# Effect of predator diet on predator-induced changes in life history and performance of anuran larvae

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# Abstract

Phenotypic plasticity allows some animals to change their behavioural, morphological, performance, and life history traits in response to changes in environmental conditions such as the presence of predators. These changes can enhance survival, but come at a cost. Some of these phenotypic changes are predator and diet specific. I examined the effects of predator diet on the performance, life-history, and morphology of developing Northern Leopard Frog (*Lithobates pipiens*) tadpoles. Tadpoles were either exposed to cues from fish free water, cues from Brown Bullhead (*Ameiurus nebulosus)* fed a diet of trout pellets, or cues from *A. nebulosus* fed a *L. pipiens* tadpoles diet. Tadpoles exposed to predatory fish cues had smaller bodies, deeper tail fins, slower growth and development rates, and better rotational performance than tadpoles that were not exposed to predatory fish cues. Moreover, tadpoles appeared to differentiate between predatory fish diet and produced diet-specific responses in tail morphology and activity, although the latter effect was only marginally significant. Hatching, metamorphosis rates, and linear performance were not affected by the treatments. These results suggest that *A. nebulosus* can induce phenotypic changes in *L. pipiens* tadpoles, with some of these changes being diet specific.

# Résumé

La plasticité phénotypique permet à certains animaux de changer leur comportement, leur morphologie, leur performance et leur biodémographie quand ils sont exposés aux signaux de prédateurs. Ces changements peuvent améliorer leurs chances de survie, mais à un certain coût. Certains de ces changements phénotypiques sont spécifiques au prédateur et à son régime alimentaire. J'ai examiné les effets du régime alimentaire du prédateur sur la performance, l'histoire de vie et la morphologie des têtards de Grenouille léopard du nord (*Lithobates pipiens*). Les têtards ont été exposés soit à aucun signal, soit aux signaux de Barbotte brune (*Ameiurus nebolusus*) sur un régime de granules, ou soit aux signaux de *A. nebulosus* sur un regime de têtards de *L. pipiens*. Les têtards exposés aux signaux du poisson eurent des corps plus petits, des nageoires et des queues plus profondes, et des taux de croissance et de développement plus lents, et une meilleure performance de rotation que les têtards qui n'avaient pas été exposés aux signaux des poissons prédateurs. De plus, les têtards peuvent différencier entre les régimes alimentaires des prédateurs. Le régime alimentaire des prédateurs peut induire des changements morphologiques et comportementaux chez les têtards, notamment au niveau de la queue même si l'effet était marginalement significatif. Le taux d'éclosion et la métamorphose, et la performance linéaire n'ont pas été affectés par les traitements. Ces résultats suggèrent que *A. Nebulosus* peut induire des changements phénotypiques aux têtards *L. pipiens*, et que certains de ces changements sont spécifiques au régime alimentaire.

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# Introduction

Predation is a strong selection force in the course of evolution (Benard 2004, Lima and Dill 1990). Adaptations; traits providing enhanced fitness (Freemans and Herron 2007); occurring during the course of evolution can enhance predation avoidance(Laurila et al. 2002, Lima and Dill 1990). Antipredator adaptations can occur when predation risks are perceived in the environment. Induced phenotypic plasticity is the ability of some organisms to obtain changes in their physiological and life history traits during development with changes in their surroundings (Schlichting 1986). Changes in temperature, feeding rates, as well as competition have been seen to induce phenotypic changes in terrestrial arthropods and aquatic amphibians (Relyea and Auld 2005, Ellers and Driessen 2011, Van Dooremalen, Koekkoek and Ellers 2011, Schoeppner and Relyea 2009c). Predator inducible defenses can occur when phenotypically plastic animals are raised in the presence of predator cues (Harvell 1990). Empirical evidence suggests that predator-induced phenotypic plasticity mainly occurs in aquatic animals (Brönmark and Miner 1992), and has been observed in several species such as Great Pond Snails (*Lymnaea stagnalis* L) (Dalesman, Rundle and Cotton 2009), Freshwater Crucian Carp (*Carassius carassius*) (Brönmark and Miner 1992), and amphibians (Capellan and Nicieza 2007, Schmidt and Van Buskirk 2005).

Usually, animals exhibiting predator-induced phenotypic plasticity incur survival benefits in predation survival tests (McCollum and VanBuskirk 1996, Relyea 2002, Hews 1988, Takahara et al. 2003). It is generally accepted that adaptations beneficial in some respects, however, incur costs in other respects (Maynard Smith 1982), and these costs tend to balance out the benefits (Tollrian 1995). This is particularly the case when induced qualities are needless (Relyea 2002, McCollum and VanBuskirk 1996). For instance, Grey

Treefrog tadpoles (*Hyla chrysoscelis*) phenotypically modified through exposure to predator cues survived better than their unmodified counterparts in the presence of odonate predators, but had higher mortality in predator free environments (McCollum and VanBuskirk 1996). Thus, predator-induced phenotypic plasticity involves trade-offs and should only occur in risky environments when it enhances fitness.

Inducible changes in phenotypically plastic animals have been well studied and documented. However, the mechanism that controls these changes has not received as much attention. The few studies available found the phenotypic changes observed to be due to genetic and/or hormonal regulations. Researchers examining the genetic aspect found variation in the regulation of certain genes causing physiological changes that alter tadpole morphology (Mori et al. 2005, Mori et al. 2009). For example, salamander predation cues up regulated the Uromodulin-like gene and down regulated the keratin gene causing the appearance of temporary bulgy morphology in *Rana pirica* tadpoles. On the other hand, those investigating the endocrine system found that the hypothalamus-pituitary-thyroid (HPT) axis regulates certain hormones such as the thyroid hormone, growth hormone, and corticotropin releasing hormone that in turn cause changes to growth, development, and morphology (Fraker 2009, Rose 2005).

In aquatic environments, chemical signals are accurate and prolonged information sources that are often crucial for prey animals to detect predation threats (Chivers and Smith 1998, Wisenden 2000), particularly when visibility is low (Gelowitz, Mathis and Smith 1993). Such cues could depend in part on the predator's diet and could be in the form of prey alarm cues (Chivers and Smith 1998) or predator cues (Petranka, Kats and Sih 1987). Alarm

cues in tadpoles were recently found to be released voluntarily by live tadpoles from secretary cells in the epidermal layer (Ferrari, Wisenden and Chivers 2010). The cues are made of two polypeptides that have not yet been genetically identified and are located in the skin and the tail of anuran larvae (Fraker 2009). These cues alone can suppress the activity of the hypothalamo-pituitary-adrenal axis, reducing the corticosterone body content and thus causing reduced activity in some aquatic animals (Fraker 2009) . Predator kairomones may be digested prey alarm cues released by the predator during digestion (Wisenden 2000). Some researchers investigating the effect of predator cues or kairomones manipulated predator diet and included a starved predator treatment. Animal responses such as activity, mass, and morphology varied with predator diets used. The intensity of those responses increased with the genetic relatedness between the prey and the induced animals. On the contrary, the starved predator treatment almost always had results similar to control, predator free treatments (Schoeppner and Relyea 2009a, Schoeppner and Relyea 2009b). That was particularly the case with anuran larvae. In fact, tadpoles exposed to starved predator cues showed no significant reduction in activity, no reduction in weight, no change in morphology, and no effect on hatching rates (Johnson et al. 2003, Marquis, Saglio and Neveu 2004, Perotti et al. 2006, Saglio and Mandrillon 2006, Schoeppner and Relyea 2009b).

Recent investigations to identify the chemical cues needed to induce plastic changes in animals found that both digestive metabolites of conspecifics, or closely related genera, and alarm signals were required (LaFiandra and Babbitt 2004, Richardson 2006). Earlier evidence showed that both types of cues can individually affect the activity of exposed animals (Wilson and Lefcort 1993, Marquis et al. 2004, Chivers, Zhao and Ferrari 2007b). In

most cases, however, both alarm cues and digestive metabolites of predators feeding on conspecifics were required to induce morphological changes (Appleton and Palmer 1988, Crowl and Covich 1990, Gelowitz et al. 1993, Schoeppner and Relyea 2009a, Laurila, Kujasalo and Ranta 1998), with only a few exceptions (Brönmark and Pettersson 1994). These cues provide prey animals with more information on predation risk than alarm cues alone, which allows for long-term behavioural changes (Schoeppner and Relyea 2009b).

Anuran larvae are the most frequently used animals in studies of predator-induced phenotypic plasticity, as they are the best to demonstrate responses to predator cues as well as fitness changes (McIntyre, Baldwin and Flecker 2004). It is now well established that tadpoles raised in the presence of predator cues change their morphology, growth rate, hatching time, hatchling development, and behaviour (Teplitsky, Plenet and Joly 2003, Teplitsky et al. 2005, Capellan and Nicieza 2007). Recent research has focused on the effect of predator-induced traits on performance of aquatic animals, including amphibians. Amphibians raised with predator cues during the juvenile stage performed better in swimming trials than those raised without (Walsh, Downie and Monaghan 2008, Stamper et al. 2009).

Tadpoles may adopt one of several performance related predator avoidance tactics. Tadpoles can use high-speed thrusts to avoid predation in nature. Although high-speed thrusts may or may not allow them to out-swim a pursuing predator, it can however give them the benefit of reaching refuge sooner (Brown and Taylor 1995, Teplitsky et al. 2005). When this strategy is not possible, tadpoles perform evasive maneuvers such as repeated sudden turns. These turns often result in tadpoles confusing the pursuing predator, escaping

its grasp, or in the predator ceasing to chase the tadpole (Brown and Taylor 1995, Warkentin 1999). In Wood Frogs (*Lithobates sylvatica*), sprint speed changes with developmental stage, with stages 30 and 38 being the fastest and stages 26 and 42 being the slowest (Brown and Taylor 1995). During stages with lowest sprint speeds, Wood Frog tadpoles perform more frequent turns with larger angles in response to predation threats to make up for their low swimming abilities (Brown and Taylor 1995).

Tactics used to evade predators also vary according to predator type. Agile Frog tadpoles (*Rana dalmatina*) raised in the presence of pursuing predators (Three-Spined Stickleback, *Gasterosteus aculeatus*) swam faster than those raised with sit and wait predators (dragonfly larva, *Aeshna cyanea*) or without predators at all (Teplitsky et al. 2005). Better swimming performance was also observed in Green Frog tadpoles (*Rana lessonae*) raised with Pumpkinseed (*Lepomis gibossus*) than with dragonfly larvae (*Aeshna cyanea*) or no predator (Wilson, Kraft and Van Damme 2005). In both experiments, predators were maintained on a tadpole conspecific diet. Given that predator diet as well as predator type have been documented to have differential induction effects independently, we should expect that similar predators fed different diets would have differential effects on the performance of anuran larvae.

