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The Effects of Leaf Litter On Predation Cue Response in Gray Tree Frog Tadpoles, Hyla Versicolor

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THE EFFECTS OF LEAF LITTER ON PREDATION CUE RESPONSE IN GRAY
TREEFROG TADPOLES, *HYLA VERSICOLOR.*

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Acknowledgements

Abstract

Plant litter is an important resource for consumers, particularly in freshwater environments where it influences the development processes of larval amphibians. Leaf litter alters the physiochemical environment by lowering dissolved oxygen while increasing nitrogen, tannin, and phenolic acid levels. Many anuran species show plastic responses to the threat of predation. This is communicated through a kairomone when a tadpole is consumed, allowing surviving tadpoles to alter their behavior and morphology to evade predators. The purpose of this study was to analyze the interactive effects of leaf litter and predation on gray treefrog tadpoles (*Hyla versicolor*). The chemical output of the leaf litter was manipulated by soaking a subset of each litter type to remove leachates. Larvae were reared in outdoor mesocosms with one of the following leaf litter types: un-soaked red maple, soaked red maple, un-soaked pin oak, soaked pin oak, or no leaf litter. Half of the tanks contained caged dragonfly larvae (*Plathemis lydia)* predators. We measured elements of water quality, tadpole growth, tail morphology, froglet size, and time to metamorphose. Maple leaf litter reduced water clarity and introduced more nitrogen than oak leaves. Predation decreased growth rate in maple tanks, and increased growth rate in oak tanks. Both maple and oak elicited bigger tails under predation, however larvae in oak leaf litter showed the greatest shift in tail morphology by developing deeper tails under predation. These results suggest the physiochemical properties of maple and oak leaf litter elicit different responses from predation threats. This study has important implications of the physiochemical relationship between leaf litter and predation cues, and the combined effect on the growth and development of *Hyla versicolor* tadpoles.

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Introduction

Plant litter is an important resource for consumers, particularly in freshwater environments where it can influence the growth and development of larval amphibians (Cohen *et al*. 2014). Leaf litter and other senescent vegetation provide biofilms and microbes that serve as a food source and provide a source of camouflage and shelter to tadpoles (Rubbo *et al.* 2008). Increased carbon, nitrogen, and phosphorus ratios potentially influence tadpole growth by altering the quantity and quality of food available. Higher nitrogen levels in particular are associated with an increased growth rate in anuran tadpoles (Cohen *et al*. 2014).

Although leaf litter is a vital ecosystem element, high concentrations of leaf litter leachates alter the physiochemical environment of freshwater ecosystems, and often have negative effects on amphibians. High leaf litter inputs elevate dissolved organic carbon (DOC) levels, increase the leaching of organic compounds creating an acidic environment, and increase phenolic acid content—all of which have been associated with low tadpole survivorship (Rubbo and Kiesecker 2004). The degree to which leaf litter alters the physicochemical environment is dependent on the litter input species. A nutrient-rich litter species (e.g., maple [*Acer spp*.]), decays at a faster rate than a species with a high lignin content (e.g., oak [*Quercus spp*.]). The fast decomposition rates in maple species can lead to high amounts of phenolic acids, lower levels of dissolved oxygen (DO), and high tannin concentrations (Stoler *et al.* 2017). Tannins reduce the quantity of bioavailable proteins for consumers, damage fish gill filaments, and cause lower oxygen intake (Earl *et al*. 2012). Recalcitrant oak litter species, on the other hand, are associated with a high level of phytoplankton and periphyton and allow for a slow release of nutrients providing grazing tadpoles a food source of lignin and cellulose over an extended period of time leading to higher tadpole performance (Stoler *et al.* 2017).

Stoler and Relyea (2013) suggest a lowered pH from the phenolic acids in leaf litter may impact predator-prey interactions by interfering with the chemical cue emitted by tadpoles under predation. In many anuran species, larvae show plastic responses to the threat of predation and communicate through a chemical known as a kairomone. This cue is vital in triggering an ecology of fear effect which induces responses in other tadpoles, allowing them to cope with the threat of predation by reducing movement or hiding (Dodson *et al.* 1994). These effects on activity can become compromised when tadpoles are reared in poor environments, suggesting there is a tradeoff in predator avoidance and need for risky foraging behavior (Kurali *et al.* 2017). In many larval anuran species, predation cues also induce morphological differences, often in the form of a deeper, more brittle tail that allows for greater burst-speed to escape from sit-and-wait predators (Dayton *et al*. 2005). However, this often results in a slower growth rate (Relyea and Hoverman 2003). Similar effects have been observed in *Hyla versicolor* tadpoles. Additionally, those exposed to heavy predation under optimal environmental conditions may produce red colored tails (Neptune and Bouchard 2017).

The goal of this study was to analyze the effects pin oak (*Quercus palutris*) and red maple (*Acer rubrum*) leaf litter have on water quality (dissolved oxygen, clarity, and nitrate/nitrite content), and determine whether or not these leaf litter species interfere with the perception of predation cues emitted by gray treefrog tadpoles. Previous studies have not been able to pinpoint the cause of variation in growth in tadpoles under predation with different litter inputs. Although there is a largely accepted understanding of what leaf litter does to water quality and tadpole growth (Earl *et al*. 2012, Stoler *et al.* 2017, Rubbo and Kiesecker 2004), there are few studies that elucidate the effect of leaf litter on perception of predation cues. Effective predation has been linked to the development of deeper tails with a reddish hue

(Neptune and Bouchard 2017). If leaf litter cues interfere with a tadpole's ability to sense

predation cues, tadpoles reared with a predator in leaf litter will likely have similar

morphological traits as those reared without a predator. We also want to analyze these effects on

tadpole growth and the rate of metamorphose. This study has the potential to provide insights

into the biochemical interactions that affect tadpole growth, development, and communication.

Materials and Methods

Red maple (*Acer rubrum*) leaf litter was collected from a maple forest in the Cleveland area, and pin oak (*Quercus palutris*) litter was collected from a nearby park near Alum creek (40°07'27"N 82°56'20"W). We collected both leaf litter species in the spring to mimic the environment to which summer breeding amphibians would be exposed (Stoler and Relyea 2013). We separately soaked a subset of both maple and oak leaf litter in tap water for 10 days, changing the water daily until there was no longer visible discoloration from leachates. This leaf litter later served as a control due to the reduced leachate quantity that will be released into the water of experimental tanks. Doing this allowed us to manipulate the chemical environment while maintaining the physical aspect of leaf litter that would be available to prey species as shelter in a natural environment.

On 14 June 2019, ten days prior to the start of the experiment, we filled forty 410-L mesocosms with tap water. We developed 10 experimental treatments and randomly assigned treatments to each mesocosm, with 4 replicates per treatment (Figure 1). Each tank contained 130 g of the following litter types: un-soaked red maple, soaked red maple, un-soaked pin oak, or soaked pin oak. We included 8 no-leaf litter tanks to serve as a control for water quality analyses. Half of the tanks contained caged predators and half contained an empty predator cage. Dragonfly larvae (*Plathemis lydia*) predators were collected from a local pond (40°10'31.9"N - 82°51'52.4"W) by sweeping aquatic vegetation with strainers. For each tank assigned to a predator, a single dragonfly larva was placed in a completely submerged, suspended cage. These consisted of 1-L plastic cups equipped with a stick for perching and mesh screening over the top secured with a rubber band (Figure 2). The mesh screening allowed the transfer of kairomones into the tank.

