). Adding a predator at the average size with higher pinewoods density had little effect on leopard frog tadpole morphology ($p = 0.5321$). At the large centroid size, adding a predator at the equal density or when there was a higher density of pinewoods did not alter leopard frog tadpole morphology ($p = 0.9427$, 0.4162 respectively). Adding a predator with a higher density of leopard frogs induced shorter, shallower bodies with taller tail fins, and a slightly longer tail (Fig. 63). The effects of adding a predator with a higher density of leopard frogs were not statistically significant ($p = 0.1385$).

Increasing pinewoods density relative to the equal density in the presence of a predator at the small centroid size did not alter leopard frog tadpole morphology ($p = 0.6283$). Increasing leopard frog density relative to the equal density in the presence of a predator generated tadpoles with longer, but shallower bodies and longer tails with taller tail fins ($p = 0.0227$, Fig. 64). Adding more leopard frogs with a predator also resulted in slightly shorter and shallower bodies, with longer tails and taller tail fins compared to increasing pinewoods density with a predator ($p = 0.0092$, Fig. 64). Despite the statistical significance, the effects of competitors on the morphological response of leopard frog tadpoles to a predator at the small centroid size appear

very slight (Fig. 64). Increasing either competitor's density had non-additive effects with the presence of a predator on leopard frog tadpole morphology ($p \leq 0.0321$). Increasing the density of pinewoods at the average centroid size relative to the equal density altered predator effects and induced longer and deeper bodies, longer tails, and shorter tail fins and tail muscles (Fig. 65). The effects of increasing pinewoods with a predator relative to the equal density at the average centroid size were not statistically significant ($p = 0.0711$). Increasing leopard frog density relative to the equal density altered predator effects by inducing shorter and shallower bodies ($p = 0.0584$, Fig. 65). Despite the statistical significance, these effects appeared small (Fig. 65). Relative to increasing pinewoods density with a predator, increasing leopard frog density with a predator induced shorter and shallower bodies, taller tail fins and tail muscles, and slightly shorter tails (Fig. 65). The differences between the two unequal density treatments with a predator at the average centroid size were not statistically significant ($p = 0.2276$). Increasing pinewoods density or increasing leopard frog density and adding a predator had additive effects at the average centroid size ($p = 0.2368$, 0.7512 respectively). Increasing pinewoods density at the large centroid size altered the effects of the predator by inducing longer and deeper bodies, taller tail fins, and slightly longer tails ($p = 0.0122$, Fig. 66). Statistically, increasing leopard frog density altered the predator effects ($p = 0.0081$), but visually the equal density with a predator and higher leopard frog density with a predator tadpole morphologies are identical (Fig. 66). Compared to increasing pinewoods density with a predator, increasing leopard frog density with a predator induced shorter and shallower bodies, shorter tails, tail muscles, and tail fins ($p = 0.0444$, Fig. 66). Increasing either competitor's density and adding a predator had additive effects ($p = 0.842$ for pinewoods, 0.1895 for leopard frogs).

Survival Comparison:

The combined survival rates for both species were still low, but were affected by treatment (28%-37%, $F_{5, 30} = 1.68$, $p = 0.1699$). Increasing pinewoods density without a predator increased survival by 12% relative to the equal density, increasing leopard frogs had no effect on survival (3% difference), and increasing pinewoods increased survival by 10% relative to increasing leopard frogs. None of these competitor only effects significantly differed from zero ($p \geq 0.2931$). Adding a predator at the equal density decreased survival by 9%, decreased survival by 6% with more pinewoods, and actually increased survival by 14% with more leopard frogs, but none of these effects significantly differed from zero ($p \geq 0.2281$). Relative to the equal density, adding a predator with more pinewoods or more leopard frogs increased survival by 27% and 25% respectively ($p \leq 0.0359$) but the increased densities with a predator did not differ from each other ($p = 0.9049$, 1% difference). Neither species had additive effects with the predator ($p \geq 0.1758$, % difference $\leq 8\%$).

Survival rates of the two species differed depending on treatment, with leopard frogs always exhibiting higher survival than pinewoods treefrogs ($F_{11, 60} = 4.14$, $p = 0.0002$). At the equal density without a predator, leopard frogs frogs exhibited 12% higher survival than pinewoods treefrogs ($p = 0.0181$, 24% survival of pinewoods, 38% for leopard frogs, Fig. 52). Adding a predator at the equal density showed similar results, with 18% higher leopard frog survival ($p = 0.0014$, 19% for pinewoods, 37% for leopard frogs, Fig. 52). The difference was not statistically significant when pinewoods density was increased without a predator ($p = 0.4931$, 34% for pinewoods, 38% for leopard frogs). When a predator was added with increased pinewoods density, there was a 9% difference in survival, but again the effect was not

statistically significant (p = 0.1471, 35% for pinewoods, 44% for leopard frogs). Increasing leopard frog density without a predator produced a 19% difference in survival (p = 0.0009, 17% for pinewoods, 36% for leopard frogs) while adding a predator with more leopard frogs altered survival between the two species by 10%, but the effect with a predator was not statistically significant (p = 0.1344, 29% pinewoods, 39% leopard frogs).

Discussion:

Predators altered the morphology and life history of both prey species and affected the abundance of algal resources that prey eat. The effects of predators tended to be stronger when densities of prey species were unequal, however, particularly when there were more pinewoods treefrogs (Fig. 50-3). Competitors also impacted resources, morphology, and life history as we expected. We hypothesized that the effects would be strongest with intraspecific competition, but that was true only around 50% of the time. Interspecific competition also had strong effects on resources, morphology, and life history.

Periphyton followed the expected pattern, decreasing with competitors relative to the control. Increasing Pinewoods Treefrog relative density decreased algae the most. This could stem from pinewoods being better competitors than leopard frogs. A study looking at competition between congeners of both *Hyla femoralis* (*H. versicolor*) and *Lithobates sphenocephalus* (*Lithobates clamitans*) showed that *L. clamitans* responded more strongly to *H. versicolor* than to its own species while *H. versicolor* responded more strongly to intraspecific competition, suggesting that hylids are better competitors than ranids (Smith et al. 2004). Another possibility is that leopard frogs are relying more on cannibalism or predation for resources. Cannibalism and predation have been documented in multiple species of tadpoles,

including hylid and ranid tadpoles (Petranka et al. 1994, reviewed in Petranka and Kennedy 1999). *Lithobates sphenocephalus* tadpoles get larger than *H. femoralis* tadpoles, and many instances of predation by tadpoles involve larger tadpoles eating smaller ones, particularly hatchlings (reviewed in Petranka and Kennedy 1999). Some other members of the genus *Lithobates* from North America have been documented to be at the same trophic level as our predator from this experiment, *Anax* dragonfly larvae (Schiesari et al. 2009). The two most similar species to *L. sphenocephalus* from that study (*L. sylvaticus* and *L. palustris*) were not found to be very predacious based on stable isotopes (Schiesari et al. 2009), suggesting that predation and cannibalism were not very likely. Further evidence against predation and cannibalism is the fact that we added the tadpoles at around the same developmental stage, so the size differences when both species were hatchlings were not very large. It is also important to note that overall survival was relatively low (Fig. 52). This suggests that when *L. sphenocephalus* was equally or more abundant, they were stronger competitors. Adding a predator increased survival with leopard frogs, suggesting it forced them to reduce their foraging to an extent that pinewoods were less disadvantaged. Leopard frog tadpoles completed metamorphosis faster when more leopard frogs and a predator were present, suggesting there was stronger impetus in that treatment to get out of the larval environment or facilitation.

Phytoplankton went up when competitors were added, and went up the most when pinewoods were more abundant than leopard frogs. Phytoplankton went down when predators were present. This seems to follow the opposite pattern of periphyton, suggesting that reduced periphyton allows more phytoplankton to grow because competition for nutrients between the two is reduced and this has been found previously (Leibold and Wilbur 1992). Adding *Anax* predators also reduced phytoplankton biomass in another study (Rudolf and Rasmussen 2013),

but their food web was more complex than ours, so it is difficult to determine what caused the decrease in phytoplankton. It is possible that it could be simply from the increase in periphyton due to reduced tadpole activity, or it could be that less active tadpoles switch to filter feeding or some other feeding strategy that targets phytoplankton over periphyton.

Leopard frog tadpoles also got larger when pinewoods density was higher, so either pinewoods are poorer competitors or have a slightly different feeding niche. The fact that pinewoods survival was lower when more leopard frogs were present supports the idea that leopard frogs are better competitors. Another hylid frog was a poorer competitor than a spadefoot toad and a true toad (Morin 1981). At equal or low leopard frog densities, pinewoods treefrogs got larger in terms of mass when a predator was added. Maybe in these scenarios, pinewoods were better at switching to utilize the phytoplankton resources, which is why phytoplankton levels went down with a predator.

Many leopard frog tadpoles did not metamorphose. This could largely be a function of the fact that they have a longer larval period. Leopard frog tadpoles may even overwinter in ponds and spend over a year as tadpoles (Hernandez and Chalcraft 2012). Leopard frog tadpoles also altered their mass and morphology in response to competitors and predators, but the morphological changes decreased in magnitude with increasing body size, and the anti-predator responses were stronger than the anti-competitor responses. Leopard frogs appeared to develop larger bodies when there was a higher density of pinewoods tadpoles present than leopard frog tadpoles, suggesting that leopard frogs are superior competitors to pinewoods treefrogs. Leopard frog tadpoles showed morphologies more consistent with anti-predator responses when there were equal or higher densities of their own species. This could be a function of the fact that we

fed predators more leopard frog tadpoles in those two treatments, so the risk of the predator was higher based on the cue present.

At least at the small and average sizes, pinewoods tadpoles altered their morphology more in response to increasing interspecific competitor density than increasing intraspecific competitor density. Similarly to leopard frogs, pinewoods tadpoles altered their morphology more in response to the presence of predators than to the presence of competitors alone, and the morphological changes were less pronounced with increasing body size. Pinewoods treefrogs also responded more strongly to predators when there were more of their own species present, lending further support to the idea that they were responding more to the amount of cue released by the predator than to competitors.

This study adds to our understanding of phenotypic plasticity and community dynamics by examining the interplay of intra- and interspecific competition with predation and examined the reciprocal responses of the two competitor species involved. We found that competitors had weaker impacts than predators, but there was a strong interaction between predation and competition. Interestingly, the effects of predation and competition on one species were not necessarily opposite to the effects on the second species (i.e. increasing pinewoods density with and without a predator increased the mass of both leopard frogs and pinewoods). Our results also supported the idea that prey respond more to a predator when that predator is eating more of the prey species (i.e. the predator is a bigger risk and releases more cue for that prey species). An interesting follow up would be to hold the two prey species densities constant and model selective foraging by the predator to see if the results are consistent. Since communities are complex systems, it is important to look at more of these reciprocal effects to determine how communities function.