There is evidence that tadpoles can differentiate between predator's diet, inducing diet specific changes in particular traits based on the level of risk. Moreover, recent experiments using fish predators instead of invertebrates found that tadpole performance is a plastic trait that can be adjusted to the predator type. What is unclear, however, is whether tadpoles can detect differences in fish diets as they do with invertebrate predators, and if

morphology can be fine tuned to enhance swimming performance (linear and rotational) in relation to the risk level in an environment. In this experiment, I raised anuran larvae with chemical cues from fish fed fish pellets or fish fed conspecific tadpoles and examined the effect of predator cues on anuran larvae morphology, life history traits, and performance. I tested the hypothesis that phenotypic plasticity functions as an anti-predatory response; as such the presence of predator cues should induce advantageous changes in life history, morphology, and performance of tadpoles. Moreover, the magnitude of such responses should be a function of the perceived risk due to the cost of inducing changes. In my case, the perceived risk should vary with the predator's diet. More specifically, I predicted that tadpoles should modify their life history traits, morphology, and performance in ways that will allow them to avoid predation. Moreover, changes that allow for a shorter larval stage and sooner emergence from the water should also be advantageous. The level of risk conveyed through the predator cues should be highest for fish feeding on tadpole conspecifics, followed by those feeding on trout pellets, and the least intense for the fish free treatment. Thus, I should observe a change in hatching, growth, and development rates, earlier metamorphosis, reduction in activity, deeper tail fins, and better performance more so in tadpoles raised with cues from fish feeding on tadpole conspecifics than those raised with cues from fish feeding on pellets or with no fish at all (Figure 1).

## Materials and Methods

### A-Study Species

On 7 May 2011, I collected a subset of each of six Northern Leopard Frog (*Lithobates pipiens)* egg masses from a pond in Old Chelsea, Québec, Canada (45º 31' 30"

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N; 75° 48' 30" W). This was done to obtain a sample representative of the population while avoiding a damaged clutch with individuals of similar genotype. The egg masses were at stages 4-8 (Gosner 1960). They were returned to the lab and placed in 2.5 l Rubbermaid plastic containers with dechlorinated water over a period of 2 hours to avoid water temperature and chemistry shocks. The next day, the eggs were separated and the experiment was initiated. The experimental design was a split clutch design with five eggs coming from each egg mass to make up 30 eggs per replicate. The eggs were pooled in 20 l plastic containers and kept in these containers until the completion of the experiment to reduce transfer stress. I collected an additional egg mass on 1 May 2011 to use as feed. I raised these animals in a 20 l plastic container until the tadpoles reached independent feeding stage (Gosner stage 25, Gosner 1960) after which I transferred them to 70 l plastic containers at a density of 1-2 tadpoles/l. All the Rubbermaid containers were water conditioned for a few months prior to use.

On 26 April 2011, I collected nine 10-15 cm long (~30 g) Brown Bullheads (*Ameiurus nebulosus*) from another pond in the Chelsea area (45° 30' 28"N, 75° 47' 26"W) using minnow traps baited with dog food pellets. I selected Bullheads to be the predator in my experiment because this fish coexisted with our Leopard frog populations, and it has been observed to coexist with Leopard frogs in general (Hecnar and McLoskey 1997). It was also the only species that did not have a palatability issue with the tadpoles and accepted them on a regular basis (Appendix 1).

## B-Animal maintenance:

During the course of the experiment, I maintained the tanks at 18ºC inside environmental chambers (Constant Temperature Control Ltd. Model ER 600; 12L: 12D). I aerated all frog eggs at least once per day by stirring the water surface to eliminate any anoxic layer formed around them. Upon reaching independent feeding, I provided the tadpoles with rabbit pellets ad libitum. I also supplemented the tanks with 50 mg of fish flakes (Nutrafin Max Complete Flake Food) twice per week. I changed 50% of the water twice a week, with wastes and excess food siphoned out and clean water poured in. After every water change, I rotated the tanks on the shelves to eliminate any shelf effect, as pilot trials showed some temperature variation between shelves.

I housed six fish in two groups of three fish each. I maintained them in 50 l opaque fiberglass tanks in the same light and temperature conditions as the tadpoles. I fed one group a 2 g tadpole diet made up of three to five tadpoles. I fed the other group a trout pellets diet of 1.25g pellets (i.e. the dry weight equivalent of the tadpole diet; (Gromko, Mason and Smith-Gill 2005, Smith-Gill and Breven 1979)). I provided this food every other day at 9h00 along with a supplement of 100 mg of fish flake once per week (Nutrafin Max Complete Flake Food). I maintained water quality through submersible carbon filters, active bacterial solutions, and 50% weekly water changes.

## C- Experimental procedure:

I used a three by 3 block design with 3 treatments replicated thrice and each replicate containing 30 frog eggs (Figure 2). The three treatments I implemented were: eggs exposed to cues of fish feeding on tadpole conspecifics (henceforth referred to as Tadpole), eggs

exposed to cues of fish feeding on trout pellets (henceforth referred to as Pellet), and eggs

exposed to cues from a fish free tank (henceforth referred to as Control). The trout pellets are

made up of 40% proteins and 11% crude fat originating from fish, avian, and plant sources.

In spite of starting with equal sample sizes in all tanks, egg and tadpole mortality were

inevitable throughout the project. Thus, the number of tadpoles per tank ranged from 23-29

tadpoles with no variation in mortality occurring among treatments. This variation in tadpole

density is minor and did not have a significant effect on growth in pilot trials. In fact, it has

been stated in previous research that a 4 fold increase in tadpole density is required to have

an effect on tadpole growth (Relyea and Auld 2005). To implement the treatments, I did not

allow contact between the fish and the tadpoles. Instead, I transferred a constant amount of

water (200 ml) daily at 12:00 from the appropriate fish tanks (Tadpole, Pellets, Control) to

the corresponding tadpole tanks. I always removed 200 ml of water from the tadpole tanks

prior to the addition of cues to maintain constant water volumes in the induction tanks. Daily

cues addition is important to make sure the animals were constantly exposed, as chemical

cues have been seen to lose their activity in two to four days (Acquistapace et al. 2005,

Peacor 2006). The mechanism for degradation of cues is believed to be either enzymatic

oxidation (cues lose their effect even in sterile conditions; (Acquistapace et al. 2005)) or

microbial (cues in pond water become inactive faster than those in well water; (Peacor

2006)). To determine the volume of cues for the daily transfers, I looked at the ratio of the

volume of cues transferred to the volume of the induction tanks used in earlier induction

experiments (for examples, see (Gall and Mathis 2010, Peacor 2006, Marquis et al. 2004)). I

selected ratios (therefore concentrations) from experiments that did observe an induced

change in life history or morphology traits of anuran larvae. I then used the average of those

ratios to determine the volume of cues that would probably induce change in my tadpoles. I exposed the animals to daily fish cues for 29 days; from early egg stages (8 May, Gosner stage 4-8) until all tadpoles started hind limb development (6 June, Gosner stage 27-34). During the induction period, I recorded hatching rate and activity per tank. At the end of this induction period, I recorded performance, final mass, final stage, and morphology. Afterwards, I added fish cues five times a week from 13 June to 11 August. I did so to maintain the same rearing conditions and thus growth and development rates for the tadpoles until they reached Gosner stages 44-46 (i.e. complete frogs). Total exposure to cues ranged from 51 - 65 days. As metamorphs emerged (emergence started 22 July), I recorded the date and mass at metamorphosis for each individual frog. All surviving animals were returned to their collection location on 11 August (Figure 3).

### i- Hatching rate

When the eggs approached hatching, I observed them at 3 hour intervals. I recorded the cumulative proportion of eggs hatched as hours from first hatch (i.e., overall first hatch is time 0). Hatching is usually recorded until half of the egg mass has hatched (Laurila, Crochet and Merilä 2001, Laurila et al. 2002). I recorded hatching until at least 50% of the eggs had hatched.

### ii- Activity

I collected data from 18 May (at 9:00. i.e. time 0) to 30 June (64 time points per replicate per treatment). I recorded the proportion of tadpoles swimming or moving their tails during a 30 sec scan of each tank. To reduce disturbance, I observed the tanks through 10 by 10 cm openings in white opaque sheets. This variable was recorded twice daily; 3 hours before the addition of fish cues (9:00) and 3 hours after (15:00).

### iii- Morphology:

I transferred tadpoles to a vertically oriented Petri dish with water, photographed them using a Canon ZR600 digital video camcorder, and staged them. I fixed a 1 mm graph paper on the backside of the dish as a scale for the measurements. Variables recorded are presented in Figure 4. I photographed each tadpole 3 times and extracted the measurements using ImageJ software (http://rsbweb.nih.gov/ij/). The average of the measurements taken was later used in the statistical analyses.

### iv- Development rate

This variable was determined using the formula:

$$Development\ rate = \frac{Final\ Stage}{Number\ of\ induction\ days}$$

(Modified from Teplitsky et al, 2003)

### v- Growth rate:

I weighed tadpoles individually using a Denver Instrument analytical balance at the end of the induction period as the final weight. Accurate measurements of initial mass were not possible without damaging the animals and thus were not done. At each measurement, I picked up the tadpole using a perforated disposable plastic spoon, dabbed the spoon dry on a paper towel through the perforations, and then dropped the tadpole in a plastic Petri dish with water after tarring the scale. The formula used to determine growth rate was:

$$Growth\ rate = e^{\left(\frac{Ln(final\ mass)}{Number\ of\ induction\ days}\right)}$$

(Modified from Teplitsky et al, 2003)

### vi- Linear swimming (Burst and Maximum) Speed:

I assessed tadpoles swimming performance in a clear circular Plexiglass race track (d = 28 cm) filled with 2 cm of water. The track was open from the top and had a grid drawn on the bottom for scale. Previous research has suggested that at low speed, animals swimming near solid walls obtain performance benefits (Webb 1993). In my experiment, all tadpoles swam along the sides of the tank standardizing the effect the walls had on all tadpoles. Therefore, wall effect was not a problem in my performance trials. I fixed a Canon ELURA 60 digital video camcorder (15 fps) below the arena and videotaped the swimming trials from the bottom to eliminate image distortions from ripples. I recorded four bursts and four swims per tadpole and used them to measure burst speed ($V_{burst}$) and maximum velocity ($V_{max}$). I initiated bursts by flicking the tadpoles' tails once and initiated maximum swimming by continuously touching the tadpoles' tails. This was done with a fine paintbrush to standardize the pressure exerted on the tadpoles. Tadpoles were given 2 min to acclimate to the racetrack before the first burst and another 2 min to rest between the burst and $V_{max}$ measurements. I fixed the number of bursts to 4 per tadpole to standardize the level of fatigue of tadpoles for the $V_{max}$ swims. The videos were later analyzed with ImageJ software extracting the distance swam during the first 0.5 sec of the burst and the time taken to cover the first 15 cm of the Vmax. These values were used to calculate $V_{burst}$ and $V_{max.}$ The maximum of the four values obtained for each individual were later used in the statistical analyses. These selected values provided good approximations of the maximum linear performance tadpoles are capable of.

### vii- Rotational swimming (Angular Velocity and Acceleration) speed:

After the linear swimming speed performance trials, I performed turning swimming speed performance trials. Tadpoles were given a 2 min rest between the last $V_{max}$ and the turning speed trials. Trials were conducted in the same performance arena described above. I used a high speed Nikon P500 digital video camera to record the trials at 250 fps. I recorded 4 turns per tadpole by touching the paintbrush tip to the nose tip of the tadpole. This elicited a c-start with the tadpole turning 90° to 180°. The videos were analyzed using Tracker 4.05 (http://www.cabrillo.edu/~dbrown/tracker/). I used the angular velocity and angular accelerations for 90 ° turns calculated by the software at every tracking point to calculate the average of those variables per tadpole per trial. I then used the maximum average calculated per trial per tadpole for the statistical analyses. These selected values provided good approximations of the maximum angular performance tadpoles are capable of.