Water chemistry data collection took place prior to introducing tadpoles into the tanks and after the leaf litter was allowed to sit in the outdoor mesocosms for at least one week. Water clarity was determined by using a Hach Dr2400 portable field spectrometer at 470 nm wavelength. We measured dissolved oxygen of each tank using the Winkler Method (Theriault 1925) titrations at the field site. We sent water samples from three non-predator mesocosms of each leaf litter type to Advanced Analytics Laboratories, Inc. in Columbus, Ohio to determine nitrate/nitrite levels.

Hyla versicolor mating pairs were collected in late evening on 16 June 2019 from the same pond as the dragonfly larvae and placed in covered 20-L containers with approximately 6 cm of pond water. We left the pairs overnight to lay their eggs. Adults were released into the vegetation around the pond the following morning and eggs were collected from all 9 pairs. We brought the egg clutches back to the lab to be hatched and reared until Gosner stage 25 when the external gills were absorbed (Gosner 1960). On 24 June 2019, we combined larvae from all clutches into a 200-L container to ensure random assortment and then counted them into groups of 20 in individually labeled cups. We took pictures of each cup with a ruler to ensure every tank had larvae with similar initial sizes. These photos were later analyzed using ImageJ (National Institute of Health; http://rsbweb.nih.gov/ij) image analysis software.

 The tadpoles were transported to Austin E. Knowlton Center for Equine Science (40°08'25.4"N 82°53'59.3"W) and placed into the outdoor mesocosms. We added 0.25 g sera micron powdered algae every three days to encourage the growth of biofilms and microbes in the tanks. Predators were fed 3-5 randomly selected *H. versicolor* hatchlings daily to allow ample kairomone diffusion into the mesocosm. This quantity of predator hatchling consumption is

sufficient to induce morphological changes due to predation in *H.versicolor* larvae (Shaffery and Relyea 2016).

We measured tadpole growth on 1 July 2019, one week after placing them in experimental mesocosms. All tadpoles in each tank were dipnetted out and placed into a 2-L, white tub with approximately 2 cm of water on the bottom to reduce movement. We placed a ruler in the tub and took dorsal view pictures of each tank with a digital camera before the tadpoles were returned to their respective tanks. Images were analyzed with ImageJ (National Institute of Health; http://rsbweb.nih.gov/ij) image analysis software to determine growth difference in body, tail, and total length.

In order to perform analysis on tail and body shape, we allowed the tadpoles to grow in their respective tanks until their head-body size reached approximately 1.5 cm in length. Due to differences in growth rate, we retrieved subsamples of 6 tadpoles on different days to size-match across treatments. On 5 July 2019, we sampled both no-predator and predator oak treatments, along with no-predator maple. On 6 July, we sampled the predator maple tanks and the no-leaf litter tanks. Tadpoles reared in soaked litter treatments were allowed to grow slightly longer in order to increase the available sample size. All soaked maple tanks were sampled on 9 July 2019, and all soaked oak tanks were sampled the following day.

Tadpoles from each tank were then over-anesthetized with MS222, blotted dry, and weighed. We took lateral photographs of each tadpole and 4 of the 6 were placed in 10% formalin for preservation. The 2 remaining tadpoles from each tank were placed in the oven at 65° C and dried until the weight remained the same for two consecutive days. These tadpoles were set aside to be analyzed for organic content at a later date. All photographs of these

subsamples were analyzed with ImageJ software to determine body length, tail length, tail muscle depth and area, and tail depth and area (Figure 3).

Tadpoles remaining in the mesocosms were allowed to metamorphose into froglets. We collected the froglets from tanks when the front legs emerged and brought them back to the lab. Here, they were kept in .25 L cups with 3 cm of water to allow the tail to completely absorb. The date of metamorphosis and snout to vent length (SVL) was recorded on the day the tail completely absorbed. We released froglets into the brush surrounding the original pond in late evening.

We analyzed water chemistry data using ANOVA, then used a pairwise comparison on SPSS statistical software to distinguish the differences between treatments. We did not look for effects of predation and included no-leaf litter tanks in water chemistry data. For tadpole morphological data, we used R to produce a linear mixed effects model to determine statistical significance. Head-body length was used as a covariate for measures of tail size and area. We set the tank as a random variable, and leaf litter and predation as fixed effects. We compared differences in tail length, tail depth, tail muscle depth, tail muscle area, and tail area between un-soaked maple and un-soaked oak with and without predator. We separately compared the same tail elements between soaked maple and soaked oak, with and without a predator. These analyses were done separately from the un-soaked treatments due to limited resources and competition between tadpoles in soaked leaf litter tanks. To analyze growth rate, we compared the effects of predation and leaf litter type on the total tadpole length of each leaf litter treatment with and without a predator.

Experimental Treatments

| Maple | Oak | Soaked Maple | Soaked Oak | No Leaf Litter |

No Predator x 4 · Predator x 4 (Maple)

No Predator x 4 · Predator x 4 (Oak)

No Predator x 4 · Predator x 4 (Soaked Maple)

No Predator x 4 · Predator x 4 (Soaked Oak)

No Predator x 4 · Predator x 4 (No Leaf Litter)

