Predator cue type and prey density alters survival strategies in larval *Ambystoma maculatum* and *Rana sylvatica.*

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Harrison Wojtas

Advisor: Jasper Leavitt

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Abstract

Predators affect prey indirectly through mere presences in a system, which can induce morphological and behavioral prey adaptations increase survival. Adaption responses may occur from visual, tactile, or chemical cues. which consists of predatory odors, diet cues, or alarm cues. In aquatic systems, chemical cues are important to induce prey antipredator morphological defenses as seen in the literature on larval *Ambystoma maculatum* and *Rana sylvatica* development. Both amphibians reside in vernal pools, and the density of *R. sylvatica* affects development rates of both. Our experiment looked at the relationship of chemical and visual cues on morphological development of *R. sylvatica* and *A. maculatum*, in addition to the relationship between interspecific densities on perceived predation risk adaptations. We performed a lab experiment using different treatments containing visual cues, predator odors, and densities to look at growth and survival ship over 29 days. We found visual and chemical cues had no effect on growth as well as, interspecific density. However, increased intraspecific density of *R. sylvatica* decreased their developmental rates while having no effect on *A. maculatum*. Additionally, survivorship decreased only in *R. sylvatica* exposed to chemical cues. Our work shows that some specific chemical cues don't have innate responses and need to be learned to prey responses, in addition to there being no relationship between prey density and perceived predation risk between *R. sylvatica* and *A. maculatum*.

Introduction

Predator-prey dynamics have complex effects on population, both directly affecting population size (**Liu 2005**) and habitat selection (**Berger 2001**) or having indirect effects due to the risk of predation (**Zanette 2011**). Perceived predation risk, or PPR, is the prey's assessment of the likelihood of being killed by a predator, which can lead to prey responding to this risk through behavioral or morphological shifts (**Zanette 2011**). Though effective in a short ecological timescale, PPR can be detrimental to the overall fitness of the prey (**Zanette 2011**). Prey survival adaptations often lead to energy use reallocations away from activities that increase fitness, such as reproduction efforts or finding resources (**Cress well 2008; Peacor 2012**). In many terrestrial habitats, survival adaptations from PPR can be seen through behavioral adaptations, such as reducing activity or altering foraging habitat (**Eilam 1999; Creel 2007**). In aquatic systems, induced antipredator defenses are often seen in morphological adaptations due to chronic predator cues in the system (**McCollum 1997**). Although it is well-documented that

PPR can be responsible for morphological changes (**Mitchell 2015; Schoeppner 2009**), the specific types of predator cues that produces these morphological adaptations are not fully understood.

In aquatic systems, kairomones play an important role in predator detection. Kairomones are chemicals emitted by one organism that are detected by another organism, providing information for the receiver **(Brown 1970)**. While prey can respond to general cues through morphological, behavioral, or genetic adaptations (**Brönmark 2011; Dawidowicz 1992; Matsunami 2015**), these cues need to be more specific to understand, the ultimate driver in prey response. For example, there are chemical cues outside of kairomones, such as intraspecific alarm cues (chemicals released from a stressed individual of the same species) or diet cues which are released as a byproduct of predator digestion (**Bairos-Novak 2017**). These chemical cues are important for predator detection by prey but are not the only cue type employed by prey; tactile cues, auditory cues, and visual cues also have an effect on predator recognition (**Middlemis 2013; Remage-Healey 2006; Oliveria 2017**). Generally, visual cues provide prey with spatial information of the predator as well as eliciting behavioral responses, as the prey is usually in immediate danger and needs to flee, hide, or defend quickly to avoid predation (**Hemmi 2005; Abrahams 1997**). In contrast, general chemical cues are useful in habitats with low visibility as they provide prey with longer time to adapt to predation, allowing for morphological defenses to develop when chronically exposed to cues (**Lima 1990**). In some prey species, chemical cues don't elicit an innate response, instead the response is learned from a previous encounter with a predator (**Gonzalo 2009**). However, when there are several different types predatory cues in a system, there is some uncertainty as to which cues are responsible for induced responses, and if combined cues impact the effectiveness of the response.

Amphibians have been a main model for studying PPR due to rapid larval development and susceptibility of chemicals (**Shaffery 2015; Holbrook 2004; Middlemis 2013**). Specifically, research focuses on salamanders in the genus *Ambystoma* and frogs in the genus *Rana* (*Lithobates*), and the interactions between these two amphibians has been looked at extensively (**Middlemis 2013; Rowe 1995; Holbrook 2004**). *Ambystoma maculatum* (Spotted Salamander) are fully aquatic during larval stage, making them vulnerable to predation and susceptible to PPR as they are often exposed to chemical cues (**Skelly 1994**). Larvae can be found in vernal pools where they experience heavy predation from invertebrates and other amphibians, resulting in high juvenile mortalities (**Stenhouse 1987**). When presented with predatory cues from dragonfly nymphs, *A. maculatum* larva develop wider heads and shorter tails (**Shaffery 2015**). Alternatively, when *Rana sylvatica* (Wood Frog) tadpoles are exposed to chronic predatory chemical cues, they reduce their growth rate and develop strong, short tails as a way to better swim away from predators (**Middlemis 2013**). *Rana sylvatica* tadpoles are often found in the same vernal pools as *A. maculatum* and are often preyed on by late larval stage *A. maculatum* as they mature and become large enough to eat the tadpoles (**Rowe 1995**). At the same time, higher densities of *R. sylvatica* result in a decreased survival rate for both species due to indirect effects on the entire ecosystem of the vernal pool (**Holbrook 2004; Wilbur 1976**). High *R. sylvatica* densities also affect their own development, as development time is increased, and it takes a longer time to metamorphosis due to decreased food availability in ponds (**Wilbur 1977**). A common predator of both amphibian larva is the dragonfly nymph of the order Odonata, which is often used a predator model in experimental studies **(Relyea 2002; Relyea 2003)**. Dragonfly nymph hunt using ambush methods, in which they sit on vegetation or rocks on the bottom of the water and wait for prey swim nearby **(Berger book)**. Although there is

research on predator effects on both species, as well as how the interspecific interactions between the two amphibians, there are few studies that look the interactions between *A. maculatum* and *R. sylvatica* while a predatory cue is in the system.