### viii- Metamorphosis:

I recorded the mass and date of metamorphosis of tadpoles that successfully metamorphosed to complete frogs (Gosner stage 44-46, Gosner 1960). I used the cumulative proportions of the metamorphs to look at rate of metamorphosis.

### ix- Statistical Analyses:

I performed all statistical analyses in S-plus 8.0 and JMP 8.When the normality and homoscadasticity assumptions of the statistical tests were not met, a log transformation usually resolved the issue. Otherwise, a non-parametric test was run on the ranked data. All non-repeated measures models contained replicates nested in treatments. This factor was treated as a random effect. I performed one-way nested univariate analysis of variance (ANOVA) for variables with one response (growth rate, development rate, froglet mass). For

variables changing in time (hatching rate, activity, metamorphosis), I ran a repeated measures one-way ANOVA. For variables with multiple responses (angular velocity and acceleration, burst and maximum speed), I ran multivariate analysis of variance (MANOVA). Finally, for morphology I performed a principal component analysis (PCA) to reduce the number of variables recorded. I found PC1 to be highly correlated with mass ($r = 0.91$) showing that PC1 represents overall size. PC2 and PC3, on the other hand, were not highly correlated with mass (PC2: $r = 0.29$, PC3: $r = 0.12$) indicating that they represent shape. All significant tests were followed with pairwise comparisons to identify the significance of the difference between the treatments. I reported $p$-values that were equal or less than 0.05 as significant, and those that were equal to or between 0.10 and 0.05 as marginally significant (Hackshaw and Kirkwood 2011, Yoccoz 1991). We reported partial $R^2$ values as a measure of effect size.

## Results

### i- Hatching rate

I found a marginally significant effect of treatment on the rate of hatching ($F_{2,6} = 4.03$, $p = 0.077$). I also found a significant effect of time ($F_{2,17} = 157.90$, $p < 0.0001$), but not time by treatment interaction ($F_{5,17} = 1.07$, $p = 0.41$). The pairwise comparisons revealed that eggs in the Pellet treatment hatched at a rate that is marginally significantly higher than those in the Tadpole treatment ($R^2$ partial $= 0.022$, $F_{1,4} = 7.11$, $p = 0.056$) and the Control treatment ($R^2$ partial $= 0.027$, $F_{1,4} = 4.64$, $p = 0.097$). However, there was no difference in hatching rate between the Tadpole and Control treatments ($R^2$ partial $= 0.00308$, $F_{1,4} = 0.09$, $p = 0.77$, Figure 5).

### ii- Activity

I found no significant effect of treatment ($F_{2,6} = 0.31$ $p = 0.74$) and no time by treatment interaction ($F_{10,30} = 1.37$, $p = 0.22$) on the ranked activity data, but a significant effect of time ($F_{5,30} = 6.82$, $p = 0.0002$). We also did not find an effect of time of day on activity ($F_{1,14} = 1.28$, $p = 0.27$). Even though the conservative Greenhouse-Giesser epsilon did not give a significant $p$-value for the time and treatment interaction effect (Greenhouse-Geisser Epsilon = 0.082, $F_{10,30} = 1.37$, $p = 0.22$), the liberal Huynh-Feldt did (Huynh-Feldt = 0.82, $F_{104,312} = 1.405$, $p = 0.013$). As I suspect the actual $p$-value to fall between these obtained values, I believe that the activity response may be changing with time. In addition, most induction experiments limited activity recording to the first 10 to 27 days of exposure, rather than the first 43 days as I did (Hamer, Lane and Mahony 2002, Monello et al. 2006, Schoeppner and Relyea 2009b). Besides, recent work found that elongated exposure to predation cues (27 days vs 10 days) dampens the reduction in activity (Hettyey et al. 2010, Van Buskirk 2001), which may suggest habituation. This is particularly the case when the exposure duration extends from pre-hatching till metamorphosis. Based on this, I decided to examine the change of activity with time, and I noticed a increase in activity after observation point 17 (after 9 days of beginning of exposure) (Figure 6).

I decided to run repeated measures ANOVA on the subset of the activity data between days 1 and 9. I obtained a marginally significant effect of treatment ($F_{2, 6} = 4.89$, $p = 0. 054$). The pairwise comparison showed that the activity in the Pellet treatment was significantly lower than in Control ($R^2$ partial = 0.059, $F_{1,4} = 10.78$, $p = 0.0304$), but there was no difference in activity levels between Pellet and Tadpole treatments ($R^2$ partial = 0.0107, $F_{1,4}$

= 2.79, $p = 0.17$) or between Control and Tadpole treatments ($R^2$ partial = 0.016, $F_{1,4} = 2.2$, $p$ = 0.21, Figure 7).

### iii- Morphology

Because our morphological data were commensurate, I ran a PCA on the covariances of the six recorded morphological variables (Timm, 2002). I retained the first three PCs. PC1 explained 95% of the variation with an eigenvalue of 0.48. I found a significant effect of treatment on PC1 ($R^2_{partial} = 0.072$, $F_{2,227} = 7.09$, $p = 0.023$). The pairwise comparisons showed that the tadpoles in the Control treatment were significantly larger than those in the Pellet ($R^2_{partial} = 0.064$, $F_{1,151} = 14.833$, $p = 0.016$) and Tadpole treatments ($R^2_{partial} = 0.093$, $F_{1,150} = 10.93$, $p = 0.027$). However, there was no difference between those in the Pellet and Tadpole treatments ($R^2_{partial} = 0.0027$, $F_{1,153} = 0.25$, $p = 0.63$, Figure 8). Tail length (TL) and total length (Total L) were the 2 variables that loaded most strongly on PC1 (Table 1).

Because predators are known to induce changes in tadpole tails (Hossie et al. 2010, Van Buskirk and McCollum 2000) and because tail shape affects swimming performance (Van Buskirk and McCollum 2000, Arendt 2010), I examined the effect of treatment on tail shape. First, I found a significant effect of treatment on PC3 ($R^2_{partial} = 0.13$, $F_{2,227} = 22.44$, $p$ = 0.0019), but not on PC2 ($R^2_{partial} = 0.000701$, $F_{2,227} = 0.044$, $p$ = 0.95). Then, I calculated the ratios of TFH and TMH to TL as well as those of TMH, TFH, and TL to Total L. I ran separate MANOVAs for these two models. I found a significant effect of treatment on measurements per TL ($F_{4,452} = 5.12$, $p = 0.0005$). Separate ANOVAs showed a significant effect of treatment on TFH:TL ($R^2_{partial} = 0.056$, $F_{2,227} = 6.71$, $p = 0.027$), but not on TMH:TL ($R^2_{partial} = 0.0044$, $F_{2,227} = 0.41$, $p = 0.67$). The pairwise comparisons showed that

tadpoles in the Tadpole treatment had significantly larger TFH:TL ratios than those in the Control treatment ($R^2_{partial} = 0.075$, $F_{1,150} = 59.97$, $p = 0.007$). However, there was only a marginally significant difference between the Tadpole and the Pellet treatment ($R^2_{partial} = 0.047$, $F_{1,153} = 5.61$, $p = 0.074$) and no difference between the Control and Pellet treatments ($R^2_{partial} = 0.0027$, $F_{1,151} = 0.26$, $p = 0.62$, Figure 9A).

For measurements per Total L, I also obtained a significant effect of treatment ($F_{6,450} = 6.44$, $p < 0.0001$). Separate ANOVAs showed a significant effect of treatment on TFH:Total L ($R^2_{partial} = 0.094$, $F_{2,227} = 16.54$, $p = 0.0034$), but not on TL:Total L ($R^2_{partial} = 0.03$, $F_{2,227} = 2.53$, $p = 0.15$) or TMH:Total L ($R^2_{partial} = 0.01$, $F_{2,227} = 1.04$, $p = 0.4$). The pairwise comparisons for TFH:Total L showed that tadpoles in the Tadpole treatment had ratios higher than those in the Control ($R^2_{partial} = 0.14$, $F_{1,150} = 220.72$, $p = 0.009$) and Pellet treatments ($R^2_{partial} = 0.056$, $F_{1,153} = 10.02$, $p = 0.033$). However, there was no difference between the ratios of the tadpoles in the Control and Pellet treatments ($R^2_{partial} = 0.019$, $F_{1,151} = 3.02$, $p = 0.15$, Figure 9B).

### iv-  Developmental rate

I found a significant effect of treatment on developmental rate ($R^2_{partial} = 0.072$, $F_{2,227} = 8.28$, $p = 0.018$). The pairwise comparisons revealed a significantly higher development rate in the Control treatment than in the Tadpole treatments ($R^2_{partial} = 0.10$, $F_{1,151} = 34.62$, $p = 0.0057$) and a marginally significant difference between the Control and Pellet ($R^2_{partial} = 0.062$, $F_{1,151} = 6.93$, $p = 0.057$) treatments. However, the there was no difference in development rate between the Pellet and Tadpole treatments ($R^2_{partial} = 0.049$, $F_{1,153} = 0.49$, $p = 0.52$, Figure 10).

### v- Growth rate

There was a significant effect of treatment on growth rate ($R^2_{partial}$ = 0.04, $F_{2,227}$ = 7.45, $p$ = 0.0208). The pairwise comparisons showed significantly higher growth rate in the Control treatment than that in the Pellet ($R^2_{partial}$ = 0.037, $F_{1,151}$ = 9.37, $p$ = 0.033) and Tadpole treatments ($R^2_{partial}$ = 0.056, $F_{1,150}$ = 18.49, $p$ = 0.0106). There was no difference in growth rate between the Pellet and Tadpole treatments ($R^2_{partial}$ = 0.00052, $F_{1,153}$ = 0.093, $p$ = 0.77, Figure 11).

### vi-    Linear swimming (Burst and Maximum) Speed

Running a MANOVA, I found a marginally significant effect of treatment on the log transformed burst ($V_{burst}$) and maximum ($V_{max}$) speed ($F_{4,448}$ = 2.32, $p$ = 0.055). I ran one way ANOVAs for the variables separately and found no effect of treatment on $V_{burst}$ ($R^2_{partial}$ = 0.34, $F_{2,225}$ = 1.49, $p$ = 0.29) or on $V_{max}$ ($R^2_{partial}$ = 0.0075, $F_{2,227}$ = 0. 43, $p$ = 0.66).

### vii-    Rotational swimming (Angular Velocity and Acceleration) speed

I found a significant effect of treatment on log transformed angular velocity ($\omega$) and angular acceleration ($\alpha$) ($F_{4,448}$ = 7.8, $p$ < 0.0001). I ran one-way ANOVAs for $\omega$ and $\alpha$ separately. I found a significant effect of treatment on $\omega$ ($R^2_{partial}$ = 0.11, $F_{2,225}$ = 10.07, $p$ = 0.012). Pairwise comparisons showed that tadpoles in the Control treatment had significantly smaller $\omega$ than those in the Pellet ($R^2_{partial}$ = 0.12, $F_{1,150}$ = 13.87, $p$ = 0.021) and the Tadpole treatments ($R^2_{partial}$ = 0.13, $F_{1,149}$ = 27.99, $p$ = 0.0057). However, there was no difference between the Pellet and Tadpole treatments ($R^2_{partial}$ = 0.00021, $F_{1,151}$ = 0.0064, $p$ = 0.94, Figure 12). For $\alpha$, I found a marginally significant effect of treatment ($R^2_{partial}$ = 0.05, $F_{2,225}$ = = 5, $p$ = 0.055). Pairwise comparisons showed that tadpoles in the Control treatment had marginally significantly smaller $\alpha$ than those in the Pellet ($R^2_{partial}$ = 0.046, $F_{1,150}$ = 7.86, $p$ =

0.094 respectively) and the Tadpole ($R^2_{partial}$ = 0.062, $F_{1,149}$= 7.87, $p$ = 0.0505) treatments.
However, there was no difference between the Pellet and Tadpole treatments ($R^2_{partial}$ = 0.000408, $F_{1,151}$ = 0.045, $p$ = 0.83, Figure 13).