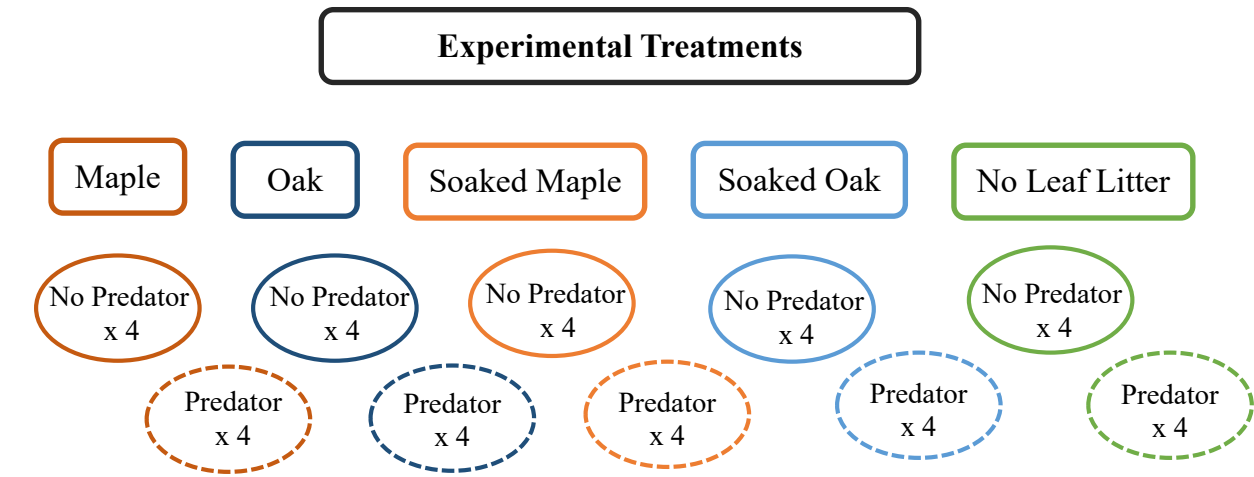Figure 1. Experimental treatments of each mesocosm, along with a photo of mesocosms set up at Austin E. Knowltin Equine Center.
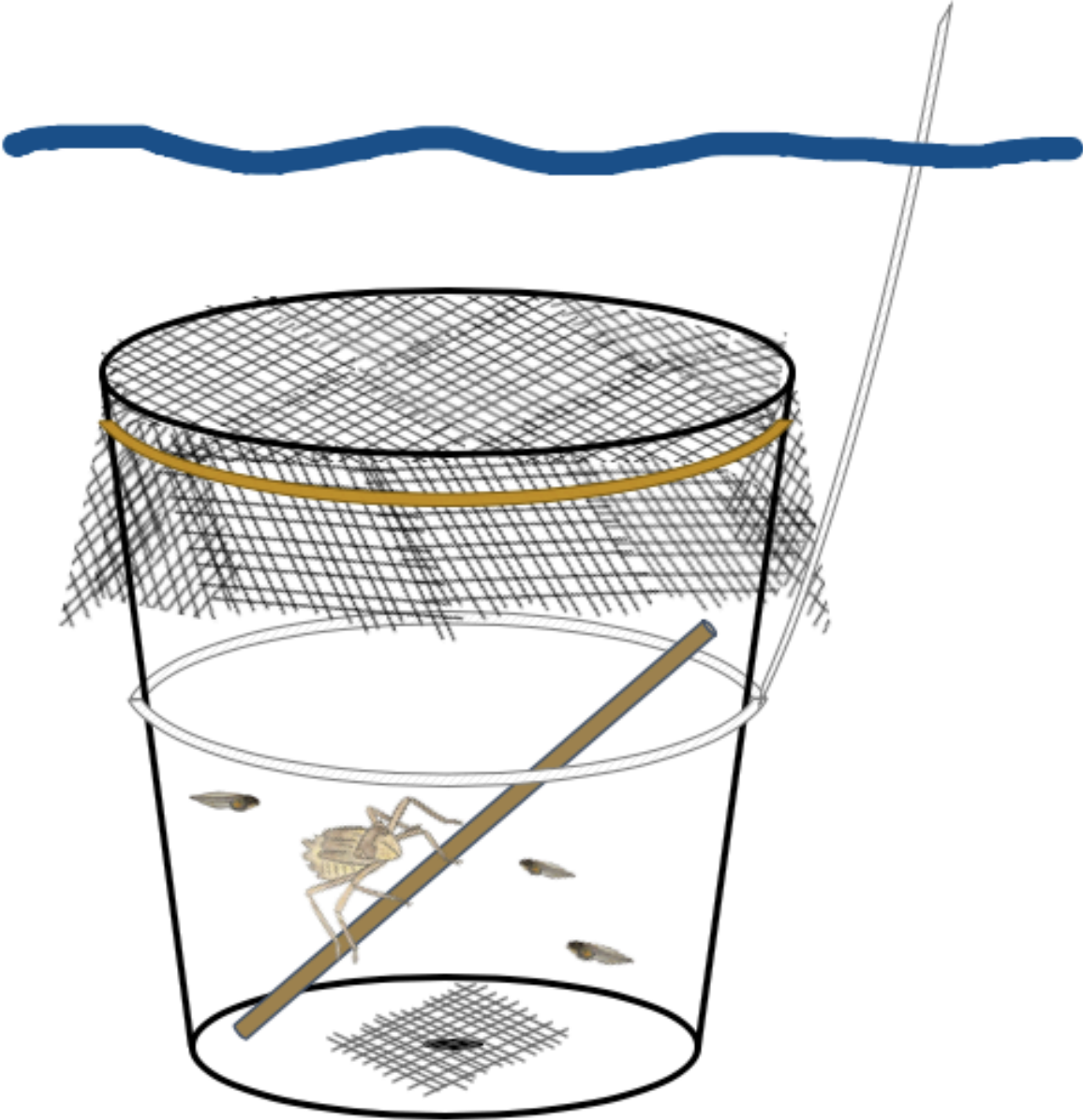
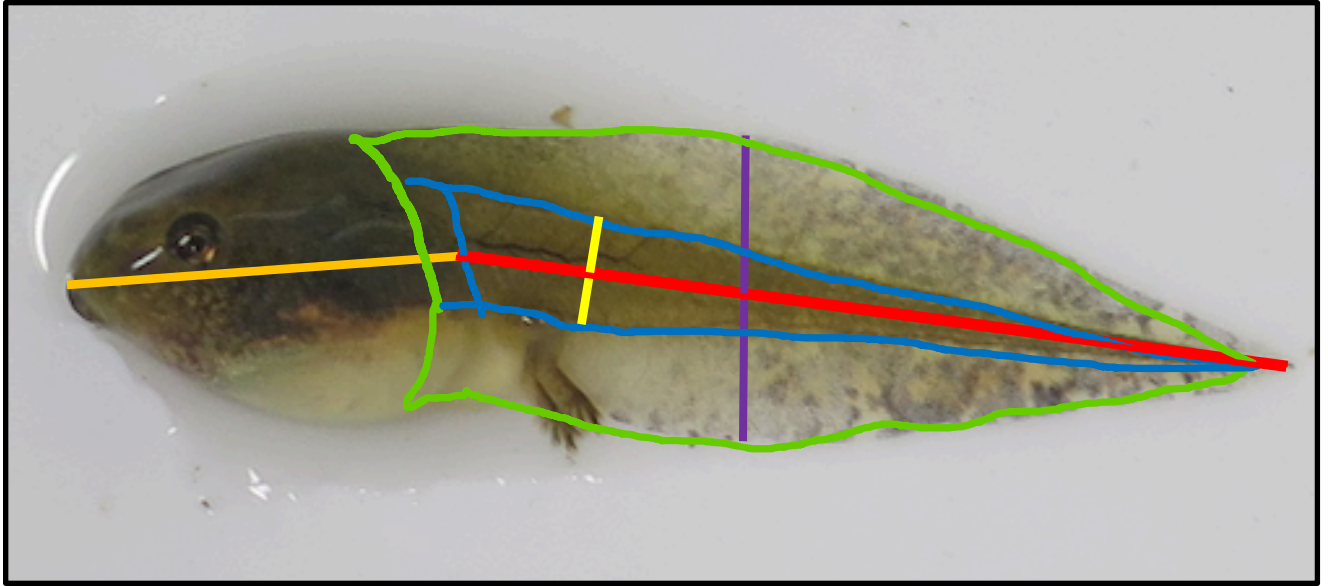Figure 2. Illustration of a dragonfly predator cage.

Figure 3. The landmarks used to determine differences in tail morphology of tadpoles were as follows: head-body length (orange), tail length (red), tail depth (purple), tail area (green), tail muscle depth (yellow), and tail muscle area (blue).

Results

Water Chemistry

Portable field spectrometer measurements indicated that leaf litter type influenced adsorption at wavelengths of 470 nm. Maple leaf litter had significantly greater absorption than every treatment except oak, indicating a darker color (Figure 4; F = 10.081, $P < 0.001$). Soaking maple leaf litter significantly reduced absorption levels (Figure 4; F = 10.081. $P < 0.001$).

All tanks containing leaf litter had significantly lower dissolved oxygen levels than those with no leaf litter (Figure 4; F = 14.949, $P < 0.001$). Tanks containing soaked oak had slightly higher dissolved oxygen levels than un-soaked maple tanks (Figure 4; F = 14.949, $P = 0.044$).

Leaf litter type also effected nitrate/nitrate levels in sampled tanks. The nitrogen content of tanks with less than 1 mg/L nitrate/nitrite was not detectable in the lab tests. All maple leaf litter tanks sampled (n=4) had nitrate/nitrite levels greater than 1 mg/L (Figure 5), and 50% of soaked maple leaf litter tanks had nitrate/nitrite levels greater than 1 mg/L (Figure 5). Tanks containing oak leaf litter did not have detectable nitrogen levels, but 25% of soaked oak tanks had nitrate/nitrite levels greater than 1 mg/L.

Growth

When measuring the effects of leaf litter on larval growth rate in terms of total tadpole length, maple and oak leaf litter had no effect during the first seven days of the experiment (Figure 6; F = 1.228, $P = 0.3105$). However, larvae reared in tanks with soaked maple and soaked oak showed significantly reduced growth rates (Figure 6; $X^2 = 1.7968$, $P = 0.1801$), though there was no significant difference in growth rate between the two soaked leaf litter treatments (Figure 6; $X^2 = 0.7118$, $P = 0.3898$).