Given this information, I performed a set of experiments to uncover some of the uncertainty on the cue type combinations and their morphological effects on larval amphibians, as well as investigating into how interspecific densities affect PPR. I hypothesized that different predator cues from the dragonfly nymphs will have different effects on morphology in larval amphibians. I predict that when in a system with chemical cues from the predator, *A. maculatum* will exhibit antipredator defenses of increasing size and weight and *R. sylvatica* will reduce in size and weight. On the other hand, visual cues will have little to no effect on morphology in larval amphibians. Additionally, *A. maculatum* and *R. sylvatica* perceived predation risk is affected by prey density and interspecific interactions. When in a denser interspecific system, *A. maculatum* will express weaker antipredator defenses, while *R. sylvatica* will express stronger antipredator defenses. Furthermore, developmental rates of prey will be affected by interspecific densities. When in a denser interspecific system, *A. maculatum* and *R. sylvatica* will experience a slower growth rate. Also, survivorship is affected by predator cue presence. When *A. maculatum* and *R. sylvatica* are exposed to visual or chemical cues, there will be a higher mortality rate.

Methods

Experimental setup

Study site- The University of Notre Dame Ecological Research Center (UNDERC) is located on the state line between Michigan's Upper Peninsula and Wisconsin. The research site is predominantly hardwood forests with many lakes, bogs, and vernal pools scattered around the

property. The site has been a field station undisturbed by the public since it was gifted to Notre dame in 1936, making it a pristine location to study natural history.

We collected 19 *Ambystoma maculatum* egg masses by hand from 11 pools (Wood Duck, Pool J, Pool V, Pool P, Pool 30a, Pool K, Pool V, Pool 9 and three unmarked pools called A_1, A_2, and A_3; Figure 1) from May 27, 2019-May 31, 2019.All egg masses were housed individually until hatching. Once eggs hatched, larvae were held in tanks organized by brood and hatch date (all larvae had hatched out by June 9th). Out of the 19 of egg masses collected, we used 112 larvae from four egg masses in our experiment (Wood Duck, J, V, and A_2), and all larvae not used were returned to their original vernal pools after experimental setup. *Rana sylvatica* tadpoles were collected using dipnets from 9 pools (Pool 30a, Pool J, Pool 5, Pool Wood Duck, Pool V, Pool P, Pool 9, Pool A_1, and Pool A_2; Figure 1) from May 27, 2019-May 31, 2019. Tadpoles were held at a maximum density of 50 per tank with water changes and feeding every other day. We used 112 tadpoles from 5 pools (Pool 30A, Pool J, Wood Duck, Pool P, and Pool A_1), and all tadpoles not used were returned to their original vernal pools after experimental setup. In order to ensure genetic variation, we used animals from multiple pools so that our results could be applied to the larger population. Twenty dragonfly nymphs from the family Aeshnidae were collected with dipnets and minnow traps at three water bodies (Wood Duck, Pool 9, and Morris Lake; Figure 1) from May 27 to June 9.

Experimental design

Salamander broods hatched between June 1 and June 9. We fed the salamander larvae *Daphnia*, which we obtained via sweep net. Tadpoles were fed PlecoWafers algae pellets. We fed dragonfly nymphs field ants that were collected by hand. Feeding and water changes occurred every other day. We used a siphon for water changes and used processed water.

Types of Cues

In order to test how different types of predator cues impact tadpoles and salamander larvae, I used four treatments: a visual and chemical (VC), a nonvisual and chemical (NVC), a visual nonchemical (VNC), and a nonvisual nonchemical (NVNC). Amphibians were held at an equal proportion with 4 tadpoles and 4 salamanders in a total of 16 tanks (dimensions: 9x30x20cm), with each treatment applied to 4 tanks. Each tank with the same treatment had different broods of salamander larvae and tadpoles in order to ensure genetics of a brood didn't affect the measurements. Predators were placed individually into 300 mL plastic cups, and the cups were secured to the side of the tanks. This allowed for the predator to be in the system without it eating the tadpoles and salamanders. We painted the cups with white paint for treatments that had no visual cues and poked 24 holes into cups that had treatments with chemical cues. We measured salamander larvae and tadpoles using calipers and weighed them using a scale at the beginning, middle, and end of the experiment. Dates of death were recorded throughout the experiment as well as the development of hind and fore limbs in tadpoles at the end of the experiment.

Densities

To test the effects of amphibian densities on predator cue response, I used 3 different combination types: an equal proportion combination with 4 tadpoles and 4 salamanders (4x4), an unequal proportion combination with 2 tadpoles and 4 salamanders (2x4), and another unequal proportion combination with 4 tadpoles and 2 salamander (4x2). The 4x2 and 2x4 combinations each had 8 tanks, with 4 VC treated tanks and 4 NVNC treated tanks. These were compared to the 4x4 VC and NVNC tanks from the cue type experiment. We collected the same measurements as the cue type experiment at the same time points.

Data analysis

All data was analyzed using the software R (R Core team 2018). We used Shapiro-Wilks test to determine the normality of the data. Non-normal data was transformed using $\log_{10}$ transformation. To determine the effects of cue type on morphological responses and on survival, we performed two-way ANOVAs with presence of visual cues and presence of chemical cues as independent variables at each time point. To look at the effect of densities for each species, we performed two-way ANOVAs with treatment and density as independent variables at each time point. We measured tadpole limb presences at the end of the experiment and ran a two-way ANOVA to determine impacts of cue type and density on development rates. All tests that had significant results were put through a post hoc Tukey Honest significant test.

Results

Types of Cues

We found no significant relationship between the cue type and the morphological development in weight or length for salamanders or tadpoles (Table 3-5).

Densities

There was a significant difference in tadpole masses between our density treatments on 6/25/19 (D.F.=2, F value=6.977, p-value=0.00663; Table 7a). Post hoc tests showed tadpoles in the 2x4 tanks, the lowest tadpole density, were significantly heavier than both the 4x4 (Difference = -0.196, p-value= 0.0179) and 4x2 (Difference= -0.207, p-value=0.00996) treatments (Table 10). We also found a significant difference between our density treatments on the lengths of tadpoles on 6/25/19 (D.F.=2, F value=3.770, p-value=0.0455; Table 7b). The post hoc tests once again showed tadpoles in 2x4 tanks were longer than those in 4x4 (Difference= -

4.839, p-value = 0.086) and 4x2 (Difference=-5.075, p-value=0.061) treatments (Table 11). All other tests showed no significant results (Tables 6-11).

