The effects of predator chemical cues on the behavior of spotted salamander larvae (*Ambystoma maculatum)*

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Abstract

The detection of kairomones, or chemical cues released by hunting predators, is an important ability of prey that allows them to exhibit anti-predator behavior, thus increasing their chance of survival and fitness. This study aims to elucidate the effect of the kairomones of two native predators, diving water beetle larvae and dragonfly larvae, on the behavior of spotted salamander larvae. Observational laboratory trials were conducted to determine the effect that the addition of kairomone-containing water had on the larvae's preference for depth and cover in their environment. I predicted that the larvae would prefer to live in a shallow environment when treated with dragonfly larvae chemical cues, a deep environment if exposed to diving water beetle chemical cues, and an even split between the two depths when exposed to the kairomones of both predators, based on the predators' different hunting methods. I also hypothesized that the salamander larvae would prefer to inhabit areas with foliage to areas with no foliage when treated with the kairomones of dragonfly larvae and/or diving water beetles. However, it was found that there was no significant difference in mean time spent in each quadrant between each of the treatments. Perhaps the effect of chemical cues is a learned trait for the salamander larvae, and thus the predator naïve larvae used in this experiment had no knowledge of the predators' effects or hunting techniques.

Introduction

The presence of predators has a direct and substantial effect on many aspects of the lives and behaviors of their prey, including where the prey chooses to live, their morphology, foraging techniques, and diet. To identify the density and types of predators present in their environment, aquatic organisms often rely on kairomones, or chemical cues released by hunting predators (Petranka et al. 1987). Some prey are born with an innate recognition of predator kairomones

(Turner 1996, Laurila et al. 1997, Berejikian et al. 2003, Mathis et al. 2003), while others learn through conditioning and experience (Mathis et al. 1996). Through knowledge of the location, type, and number of predators, prey can formulate an appropriate response to increase their chance of survival. The response of prey aids in keeping the predator-prey population in balance (Reynolds and Bruno 2013), which is important for maintaining the diversity that is required for the intricate aquatic trophic cascades that all aquatic organisms rely upon (Jackson et al. 2001).

Amphibian larvae have often been used to analyze the effects of predators in aquatic environments. *Ambystoma maculatum*, the spotted salamander, has been studied many times, and has been found to be a voracious forager; especially when in competition with and in the presence of other predators and can either exacerbate prey (such as zooplankton) declines or contribute to the diversity of their prey (Urban 2013). *Ambystoma maculatum* themselves are also significantly affected by the presence of intra-guild predators, and have significantly less biomass when in the presence of competition, due to predation as embryos and intraguild competition (Walls and Williams 2001). Therefore, it would be beneficial to the salamander larvae, especially the smaller ones in ponds with competitive predators, to be able to sense possible threats and to exhibit anti-predator behavior.

Two common insect predators in the vernal pools where spotted salamander larvae inhabit are dragonfly larvae and diving water beetle larvae, and each has an individual hunting technique. Dragonfly larvae use an ambush style of hunting in which they bury themselves at the bottom of the water and wait for their prey to swim by (Berger and Hansen 2004). Diving water beetle larvae swim through the water, latching on to and attacking prey with mandibles containing digestive enzymes (Johansson and Nilsson 1992). Salamander larvae are very small and often live in the litter of vernal ponds (Petranka 1998). Being that about 90% of salamander

larvae die before they transform (Petranka 1998), the detection of chemical cues produced by these predators could aid the salamander larvae by inducing life-protecting behavior. Predator chemical cues produced by the diving beetle and dragonfly naiads have already been found to have significant effects on phenotypically plastic responses of salamander larvae (Storfer and White 2004), especially looking at their size. The presence of chemical cues not only affects amphibian morphology, but behavior as well. Looking at another amphibian, Schoeppner and Relyea (2005) found that alarm cues induced in prey by predators caused tadpoles to seek refuge in leaf litter more often. Previous studies have found that salamander larvae increase the use of refuges in the presence of predators (Sih et al. 1992), but no studies have examined the behavior of the larvae in terms of depth preference. Therefore, this study poses a unique question in determining the larvae's preference in both depth and cover in the presence and absence of predators.