Predation also affected larval growth. There was an effect of predation on growth, but it was dependent on leaf litter type (Figure 6; $X_2 = 10.72$, $P < 0.001$). Larvae reared in maple leaf litter under predation grew significantly slower than larvae reared in maple litter with no predator present (Figure 6; $F = 8.401$, $P = 0.0298$). Larvae reared in oak leaf litter showed slightly faster growth rates under predation (Figure 6; $F = 4.684$, $P = 0.0486$). Conversely, predation had no effect on growth rate in soaked leaf litter treatments (Figure 6; $X_2 = 1.4069$, $P = 0.3898$).

Tail Morphology

Not all aspects of tail morphology were influenced by treatment group. There was no significant difference in the following aspects of tail morphology in maple leaf litter treatments with and without predation: tail depth (Figure 7; $X_2 = 0$, $P = 0.9987$), tail area (Figure 7; $X_2 = 0.7442$, $P = 0.3883$), tail muscle depth (Figure 7; $X_2 = 3.8458$, $P = 0.5618$), and tail muscle area (Figure 7; $X_2 = 0.2843$, $P = 0.5939$). For oak leaf litter treatments, there were no significant differences in the following with and without predation: tail muscle area (Figure 7; $X_2 = 3.0714$, $P = 0.0797$), tail area (Figure 7; $X_2 = 0.937$, $P = 0.333$), and tail length (Figure 7; $X_2 = 0.0072$, $P = 0.9326$).

For larvae reared in maple leaf litter, predation increased tail length (Figure 7; $X_2 = 4.4175$, $P = 0.0356$). Larvae reared in oak leaf litter had significantly greater tail muscles (Figure 7; $X_2 = 7.3799$, $P = 0.0066$) and tail depths (Figure 7; $X_2 = 4.8439$, $P = 0.02776$). than those reared in maple leaf litter (Figure 7; $X_2 = 4.8429$, $P = 0.0277$). Overall, there was an increasing trend in tail dimension under predation for larvae reared in both maple and oak leaf litter. However, the same was not true for larvae reared in tanks with soaked maple or soaked oak leaf

litter. There were no trends in data or significant differences between treatment groups (Figure 8).

Froglet Size

Measurements of froglet size were also influenced by treatment type. Without predation, there was a significant effect of leaf litter type on froglet snout to vent length (SVL) at time of metamorphosis, with oak litter tanks producing smaller froglets than maple tanks (Figure 9; F = 8.8762, $P = 0.0312$). There was a negative effect of predation on froglet size in un-soaked maple and soaked oak treatments (Figure 9; Maple: $X_2 = 7.6192$, $P < 0.001$, Soaked oak: $X_2 = 7.1404$, P = 0.007). Predation had no effect on oak or soaked maple treatments (Figure 9; Oak: $X_2 = 0.666$, $P = 0.7972$, Soaked maple: $X_2 = 3.6863$, P = 0.0549).

Timing of Metamorphosis

The timing of larvae to froglet metamorphosis was mostly unaffected by treatment. Soaking had an effect on metamorphic timing in oak treatments, but not in maple. Tadpoles reared in un-soaked oak metamorphosed significantly earlier than tadpoles reared in soaked oak leaf litter (Figure 10; $X_2 = 4.2144$, $P = 0.0401$). Tadpoles reared in soaked maple leaf litter, however, metamorphosed the same time as those reared in un-soaked maple leaf litter (Figure 10; $X_2 = 1.1103$, $P = 0.292$).

Predation had no effect on metamorphic timing across all treatment types. There was no effect of predation in un-soaked maple treatments (Figure 10; $X_2 = 0.9161$, $P = 0.3385$) or un-soaked oak treatments (Figure 10; $X_2 = 0.003$, $P = 0.9562$). There was also no difference in metamorphic timing between tadpoles reared with predators in un-soaked oak and un-soaked