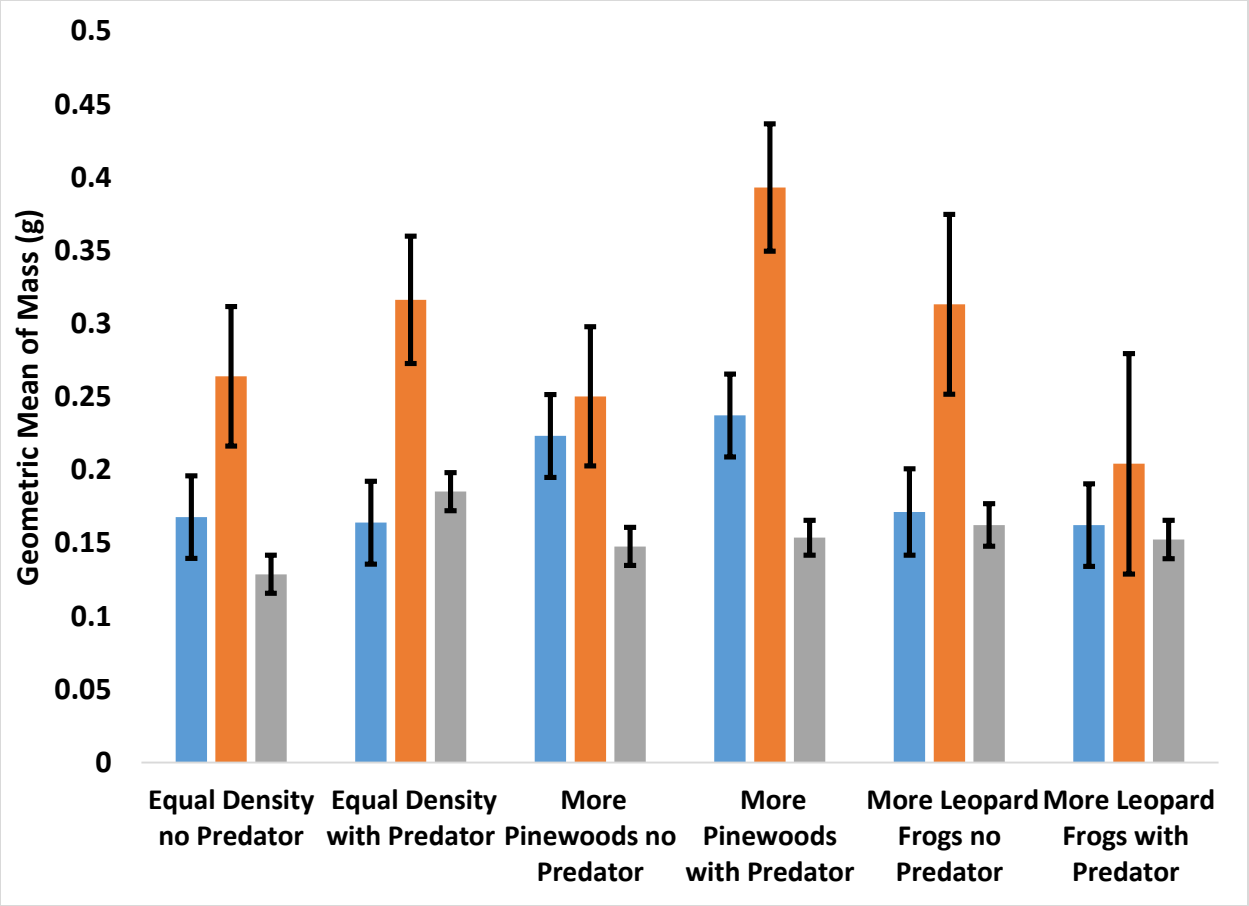

Figure 50. Geometric mean of mass of pinewoods treefrog tadpoles at 3 weeks after hatching (blue), 8 weeks after hatching (orange), and metamorphosis (gray). Means are least square means and error bars represent 1 standard error of the mean. See text for significant differences. Only treatments at a single time step were compared.

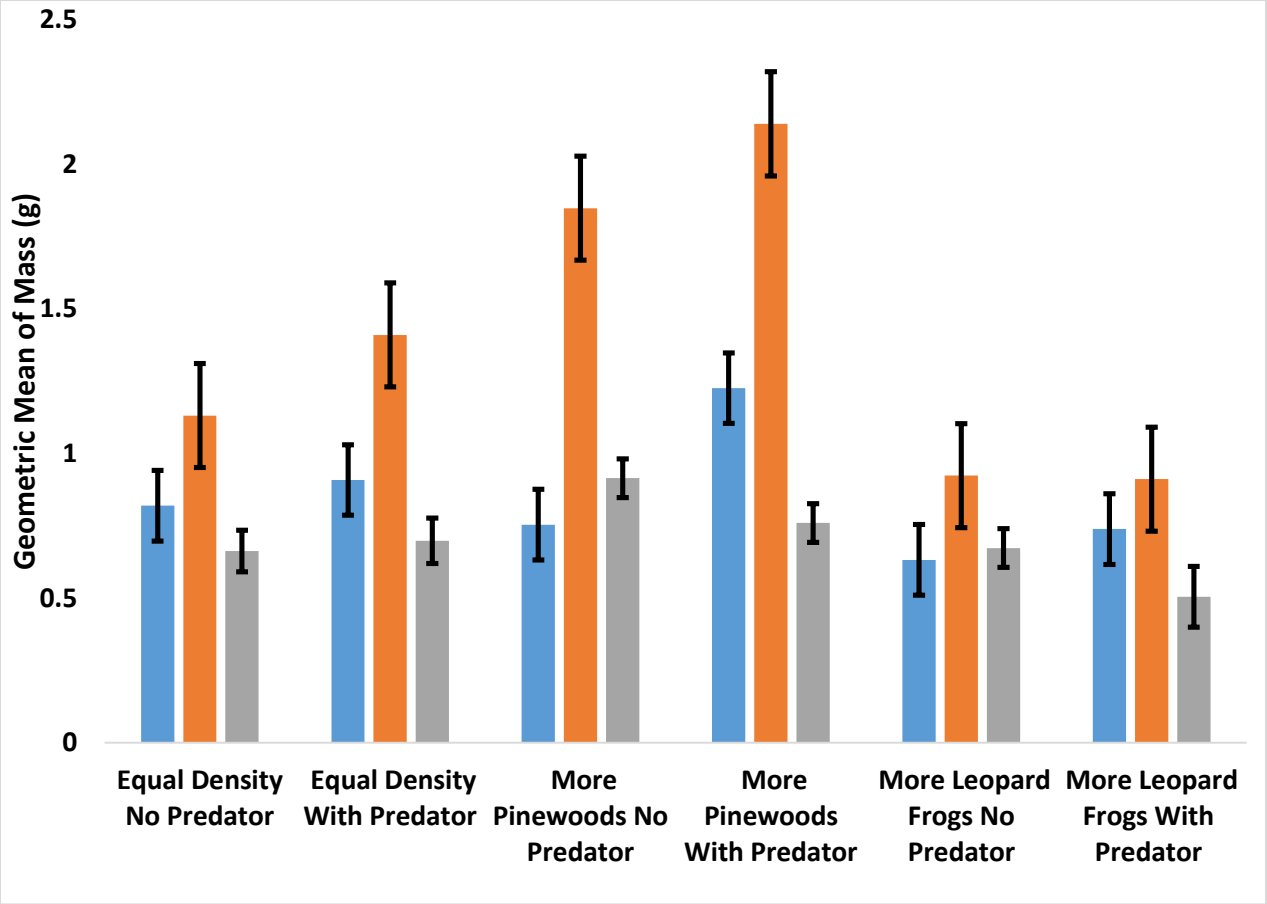

Figure 51. Geometric mean of mass of southern leopard frog tadpoles at 3 weeks after hatching (blue), 8 weeks after hatching (orange), and metamorphosis (gray). Means are least square means and error bars represent 1 standard error of the mean. See text for significant differences. Only treatments at a single time step were compared.

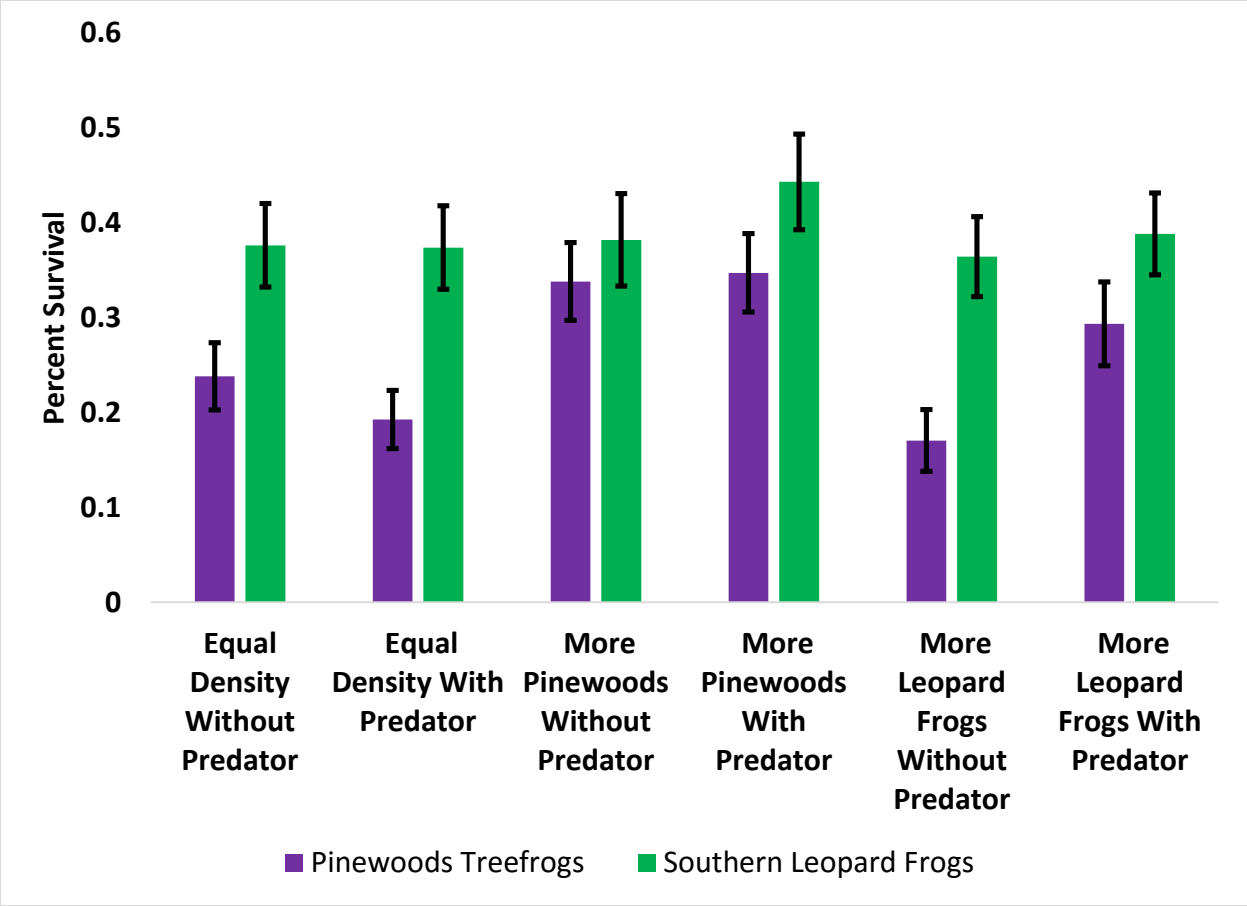

Figure 52. Percent survival at the end of the experiment of southern leopard frogs and pinewoods treefrogs. This includes tadpoles collected at the end of the experiment as well as individuals that successfully emerged as metamorphs. Means are least square means and error bars represent 1 standard error of the mean. See text for significant differences.

Figure 53. Shape comparison between pinewoods treefrog tadpoles at the small size in the Equal Density No Predator treatment (orange), the More Pinewoods No Predator Treatment (purple), and the More Leopard Frogs No Predator Treatment (blue). Axes are relative deformation values.