To identify if the differences between treatments are a function of shape or size, I examined the effect of treatments, as well as mass, PC2, and PC3 on angular performance. I found a significant effect of PC3 ($R^2_{partial}$ = 0.011, $F_{1,222}$ = 4.502, $p$ = 0.032) and mass ($R^2_{partial}$ = 0.208, $F_{1,222}$ = 82.34, $p$ < 0.0001) and a marginally significant effect of treatment ($R^2_{partial}$ = 0.059, $F_{2,222}$ = 11.73, $p$ = 0.062) on ω. I also found a significant effect of mass ($R^2_{partial}$ = 0.078, $F_{1,222}$ = 21.86, $p$ < 0.0001) on α. This indicates that differences in angular velocity are a function of shape as well as size.

### viii- Metamorphosis
There was no effect of treatment on final weight of froglets ($R^2_{partial}$ = 0.081, $F_{2,22}$= 1.09, $p$ = 0.35). For the metamorphosis rate, I did not find an effect of treatment ($F_{2,6}$ = 2.04, $p$ = 0.209) or a time by treatment interaction ($F_{5,16}$ = 1.03, $p$ = 0.43) on ranked metamorphosis rate, but I did find an effect of time ($F_{2,16}$ = 70.97, $p$ < 0.0001).

## Discussion
The timing of hatching of amphibian eggs has been demonstrated to be an inducible trait (Warkentin 2011). It is believed that egg predators (such as leeches) induce earlier hatching, whereas larval predators (such as fish) induce later hatching (Warkentin 2011). My results show that the eggs in the Pellet treatment tended to hatch earlier than those in the other treatments; however this tendency was not significant. This may suggest that *A. nebulosus* is both an egg and a larval predator. However, this is not a definite conclusion as

results are not always consistent and tend to vary with the specificity of the conditions involved (Laurila et al. 2002, Schalk, Forbes and Weatherhead 2002, Anderson and Brown 2009). In addition, the lack of a starved predator treatment in my experiment renders me unable to make that assumption. It may also suggest that hatching rate is not an inducible defense in *L. pipiens*. Lack of significance of my results may be due to the inaccuracy of the representation of the risk level involved. Alarm signals and digestive kairomones of fish feeding on tadpoles rather than eggs might act as confusing signals to the embryos. It has been suggested that inaccurate information from which animals cannot identify the risk or the prey involved may lead to no or even maladaptive responses (De Witt, Sih and Wilson 1998, Warkentin 2011). Moreover, tactile stimulation along with predator cues in the water have been seen to cause faster hatching than predator cues alone (Smith and Fortune 2009). This is because tactile stimulation is translated to direct risk to the eggs in question that would require instantaneous measures such as hatching earlier (Warkentin 2011).

Previous research that exposed tadpoles to predator cues feeding on a conspecific or closely related prey diet found a decrease in activity in the presence of predators (Schoeppner and Relyea 2009a). A reduction in activity serves crypsis, thus helping tadpoles escape predation. My results reveal no consistent effect of treatment on tadpole activity, with no effect being observed for the whole data set. However, during the first 17 time points, tadpoles in the Pellet treatment had a significant tendency to be less active than those in the Control treatment. It seems that the tadpoles are able to detect the risk of predation and provide the proper response in the first days of exposure, however this response wanes with extended exposure as suggested earlier (Hettyey et al. 2010, Hettyey et al. 2011). These results are compatible with most experiments that used starved predators or predators that

were fed a non conspecifics diet (Lane and Mahony 2002, Orizaola and Braña 2003, Hamer et al. 2002) with some exceptions (Nicieza 2000). They are also compatible with the findings of Dayton and Fitzgerald (2011) who found caged hydrophilid larvae fed on a conspecifics tadpoles diet to have no effect on tadpole activity. They explained that it is too costly for tadpoles growing in ephemeral ponds to exhibit behavioural changes to avoid predation when the main threat they face is pond desiccation and limited resources (Dayton and Fitzgerald 2011). Moreover, earlier studies have suggested that tadpole size can affect their behavioural response to predation (Hettyey et al. 2011). With experimental conditions similar to mine, Hettyey et al. (2011) found larger tadpoles to reduce their activity with predators. On the contrary, smaller tadpoles registered activity levels higher than control treatments. Even though survival and activity are related (Van Buskrik and McCollum 2000), Hettyey et al. (2011) explained that smaller tadpoles may benefit more by enhancing foraging and therefore growth. In my experiment, tadpole feed was provided ad libidum indicating that tadpoles did not need to be active to obtain better feeding opportunities. They could benefit from the crypsis of inactivity without missing out on feeding opportunities. In such conditions where tadpoles are satiated, swimming behaviour is not affected by the presence of predators (Van Buskrik and McCollum 2000). Finally, some researchers suggest that animals investing in morphological changes that enhance predation avoidance do not require and will not elicit major behavioural changes (Abrahams 1995, Chivers, Zhao and Ferrari 2007a)

Examining the effect of treatment on the six morphological variables recorded, I found the tadpoles in the Control treatment to be the largest. Tadpoles in the Tadpole treatment had the deepest tail fins. Those in the Pellet treatment also had deeper tail fins than

those in the Control treatment. It is not uncommon for predator cues to induce smaller bodies as well as a change in tail shape, particularly deeper tail fins (Van Buskirk 2001, Richardson 2006, Hettyey et al. 2010). What is novel in my results is that tadpoles appeared to differentiate between fish predators fed different diets. They detected the higher risk involved when the predatory fish were on a tadpole conspecifics diet vs a pellet diet. As such, they exhibited a graded tail fin depth responses. To the best of my knowledge, only two other experiments have examined the effect of predator diet on tadpole morphology (Schoeppner and Relyea 2009b, Schoeppner and Relyea 2005). Those experiments used dragonfly nymph and limited their analysis to two morphological variables; tail depth and body length. Their results are compatible with my own in that different diets induce graded responses with the predator's conspecifics diet inducing the most marked changes. However, my experiment goes a step further providing some evidence that other predators can induce deeper tails, and that this increase in depth is at the tail fin level.

Predator treatments had significant effects on growth and development rates. Tadpoles in the Control treatments grew the fastest. They also developed faster than those in the Tadpole treatment and marginally faster than those in the Pellet treatment. Earlier experiments using different predators suggested that growth and development rates were not predator specific responses (Gómez and Kehr 2011). My results are compatible with those found in the literature review by Tejedo et al. (2010). Reviewing 11 experiments with dragonfly larvae as predators, they found that tadpoles respond to predation risk by reducing their growth and development rates. Reduction in growth and development rates can be attributed to reduced foraging (Higginson and Ruxton 2010). However, some researchers examining foraging and gut evacuation in induced tadpoles were able to decouple feeding

and development rates showing that induced tadpoles increase gut evacuation and maintain

constant feeding rates thus reducing conversion rates (Steiner 2007). Another explanation

could be that the elevated investment in morphological changes comes at the cost of reduced

growth and development in predator exposed treatments. As all predators will likely induce

differential investment in morphology versus growth, predator exposed tadpoles will show a

general response of reduced growth and development rates rather than a diet or predator

specific response. Experiments using *L. pipiens* to examine predator induced phenotypic

plasticity are scarce. However, reduced growth rate is a common cost of induced

morphological changes in anuran larvae (Van Buskirk and Relyea 1998, LaFiandra and

Babbitt 2004).

Smaller tadpoles with deeper tail fins have been shown to have enhanced swimming

performance (Dayton et al. 2005). Even though fish exposed tadpoles in my experiment did

possess such morphological traits, I did not find an effect of treatment on burst and

maximum speed. My results go against the findings of other experiments that examined the

effect of fish predators on tadpole performance (Teplitsky et al. 2005, Wilson et al. 2005).

These experiments detected enhanced swimming performance in *Rana dalmatina* and *Rana*

*lessonae* tadpoles exposed to fish feeding on tadpole conspecifics. As I mentioned earlier,

tadpoles at different stages can utilize different predator avoidance tactics. Watkins (1996)

discovered that surviving Pacific Tree Frogs (*Pseudacris regilla*) tadpoles at stages 26-36

performed significantly more evasive manoeuvres to escape a pursuing Garter Snake

(*Thamnophis sirtalis*). In addition, Wood Frog tadpoles (*L. sylvatica*) depended on more

evasive manoeuvres to escape predation at stages 26 and 42 (Brown and Taylor 1995). In my

experiment, I examined turning speed and acceleration as an alternative antipredator

behaviour in *L. pipiens*. Fish exposed tadpoles turned faster than those in the Control

treatment. Although marginally significant, fish exposed tadpoles showed the same trend in

terms of angular acceleration. As fish exposed tadpoles are smaller and therefore cannot rely

on linear performance to escape predation, their ability to make faster turns may be

advantageous. A Painted Turtle (*Chrysemys picta*) predator chasing a Rio Grande Leopard

Frog tadpole (*Rana berlandieri*) in a linear pursuit tends to catch up to it in spite of the

latter's faster burst speed (Feder 1983). However, a sudden rapid turn allows the tadpole to

abruptly exit the turtle's trajectory. The turtle would then either lose sight of the tadpole or

not be able to turn fast enough to continue the pursuit, which would result in the tadpole's

escape (Feder 1983). To the best of my knowledge, only one other study examined the effect

of predation on turning performance of tadpoles. This study found that *R. sylvatica* tadpoles

exposed to an *Anax* predator do not have enhanced turning speed or acceleration (Eidietis

2005). It seems that rotational performance is an inducible trait that is specific to the predator

but not to the predator's diet.

       Tadpoles are expected to metamorphose earlier in the presence of predators as a

means to escape aquatic predation threats. Previous experiments using insect predators found

tadpoles to metamorphose faster and at larger size (Barry 2011, Hettyey et al. 2011, Dayton

and Fitzgerald 2011). This phenomenon may be due to the variation of insect predator's

detection rate with prey size, as suggested by Higginson and Ruxton's (2010) theoretical

model. On the contrary, fish predators do not seem to affect metamorphosis rate (Hamer et

al. 2002, Resetarits, Rieger and Binckley 2004, Nicieza 2000). In my experiment, fish

predators neither affected the rates at which tadpoles metamorphosed nor their weight at

metamorphosis. My results are in accordance with those of Relyea (2007). Relyea (2007)

suggests that tadpoles invest in more immediate responses, such as deeper tail fins in my case. Such changes incur costs prohibiting sooner emergence, but allows for better survival during the larval period (Relyea 2007). My results also agree with Orizaola and Braña (2005) who found Palmate Newts (*Triturus helveticus*) to metamorphose earlier and at smaller sizes when exposed to Brown Trout (*Salmo trutta*) predation cues during embryonic stages, but not during larval stages.