maple leaf litter (Figure 10; $X_2 = 0.9161$, $P = 0.3385$). Similarly, there was no effect of predation within soaked maple (Figure 10; $X_2 = 1.8837$, $P = 0.1699$) or soaked oak (Figure 10; $X_2 = 0.5989$, $P = 0.439$). There was also no difference in metamorphic timing between soaked oak and soaked maple under predation litter (Figure 10; $X^2 = 1.8837$, $P = 0.1699$).
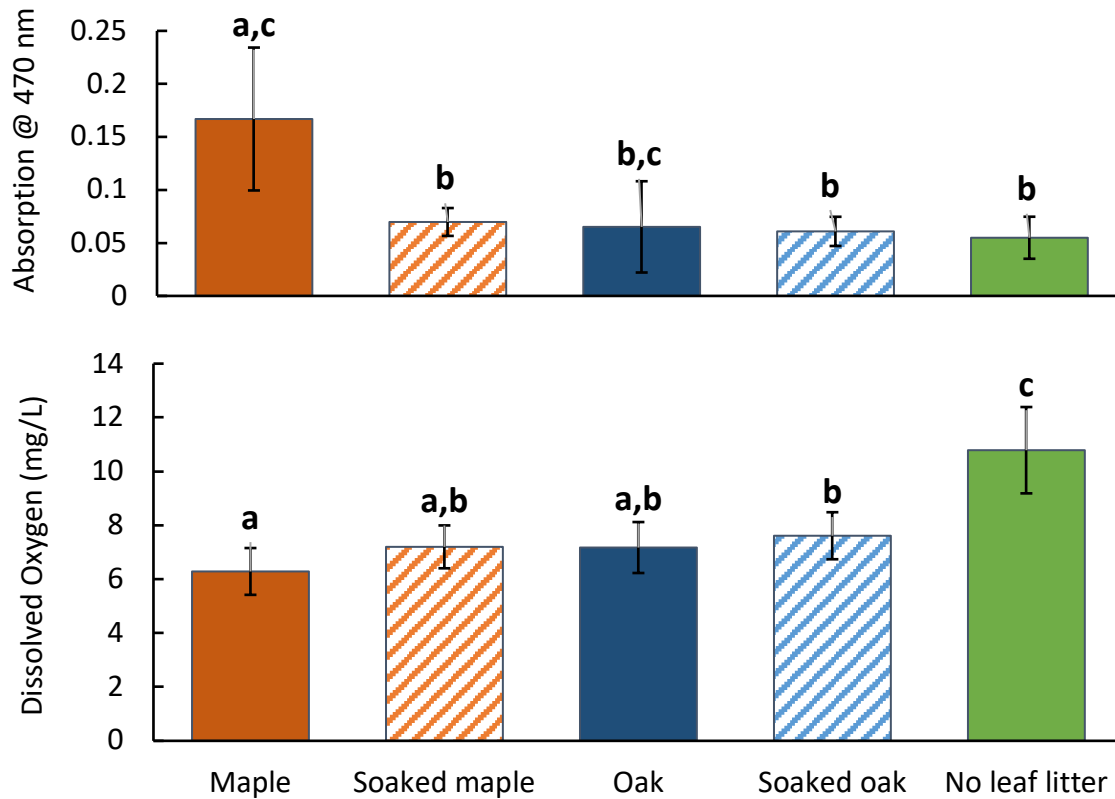
Figure 4. Comparison of the water clarity (absorption of wavelength at 470 nm) and dissolved oxygen levels of each leaf litter treatment. Leaf litter type and soaking had a significant effect on water clarity (Litter type: F = 11.97, $P < 0.002$; Soaking: F = 10.06, $P < 0.004$). All treatments containing leaf litter had significantly lower dissolved oxygen levels than tanks with no leaf litter (F = 21.57, $P < 0.001$). Soaking leaf litter had no effect on dissolved oxygen levels (F = 3.35, $P < 0.08$). Data with the same letter label are statistically the same. Data are means ± 95% CI
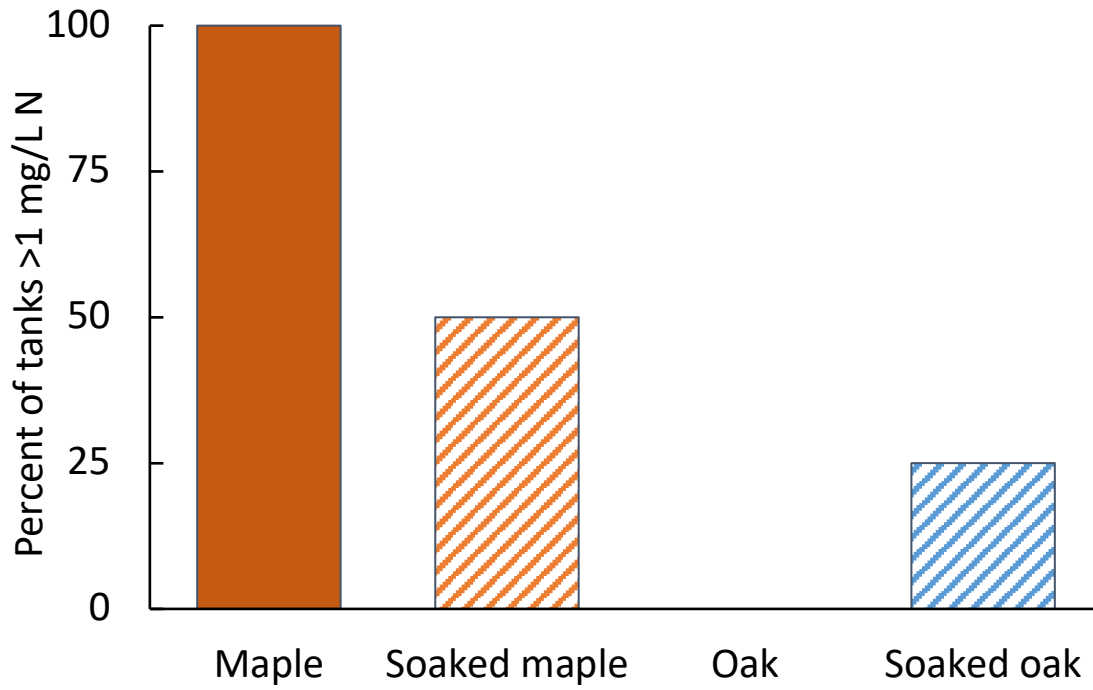
Figure 5. Percentage of tanks sampled (n=4) containing nitrate/nitrite levels greater than 1 mg/L. All maple leaf litter tanks had nitrate/nitrite levels greater than 1 mg/L, and all oak leaf litter tanks had less than 1 mg/L nitrate/nitrite levels.
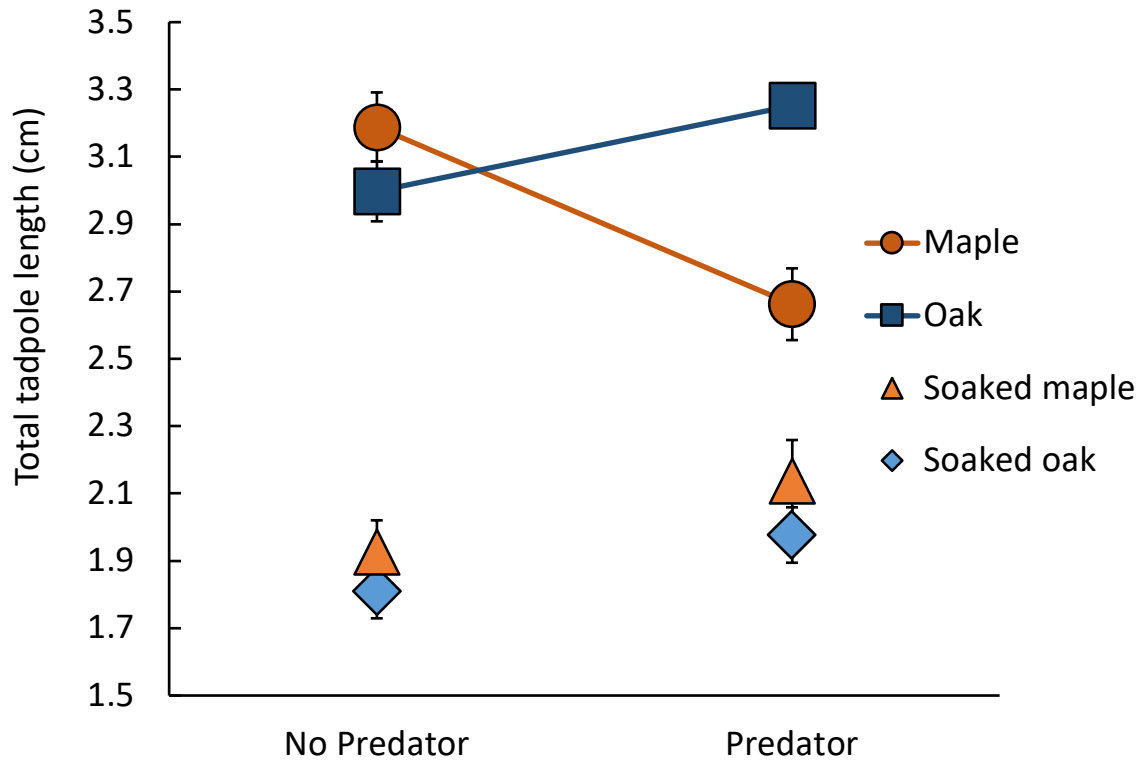
Figure 6. One-week growth of tadpoles reared in tanks under each leaf litter treatment with or without a predator. Maple leaf litter tanks exposed to predation had a slower growth rate after one-week (F = 8.401, P < 0.03), while oak litter tanks had a faster growth rate (F = 4.684, P = 0.0486). Trend lines represent significant differences. Data are means ± 95% CI.

Figure 7. Tail morphology of tadpoles reared in tanks containing either maple or oak leaf litter with or without a predator. There is a positive trend in tail dimensions under predation in both leaf litter species. Tadpoles reared in oak leaf litter under predation had significantly greater tail depth ($X^2 = 4.8429$, $P = 0.0278$) tail muscle depth ($X^2 = 7.3799$, $P= 0.0066$). Trend lines represent significant differences. Data are means ± 95% CI.