Figure 54. Shape comparison between pinewoods treefrog tadpoles at the average size in the Equal Density No Predator treatment (orange), More Pinewoods No Predator Treatment (purple), and the More Leopard Frogs No Predator Treatment (blue). The lower Figure is a comparison between More Leopard Frogs No Predator and More Pinewoods No Predator, but differences were small and lines largely overlap for both comparisons. Axes are relative deformation values.

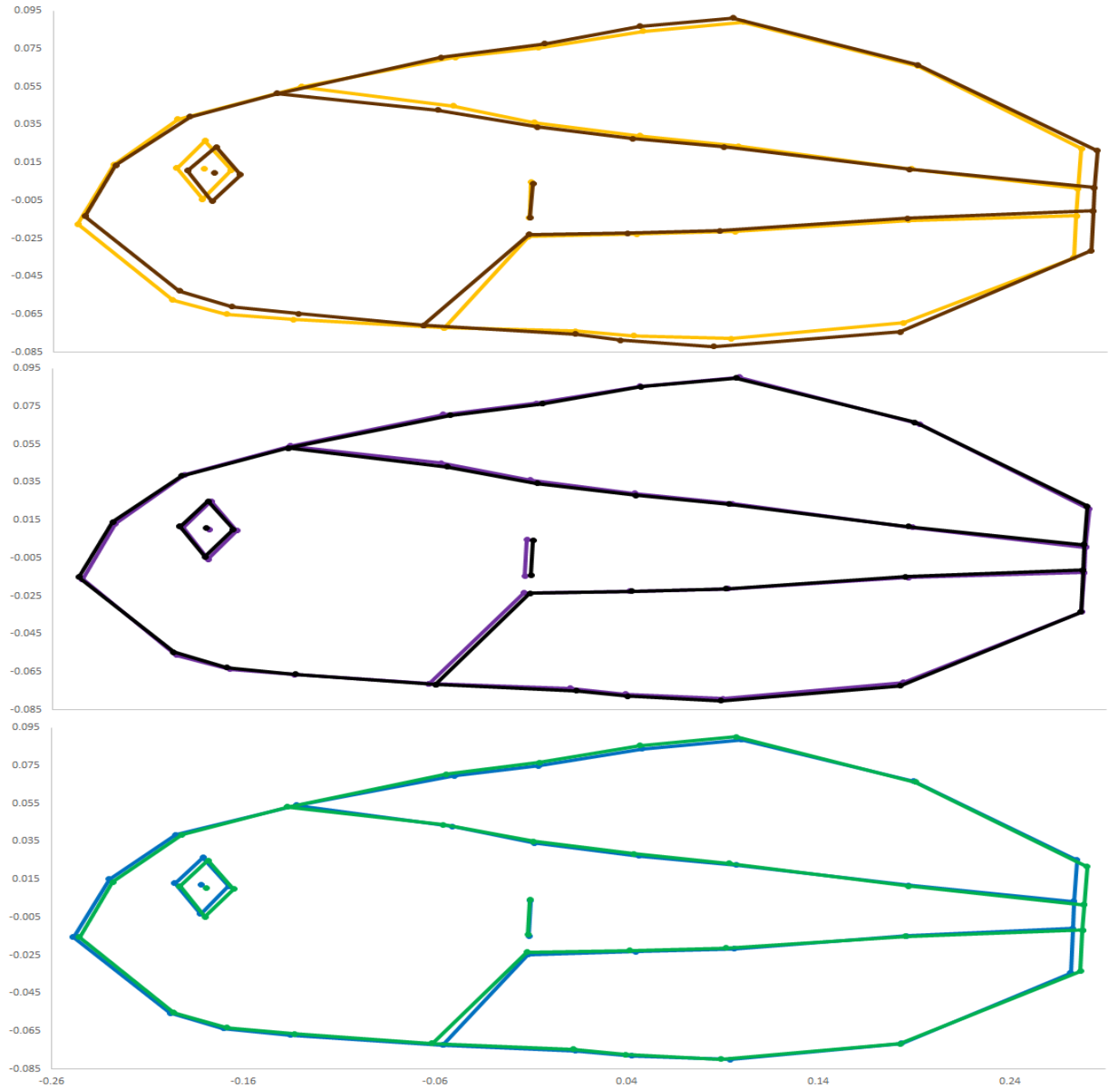

Figure 55. Shape comparison between pinewoods treefrog tadpoles at the small size in the Equal Density No Predator (orange), Equal Density And Predator (brown), More Pinewoods No Predator (purple), More Pinewoods And Predator (black), More Leopard Frogs No Predator Treatment (blue) and More Leopard Frogs And Predator treatment (green). Axes are relative deformation values.

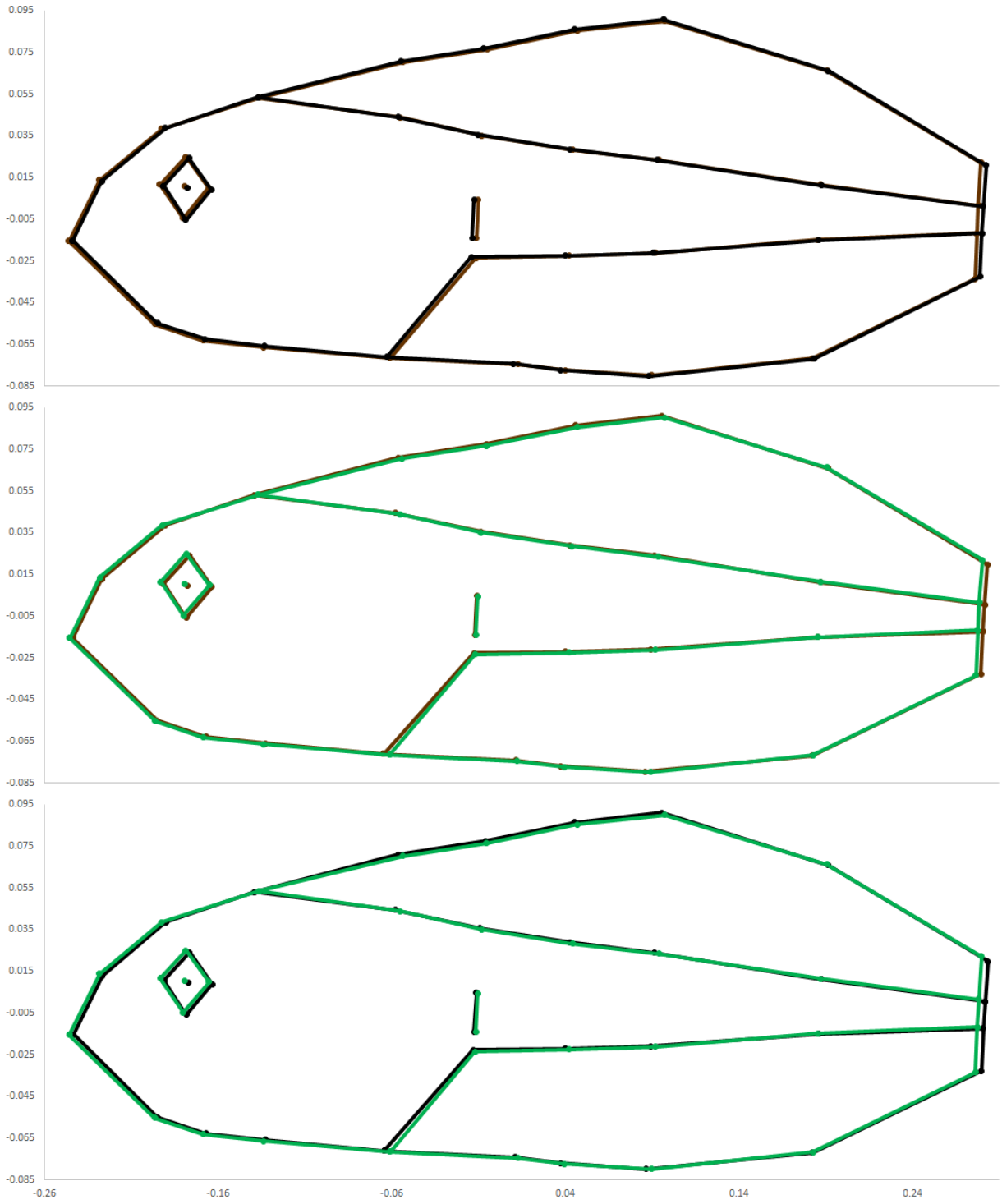

Figure 56. Shape comparison between pinewoods treefrog tadpoles at the small size in the Equal Density And Predator (brown), More Pinewoods And Predator (black), and More Leopard Frogs And Predator treatments (green). Axes are relative deformation values.

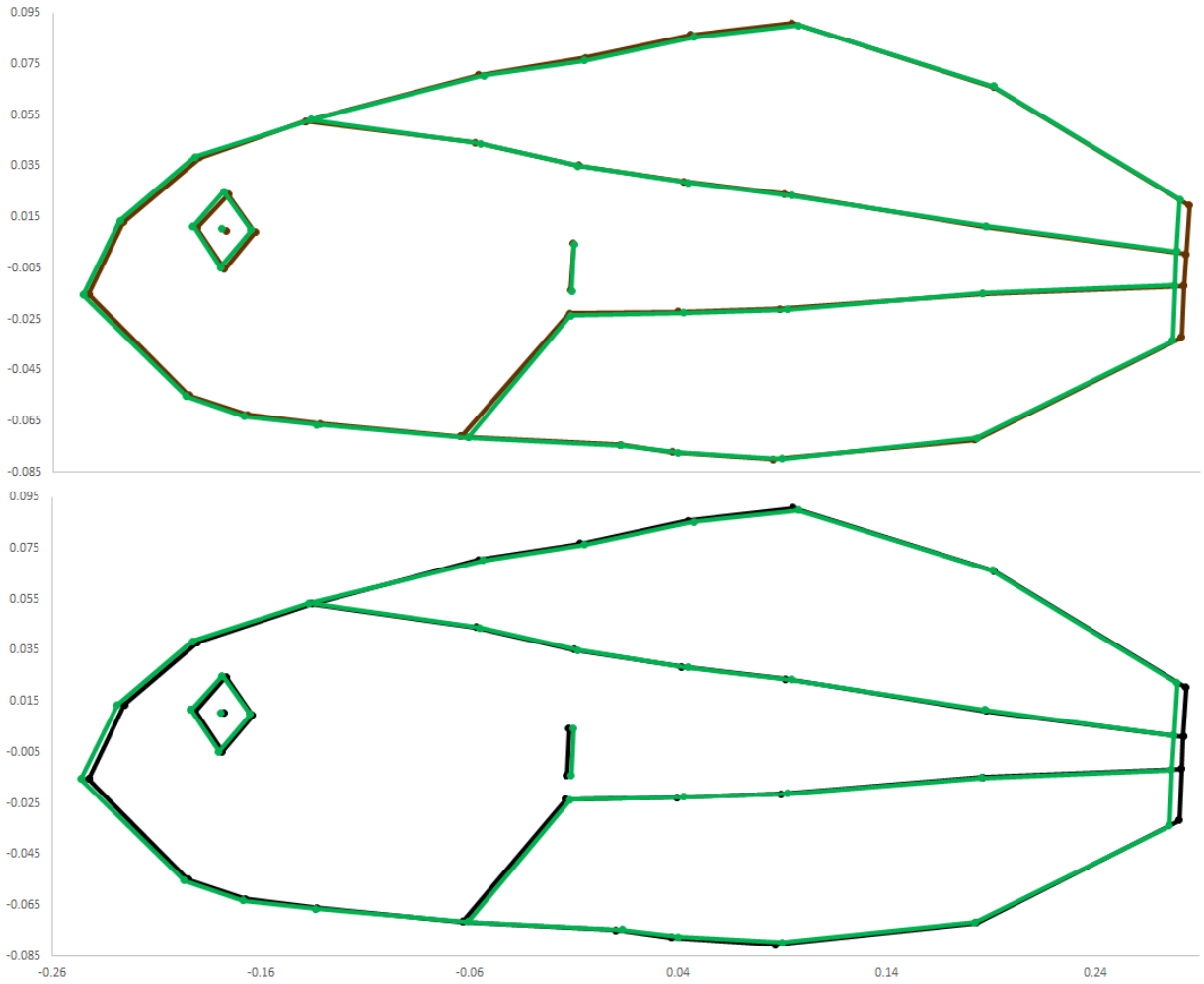

Figure 57. Shape comparison between pinewoods treefrog tadpoles at the large size in the Equal Density And Predator (brown), More Pinewoods And Predator (black), and More Leopard Frogs And Predator treatments (green). Axes are relative deformation values.