The purpose of this study is to determine whether the behavior of *A. maculatum* is affected by the presence of chemical cues produced by two predators in their natural habitat: the diving water beetle of the family Dytiscidae and Odonate naiads of the family Libellulidae. In particular, this study will look at what depths the salamander larvae prefer to inhabit and the amount of foliage they prefer. I hypothesize the larvae will prefer to live in a shallow environment when treated with dragonfly larvae chemical cues, a deep environment if exposed to diving water beetle chemical cues, and they will split evenly between the two depths when exposed to the kairomones of both predators. I also hypothesize that the salamander larvae will prefer to inhabit areas with foliage to areas with no foliage when treated with the kairomones of dragonfly larvae and/or diving water beetles.

Materials and Methods

Study Organisms

Salamander larvae of the species *Ambystoma maculatum* were collected as a single egg mass from vernal pond 5 (Figure 1) at the University of Notre Dame Environmental Research Center (UNDERC). Because all the salamanders used in the study were hatched from the same egg mass, they were half-sibs and thus share at least half of their genetic make up. This removes a possible genetic variable influencing behavior. The larvae were fed zooplankton obtained in the pond from which they were collected. The zooplankton were rinsed with tap water to prevent the introduction of foreign chemical cues.

Dragonfly larvae in the family Libellulidae and diving water beetle larvae in the family Dytiscidae were obtained through dip netting in vernal ponds P and 9 (Figure 1). They were fed ants to ensure that the kairomone water did not contain alarm cues from the *Ambystoma maculatum* species, or any other aquatic species.

Experimental Design

Tanks were set up on a shelving unit with 5 replicate tanks for each of the 4 treatments: a tap water control, a diving water beetle larvae kairomone treatment, a dragonfly larvae kairomone treatment, and a treatment containing kairomones from both predators. Kairomones were added to the tank by taking water from the tanks in which the predators were kept (7 of each predator in their respective tanks with about 8 liters of water) and adding this 125mL of kairomone-containing water to the corresponding treatment tanks, to ensure the safety and humane treatment of the salamander larvae (McCarthy and Fisher 2000). For the control treatments, 125mL of aerated tap water was added to the tanks, and for the treatment with both

predator kairomones, around 60mL of each predator's water were added to the corresponding tanks.

The tanks were divided into four equally sized quadrants, two deep and two shallow. One of each of the depths contained foliage, resulting in four different quadrants (Figure 2). The different depths were achieved using 5 quarts of sand and creating an incline at the middle of the tank. The sand in the deep side was 0.5 inches deep, while the sand in the shallow side was 2 inches deep. Water was added until the surface was about 4 inches above the sand in the deep quadrants and 2.5 inches above in the shallow quadrants. Leaves were collected from vernal pond 5 and rinsed and dried, and about 10 grams of leaves of about equal size were added to the quadrants with foliage.

The study began on July 3rd, 2013; about 3 weeks after the larvae began hatching (the first one was seen on June 12th). A single larva was placed in each treatment tank and observed for 5 minutes during each of 3 observation periods, between 0800-1000 h, 1200-1400 h, and 1800-2000 h, over the course of 7 days, imitating the study done by Brodman and Jaskula (2002). Kairomone water was added 10 minutes before the first and third observation periods each day. The amount of time the larva of each tank spent in a certain quadrant out of the 5-minute observation periods was recorded.

Statistical Analyses

A one-way ANOVA was run for each treatment (control, dragonfly, beetle, both predators) to ascertain if there was a difference in mean time spent in each quadrant. If there was a significant difference, a Tukey's HSD was run to elucidate between which quadrants the means were different. The data was also pooled based on depth and cover, and a one-way ANOVA was run with each pooled data set for every treatment.