Survivorship

We found a significant difference in tadpole survival driven by chemical cues (D.F.=1, F value=6.231, p-value=0.0362; Table 12a. The post hoc test showed that tadpoles exposed to chemical cues had higher mortality rates than those not exposed to chemical cues (Difference=-1.125, p-value= 0.0281; Table 13). We also found a significant difference in tadpole survival between the cue treatments in our density experiment (D.F. =1, f value= 5.174, p value=0.0362; Table 12b). The post hoc test showed that tadpoles exposed to visual and chemical cues had higher mortality rates than those without exposure to predator cues (Difference= 0.308, p value=0.0365; Table 14). All other test showed no significant results (Tables 12-14).

Tadpole Developmental

We found a significant in forelimb presence when comparing tadpole densities (X^2=22.383, D.F.=5, p-value=0.000443). By looking at the data, we concluded there is a higher proportion of individuals with forelimbs present in the 2x4 densities than 4x2 densities.

Discussion

We found predatory chemical cues and visual cues from dragonfly nymphs do not affect the morphology of *A. maculatum* and *R. sylvatica* which doesn't support my hypothesis that there will be a difference in prey responses. However, the cue type did have an effect on the survival hood, as tadpoles exposed to chemical cues experienced more deaths by the end of the experiment than tadpoles not exposed to chemical cues, which supports part of my hypothesis. Additionally, the intraspecific densities of the system affect the growth rate of *R. sylvatica*

tadpoles, which supports my hypothesis, as tadpoles in lower densities had larger mean masses and mean lengths than more dense tanks. In addition to that, *R. sylvatica* tadpoles in lower densities developed forelimbs faster than *R. sylvatica* tadpoles in higher densities. Visual and predator odor cues had no effect on the larval amphibian's morphological development, possibly as the chemical cues we used are not innate to a specific response and must be learnt. However, the exposure chemical cues could have still caused stress to the *R. sylvatica* tadpoles, resulting in higher mortalities. Low densities of tadpoles increase the developmental rate of the tadpoles through lack of intraspecific competition.

Our results illustrate dragonfly nymph visual and chemical cues don't lead to increases in length or mass of *A. maculatum* larvae or *R. sylvatica* tadpoles, which agrees with previous findings (**Shaffery & Relyea 2015; Chivers 2015**). The type of predatory cue used in our experiment may be the explanation of no effect. Both *R. sylvatica* and *A. maculatum* natural habitats are in vernal pools, which typically harbor low visibility and an abundance of leaf litter that provides cover (**Williams 1987**), making visual cues not as important in their evolutionary history. As for the chemical cues, we used only predator odors, rather than diet cues and predator odors. However, through using only predator odor cues, our results reinforce some of the findings issued by past researchers that found predator odors elicit different effects when prey diet cues are absent or present (**Schoeppner & Relyea 2005; Stabell 2003**). **Middlemis (2013**) found that diet cues, and not predator odors, delayed the growth rate of *R. sylvatica* tadpoles while we found similar results as tadpoles exposed to predator odors had reduced growth. In addition, our study highlights that dragonfly nymph predator odors aren't innate in eliciting antipredator responses in *A. maculatum* and *R. sylvatica* larvae, which agrees the findings of

Dalesman (2006) who found predator odors have no behavioral effect on snails, until they are associated with odors of crushed conspecific snails.

Our results show *R. sylvatica* tadpoles chronically exposed to predator chemical cues have a higher proportion of mortality in comparison to no exposure, which is similar to the findings of Relyea (2004) who found chronic exposure of dragonfly nymph predatory cues are amplified by roundup weed killer, resulting in higher mortalities of *R. sylvatica* tadpoles. **Middlemis et al (2013**) had opposite results than ours, as they found chronic exposure to chemical cues caused higher survival rates in tadpoles than those exposed to acute chemical cues. However, they used predatory odors paired with diet cues as their chemical cues and their chronic exposure lasted for 8 days, while ours lasted for 29 days. **Middlemis et al (2013**) additionally looked at the produced stress hormone, and they found it present in tadpoles when exposed to dietary and predator odor cues. There is a possibility that the stress hormone response can be induced by only predator odor cues, making the *R. sylvatica* stressed for the entirety of the experiment, causing more of their deaths from chemical cue exposure. One possible reason for chemical cues only affected the *R. sylvatica* survivorship instead of *A. maculatum* survivorship is because we collected tadpoles after they briefly lived in their natural environment, where predatory cues could have been present, and they could have learned to associate those cues with stress. Salamander however, hatched in a lab setting, where they weren't exposed to predators during the larval time of their life. Additionally, we used 6-day old *A. maculatum* larvae for our experiment, in comparison to other researchers that waited for larvae to be more developed before experiments (**Relyea 2004**). Running experiments on such young larvae may be the reason predator cues caused no noticeable morphological response.

We found that density effects tadpole development and growth rate of tadpoles. Decreased densities of *R. sylvatica* tadpoles results in faster growth and development rates, which agrees with **Wilbur (1977)** who found that higher densities of *R. sylvatica* and *A. maculatum* in a pond results in smaller mean body size. That study was in a field setting, while our experiment was set in a lab, where we had more control over environmental variables. So, our results highlight that there is a direct effect between *R. sylvatica* density and growth. Our results also found that salamander larvae weren't affected by the combination of densities which conflicts with the findings of **Wilbur (1977)** as he found that increased *R. sylvatica* density increases, the survival and growth rate of *A. maculatum* larval decreases.

Not all chemical cues are the same, as different types of chemical cues elicit different prey responses as well as some do not induce a prey response. Further research should investigate diet cues and their prey responses as well as the ability for *A. maculatum* and *R. sylvatica* to associate diet cues with predator odors. However, there can be an association with some chemical cue types, that allow for antipredator response be learnt for non-innate cue types. In addition, interspecific density doesn't amplify the chemical cues exposed in our experiment, however increased *R. sylvatica* tadpole densities does reduce the development and growth rate of its own population. Our study suggests not all chemical cues produce the same prey response, as some, such as predator odors, need to be learned through association while other cues are innate in prey response. Additionally, density of interspecific communities doesn't have an effect on PPR when visual and chemical cues are present and instead affects intraspecific growth and development, which may hinder the fitness and survival of prey.