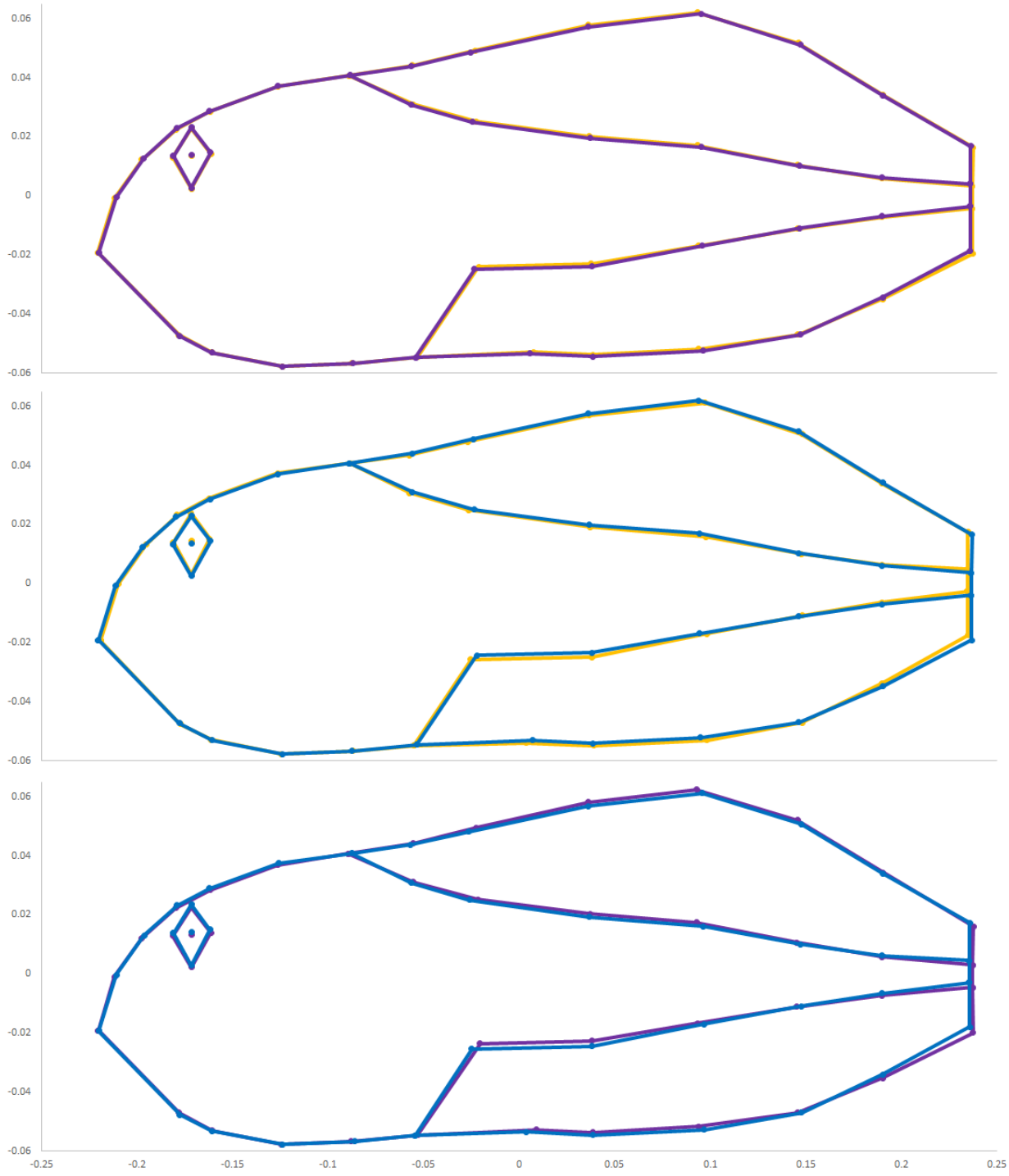

Figure 58. Shape comparison between southern leopard frog tadpoles at the small size in the Equal Density No Predator (orange), More Pinewoods No Predator (purple), and More Leopard Frogs No Predator treatments (blue). Axes are relative deformation values.

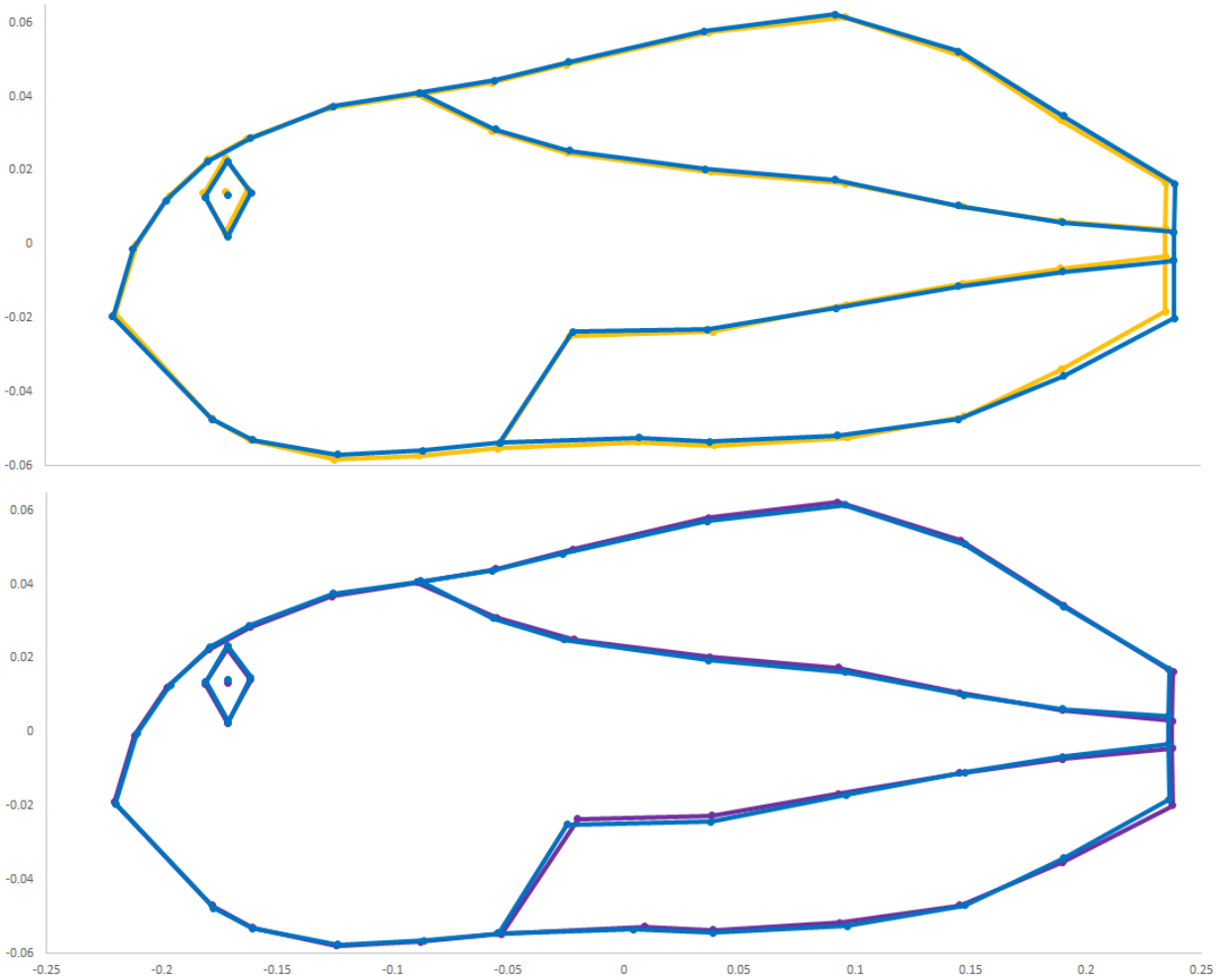

Figure 59. Shape comparison between southern leopard frog tadpoles at the average size in the Equal Density No Predator (orange), More Pinewoods No Predator (purple), and More Leopard Frogs No Predator treatments (blue). Axes are relative deformation values.