Results

There was a significant difference in mean time spent in each quadrant for the control

treatment Fig. 3) and a Tukey's HSD test revealed a significant difference (P=0.0402) between

quadrant two (shallow; no leaves) and quadrant four (shallow; leaves). However, there was no

significant difference in mean time spent in each quadrant for the beetle larvae treatment

(F=0.136; df=3, P=0.937), the dragonfly larvae treatment (F=0.691; df =3, P=0.583), or the

treatment with both predator kairomones (F=2.014; df =3 , P=0.166).

There was no significant difference between time spent in shallow versus deep quadrants

for the control treatment (F=2.769; df=1, P=0.135), the beetle larvae treatment (F=0.158; df =1,

P=0.705), the dragonfly larvae treatment (F=0.652; df=1, P=0.465) or the treatment with both

predator kairomones (F=3.261; df=1, P=0.121).

Finally, the data was pooled based on cover to determine if there was a difference in

mean time spent in either quadrants with cover or quadrants with no cover. There was no

significant difference between time spent in cover versus no cover for the control treatment

(F=1.09; df=1, P=0.327), the beetle larvae treatment (F=1.856; df=1, P=0.222), or the dragonfly

larvae treatment (F=0.017; df=1, P=0.903). However, there was a significant difference in mean

time spent in cover vs. no cover quadrants in the treatment with both sets of kairomones (F=9.6;

df=1, P=0.0212, Figure 4). The larvae averaged 45 minutes in quadrants with no cover (1 and 2),

and only 25 minutes in quadrants with cover (3 and 4).

Discussion

Contrary to my hypothesis, there was no difference in mean time spent in each quadrant

for the different predator treatments. There also was no significant difference in preference for

shallow or deep quadrants among the different treatments. No significant difference was seen in

preference for covered or non-covered quadrants as well, except for a significant preference for non-covered quadrants in the treatment with both predator kairomones.

   Arguably the most obvious and broad assertion that could be made from these results is that predator kairomones do not have an effect on the behavior of spotted salamander larvae. While literature suggests the opposite, specifically in regards to the morphology of salamander larvae (Storfer and White 2004), their preference for refuges, and amount of activity in the presence of predator chemical cues (Sih and Kats 1991), this study found that the addition of predator kairomones had no effect on salamander larvae behavior. One reason for the larvae's lack of responses could be due to the fact that they were hatched in captivity. Hepper and Waldman (1992) found that embryonic olfactory learning plays a part in tadpole preferences. Because salamander and frog egg masses are very similar, the two amphibians' larval development can be compared. If the egg masses obtained for this study were not in the pond for very long, the larvae might not have had time to learn the kairomones of the predators used in this study. Other amphibians have been seen to acquire recognition of their predators in their habitats (Chivers et al. 2001), and so a response may not have been seen because the larvae were raised in captivity and never introduced to any predators.

   Another reason for the lack of response could be a low population of the predators used in this study in vernal pond 5, the pond from which the egg mass was obtained. When the egg mass was found, beetle and dragonfly larvae were also sighted, but not in the same abundance as in other vernal ponds. Thus they were caught in vernal ponds P and 9. Ferrari and Brown found that with increasing concentration of cues, there is an increase in prey response (2009). Therefore, drawing from what was mentioned above about olfactory learning, if there was only a

small population of predators in vernal pond 5, the un-hatched larvae may not have had a high enough concentration of chemical cues to learn a predator response for.

An absence of response to the predator chemical cues could also be because the predators were not physically in the tanks with the salamander larvae. Maybe sight plays more of a role than kairomones in the response of the larvae to the presence of predators. It has been found that flathead minnows have learned to generalize their anti-predator responses based on the sight of a potential predator (Ferrari et al. 2010). Perhaps larval salamanders base their anti-predator responses in the same way, and without a visual cue the larvae generalized their reactions and behaved in response to a general, unknown predator, and therefore there was no difference seen in quadrant preference between the treatments of different predators.