Graphs & Figures

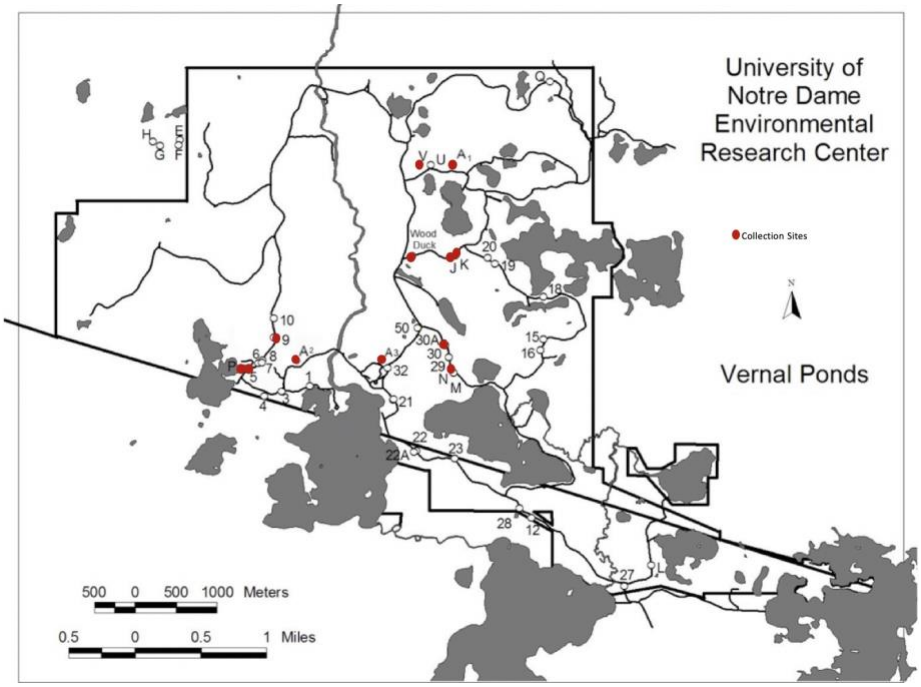

Figure 1. Collection sites of *Ambystoma maculatum* egg masses and *Rana sylvatica* tadpole.

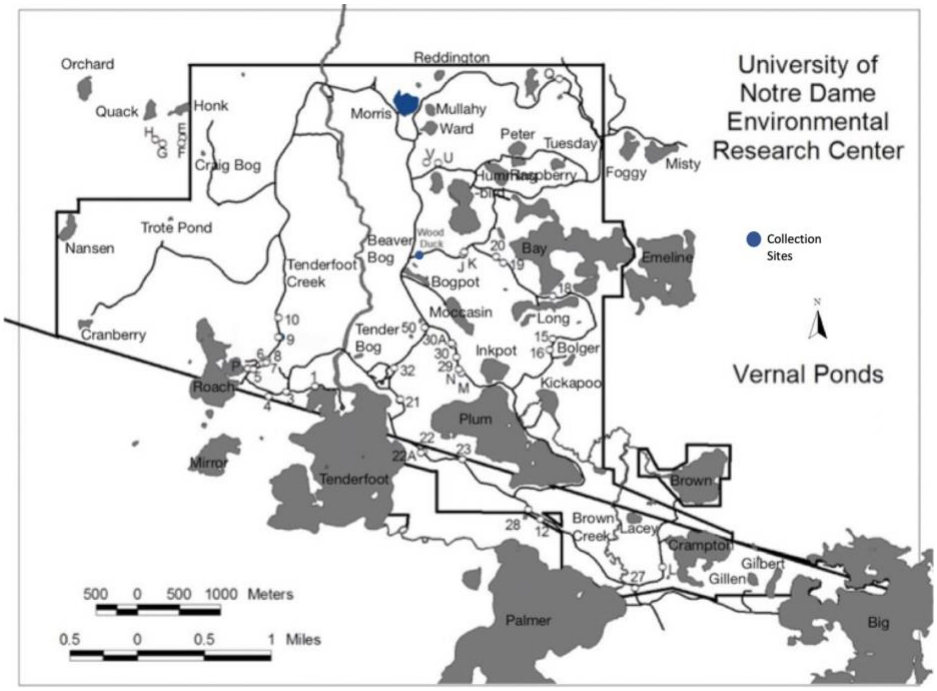

Figure 2. Collection sites of dragonfly nymphs.

Table 1. Shapiro-Wilks test for normality for the density and treatment experiment's mass data sets. * transformed with $\log_{10}$.

	p values			
Date	Tadpole Density	Salamander Density	Tadpole Treatment	Salamander Treatment
06/15/19	0.2004*	0.2045*	0.6316	0.0925
06/29/19	0.3332	5.822e-05*	0.0001158	0.1252
07/13/19	0.625	0.7594	0.6861	0.2169

Table 2. Shapiro-Wilks test for normality for the density and treatment experiment's length data sets. * transformed with $\log_{10}$.

	p values			
Date	Tadpole Density	Salamander Density	Tadpole 4x4	Salamander 4x4
06/15/19	.7093	0.5361	0.7202	0.3181
06/29/19	0.9679	0.06077	0.8209	0.06175
07/13/19	0.0001158*	0.7524	0.3251	0.1419

Treatment type experiment

Table 3. ANOVA results of 6/15/19 comparing visual cues treatments to chemical cues treatment. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*	**b.**	*D.F.*	*F value*	*p value*
Visual	1	0.999	0.336	*Visual*	1	0.278	0.607
Chemical	1	1.384	0.261	*Chemical*	1	0.048	0.831
Visual X Chemical	1	0.204	0.659	*Visual X Chemical*	1	0.113	0.742
c.	*D.F.*	*F value*	*p value*	**d.**	*D.F.*	*F value*	*p value*
Visual	1	0.101	0.756	*Visual*	1	0.672	0.428
Chemical	1	0.186	0.674	*Chemical*	1	0.023	0.881
Visual X Chemical	1	0.272	0.659	*Visual X Chemical*	1	0.209	0.656

Table 4. ANOVA results of 6/25/19 comparing visual cues treatments to chemical cues treatment. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*
Visual	1	0.018	0.895
Chemical	1	1.325	0.274
Visual X Chemical	1	0.317	0.585

b.	*D.F.*	*F value*	*p value*
Visual	1	1.081	0.321
Chemical	1	0.935	0.354
Visual X Chemical	1	0.002	0.961

c.	*D.F.*	*F value*	*p value*
Visual	1	4.188	0.0654
Chemical	1	0.650	0.4371
Visual X Chemical	1	0.041	0.8434

d.	*D.F.*	*F value*	*p value*
Visual	1	0.000	0.988
Chemical	1	1.280	0.282
Visual X Chemical	1	0.643	0.440