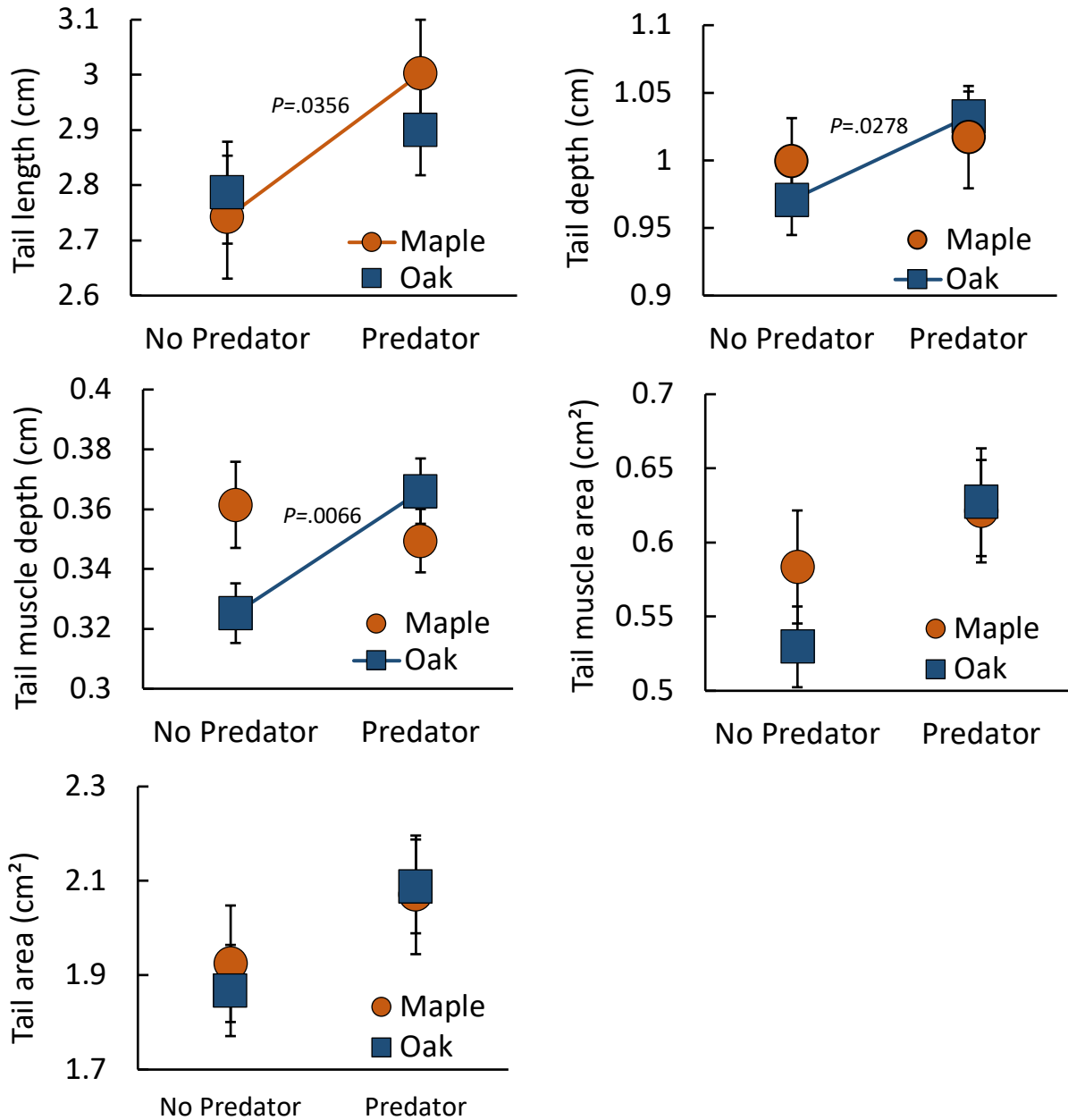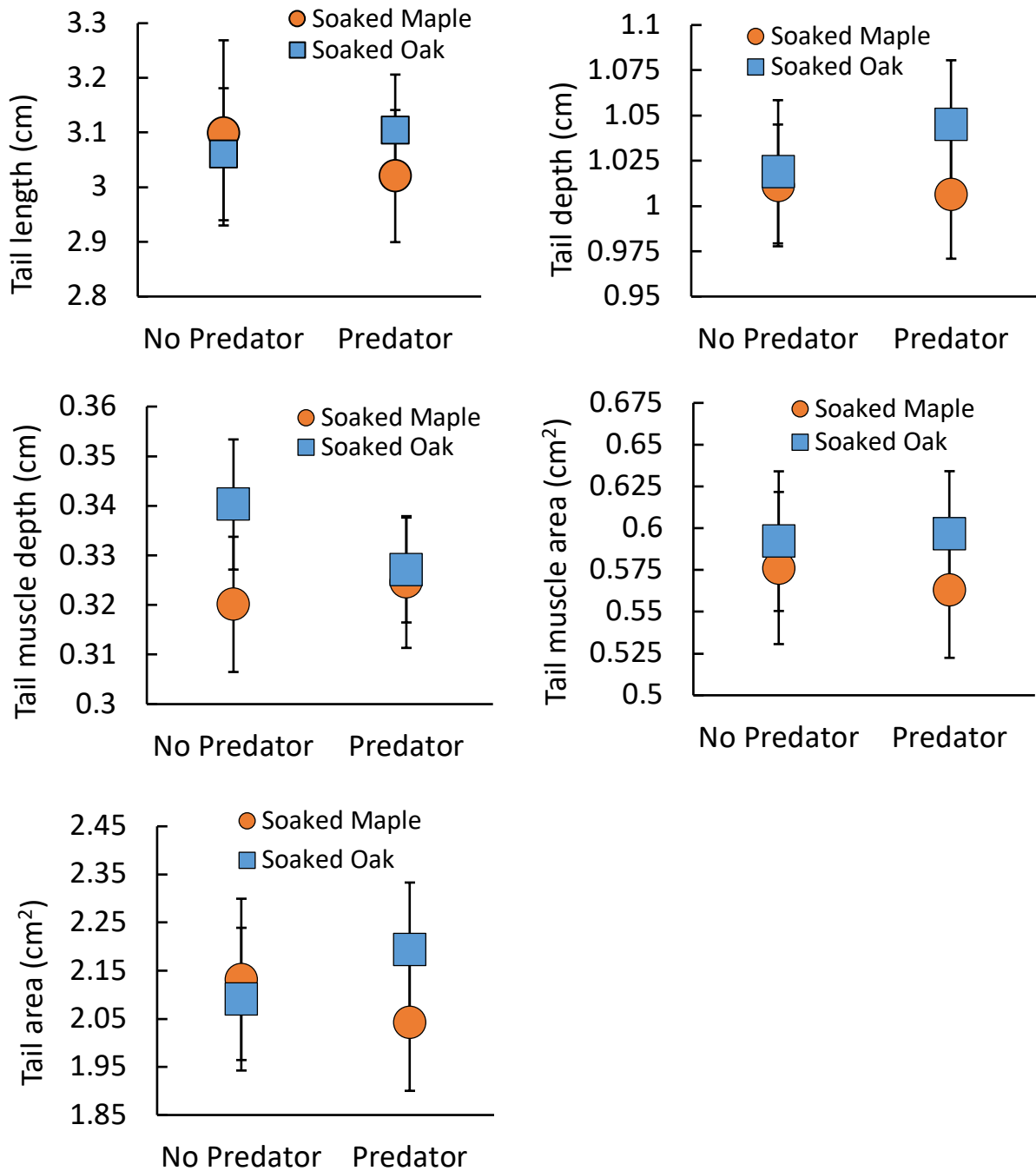Figure 8. Tail morphology of tadpoles reared in tanks containing either soaked maple or soaked oak leaf litter with or without a predator. There was no significant effect of leaf litter or predation on tail morphology. Data are means ± 95% CI.
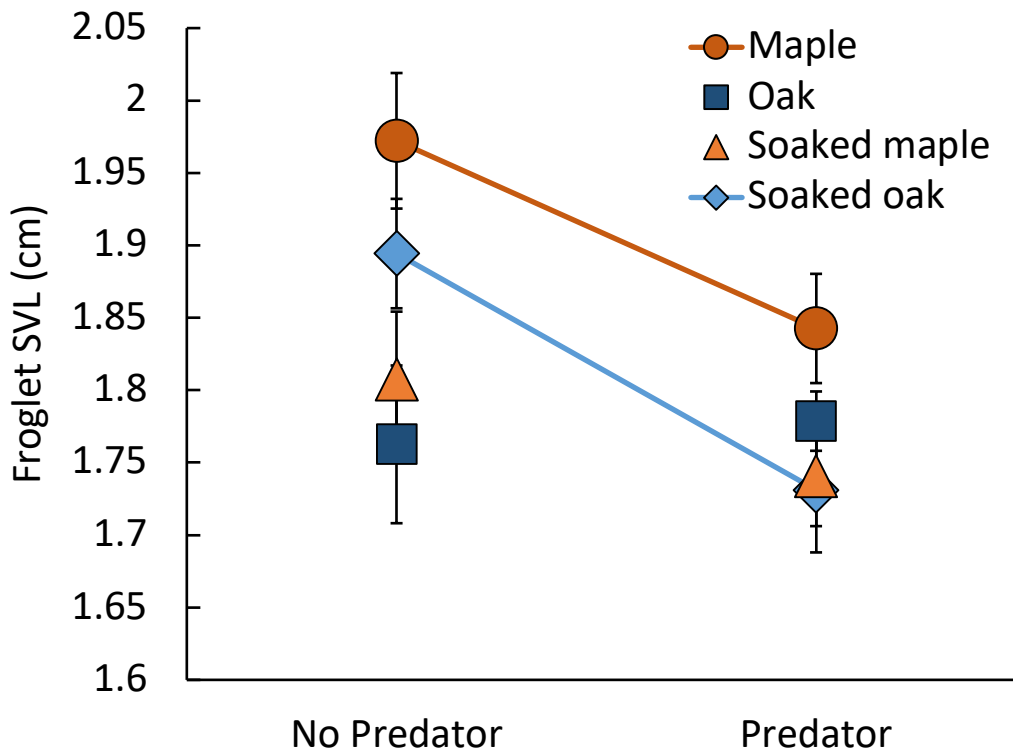
Figure 9. Average snout to vent length (SVL) of froglets at time of metamorphosis for each leaf litter treatment, with or without a predator. Predation significantly decreased froglet size in tanks containing maple and soaked oak (Maple: $X^2 = 7.6192$, $P < 0.001$, Soaked oak: $X^2 = 7.1404$, $P = 0.007$). Trend lines represent significant differences. Data are means ± 95% CI.
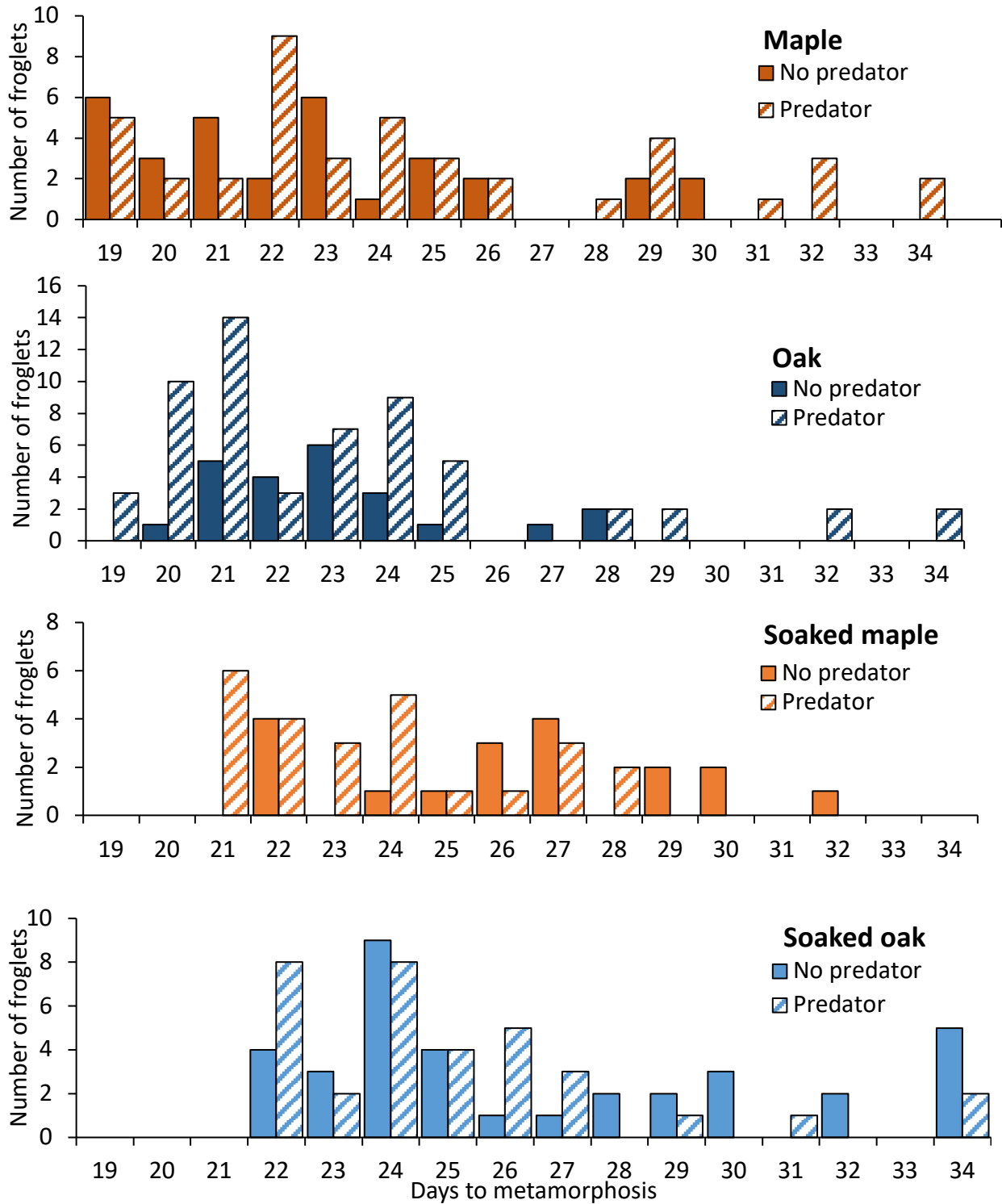
Figure 10. Frequency of froglets reared in soaked maple or soaked oak leaf litter with or without a predator metamorphosing on a given day. Tadpoles reared in oak metamorphosed significantly earlier than tadpoles reared in soaked oak leaf litter ($X^2 = 4.2144$, $P = 0.0401$).