However, the larvae did prefer non-covered quadrants to covered quadrants when treated with kairomones of both predators. One previous study looking at the effects of multiple predator guilds in aquatic ecosystems found that where predator foraging domains do not overlap, thus giving prey no effective refuge, there is an increase risk of predation for the prey (Ford and Swearer 2012). The prey aim to avoid the habitats preferred by the predators, and are sandwiched between the territories in their environment. Conversely, when predators share narrow domains in an environment while the prey are able to inhabit multiple habitat domains, there is less risk for the prey and more competition between the predators (Schmitz 2007). Perhaps the salamanders preferred to inhabit domains less inhabited by both predators (the covered domains) in order to avoid the predators' habitat.

Further studies could be conducted to determine if a response to kairomones released by diving water beetle and dragonfly larvae is a learned behavior in salamander larvae. This could be done by comparing the responses of predator naïve larvae laid and hatched in captivity to the

responses and behavior of already hatched salamander larvae caught in the wild in a location with known predators. Another study to be conducted could involve the role of sight in salamander response to predators. A predator could be added to the tank in a separate compartment to keep it away from the larva, but still present, seen, and releasing kairomones into the environment.

The ability for prey to detect and recognize the presence of potential predators can aid their survival considerably. Nevertheless, without the appropriate response to the detected predator(s), the ability to sense the attacker is useless. Learning the effects and dangers of predators through sight and other sensory experiences such as alarm cues may play a larger part in the anti-predator behavior of prey than the innate predator responses of naïve prey.

Figures
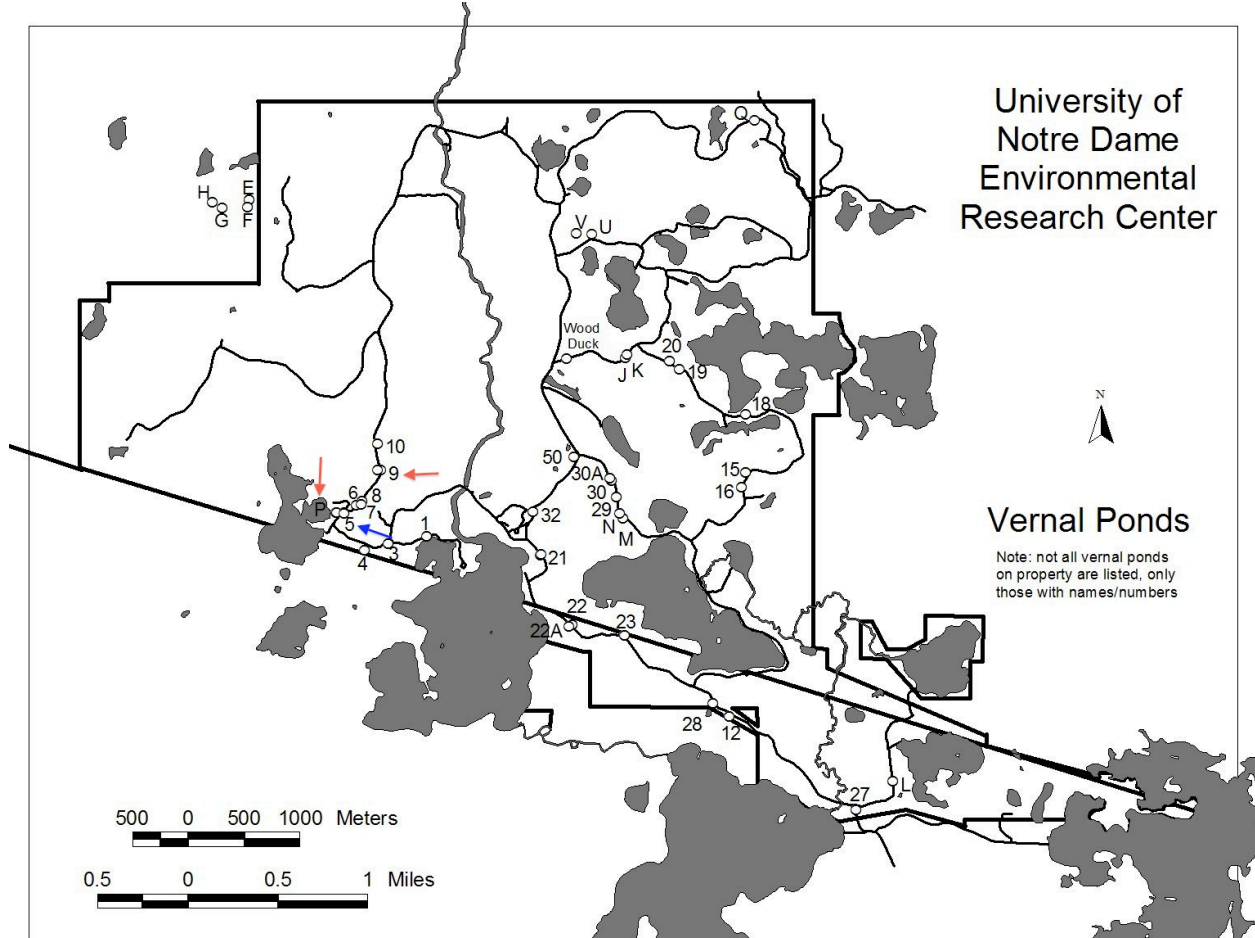
Figure 1. The location of vernal ponds on UNDERC property; red arrows point to the vernal ponds where the predators were obtained, and the blue arrow points to where the salamander egg mass was obtained.