Table 5. ANOVA results of 6/13/19 comparing visual cues treatments to chemical cues treatment. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*
Visual	1	0.764	0.401
Chemical	1	0.030	0.866
Visual X Chemical	1	1.814	0.205

b.	*D.F.*	*F value*	*p value*
Visual	1	0.563	0.469
Chemical	1	0.410	0.535
Visual X Chemical	1	1.268	0.284

c.	*D.F.*	*F value*	*p value*
Visual	1	0.235	0.712
Chemical	1	0.491	0.611
Visual X Chemical	1	0.038	0.878

d.	*D.F.*	*F value*	*p value*
Visual	1	1.086	0.487
Chemical	1	0.188	0.739
Visual X Chemical	1	0.371	0.652

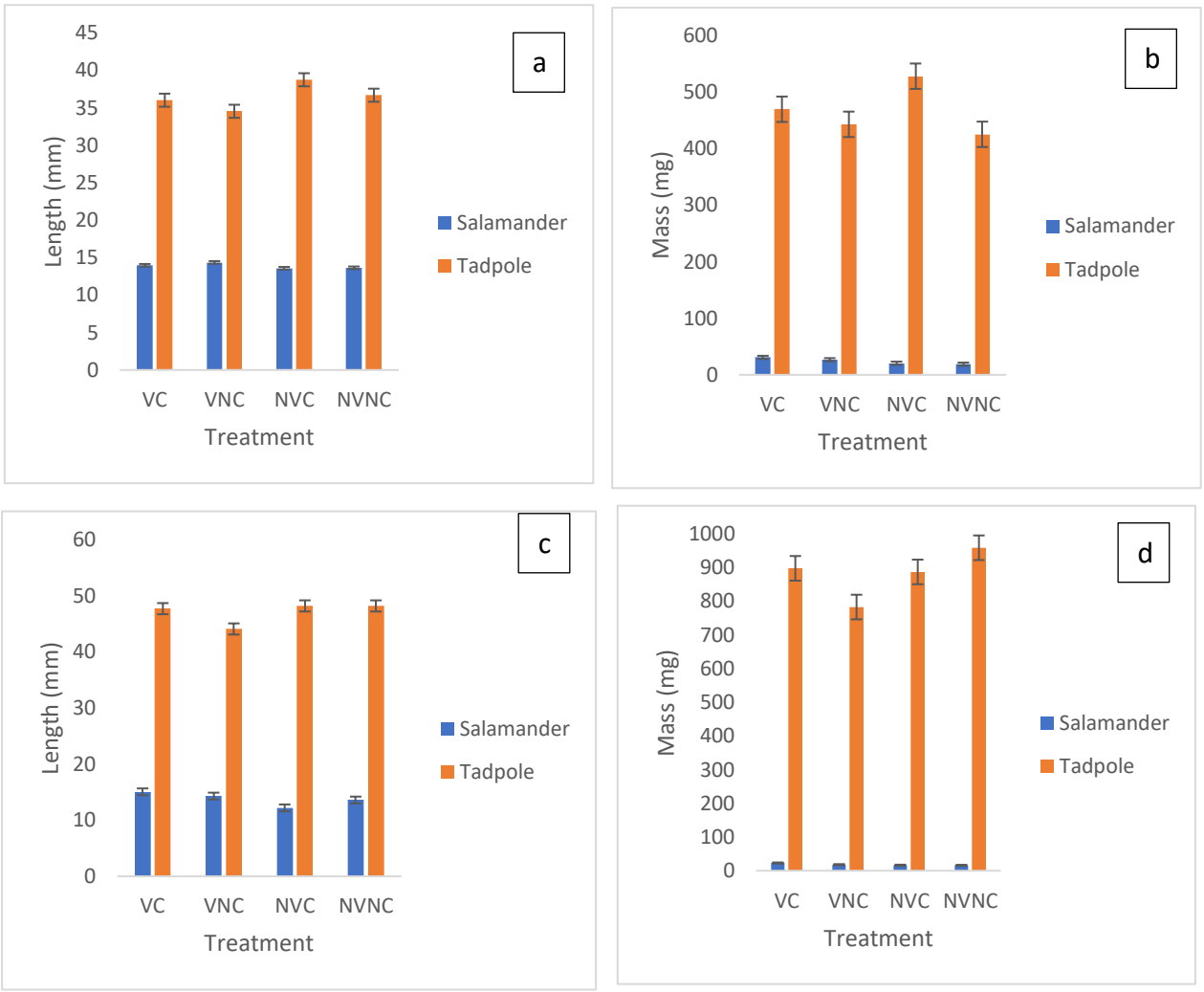

Figure 3a. Comparison of cue treatment types to mean (SE) lengths in 4x4 combination treatment on 6/29/19. (b) Comparison of cue treatment types to mean (SE) masses in 4x4 combination tanks on 6/29/19. (c) Comparison of cue treatment types to mean (SE) lengths in 4x4 combination tanks on 7/13/19. (d) Comparison of cue treatment types to mean (SE) masses in 4x4 combination tanks on 7/13/19.

Density Experiment

Table 6. ANOVA results of 6/15/19 comparing treatment types to combination type. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*
Combination	2	0.410	0.670
Treatment	1	2.638	0.122
Treatment X Combination	2	0.897	0.425

b.	*D.F.*	*F value*	*p value*
Combination	2	1.768	0.199
Treatment	1	0.989	0.333
Treatment X Combination	2	0.014	0.986

c.	*D.F.*	*F value*	*p value*
Combination	2	4.689	0.023
Treatment	1	.092	0.765
Treatment X Combination	2	0.222	0.803

d.	*D.F.*	*F value*	*p value*
Combination	2	2.219	0.138
Treatment	1	0.685	0.419
Treatment X Combination	2	0.057	0.945

Table 7. ANOVA results of 6/29/19 comparing treatment types to combination type. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*
Combination	2	6.977	0.00663*
Treatment	1	0.651	0.43171
Treatment X Combination	2	1.038	0.37696

b.	*D.F.*	*F value*	*p value*
Combination	2	3.770	0.0455*
Treatment	1	1.981	0.1784
Treatment X Combination	2	0.749	0.4885

c.	*D.F.*	*F value*	*p value*
Combination	2	1.169	0.3359
Treatment	1	0.013	0.9091
Treatment X Combination	2	2.754	0.0938

d.	*D.F.*	*F value*	*p value*
Combination	2	0.638	0.541
Treatment	1	0.000	0.989
Treatment X Combination	2	0.066	0.936