This work shows some evidence of the ability of phenotypically plastic animals to fine tune their responses to the level of predation risk. Future research should examine the ability of anuran larvae to fine tune their responses to other environmental factors as well as examine the cumulative effects of multiple stresses on these responses. As most of our knowledge on phenotypic plasticity stems from anuran larvae, more work should be done on other taxa to explore the commonality of the responses between phenotypically plastic animals.

## Conclusion

This thesis provides evidence that *Lithobates pipiens* tadpoles recognize the different diets of *Ameiurus nebulosus*. This recognition induced phenotypic changes that allowed the tadpoles to change their morphological and rotational performance traits. Moreover, tadpoles were able to differentiate between predators diet and induce changes in their tail morphology of different magnitudes based on the detected level of risk. Even though performance did vary with treatments, it did not show this graded response. Moreover, tadpoles seem to lack the ability to modulate their hatching rates, metamorphosis rates, activity, and linear performance in the presence of predators. This work opens new realms for future

investigations aiming to assess how well phenotypically plastic animals can fine tune their various responses to the level of risk of the predators, along with the efficiency of these responses in avoiding predation.

**Table 1**. The factor loadings or eigen vectors of the six recorded morphological variables on the first three principle components. BH: Body height, BL: Body length, TL: tail length, TFH: Tail fin height, TMH: Tail muscle height, Total L: Total length

| Variables | PC1 | PC2 | PC3 |
|-----------|-------|-------|-------|
| BH | 0.503 | -0.64 | 0.41 |
| BL | 0.26 | 0.67 | 0.064 |
| TL | 0.14 | 0.34 | 0.68 |
| TFH | 0.062 | 0.047 | 0.064 |
| TMH | 0.075 | 0.074 | 0.37 |
| Total L | 0.801 | 0.101 | -0.45 |

**Figure 1**. Summary of all the response variables with their corresponding predicted and observed results. ( - - - ) or ( ■ ) represents the Control treatment, ( ⸱⸱⸱⸱⸱⸱ ) or ( ■ ) represents the Pellet treatment, and (− ⸳⸳ −) or ( ▢ ) represents the Tadpole treatment. TFH: Tail fin height, TL: Tail length, Total L: Total length.
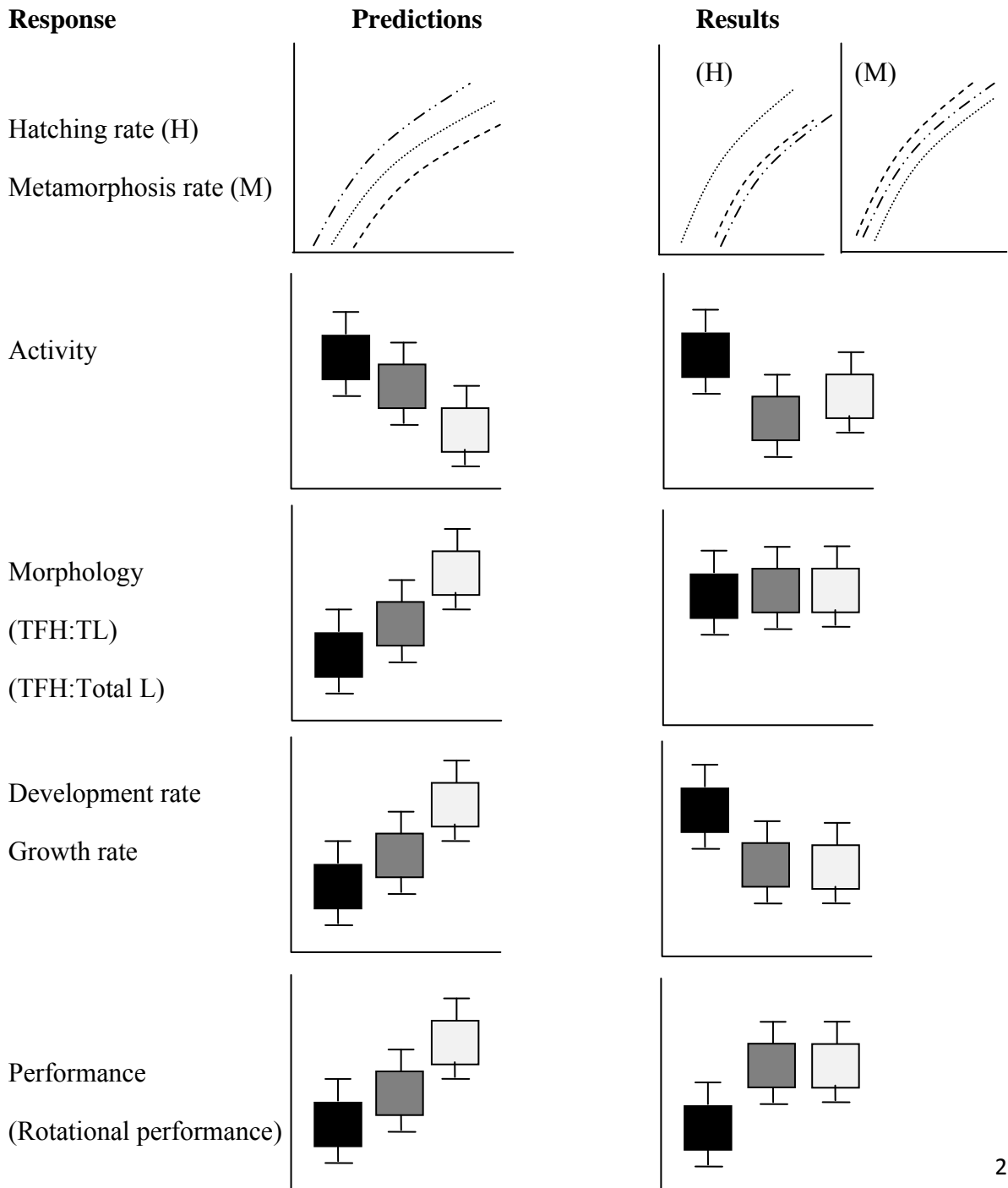
**Figure 2**. Schematic representation of the experimental procedures followed in the experiment to determine whether predator diet induce adaptive changes in the phenotypes of tadpoles.
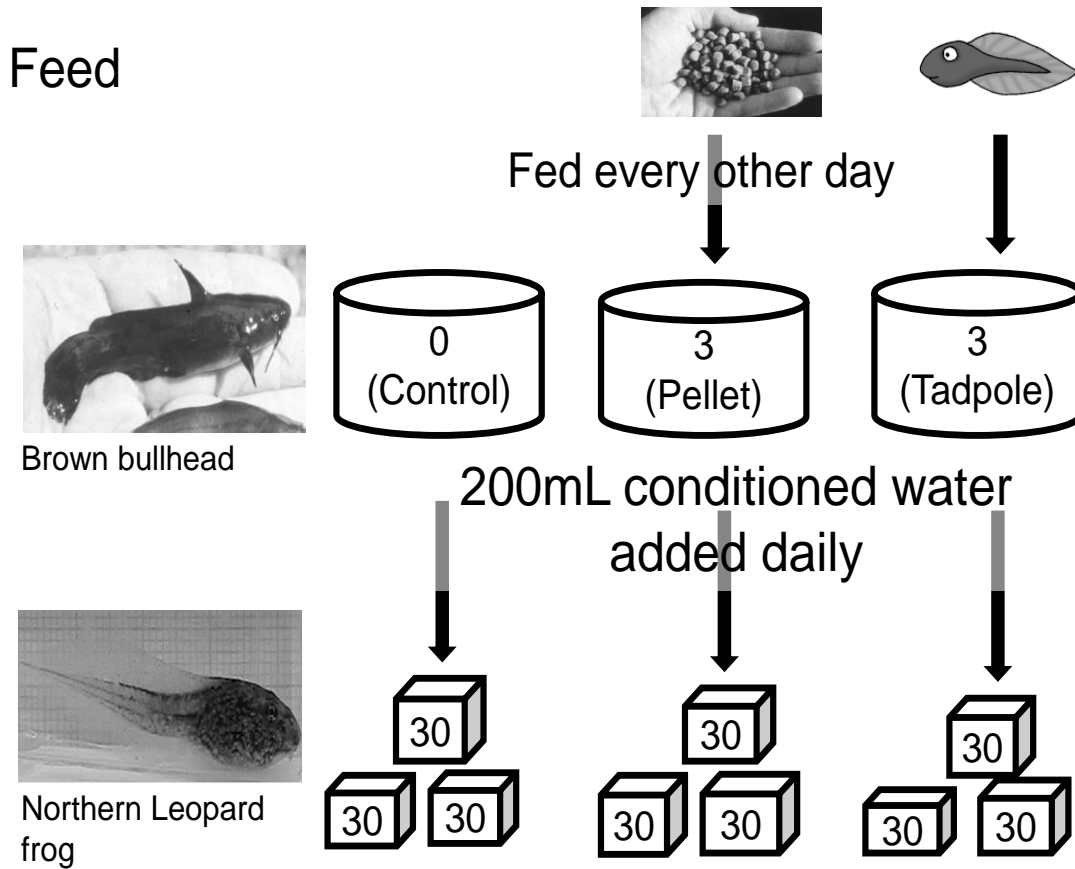
**Figure 3**. Summary of the major events of the experiment arranged on a time line indicating the day on which they occurred/were initiated.
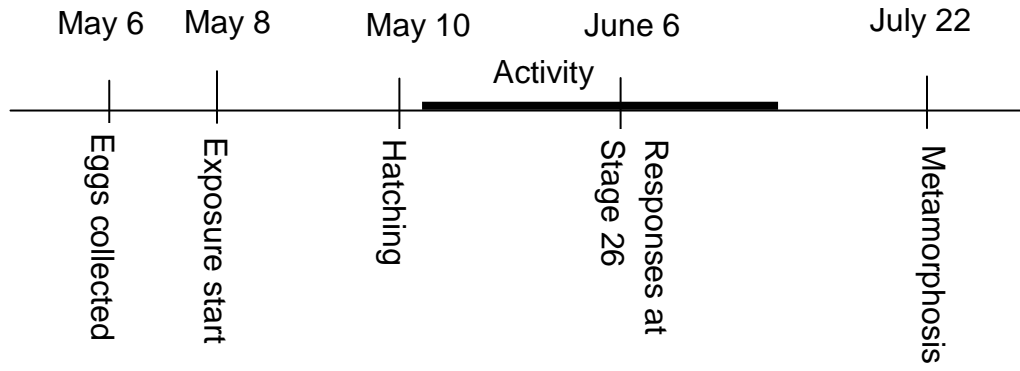
**Figure 4**. The six morphological variables measured for each tadpole in the three experimental treatments are:
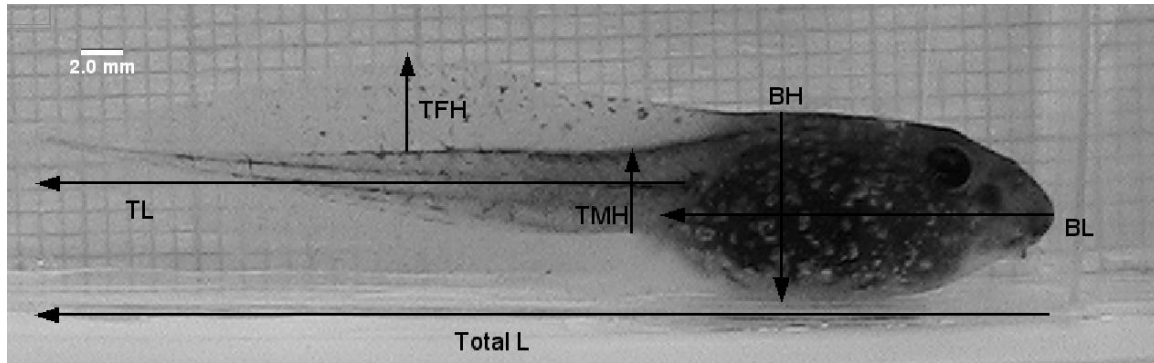


**Body Height** (**BH**): the height of the head from the intersection of the tail fin with the head to the ventral side.