Quadrant 4 Shallow with foliage	Quadrant 2 Shallow without foliage
Quadrant 3 Deep with foliage	Quadrant 1 Deep without foliage

Figure 2. The tanks were set up with equal quadrants, with two deep quadrants and two shallow quadrants. One quadrant from each of the depths contained foliage, and one did not.
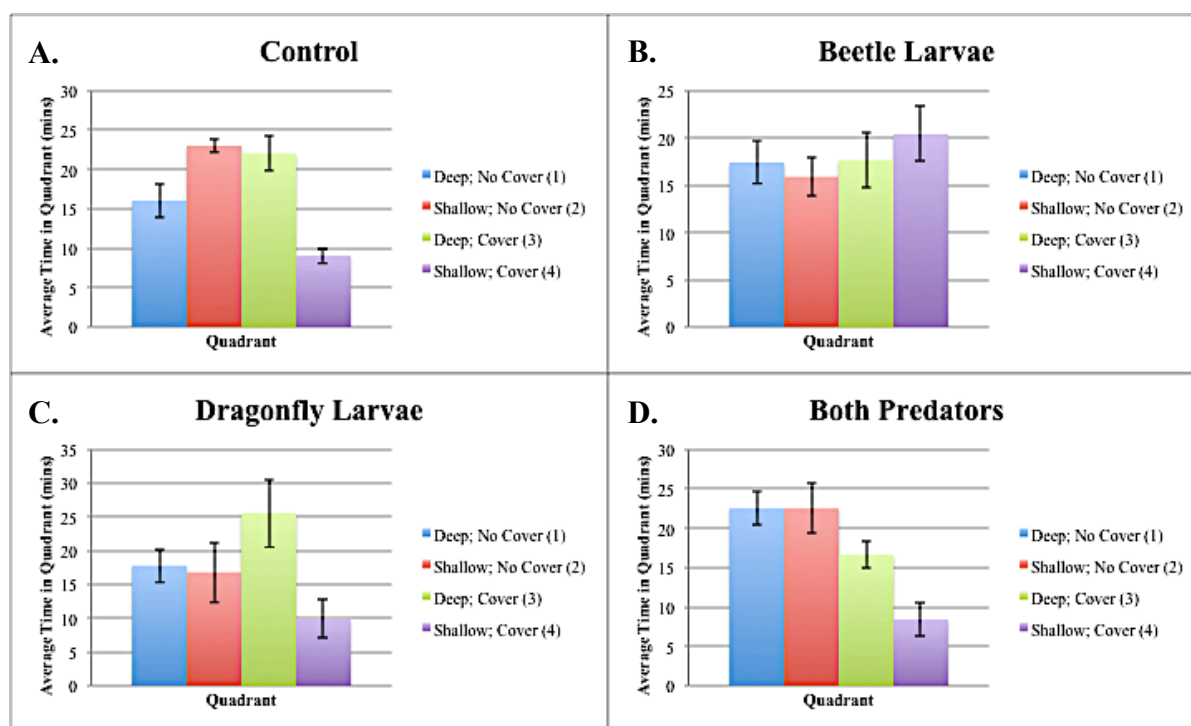
Figure 3. An ANOVA was run for each treatment to compare the difference in mean time spent in each quadrant. **A.** There was a significant difference in mean time spent in each quadrant for the control treatment (F=3.768; df =3, P=0.0321), and a Tukey's HSD revealed a difference between quadrants 2 and 4 (P=0.0402). **B.** There was no significant difference in mean time spent in each quadrant for the beetle larvae treatment (F=0.136; df=3, P=0.937). **C.