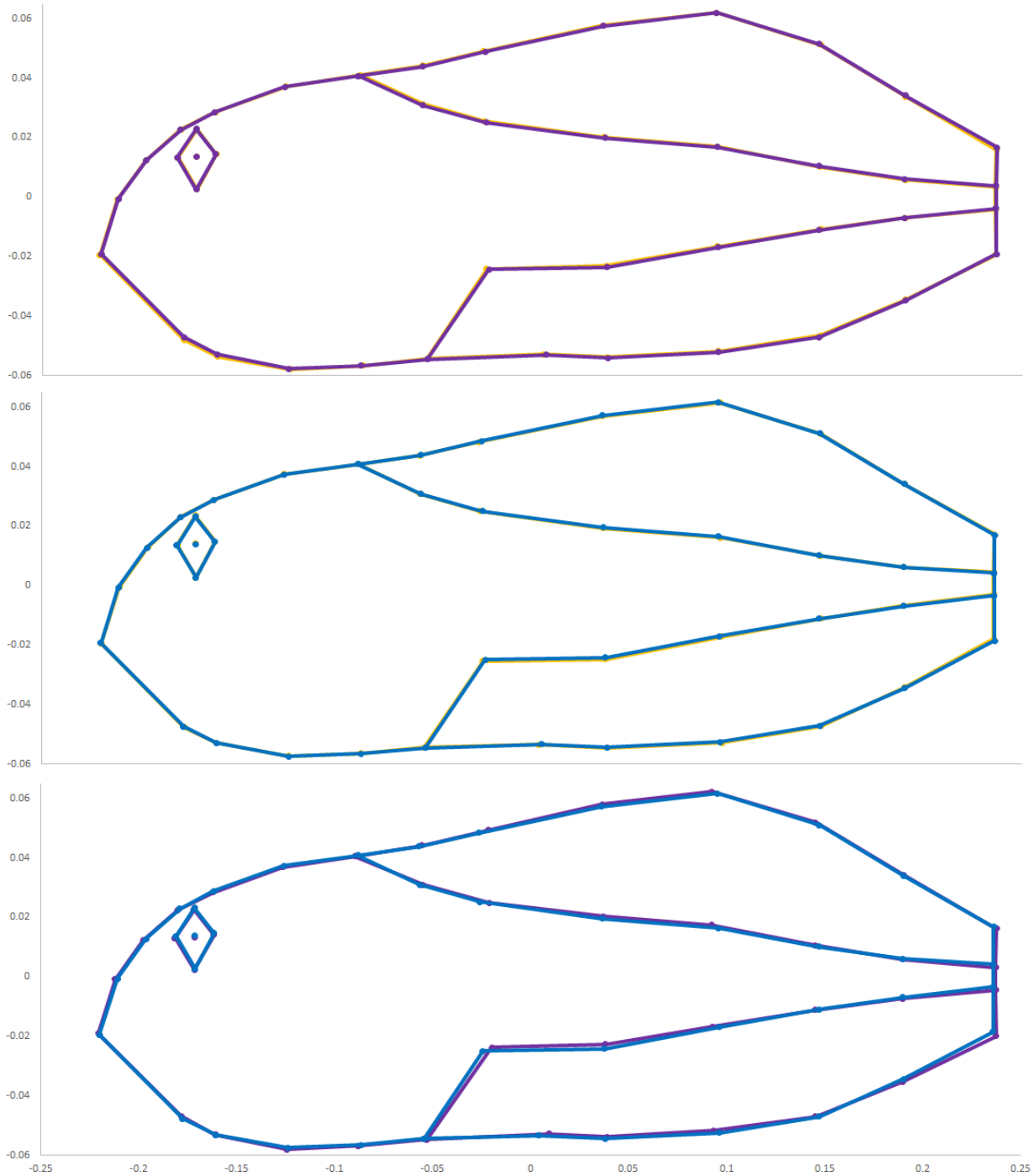

Figure 60. Shape comparison between southern leopard frog tadpoles at the large size in the Equal Density No Predator (orange), More Pinewoods No Predator (purple), and More Leopard Frogs No Predator treatments (blue). The orange Equal Density No Predator lines largely overlap with the other two treatment lines. Axes are relative deformation values.

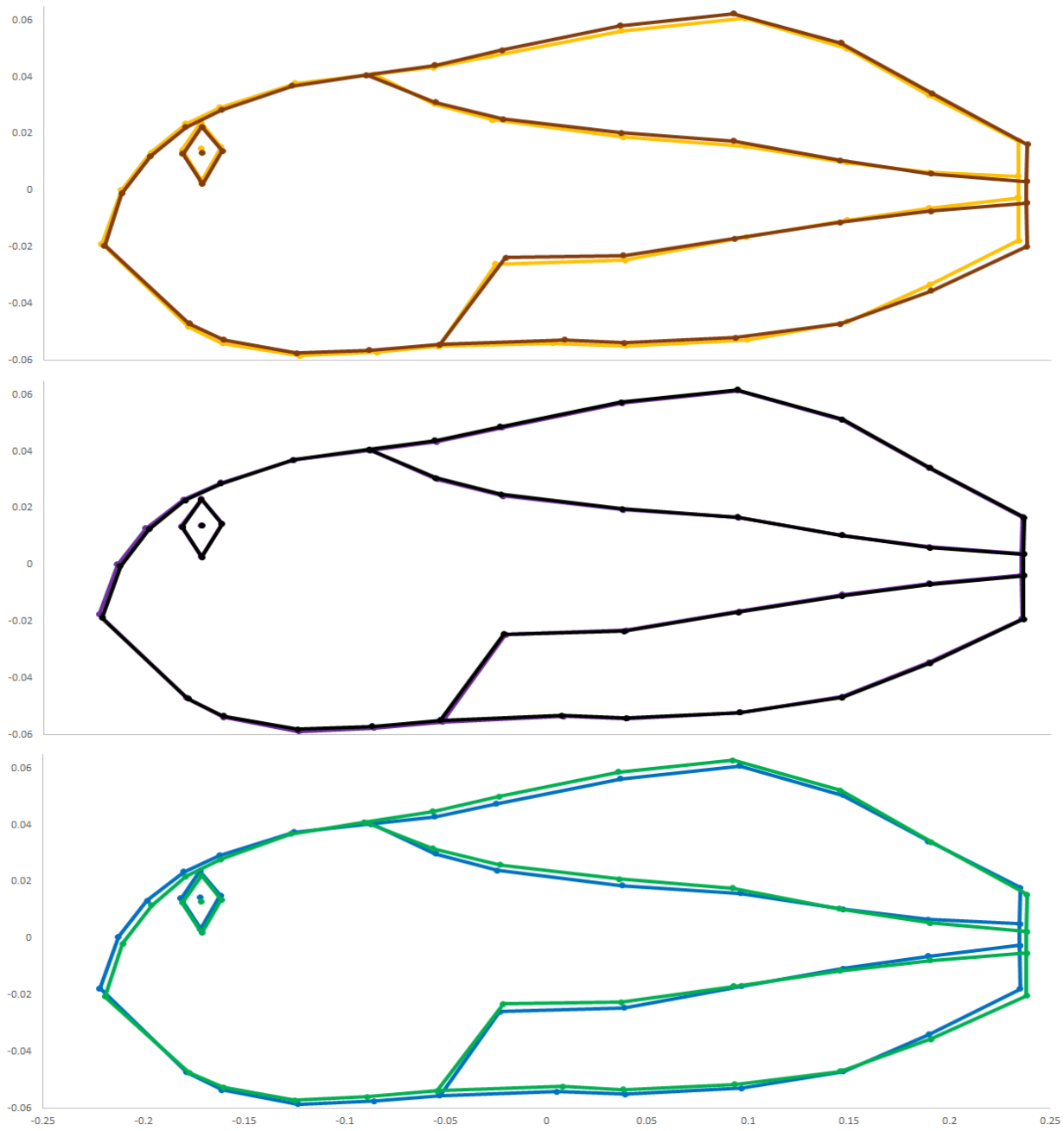

Figure 61. Shape comparison between southern leopard frog tadpoles at the small size in the Equal Density No Predator (orange), Equal Density And Predator (brown), More Pinewoods No Predator (purple), More Pinewoods And Predator (black), More Leopard Frogs No Predator Treatment (blue), and More Leopard Frogs And Predator treatment (green). Black and purple lines largely overlap. Axes are relative deformation values.

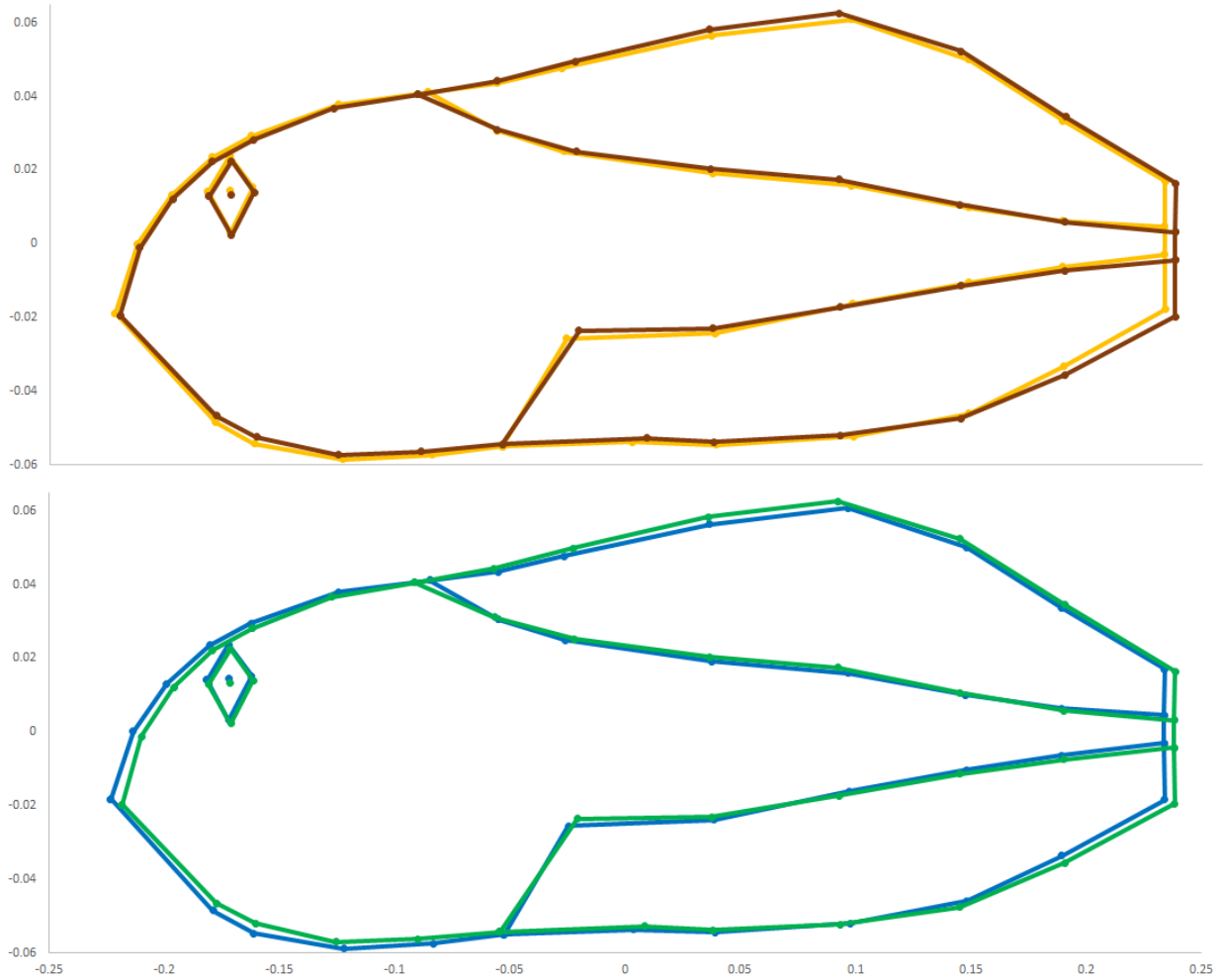

Figure 62. Shape comparison between southern leopard frog tadpoles at the average size in the Equal Density No Predator (orange), Equal Density And Predator (brown), More Leopard Frogs No Predator Treatment (blue), and More Leopard Frogs And Predator treatment (green). Axes are relative deformation values.

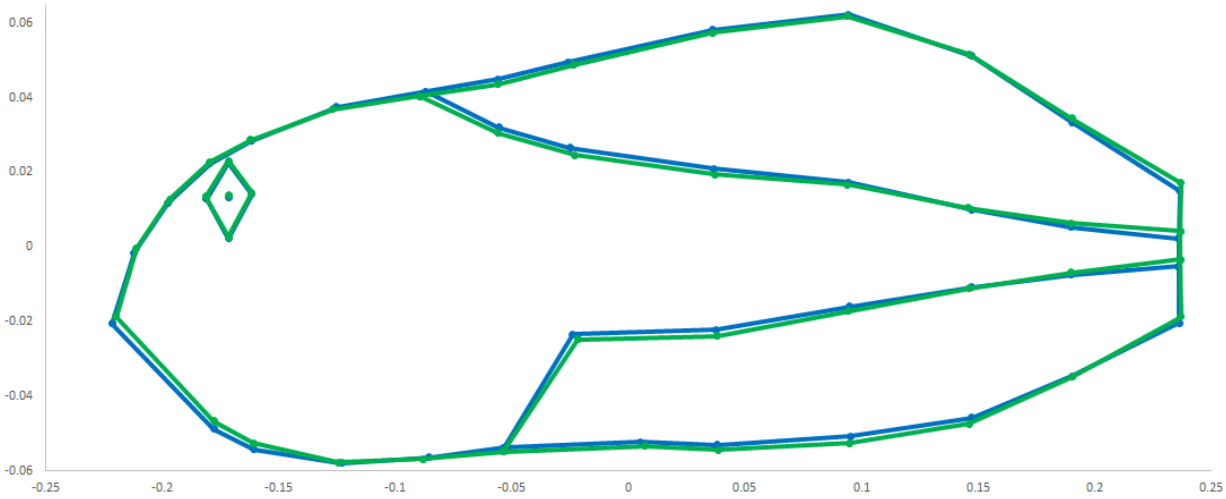

Figure 63. Shape comparison between southern leopard frog tadpoles at the large size in the More Leopard Frogs No Predator Treatment (blue) and More Leopard Frogs And Predator treatment (green). Axes are relative deformation values.