Table 8. ANOVA results of 7/13/19 comparing treatment types to combination type. (a) Comparison of *R. sylvatica* mean masses. (b) Comparison of *R. sylvatica* mean lengths. (c) Comparison of *A. maculatum* mean masses. (d) comparison of *A. maculatum* mean lengths.

a.	*D.F.*	*F value*	*p value*
Combination	2	3.176	0.0729
Treatment	1	0.001	0.9771
Treatment X Combination	2	0.309	0.7394

b.	*D.F.*	*F value*	*p value*
Combination	2	2.581	0.111
Treatment	1	0.955	0.345
Treatment X Combination	2	0.917	0.422

c.	*D.F.*	*F value*	*p value*
Combination	2	-	-
Treatment	1	-	-
Treatment X Combination	2	-	-

d.	*D.F.*	*F value*	*p value*
Combination	2	-	-
Treatment	1	-	-
Treatment X Combination	2	-	-

Post hoc test

Table 9. TukeyHSD test on comparing density on mean masses of *A. maculatum* on 6/15/19

Combination	*Difference*	*Lower*	*Upper*	*P value*
4x2-2x4	0.2483635	-0.1168748	0.61360175	0.2196172
4x4-2x4	-0.1885280	-0.5537662	0.17671034	0.4040648
4x4-4x2	-0.4368914	-0.8021297	-0.07165311	0.0178936

Table 10. TukeyHSD test on comparing density on mean masses of *R. sylvatica* on 6/25/19

Combination	*Difference*	*Lower*	*Upper*	*P value*
4x2-2x4	-0.19550000	-0.3582486	-0.03275144	0.0178704
4x4-2x4	-0.20677976	-0.3643604	-0.04919915	0.0099578
4x4-4x2	-0.01127976	-0.1688604	0.14630085	0.9813904

Table 11. TukeyHSD test on comparing density on mean lengths of *R. sylvatica* on6/25/19

Combination	*Difference*	*Lower*	*Upper*	*P value*
4x2-2x4	-4.8392857	-10.292380	0.6138087	0.0863297
4x4-2x4	-5.0754464	-10.355382	0.2044896	0.0605164
4x4-4x2	-0.2361607	-5.516097	5.0437753	0.9926867

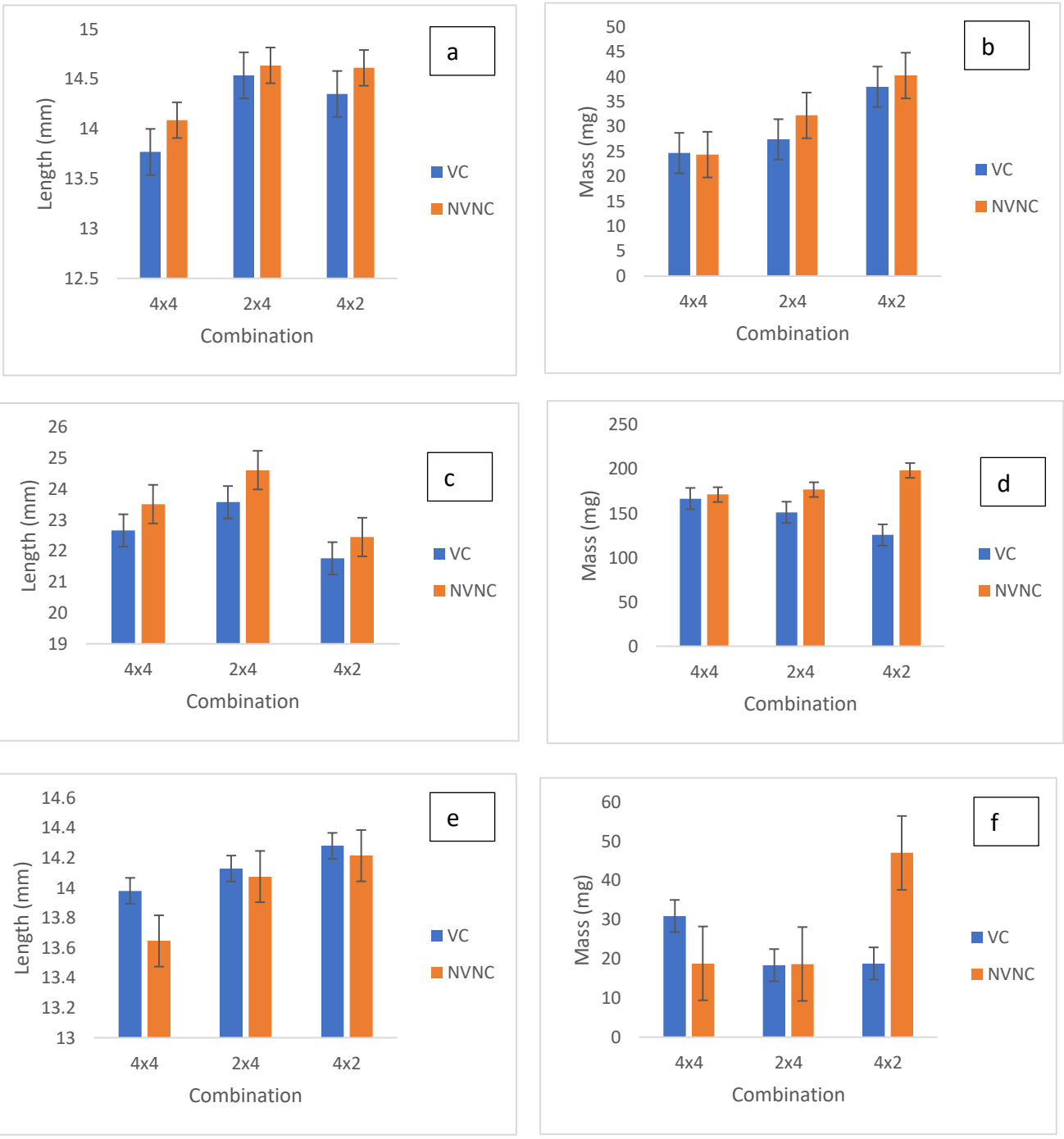

Figure 7(a). Comparison of *A. maculatum* densities and mean (± SE) lengths on 6/15/19. (b) Comparison of *A. maculatum* densities and mean (± SE) masses on 6/15/19. (c) Comparison of *R. sylvatica* densities and mean (± SE) lengths on 6/15/19. (d) Comparison of *R. sylvatica* densities and mean (± SE) masses on 6/15/19. (e) Comparison of *A. maculatum* densities and mean (± SE) length on 6/29/19. (f) Comparison of *A. maculatum* densities and mean (± SE) masses on 6/29/19.