** There was no significant difference in mean time spent in each quadrant for the dragonfly larvae treatment (F=0.691; df =3, P=0.583). **D.** There was no significant difference in mean time spent in each quadrant for the treatment with both predators' kairomones (F=2.014; df =3, P=0.166).
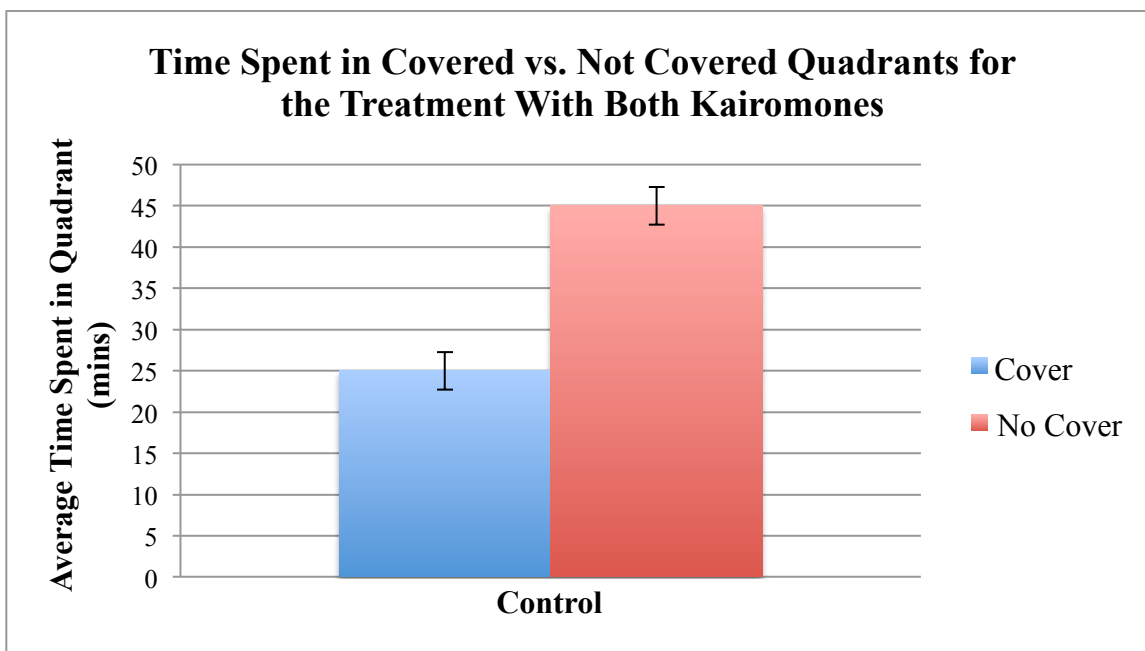
Figure 4. An ANOVA for the pooled data comparing mean time spent in covered vs. not covered quadrants for the treatment with both predator kairomones resulted in a significant difference between the two ($F=9.6$; $df=1$, $P=0.0212$). The larvae averaged 45 minutes in quadrants with no cover (1 and 2), and only 25 minutes in quadrants with cover (3 and 4).

Acknowledgements

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