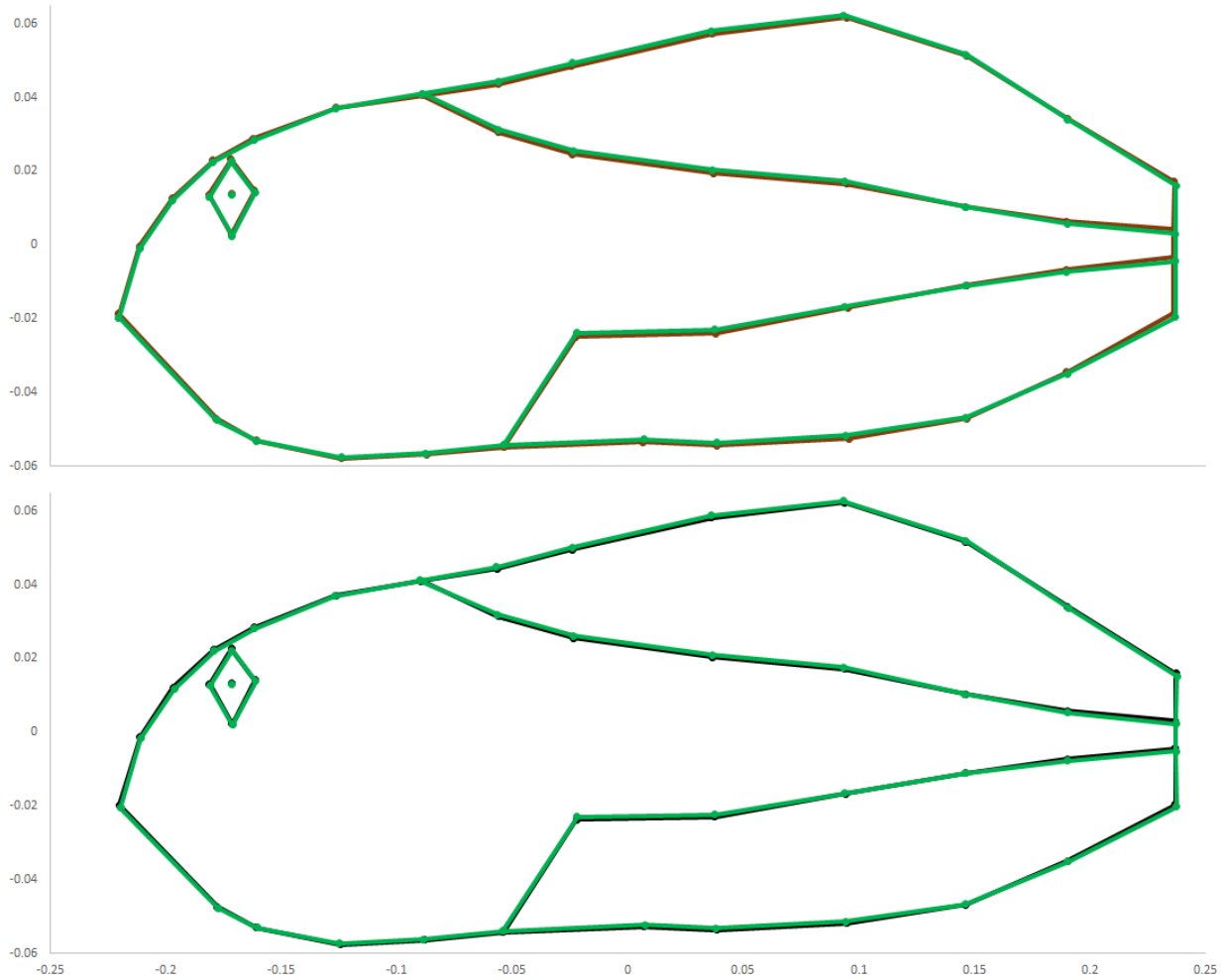

Figure 64. Shape comparison between southern leopard frog tadpoles at the small size in the Equal Density And Predator (brown), More Pinewoods And Predator (black), and More Leopard Frogs And Predator treatment (green). Lines largely overlap for both comparisons. Axes are relative deformation values.

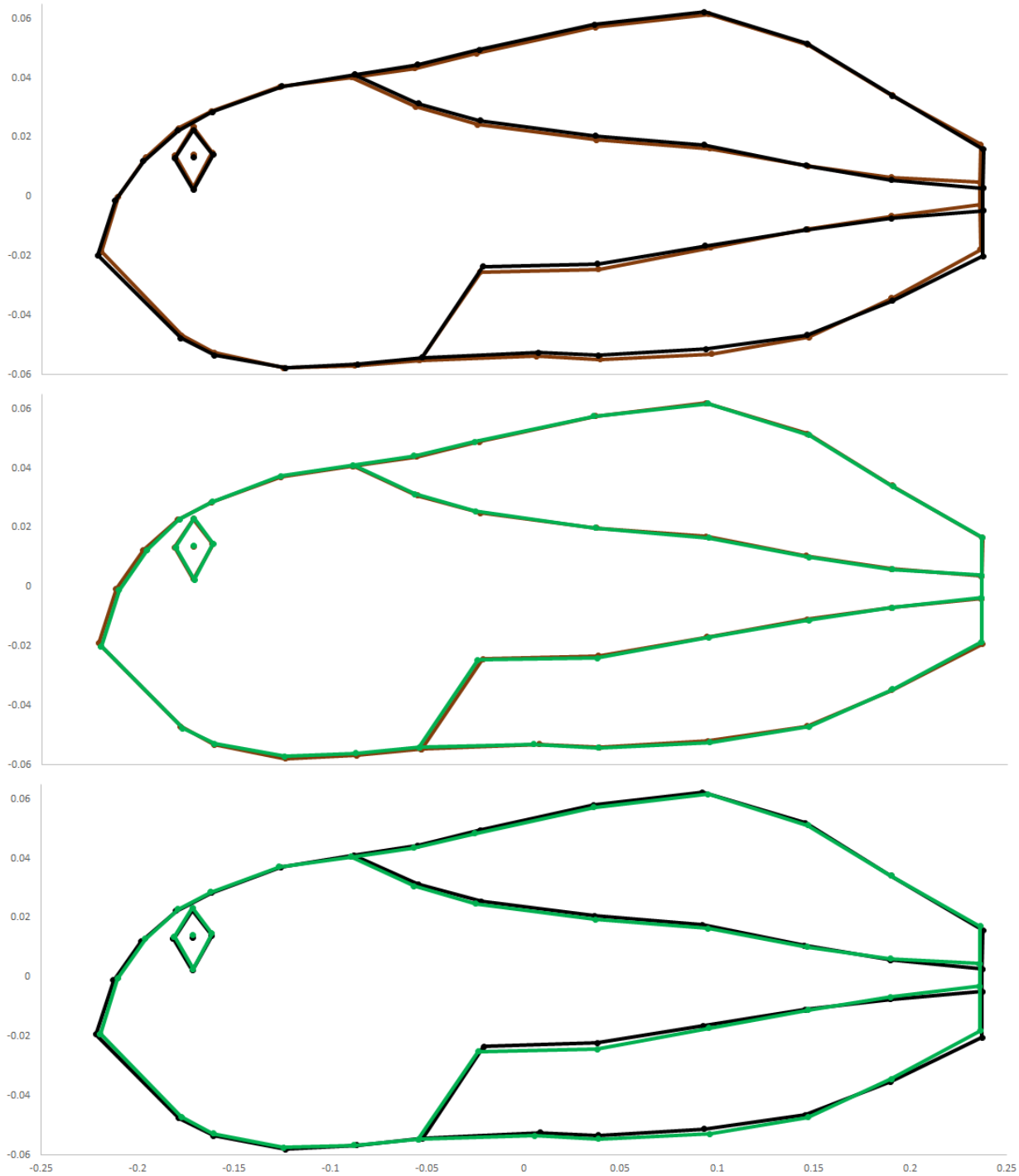

Figure 65. Shape comparison between southern leopard frog tadpoles at the average size in the Equal Density And Predator (brown), More Pinewoods And Predator (black), and More Leopard Frogs And Predator treatment (green). Axes are relative deformation values.

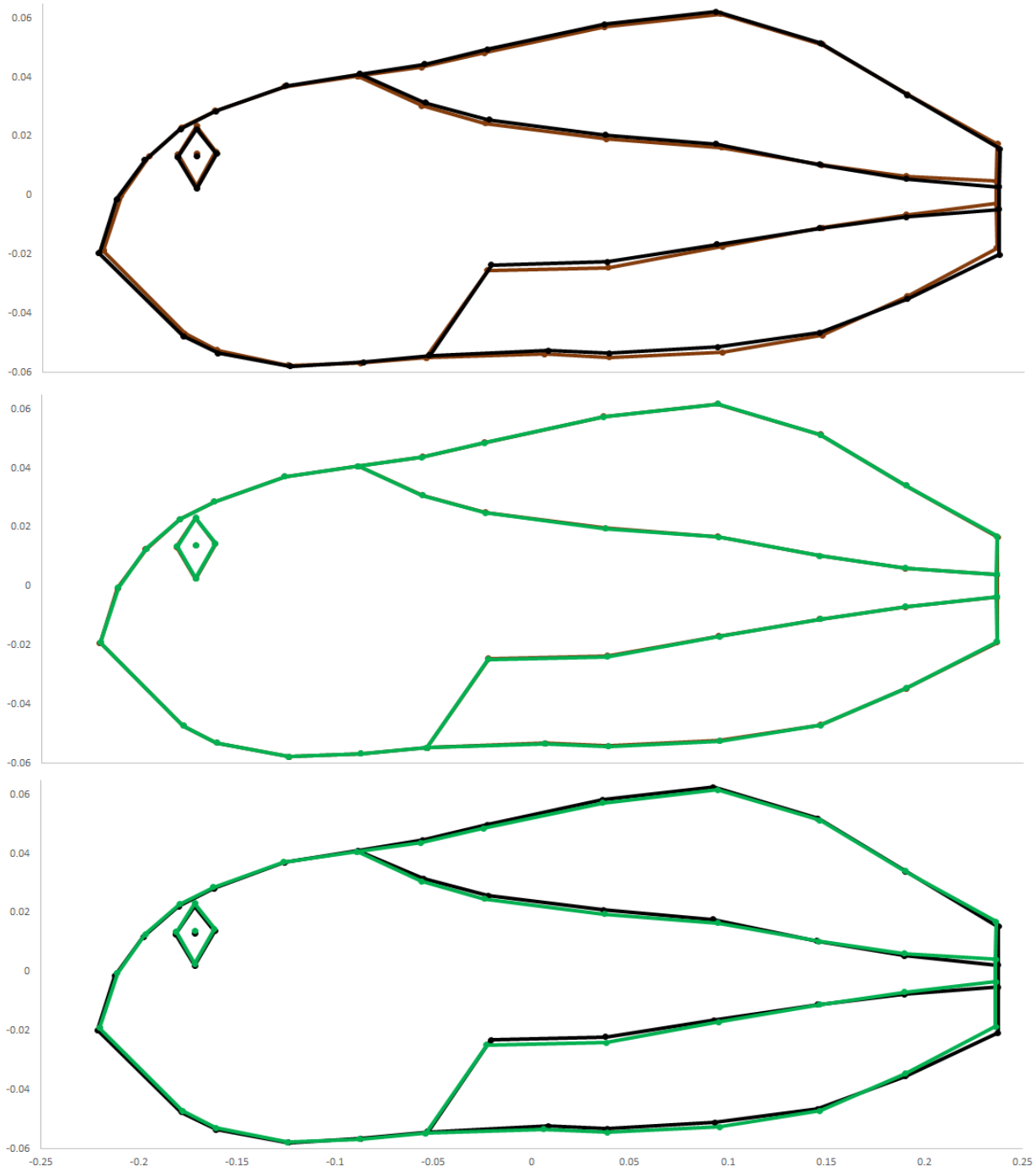

Figure 66. Shape comparison between southern leopard frog tadpoles at the large size in the Equal Density And Predator (brown), More Pinewoods And Predator (black), and More Leopard Frogs And Predator treatment (green). The brown line is obscured by the green line in the middle panel. Axes are relative deformation values.