Discussion

Leaf litter type played a significant role in the response of *Hyla versicolor* tadpoles to predation cues. Past research has demonstrated that two predator evasion tactics predominate: altering growth rate and increased tail depth (Earl *et al*. 2012, Gómez and Kehr 2013 Relyea 2002a, Relyea 2002b, Relyea and Hoverman 2003, Rubbo and Kiesecker 2004, Smith and Van Buskirk 1995, Stoler *et al.* 2017). In this study, tadpoles reared in red maple leaf litter had a slower growth rate under predation while tadpoles reared in pin oak had a slightly increased growth rate under predation. Both maple and oak leaf litter allowed tadpoles to respond with slightly larger tails under predation compared to treatments without predation. Tadpoles reared in maple leaf litter increased tail length in response to predation. Those reared in oak increased tail muscle depth and tail depth, indicating a more expensive response to predation.

Studies examining the effect of predation on growth rate in anuran tadpoles often have contradicting results (Earl *et al.* 2014, Gómez and Kehr 2013, Relyea 2004, Rubbo and Keisecker 2004). For example, Gómez and Kehr (2013) found *Scinax nasicus* increase or decrease activity levels depending on the lethality of the predator present, while Relyea (2004) found *Rana sylvatica* increase activity as predators increase in density. It is possible that larvae only respond to predation cues with a decrease in activity if the larvae deem the predator effective (Gómez and Kehr 2013). Our results suggest leaf litter could influence how the predators perceive the effectiveness of a predator. It is also important to note that growth responses early in ontogeny indicate leaf litter can play a large role in tadpole behavioral and phenotypic plasticity. Rubbo and Keisecker (2004) performed a study on *Rana sylvatica* tadpoles

and discovered tradeoffs in development are largely based on the physiochemical environment; leaf litter inputs in particular.