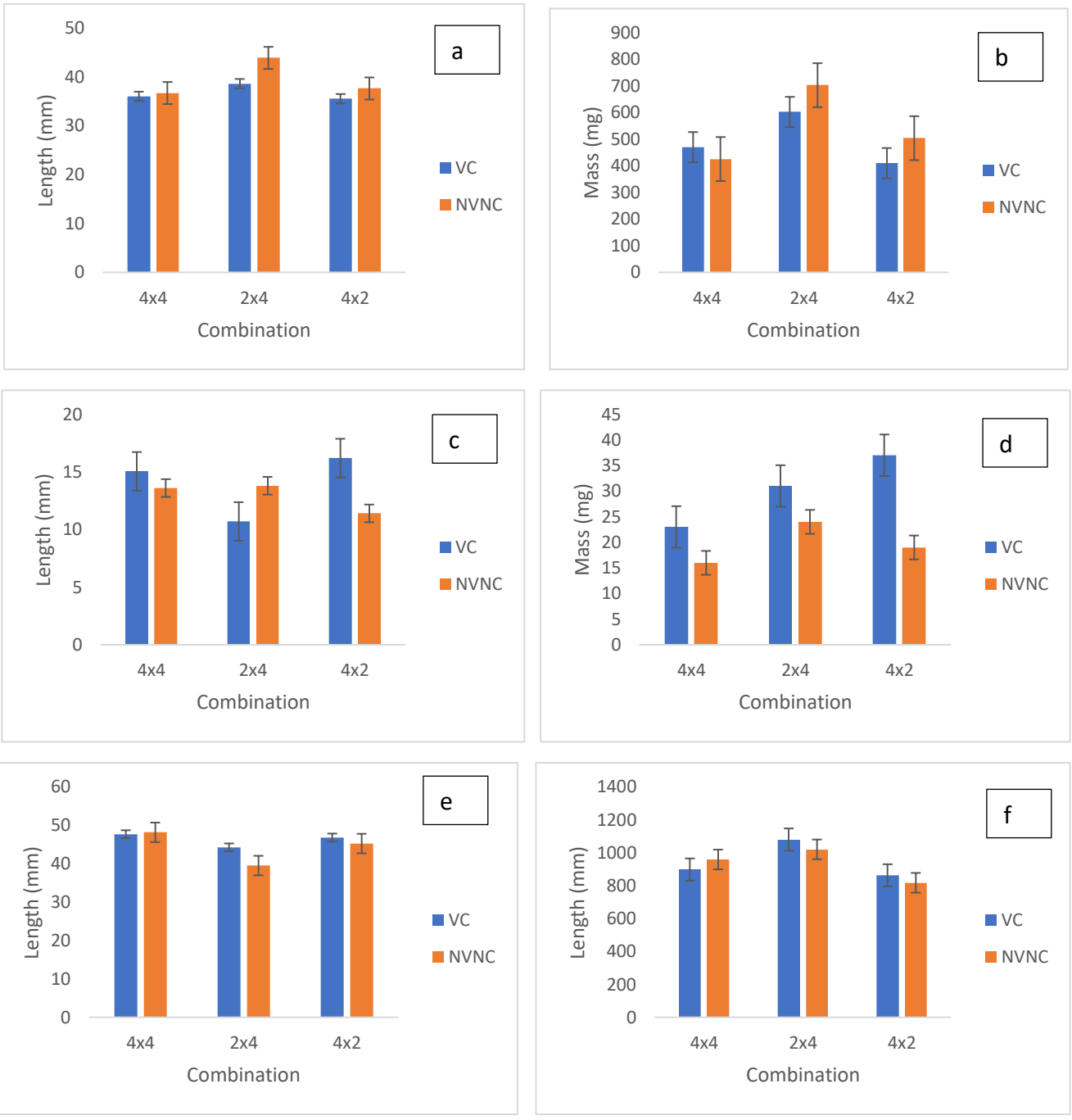

Figure 8(a). Comparison of *R. sylvatica* densities and mean (± SE) lengths on 6/29/19. (b) Comparison of *R. sylvatica* densities and mean (± SE) masses on 6/29/19. (c) Comparison of *A. maculatum* densities and mean (± SE) lengths on 7/13/19. (d) Comparison of *A. maculatum* densities and mean (± SE) masses on 7/13/19. (e) Comparison of *R. sylvatica* densities and mean (± SE) lengths on 7/13/19. (f) Comparison of *R. sylvatica* densities and mean (± SE) masses on 7/13/19

Mortalities

Table 12. ANOVA results of mortalities at the end of experiment,7/13/19. (a) Treatment type comparison of *R. sylvatica* mean mortalities. (b) Density and treatment type comparison of *R. sylvatica* mean mortalities. (c) Treatment type comparison of *A. maculatum* mean mortalities. (d) Density and treatment type comparison of *A. maculatum* mean mortalities.

a.	*D.F.*	*F value*	*p value*
Visual	1	1.923	0.1907
Chemical	1	6.231	0.0281
Visual X Chemical	1	1.923	0.1907

b.	*D.F.*	*F value*	*p value*
Combination	2	1.204	0.3243
Treatment	1	5.174	0.0362
Treatment X Combination	2	0.512	0.6083

c.	*D.F.*	*F value*	*p value*
Visual	1	0.686	0.424
Chemical	1	0.686	0.424
Visual X Chemical	1	0.171	0.686

d.	*D.F.*	*F value*	*p value*
Combination	2	0.247	0.784
Treatment	1	0.067	0.799
Treatment X Combination	2	0.026	0.974

Post hoc tests

Table 13. TukeyHSD test on comparing *R. sylvatica* mean mortalities between treatment types on 7/13/19.