Table 12. Planned contrasts for statistical analyses. Contrasts 1-11 were used for all tadpole and metamorph response variables as well as the phytoplankton and periphyton. Contrasts 12-17 were only used with the phytoplankton and periphyton analyses. Contrasts 18-23 were only used when comparing survival between the two species within a treatment.

Contrast #	Contrast Description	Treatments Compared
1	Does adding more pinewoods affect competition?	Equal No Pred vs. More Hf No Pred
2	Does adding more leopard frogs affect competition?	Equal No Pred vs. More Ls No Pred
3	Are the effects of adding more leopard frogs and adding more pinewoods the same?	More Ls No Pred vs. More Hf No Pred
4	Does adding a predator affect the equal density?	Equal No Pred vs. Equal Pred
5	Does adding a predator affect when more pinewoods are present?	More Hf No Pred vs. More Hf Pred
6	Does adding a predator affect when more leopard frogs are present?	More Ls No Pred vs. More Ls Pred
7	Do more pinewoods change the effect of the predator?	Equal Pred vs. More Hf Pred
8	Do more leopard frogs change the effect of the predator?	Equal Pred vs. More Ls Pred
9	Are the effects of adding leopard frogs and adding pinewoods on the predator the same?	More Ls Pred vs. More Hf Pred
10	Are the effects of adding more pinewoods and adding predators additive?	Equal No Pred and More Hf Pred vs. Equal Pred and More Hf No Pred
11	Are the effects of adding more leopard frogs and adding predators additive?	Equal No Pred and More Ls Pred vs. Equal Pred and More Ls No Pred
12	Does adding equal numbers of both species change tank conditions?	Algae Control vs. Equal No Pred
13	Does adding equal numbers of both species and a predator change tank conditions?	Algae Control vs. Equal Pred
14	Does adding more pinewoods change tank conditions?	Algae Control vs. More Hf No Pred
15	Does adding more pinewoods and a predator change tank conditions?	Algae Control vs. More Hf Pred

Table 12. Cont.

16	Does adding more leopard frogs change tank conditions?	Algae Control vs. More Ls No Pred
17	Does adding more leopard frogs and a predator change tank conditions?	Algae Control vs. More Ls Pred
18	Does adding equal numbers of pinewoods and leopard frogs affect survival of pinewoods and leopard frogs differently?	Hf Equal No Pred vs. Ls Equal No Pred
19	Does adding a predator with equal numbers of pinewoods and leopard frogs affect survival of pinewoods and leopard frogs differently?	Hf Equal Pred vs. Ls Equal Pred
20	Does adding more pinewoods affect survival of pinewoods and leopard frogs differently?	Hf More Hf No Pred vs. Ls More Hf No Pred
21	Does adding a predator and more pinewoods affect survival of pinewoods and leopard frogs differently?	Hf More Hf Pred vs. Ls More Hf Pred
22	Does adding more leopard frogs affect survival of pinewoods and leopard frogs differently?	Hf More Ls No Pred vs. Ls More Ls No Pred
23	Does adding a predator and more leopard frogs affect survival of pinewoods and leopard frogs differently?	Hf More Ls Pred vs. Ls More Ls Pred

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APPENDIX A: AUP Approval Letters

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

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

April 26, 2012

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

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "How Long do the Costs of Phenotypic Plasticity Incurred as Larvae Impact Adults?" (AUP #D275) was reviewed by this institution's Animal Care and Use Committee on 4/26/12. The following action was taken by the Committee:

"Approved as submitted"

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

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

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

April 10, 2014

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

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "How Does the Timing of the Arrival of a Competitor Alter the Non-Lethal Effects of Predators on Prey?" (AUP #D309) was reviewed by this institution's Animal Care and Use Committee on 4/10/14. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

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

Sincerely yours,

SBMcRae

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

SM/jd

Enclosure

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

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

April 14, 2015

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

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, "How Does Variation in the Relative Strength of Intraspecific and Interspecific Competition Alter the Extent to Which Prey Alter Their Morphology in Response to the Threat of Predation?" (AUP #D327) was reviewed by this institution's Animal Care and Use Committee on April 13, 2015. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

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

Sincerely yours,

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

SM/jd

Enclosure

APPENDIX B: Supplemental Tables and Figures

Supplemental Table S1. ANOVA table for tadpoles at two weeks post hatching. The first number is the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Mass	$0.55_{2,8}$ (0.595)	N/A	N/A
Body Length	$0.57_{2,7}$ (0.5901)	$12.49_{1,7}$ (0.0095)	N/A
Body Depth	$2.12_{2,5}$ (0.2152)	$1.42_{1,5}$ (0.2872)	$1.79_{2,5}$ (0.2590)
Tail Length	$0.48_{2,7}$ (0.6360)	$5.84_{1,7}$ (0.0464)	N/A
Maximum Tail Fin Height	$0.52_{2,7}$ (0.6179)	$9.52_{1,7}$ (0.0177)	N/A
Tail Muscle Depth	$1.46_{2,7}$ (0.2944)	$16.56_{1,7}$ (0.0048)	N/A
Body Width	$4.87_{2,5}$ (0.0671)	$19.13_{1,5}$ (0.0072)	$4.89_{2,5}$ (0.0666)
Tail Muscle Width	$1.91_{2,7}$ (0.2173)	$39_{1,7}$ (0.0004)	N/A

Supplemental Table S2. ANOVA table for tadpoles at four weeks post hatching. The first number if the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Mass	$1.05_{2, 8}$ (0.3951)	N/A	N/A
Body Length	$14.25_{2, 5}$ (0.0086)	$74.83_{1, 5}$ (0.0003)	$12.58_{2, 5}$ (0.0112)
Body Depth	$0.88_{2, 7}$ (0.4568)	$51.57_{1, 7}$ (0.0002)	N/A
Tail Length	$5.39_{2, 5}$ (0.0565)	$42.22_{1, 5}$ (0.0013)	$4.05_{2, 5}$ (0.0901)
Maximum Tail Fin Height	$0.21_{2, 7}$ (0.8165)	$17.74_{1, 7}$ (0.0040)	N/A
Tail Muscle Depth	$13.99_{2, 5}$ (0.0090)	$46.10_{1, 5}$ (0.0011)	$16.33_{2, 5}$ (0.0064)
Body Width	$8.24_{2, 8}$ (0.0114)	N/A	N/A
Tail Muscle Width	$34.39_{2, 7}$ (0.0002)	$22.76_{1, 7}$ (0.0020)	N/A

Supplemental Table S3. ANOVA table for toads at metamorphosis (5-7 weeks post hatching). The first number if the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Survival	$7.81_{2, 12}$ (0.0067)	N/A	N/A
Mass	$1.44_{2, 8}$ (0.2931)	N/A	N/A
Average Emergence Time	$1.44_{2, 8}$ (0.2931)	N/A	N/A
Snout-urostyle Length	$2.40_{2, 5}$ (0.1862)	$674.51_{1, 5}$ (<0.0001)	$1.89_{2, 5}$ (0.2442)
Cranial Width	$3.67_{2, 7}$ (0.0813)	$757.94_{1, 7}$ (<0.0001)	N/A
Tibio-fibula Length	$0.99_{2, 7}$ (0.4166)	$391.47_{1, 7}$ (<0.0001)	N/A
Femur Length	$2.28_{2, 5}$ (0.1978)	$109.96_{1, 5}$ (0.0001)	$2.04_{2, 5}$ (0.2244)
Average Jump Distance	$8.36_{2, 5}$ (0.0255)	$8.20_{1, 5}$ (0.0352)	$10.22_{2, 5}$ (0.0171)

Supplemental Table S4. ANOVA table for toads at 3-5 weeks after metamorphosis (8-10 weeks post hatching). The first number if the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Mass	$0.66_{2, 7}$ (0.5455)	N/A	N/A
Snout-urostyle Length	$1.60_{2, 6}$ (0.4049)	$266.18_{1, 6}$ (<0.0001)	N/A
Cranial Width	$0.95_{2, 6}$ (0.4397)	$268.79_{1, 6}$ (<0.0001)	N/A
Tibio-fibula Length	$0.46_{2, 6}$ (0.6512)	$143.48_{1, 6}$ (<0.0001)	N/A
Femur Length	$5.59_{2, 4}$ (0.0695)	$415.88_{1, 4}$ (<0.0001)	$7.08_{2, 4}$ (0.0485)
Average Jump Distance	$1.66_{2, 6}$ (0.2666)	$32.09_{1, 6}$ (0.0130)	N/A

Supplemental Table S5. ANOVA table for toads at 5-8 weeks after metamorphosis (10-12 weeks post hatching). The first number if the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Survival	$1.97_{2, 12}$ (0.1827)	N/A	N/A
Mass	$1.55_{2, 7}$ (0.2762)	N/A	N/A
Snout-urostyle Length	$1.00_{2, 6}$ (0.4206)	$99.53_{1, 6}$ (<0.0001)	N/A
Cranial Width	$0.60_{2, 6}$ (0.5802)	$46.58_{1, 6}$ (0.0005)	N/A
Tibio-fibula Length	$1.40_{2, 6}$ (0.3164)	$29.30_{1, 6}$ (0.0016)	N/A
Femur Length	$0.94_{2, 4}$ (0.4637)	$27.87_{1, 4}$ (0.0062)	$1.80_{2, 4}$ (0.2772)
Average Jump Distance	$1.87_{2, 4}$ (0.2673)	$3.93_{1, 4}$ (0.1183)	$2.79_{2, 4}$ (0.1743)