Conflicting results in growth rate can be due to two different strategies tadpoles employ to avoid predation. They can either decrease growth rate by reducing foraging behavior to avoid predator encounters (Dodson *et al.* 1994) or increase growth rate to reach a size refuge from predators (Reylea 2003). Although reducing growth rate maintains larvae at a more vulnerable developmental stage for longer, it does have some notable advantages. A slower growth rate can increase burst speed, allowing for better predator evasion and fitness (Arendt 2003). It is also less physiologically taxing on anuran larvae due to the minimal effort involved in hiding (Dodson *et al.* 1994).

It is likely the physiochemical properties of maple leaf litter caused the reduction in foraging behavior early in ontogeny. This behavior can be linked to poor water quality, as it is less costly to reduce movement to evade predation than it is to get larger to reach a size refuge from predator attacks (Arendt 2003). Red maple leaf litter is nutrient dense and decomposes quickly, but also has a complex composition of harsh chemical leachates, making it a suboptimal food source for anuran larvae (Earl *et al*. 2014). Our water chemistry analyses show maple leaf litter resulted in low water clarity and high nitrogen levels, which have both been associated with high tannin concentrations and increased mortality rates (Earl *et al*. 2014, Stoler and Relyea 2013). However, we did not observe detrimental effects maple litter on tadpoles in our study. Tadpoles reared in maple litter under no predation had the same growth rate as oak, signaling similar food supplies within the first week of ontogeny.

Stoler and Relyea (2013) found the level to which maple leaf litter negatively affects *Hyla versicolor* tadpole growth is highly dependent on the level of leaf litter input. In their study,

low levels of red maple leachates did not affect tadpole growth and decreased the rate of mortality by predation. The leaf litter used in our experiment was spring collected and therefore can be associated with the low-leachate treatments of that study as it has already been broken down to some extent. At low leachate levels, it is possible maple leaf litter was not detrimental to tadpole growth due to the associated levels of dissolved organic carbon (Stoler and Relyea 2013) which can increase microbial growth (Rubbo and Keisecker 2004). Despite this, the stressor of having high tannin leachates in the water could have caused tadpoles to reduce movement at the addition of a second stressor in the form of a predation cue.

While tadpoles reared in maple leaf litter decreased growth rate under predation, the growth rate of tadpoles reared in oak litter slightly increased under predation. This leads us to believe early conditions allowed for optimal predation-induced behavioral plasticity. Studies have shown anuran larvae reared in resource rich, low competition environments can endure the cost of an increased growth rate (Reylea 2003). Water chemistry results indicated similarities in water clarity and dissolved oxygen levels with maple leaf litter treatments, but unlike maple leaf litter, nitrogen levels were undetectable in oak treatments. Soaked oak treatments, however, had detectable levels of nitrogen. This indicates soaking oak litter aids in decomposition and nutrient release over time. Slow decomposition of oak litter is due to high levels of lignin and cellulose which delay microbe colonization—offering a constant source of food to consumers over a longer period of time (Earl *et al.* 2014, Stoler *et al.* 2017). This leads us to believe the tadpoles reared in oak leaf litter had enough lignin and cellulose dense food to maintain the same growth rate as maple under no predation. Consequently, tadpoles reared in oak could increase foraging effort in an attempt to reach a size refuge from predators.

Both maple and oak reared tadpoles altered their tail shape in response to predation. There was a general trend of longer, deeper tails under predation and there were few differences between tadpoles from maple and oak treatments. The similarities in tail morphology indicate both treatments had adequate resources to induce morphological plasticity in response to predation. In the absence of predation, however, tadpoles reared in oak had significantly smaller tail muscle depths than maple tadpoles. This is likely because as tadoles reared in oak grew, they became more food limited because of slow oak decomposition rates. In response to competition, they reduced tail size. However, in the presence of a predator the sacrifice of tail size was too risky, so they developed deeper tails. Kurali *et al.* (2018) suggests *Hyla versicolor* tadpoles are capable of producing deeper tails without predation, but it does not take priority when resources need to be allocated elsewhere to improve fitness. Competition among tadpoles in oak litter could also explain the small tail muscle depth. Several studies have suggested the responses to competition are dependent on the presence of predators, typically resulting in a decrease in predation evasion responses with high competition (Neptune and Bouchard 2017, Relyea2004, Relyea and Hoverman 2003). The more drastic difference in tail depth of oak-reared tadpoles potentially indicates better resource quality later in ontogeny, as large morphological changes to predation are indicators of a quality environment (Costello and Michel 2013, Shaffery and Relyea 2016).

The variation in froglet snout-vent length across treatments suggests a tradeoff between growth rate and froglet size. The negative effect of predation on maple froglet size is likely translated from the stunted growth within the first week of the experiment. Oak froglets, on the other hand, were significantly smaller than maple froglets with and without predation. This suggests oak froglet size was driven by competition and resources limitation later in ontogeny.

The slow decomposition of oak leaf litter potentially created resource limitations, as it could not keep up with the demand of faster growing tadpoles. Studies have found that predation influences morphological and behavioral changes in the early stages of development, and competition dominates the later stages (Bennett *et al.* 2013, Gomez and Kehr 2013, Van Buskirk and Yurewicz 1998.) As we would expect, increased growth rate was associated with faster times to metamorphose. Although we did not pick up statistical differences between maple, oak, and soaked maple treatments, there is a general trend showing oak and maple froglets metamorphosed first with or without predation.

We expected to demonstrate the extremes of morphological responses to predation cues through our soaked maple, soaked oak, and no-leaf litter treatments. We refuted our predictions that removing the leachates in leaf litter would allow for greater responses to predation cues in growth rate and tails. Even though the soaked leaf litter and no-leaf litter tanks had biofilm growth, there was an obvious lack of available nutrients. This most likely created a highly competitive environment that negatively affected growth rate and tail size. When competition is high, there is a threshold of resources anuran species need to meet in order to grow and develop properly (Gómez and Kehr 2013, Teplitsky and Laurila 2007). The significant lag in growth rate and large error in tail morphology analyses led us to compare control treatments separately from un-soaked maple and oak. Despite this, these treatments still provided valuable information in analyzing water quality and metamorphic data.

In short, the combined effects of predation and leaf litter type effected tadpoles in different ways during their morphological development. The cost-benefit balance of both foraging for nutrients and hiding from predators was tipped based on the leaf type present. The

more slowly decomposing oak provided sufficient nutrients for tadpoles to maintain early growth rates. However, as they grew larger, they became nutrient limited.

Further work should aim to isolate competition from resource supply and predation effects while manipulating leaf litter type and decomposition. Although this study provided insightful information on the trade-offs between resource allocation and predation responses, it would be beneficial to know the physiochemical effects of leaf litter on responses to predation cues without potential resource limitations. In this study, we supplied the tadpoles with very low levels of additional food simply just to stimulate the growth of periphyton and microbes which resulted in an unaccounted-for competition factor.

Based on our research, there are three areas for further exploration. First, isolating the chemical leachates present in different leaf litter types and identifying how they interact with kairomones could help researchers understand the distinct effects of litter type on predation in tadpoles. Second, researchers could use morphometrics to more distinctly identify the effects of predation and leaf litter on tail shape in tadpoles. Third, future studies could examine the organic matter composition of dehydrated tadpole samples reared in different leaf litter types. Though we dehydrated tadpoles from the populations in our study, we did not have time to examine their organic matter content in order to understand how they used leaf litter for nutrition. This study lays the foundation for further exploration of these topics.

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