**Body Length** (**BL**): the maximum length of the head excluding the anus.

**Tail Length** (**TL**): the length of the tail from tail body intersection till the tail tip.

**Tail Fin height** (**TFH**): the height of the tail fin at the midpoint of the tail.

**Tail Muscle height** (**TMH**): the height of the tail muscle measured at the ventral dent of the tail muscle.

**Total Length** (**Total L**): total length of the tadpole from the snout tip till the tail tip.

**Figure 5**. The rate of hatching of *Lithobates pipiens* eggs. The graph displays the cumulative proportion of eggs hatched per treatment. This proportion is averaged across replicates. It is presented as a function of time delay (in hours) from the first egg hatched. (SE: Control = 0.04; Pellet = 0.039; Tadpole = 0.04).
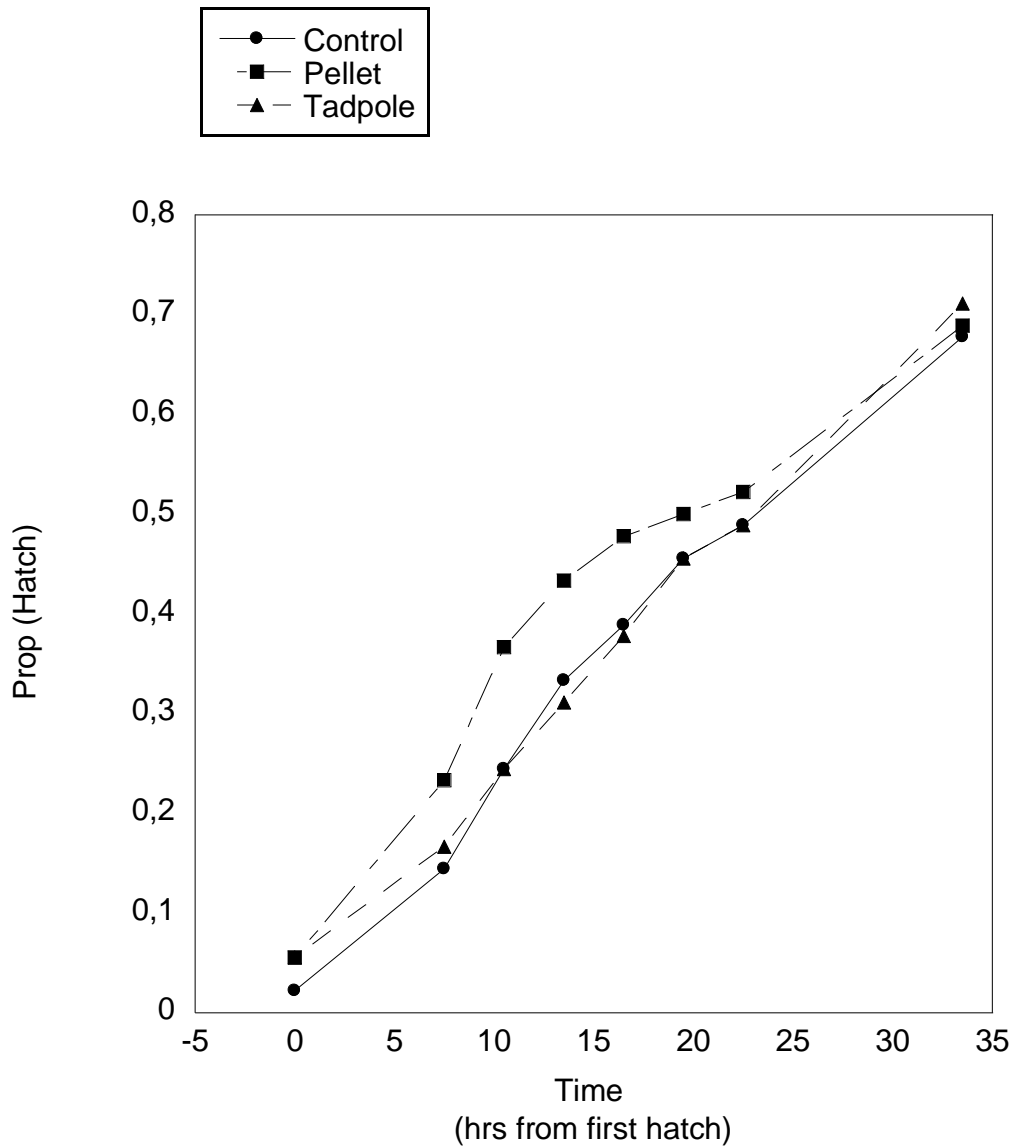
**Figure 6**. Activity levels of *Lithobates pipiens* tadpoles. The graph shows the mean proportion (across replicates) of moving tadpoles per treatment as a function of observation times (two observations per day). (SE: Control = 0.0091; Pellet = 0.0097; Tadpole = 0.01).
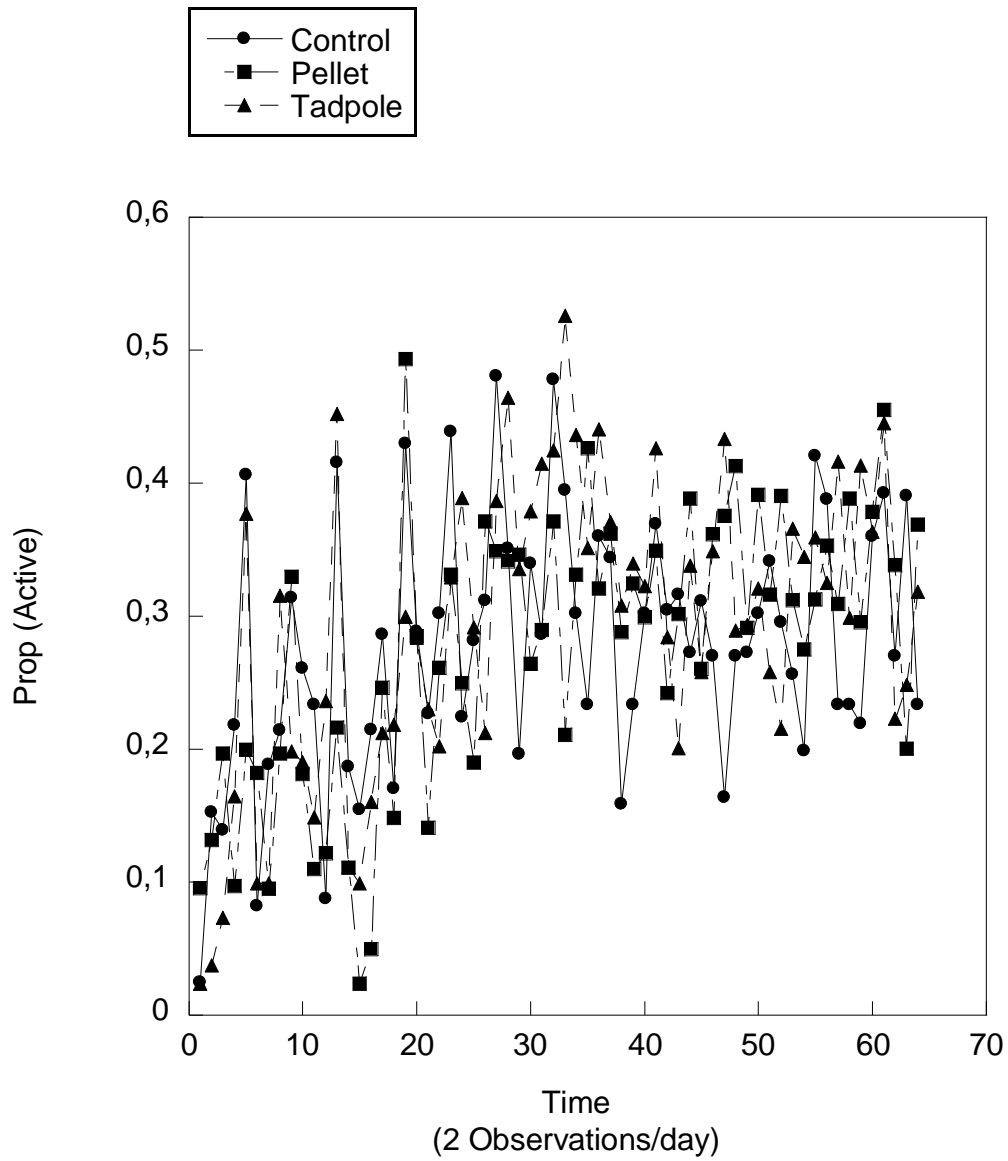
**Figure 7**. Box plot (means, 25th & 75th percentiles and data range) of the mean activity levels of *Lithobates pipiens* tadpoles per treatment recorded during the first 9 days of the experiment. *p*-values are displayed above the lines connecting the compared treatments. (SE: Control = 0.018; Pellet = 0.014; Tadpole = 0.018).

**Figure 8**. Box plot (means, 25th & 75th percentiles and data range) showing the first principal component of the principal component analysis run on the six morphological variables measured for *Lithobates pipiens* tadpoles as a function of treatments. *p*-values are displayed above the lines connecting the compared treatments. (SE: Control = 0.079; Pellet = 0.075; Tadpole = 0.07).