Treatment	*Difference*	*Lower*	*Upper*	*P value*
V-NV	-0.625	-1.606978	0.3569777	0.1907394
NC-C	-1.125	-2.106978	-0.1430223	0.0281132

Table 14. TukeyHSD test on comparing *R. sylvatica* mean mortalities between densities and treatment types on 7/13/19.

Combination	*Difference*	*Lower*	*Upper*	*P value*
4x2-2x4	-0.004464286	-0.4361164	0.4271879	0.9996120
4x4-2x4	-0.223214286	-0.6548664	0.2084379	0.4002828
4x4-4x2	-0.218750000	-0.6357656	0.1982656	0.3903794
Treatment	*Difference*	*Lower*	*Upper*	*P value*
VC-NVNC	0.3080357	0.02171293	0.5943585	0.0365184

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Citations

Abrahams, M. V., and M. G. Kattenfeld. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behavioral Ecology and Sociobiology 40:169–174.

Bairos-Novak, K. R., M. D. Mitchell, A. L. Crane, D. P. Chivers, and M. C. O. Ferrari. 2017. Trust thy neighbour in times of trouble: background risk alters how tadpoles release and respond to disturbance cues. Proceedings of the Royal Society B: Biological Sciences 284:20171465.

Berger, J., P. B. Stacey, L. Bellis, and M. P. Johnson. 2001. A Mammalian Predator-Prey Imbalance: Grizzly Bear and Wolf Extinction Affect Avian Neotropical Migrants. Ecological Applications 11:947.

Berger, Cynthia. *Dragonflies.* 2004. Stackpole Books.

Brown, W. L., Jr., T. Eisner, and R. H. Whittaker. 1970. Allomones and Kairomones: Transspecific Chemical Messengers. BioScience 20:21–22.

Brönmark, C., T. Lakowitz, and J. Hollander. 2011. Predator-Induced Morphological Plasticity Across Local Populations of a Freshwater Snail. PLoS ONE 6:e21773.

Chivers, D. P., A. Mathiron, J. R. Sloychuk, and M. C. O. Ferrari. 2015. Responses of tadpoles to hybrid predator odours: strong maternal signatures and the potential risk/response mismatch. Proceedings of the Royal Society B: Biological Sciences 282:20150365.

Cresswell, W. 2008. Non-lethal effects of predation in birds. Ibis 150:3–17.

Creel, S., D. Christianson, S. Liley, and J. A. Winnie. 2007. Predation Risk Affects Reproductive Physiology and Demography of Elk. Science 315:960–960.

Dawidowicz, P., and C. J. Loose. 1992. Metabolic costs during predator-induced dielvertical migration of Daphnia. Limnology and Oceanography 37:1589–1595.

Eilam, D., T. Dayan, S. Ben-Eliyahu, I. Schulman, G. Shefer, and C. A. Hendrie. 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. Animal Behaviour 58:1085–1093.

Gonzalo, A., P. López, and J. Martín. 2009. Learning, memorizing and apparent forgetting of chemical cues from new predators by Iberian green frog tadpoles. Animal Cognition 12:745–750.

Holbrook, C. T., and J. W. Petranka. 2004. Ecological Interactions between Rana sylvatica and Ambystoma maculatum: Evidence of Interspecific Competition and Facultative Intraguild Predation. Copeia 2004:932–939.

Hemmi, J. M. 2005. Predator avoidance in fiddler crabs: 2. The visual cues. Animal Behaviour 69:615–625.

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

Liu, B., Y. Zhang, and L. Chen. 2005. The dynamical behaviors of a Lotka–Volterra predator–prey model concerning integrated pest management. Nonlinear Analysis: Real World Applications 6:227–243.

Matsunami, M., J. Kitano, O. Kishida, H. Michimae, T. Miura, and K. Nishimura. 2015. Transcriptome analysis of predator- and prey-induced phenotypic plasticity in the Hokkaido salamander (Hynobius retardatus). Molecular Ecology 24:3064–3076.

McCollum, S. A., and J. D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. Oecologia 109:615–621.

Mitchell, M. D., D. P. Chivers, M. I. McCormick, and M. C. O. Ferrari. 2015. Learning to distinguish between predators and non-predators: understanding the critical role of diet cues and predator odours in generalisation. Scientific Reports 5.

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

Oliveira, T. A., R. Idalencio, F. Kalichak, J. G. dos Santos Rosa, G. Koakoski, M. S. de Abreu, A. C. V. Giacomini, D. Gusso, D. B. Rosemberg, R. E. Barreto, and L. J. G. Barcellos. 2017. Stress responses to conspecific visual cues of predation risk in zebrafish. PeerJ 5:e3739.

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

Remage-Healey, L., D. P. Nowacek, and A. H. Bass. 2006. Dolphin foraging sounds suppress calling and elevate stress hormone levels in a prey species, the Gulf toadfish. Journal of Experimental Biology 209:4444–4451.

Relyea, R. A. 2002. Costs of Phenotypic Plasticity. The American Naturalist 159:272–282.

Relyea, R. A. 2003. Predators come and predators go: the reversibility of predator-induced traits. Ecology 84, 1840-1848.

Relyea, R. A. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological Applications 15:1118–1124.

Schoeppner, N. M., and R. A. Relyea. 2009. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. Functional Ecology 23:1114–1121.

Shaffery, H. M., and R. A. Relyea. 2015. Predator-Induced Defenses in Five Species of Larval Ambystoma. Copeia 103:552–562.

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

Stabell, O. B. 2003. Inducible Defences in Daphnia Depend on Latent Alarm Signals from Conspecific Prey Activated in Predators. Chemical Senses 28:141–153.

Stenhouse SL. 1987. Embryo mortality and recruitment of juveniles of juveniles of *Ambystoma maculatum* and *Ambystoma opacum* in Norther Carolina. Herpetologica 43: 496-501.

Wilbur, H. M. 1976. Density-Dependent Aspects of Metamorphosis in Ambystoma and Rana Sylvatica. Ecology 57:1289–1296.

Wilbur, H. M. 1977. Interactions of Food Level and Population Density in Rana Sylvatica. Ecology 58:206–209

Williams, D.D. 1987. The Ecology of Temporary Waters. Timber Press, Portland, OR

Zanette, L. Y., A. F. White, M. C. Allen, and M. Clinchy. 2011. Perceived Predation Risk Reduces the Number of Offspring Songbirds Produce per Year. Science 334:1398–1401.

Appendix

Software for statistical analysis:

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.