Supplemental Table S6. ANOVA table for toads at 8-11 weeks after metamorphosis (12-14 weeks post hatching). The first number if the F-value, the subscripts are the degrees of freedom, and the values in parentheses are the unadjusted *p*-values. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Survival	$0.03_{2, 12}$ (0.9688)	N/A	N/A
Mass	$1.48_{2, 5}$ (0.3130)	N/A	N/A
Snout-urostyle Length	$2.03_{2, 4}$ (0.2469)	$2.03_{1, 4}$ (0.2469)	N/A
Cranial Width	$0.84_{2, 4}$ (0.4957)	$71.38_{1, 4}$ (0.0011)	N/A
Tibio-fibula Length	$3.11_{2, 2}$ (0.2434)	$351.26_{1, 2}$ (0.0028)	$6.30_{2, 2}$ (0.1370)
Femur Length	$8.01_{2, 2}$ (0.1110)	$116.89_{1, 2}$ (0.0084)	$5.23_{2, 2}$ (0.1606)
Average Jump Distance	$2.42_{2, 2}$ (0.2921)	$15.96_{1, 2}$ (0.0573)	$3.91_{2, 2}$ (0.2036)

Supplemental Table S7. ANOVA Tables for Tadpole Traits. The first number is the F statistic, the subscript numbers are the numerator and denominator degrees of freedom and the value in parentheses is the unadjusted *p* value. Mass in this case was the natural log of the geometric mean of mass. We used the geometric mean of mass as this is typically a better fit for mass data than the arithmetic mean as mass often follows a lognormal distribution. We used the natural log of the geometric mean to meet the assumptions of normality when mass was included in the model as a covariate, as data tended to be non-normally distributed without log transforming the mass. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

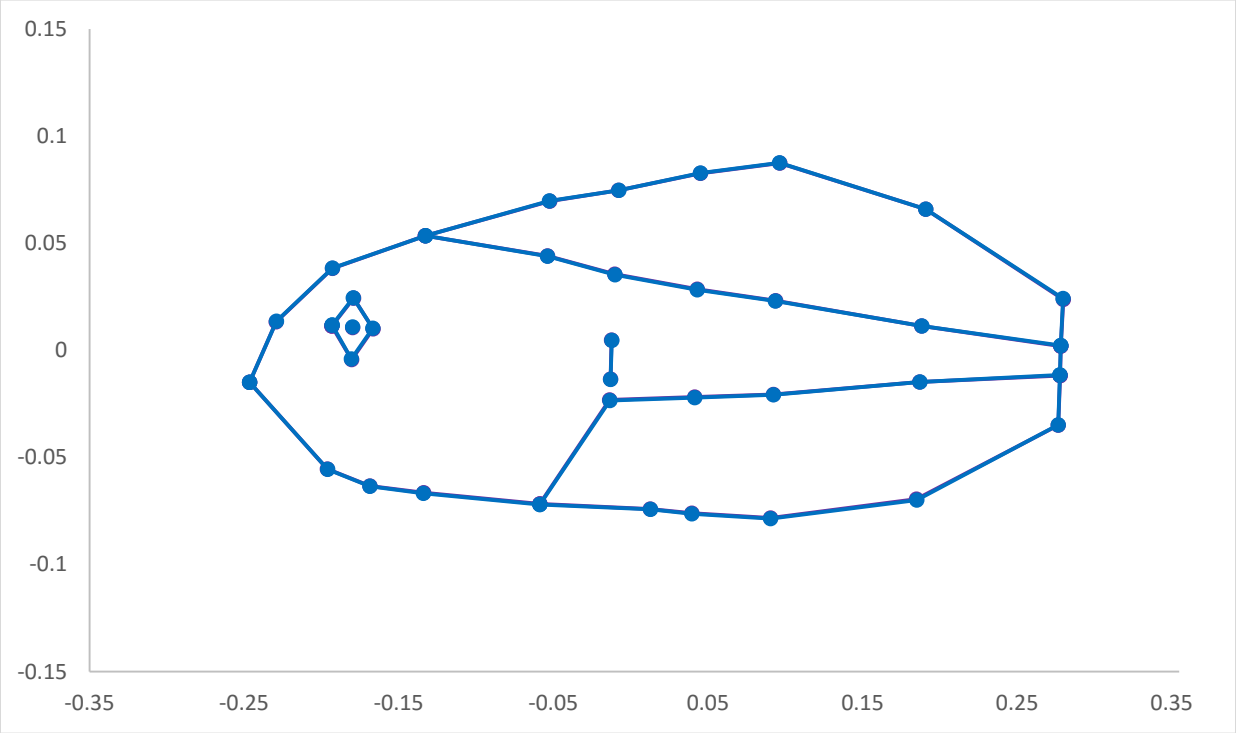
Response	Treatment	Mass	Treatment X Mass
Mass	$0.54_{9, 25}$ (0.8337)	N/A	N/A
Eye Width	$1.86_{9, 15}$ (0.1390)	$13.82_{1, 15}$ (0.0021)	$1.70_{9, 15}$ (0.1738)
Body Length	$3.91_{9, 15}$ (0.0099)	$37.53_{1, 15}$ (<0.0001)	$4.40_{9, 15}$ (0.0058)
Body Depth	$4.72_{9, 15}$ (0.0042)	$31.45_{1, 15}$ (<0.0001)	$5.25_{9, 15}$ (0.0025)
Tail Length to End of Pigment on Tail (TPL)	$3.91_{9, 15}$ (0.0099)	$54.96_{1, 15}$ (<0.0001)	$3.99_{9, 15}$ (0.0089)
Tail Origin to Tail Tip Length (HTL)	$3.75_{9, 15}$ (0.0117)	$61.41_{1, 15}$ (<0.0001)	$3.90_{9, 15}$ (0.0099)
Maximum Tail Height	$3.76_{9, 15}$ (0.0117)	$48.04_{1, 15}$ (<0.0001)	$4.30_{9, 15}$ (0.0064)
Tail Muscle Height	$4.58_{9, 15}$ (0.0048)	$24.08_{1, 15}$ (0.0002)	$4.80_{9, 15}$ (0.0038)
Tail Stripe Height	$2.28_{9, 15}$ (0.0766)	$16.95_{1, 15}$ (0.0009)	$2.65_{9, 15}$ (0.0457)
Mouth Width	$5.91_{9, 15}$ (0.0014)	$74.90_{1, 15}$ (<0.0001)	$5.13_{9, 15}$ (0.0028)
Inter Eye Distance	$2.52_{9, 14}$ (0.0585)	$24.98_{1, 14}$ (0.0002)	$2.91_{9, 14}$ (0.0360)
Body Width in Front of the Spiracle	$3.59_{9, 15}$ (0.0142)	$82.22_{1, 15}$ (<0.0001)	$4.19_{9, 15}$ (0.0072)
Body Width Behind the Spiracle	$3.62_{9, 15}$ (0.0137)	$41.40_{1, 15}$ (<0.0001)	$4.33_{9, 15}$ (0.0062)
Length from Midpoint of Gut to Center of Base of Tail	$4.48_{9, 15}$ (0.0053)	$70.94_{1, 15}$ (<0.0001)	$4.40_{9, 15}$ (0.0058)

Supplemental Table S7 Cont.

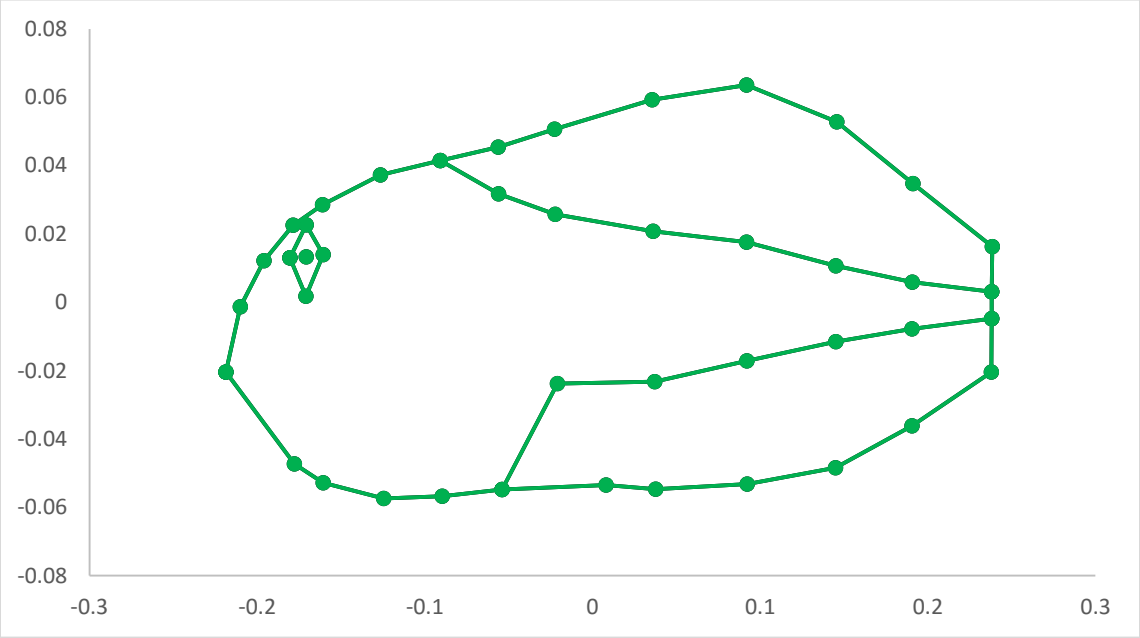
Tail Width	$3.82_{9, 15}$ (0.0109)	$32.21_{1, 15}$ (<0.0001)	$4.21_{9, 15}$ (0.0071)
Tail Length from Base of Tail to Tail Tip	$3.40_{9, 15}$ (0.0177)	$69.20_{1, 15}$ (<0.0001)	$3.48_{9, 15}$ (0.0162)

Supplemental Table S8. ANOVA Tables for Focal Metamorph Traits. The first number is the F statistic, the subscript numbers are the numerator and denominator degrees of freedom and the value in parentheses is the unadjusted *p* value. Mass in this case was the natural log of the geometric mean of mass. We used the geometric mean of mass as this is typically a better fit for mass data than the arithmetic mean as mass often follows a lognormal distribution. We used the natural log of the geometric mean to meet the assumptions of normality when mass was included in the model as a covariate, as data tended to be non-normally distributed without log transforming the mass. The term N/A indicates that F-value, degrees of freedom, and *p*-values were not applicable for that response column (i.e. the interaction term between treatment and mass was not significant so the interaction term was not included, or there is no mass or interaction term as with the response variable Mass).

Response	Treatment	Mass	Treatment X Mass
Average Emergence Time	$1.24_{9,25}$ (0.3143)	N/A	N/A
Geometric Mean of Mass	$0.85_{9,25}$ (0.5764)	N/A	N/A
Survival to Metamorphosis	$0.86_{9,29}$ (0.5686)	N/A	N/A
Survival Including End of Experiment Tadpoles	$0.94_{9,29}$ (0.5079)	N/A	N/A
Femur Length	$2.70_{9,24}$ (0.0249)	$788.34_{1,24}$ (<0.0001)	N/A
Tibio-fibula Length	$0.68_{9,24}$ (0.7226)	$1068.34_{1,24}$ (<0.0001)	N/A
Average Jump Distance	$1.44_{9,24}$ (0.2271)	$171.26_{1,24}$ (<0.0001)	N/A
Snout-Urostyle Length	$1.83_{9,15}$ (0.1441)	$478.94_{1,15}$ (<0.0001)	$2.05_{9,15}$ (0.1057)
Cranial Width	$1.68_{9,15}$ (0.1795)	$241.63_{1,15}$ (<0.0001)	$1.72_{9,15}$ (0.1691)



Supplemental Figure 1. Landmarks used for geometric morphometrics for pinewoods treefrog tadpoles (Landmark number is 38).



Supplemental Figure 2. Landmarks used for geometric morphometrics for southern leopard frog tadpoles (Landmark number is 43).