**Figure 9**. Box plots (means, 25th & 75th percentiles and data range) of the ratios of *Lithobates pipiens* tadpole tail measurements per treatments (tail shape). The ratios are those of (A) tail fin height (TFH) to tail length (TL) (SE: Control = 0.0016; Pellet = 0.0018; Tadpole = 0.0017) and (B) tail fin height (TFH) to total length (Total L). (SE: Control = 0.00089; Pellet = 0.001; Tadpole = 0.00097). *p*-values are displayed above the lines connecting the compared treatments.
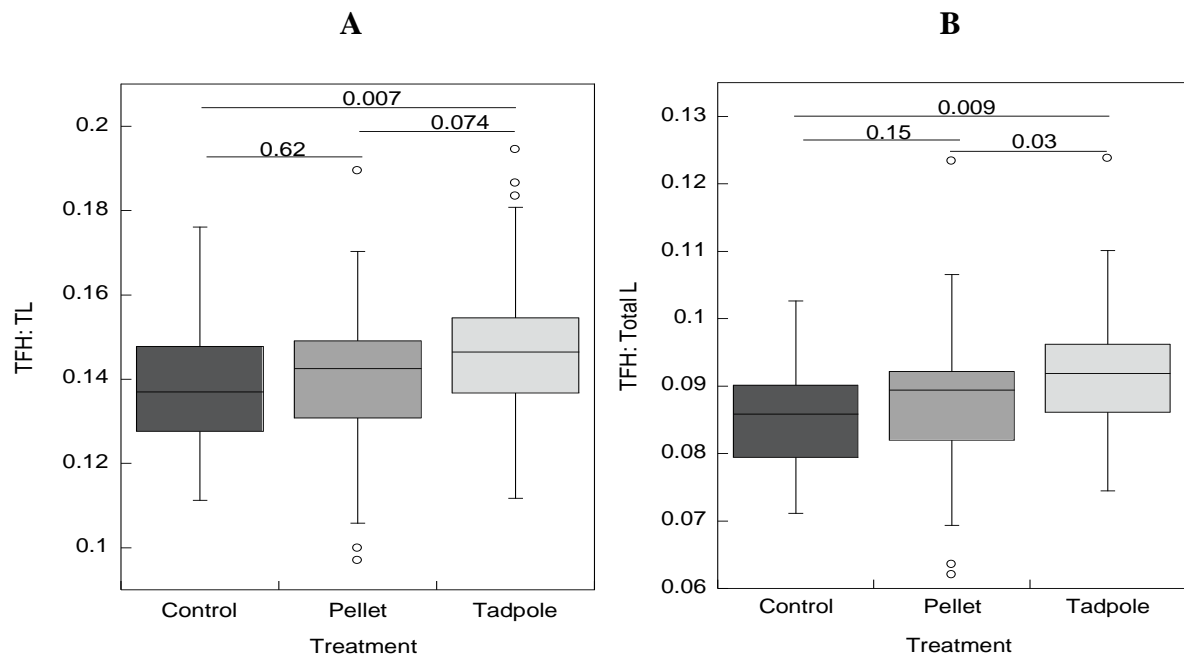
**Figure 10**. Box plot (means, 25$^{th}$ & 75$^{th}$ percentiles and data range) of the development rate

of *Lithobates pipiens* tadpoles per treatment. Development rate is the ratio of tadpole stage to

days of induction. *p*-values are displayed above the lines connecting the compared

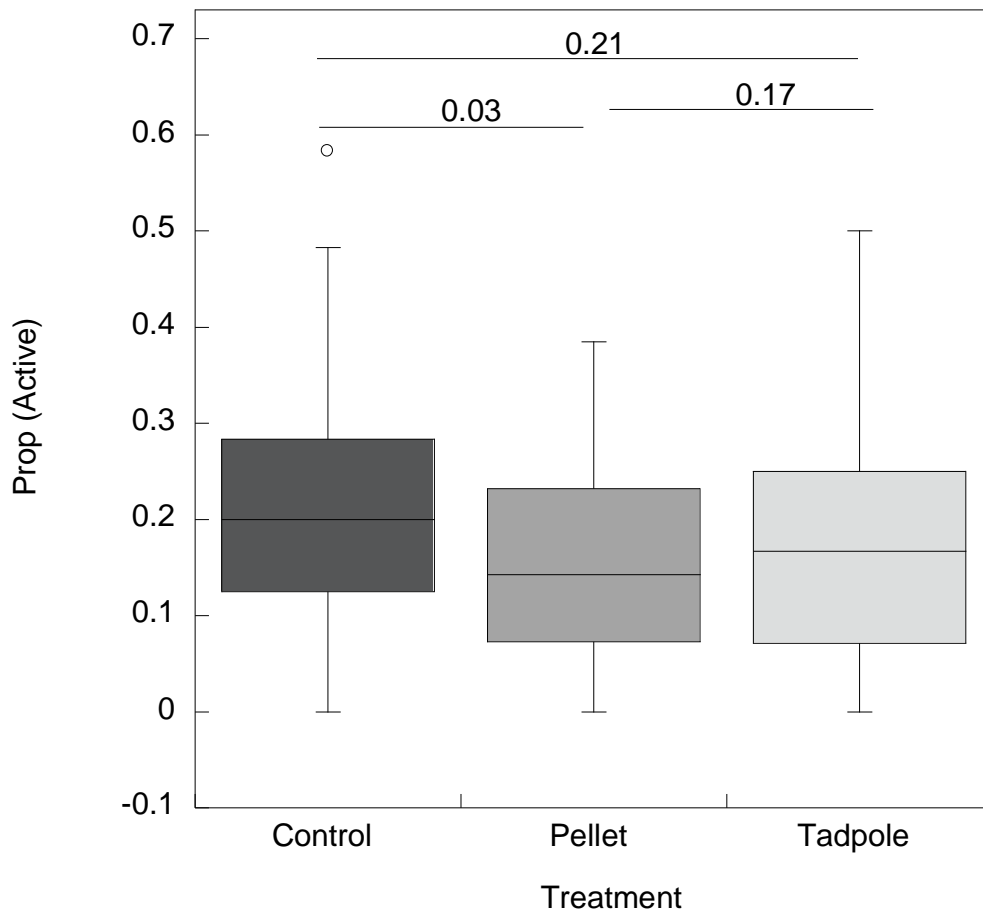treatments. (SE: Control = 0.0064; Pellet = 0.0072; Tadpole = 0.007).
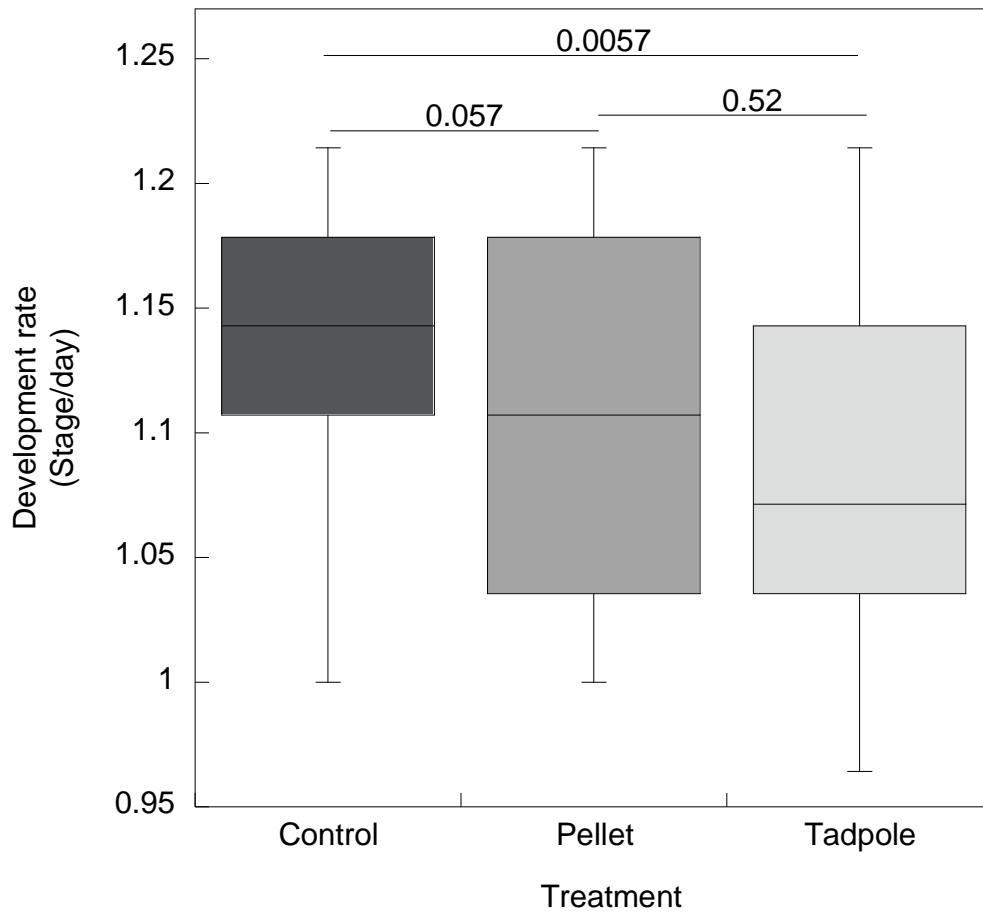
**Figure 11**. Box plot (means, 25th & 75th percentiles and data range) of the growth rate of

*Lithobates pipiens* tadpoles in each treatment. Growth rate is the ratio of final tadpole weight

to days of induction. *p*-values are displayed above the lines connecting the compared

treatments. (SE: Control = 0.0021; Pellet = 0.0023; Tadpole = 0.0019).
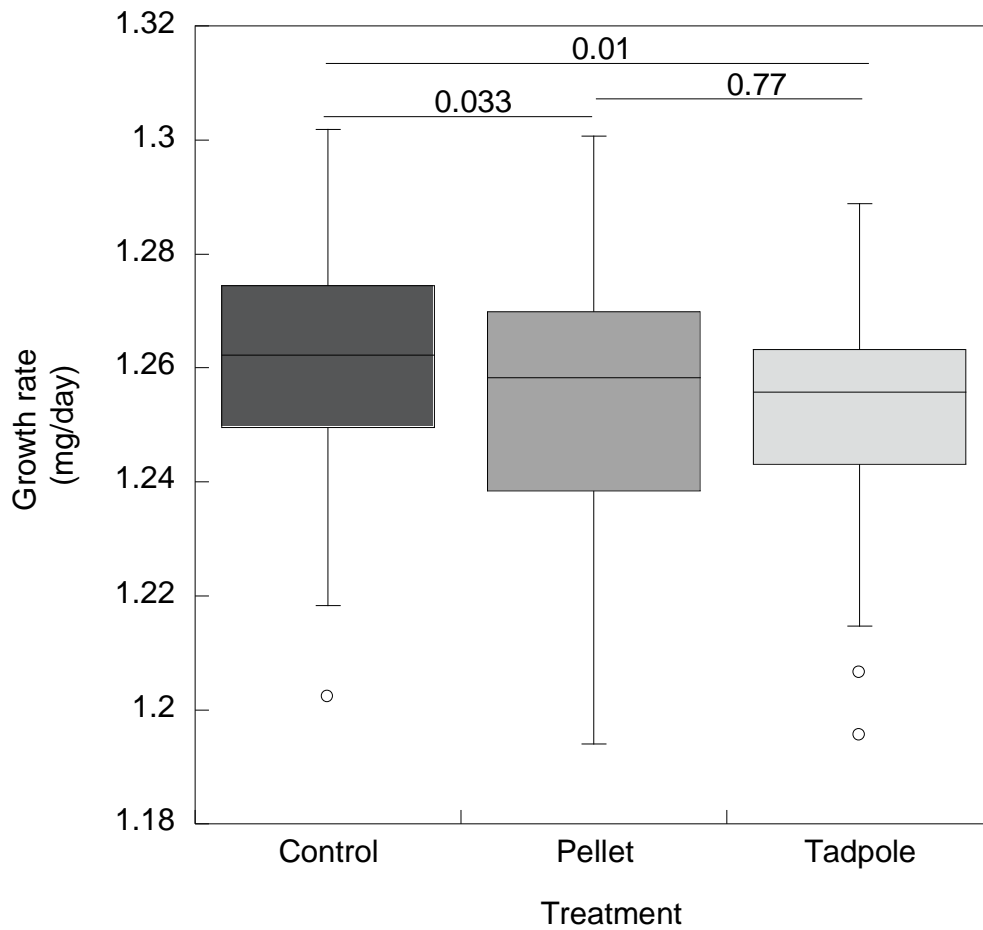
**Figure 12**. Box plot (means, 25th & 75th percentiles and data range) of the mean angular

velocity of *Lithobates pipiens* tadpoles per treatment plotted on a log scale. The mean is of

all angular velocities recorded for each video frame per tadpole while performing a 90° turn.

