

Palatability and Defense of Eastern Newt (*Notophthalmus viridescens*) Larvae and Metamorphic Juveniles against Predatory Dragonfly Nymphs

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Abstract - We examined the predator–prey relationship between nymphs of the predatory dragonfly *Anax junius* (Common Green Darner) and larval and metamorphic *Notophthalmus viridescens* (Eastern Newt), some of which may contain the potent neurotoxin, tetrodotoxin. First, we conducted a palatability study to determine which life-history stages were palatable to dragonflies. We also tested the metamorphosis and survival rates of larval newts when exposed to predatory dragonflies in small microcosms. Finally, we tested the predator avoidance behavior of larval newts in response to chemical cues from a control, food stimulus, and predatory dragonflies. All life-history stages (small and large larvae, and recent metamorphs) were palatable to dragonflies. In microcosm trials, we found that newt larvae had a lower chance of surviving and transforming when dragonflies were present compared to a control. Finally, newt larvae decreased movement significantly when exposed to predatory dragonfly stimulus compared to either a control or food stimulus. These results suggest dragonflies are effective predators of newts from hatching through metamorphosis. However, the larvae do possess behavioral avoidance mechanisms that likely reduce the risk of predation by dragonflies.

Introduction

The act of predation can be viewed as a series of stages culminating in the death of a prey organism (Endler 1986). For prey, the sequence progresses from stages entailing relatively high probabilities of escape (low risk) to those in which the risk of death is high and subsequent probability of escape is low (Endler 1986, Hopkins et al. 2011). This predation risk continuum has led to the evolution of numerous morphological adaptations, behavioral strategies, and chemical means by which prey can reduce their risk of predation throughout the various stages (Edmunds 1974). Species often use a mixture of these traits and behaviors in concert to maximize their probability of escape at any particular point within the interaction (Edmunds 1974, Endler 1986, Hopkins et al. 2011).

For prey, an effective strategy is often to avoid contact with a predator by exhibiting predator-avoidance mechanisms, thus preventing the initiation of a predation event in the first place (Brodie et al. 1991). For example, organisms in aquatic environments commonly use chemical stimuli to determine if a potential predator is in close proximity (Solomon 1977). In these circumstances, prey often respond with reduced activity, spatial avoidance, or temporal avoidance (Brodie and Brodie 1991, Lima and Dill 1990), all of which can reduce the probability of being detected

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by a predator and preventing the predation event from initiating (Edmunds 1974, Sih 1985). Nevertheless, prey that respond too frequently with predator avoidance behaviors may also be at a selective disadvantage due to trade-offs with fitness-enhancing activities such as foraging or mating (Lima and Dill 1990). Therefore, prey often also rely on morphological or chemical defenses thus ensuring predation risk is minimized during these circumstances. For example, the Eastern Newt possesses the neurotoxin tetrodotoxin (TTX) in its skin (Brodie 1968, Yotsu-Yamashita et al. 2012). Adult newts are aquatic, and the toxin appears to be an effective deterrent against predation by some aquatic invertebrates, crayfish, fishes, and water snakes (Brodie 1968, Marion and Hay 2011). However, *Chelydra serpentina* (L.) (Common Snapping Turtle) are not deterred by the toxin, and newts respond with predator avoidance behaviors in response to these turtles but not other species that do not eat newts (e.g., water snakes; Chapman et al. 2017).

For other developmental stages of the Eastern Newt, the role of toxicity and behavior in reducing predation risk is not as well understood. Eastern Newts possess a complex life-history, with adult newts depositing eggs in early spring (Petranka 1998). After hatching, the aquatic larvae develop for several months before metamorphosing into the terrestrial eft stage (Petranka 1998). The efts develop on land for several years, after which they transform again into the aquatic adult (Petranka 1998). When depositing eggs, females provision the yolk of their eggs with TTX, which makes them unpalatable to some predators (Marion and Hay 2011). The eft stage is highly toxic, which makes them unpalatable to numerous terrestrial predators (Brodie 1968, Brodie and Brodie 1980, Mebs et al. 2016). However, the role of behavior and toxicity in shaping the predation-risk environment for larval newts is less clear. Maternally derived TTX does appear to protect recently hatched larvae (Marion and Hay 2011) and may protect them for several weeks until the TTX within the yolk is fully metabolized (Brossman et al. 2014, Gall et al. 2011). However, the role of TTX in influencing palatability of older larvae, along with recently metamorphosed individuals, is unknown.