*p*-values are displayed above the lines connecting the compared treatments. (SE: Control =
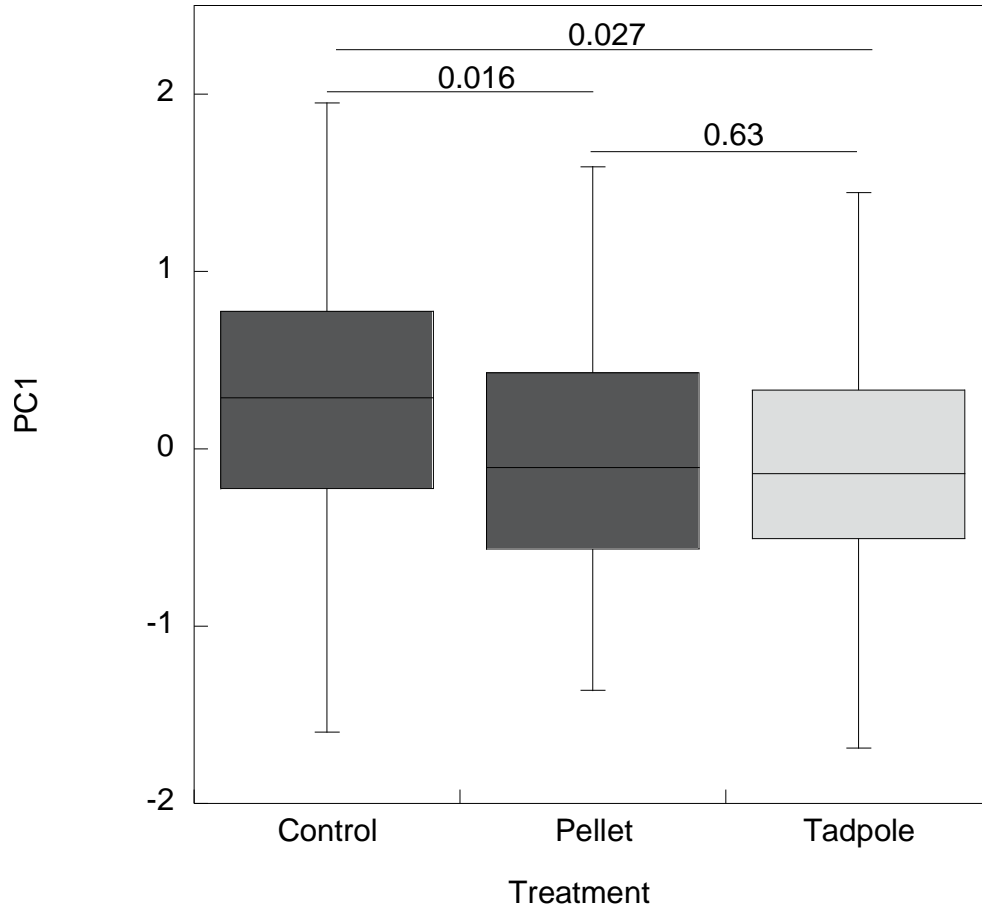
0.76; Pellet = 0.8; Tadpole = 0.83).

**Figure 13**. Box plot (means, 25$^{th}$ & 75$^{th}$ percentiles and data range) of the mean angular acceleration of *Lithobates pipiens* tadpoles per treatment plotted on a log scale. The mean is of all angular accelerations recorded for each video frame per tadpole while performing a 90° turn. *p*-values are displayed above the lines connecting the compared treatments. (SE: Control = 101.90; Pellet = 99.87; Tadpole = 78.98).
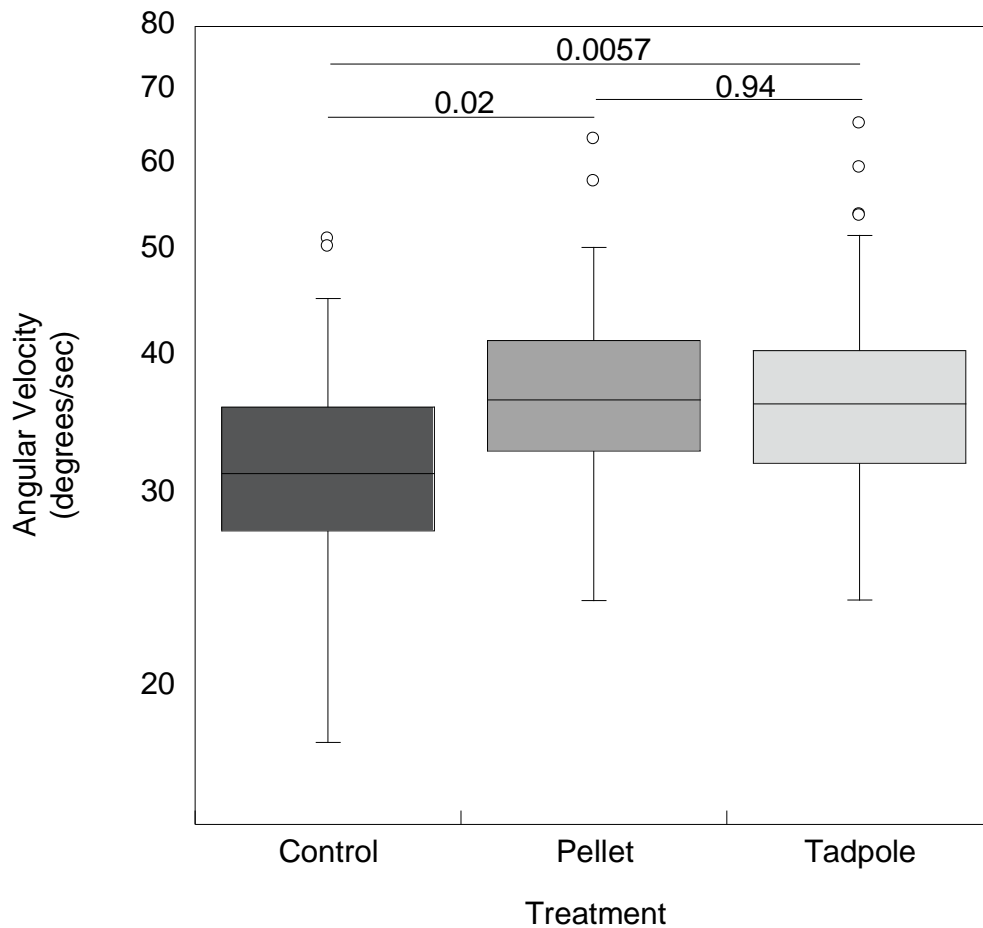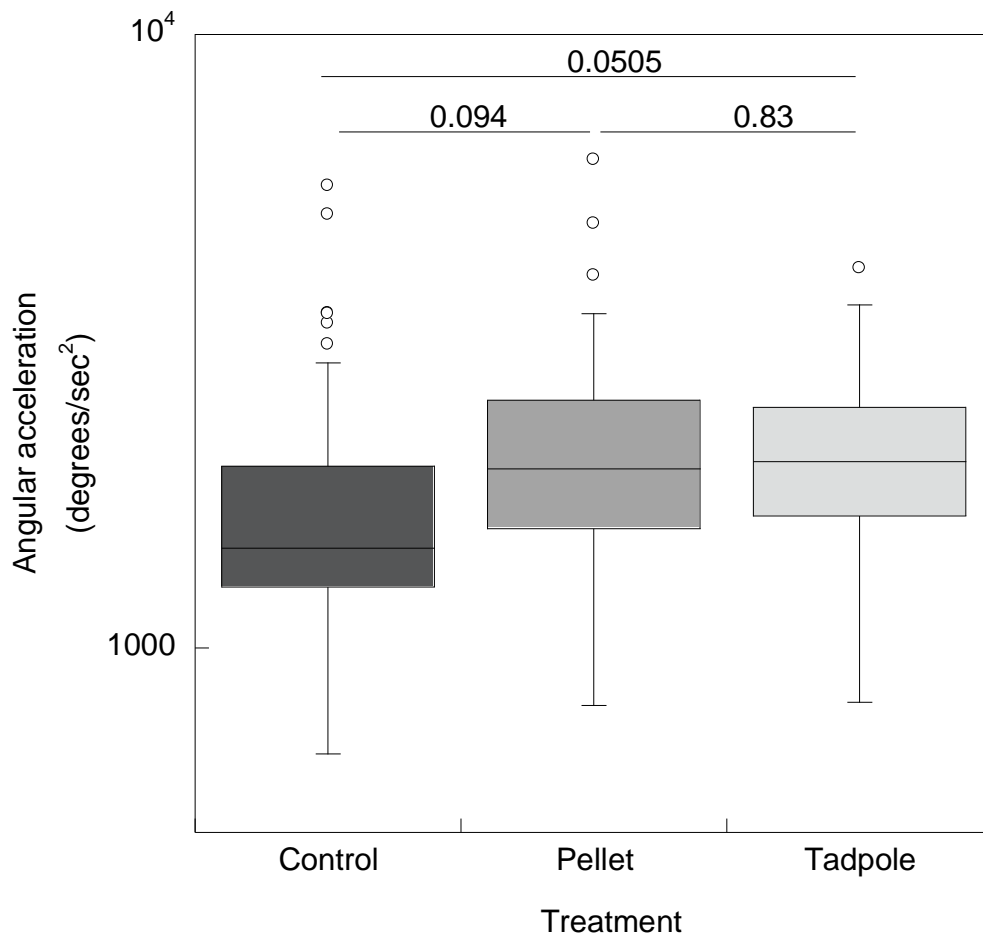
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# Appendix I

El Balaa R & Blouin-Demers G. 2011. Unpalatability of northern leopard frog *Lithobates pipiens* Schreber, 1782 tadpoles. Herpetology Notes 4: 159.

Frogs breeding in ponds with fish tend to be unpalatable to them (Gunzburger and Travis 2005). Such characteristic has been mostly seen in Bufo species and a few Rana species such as *Rana arvalis*, *R. Catesbeiana*, and *R. Clamitans* (Gunzburger and Travis 2005). *R. lithobates (pipiens)* usually breed in semi-permanent fishless ponds and the juveniles move to permanent ponds that contain predatory fish like brown bullhead (*Ameiurus nebulosus*), bluegill (*Lepomis macrochirus*) pumpkinseed (*Lepomis gibbosus*), black crappie (*Pomoxis nigromaculatus*), and yellow perch (*Perca flavescens*) in North America (Germaine and Hays 2009, Hecnar and McLoskey 1997, Collins and Wilbur 1979). To the best of my knowledge, distastefulness has not been suggested for *R. pipiens*. In fact, a thorough literature review by Gunzburger & Travis (2005) on 603 predator-prey trials found 1 such incident where *R.pipiens* were not palatable to Bluegill sunfish (but see (Parris, Reese and Storfer 2006). Trying to select a fish predator for my experiments, I found that the *R. Pipiens* tadpoles I used were not accepted by five different fish species, namely bluegill, black crappie, pumpkinseed, yellow perch, and creekchub minnow. Juvenile fish would consume a tadpole once and then reject other tadpoles given. Adult fish would orally capture the tadpole and then release it immediately without swallowing it. However, Brown bullhead was present in the same habitat as the tadpoles and accepted them on regular basis. Brown bullhead's home range overlaps with that of *Rana pipiens* in North America.