We conducted a series of studies to investigate the palatability, predator avoidance behavior, and propensity for survival and metamorphosis of *Notophthalmus viridescens* (Rafinesque) (Eastern Newt; hereafter referred to generically as newts) during interactions with a predatory dragonfly, *Anax junius* (Drury) (Common Green Darner; hereafter referred to generically as dragonflies). Dragonflies are common predators in the same ponds in which newts develop and are one of the top-predators in fishless systems (Crumrine 2006, Hopkins et al. 2011). In the first study, we tested the palatability of several life stages of newt larvae to determine how willing dragonflies would be to prey on newts. In a second experiment, we tested predator-avoidance behavior in newt larvae when exposed to chemical stimuli from a control (water), food source (worms), and predator (dragonflies). In the final experiment, we exposed larval newts to dragonflies in a microcosm to observe the role of this predator on the survival and metamorphosis rate of larval newts.

Methods

Animal collection and housing

We collected dragonflies and newt larvae via dipnetting from a fishless pond in Switzerland County, IN, in July and August 2017. We housed newts individually in a plastic container (9.5 cm × 11.0 cm) filled with ~0.8 L of dechlorinated tap water (henceforth, water) and housed dragonflies individually in 275-ml glass bowls filled with ~1.6 L of water and a small rock (for perching). All larval newts and dragonflies were fed ~10–15 *Lumbriculus variegatus* (Müller) (Blackworm) 3 times per week. We kept the newts and dragonflies in an environmental chamber at 18 °C on a 12-h light:12-h dark cycle.

Palatability trials

We tested the palatability of small (<25 mm) and large (>30 mm) larval newts and recently metamorphosed newts against predatory dragonflies. We withheld food from each dragonfly for 24 hrs prior to the start of a trial. At the start of a trial, a newt larva of the appropriate size was removed from its holding container with a pipette and introduced directly in front of a randomly chosen dragonfly. We recorded the number of times the dragonfly struck at the newt, the number of strikes in which the dragonfly was successful in grasping the newt, and whether the newt escaped or survived each interaction. Each trial continued for 20 minutes. If a dragonfly was in the process of consuming a newt at the end of the 20 min trial, it was monitored until the dragonfly stopped eating or the newt was entirely consumed. We visually buffered concurrent trials with opaque partitions between each test chamber.

Chemical cue trials

To determine whether newt larvae respond to chemical stimuli from dragonflies with predator-avoidance behavior, we exposed newt larvae (>30 mm) to chemical stimuli from 1 of 3 different treatments: a control (dechlorinated tap water), a food stimulus (chemical cues from Blackworms), and a predator cue (chemical cues from dragonflies). We prepared the chemical cues 48 hrs prior to the start of trials. The dragonfly cue was prepared by placing 3 dragonflies in separate plastic containers (9.5 cm × 11.0 cm) with 50 ml water per 0.1 g body mass. The Blackworm stimulus was prepared by weighing a group of Blackworms and placing them in a plastic container with 50 ml water per 0.1 g mass. Control containers were also prepared in an identical manner, but lacked a stimulus animal. Preparing chemical-cue solutions in this manner is a common technique to test the predator-avoidance behavior of prey organisms in response to both attractive (i.e., food) and aversive (i.e., predator) stimuli (e.g., Chapman et al. 2017, Gyssels and Stoks 2005, Spivey et al. 2015, Wudkevich et al. 1997). We removed the stimulus animals from the containers 48 hrs after their introduction and combined and mixed thoroughly the water separately for each stimulus type to remove variation from individual donor animals. These freshly prepared stimulus cues were used for all trials.

We performed the chemical cue trials in September 2017 between 1600 and 2100 hours. The experimental chamber consisted of a plastic container (6 cm × 11

cm) filled with 200 ml of water. To minimize disturbance to the newt larvae, we removed excess water from each larvae's home container before dumping the larva into the experimental chamber. We then initiated a 20-min acclimation period, followed by a 5-min pre-stimulus observation period during which we recorded the time the larva spent moving; we considered movement to be any motion by the larvae, regardless of intensity. After the pre-stimulus period, we injected 30 ml of a randomly chosen stimulus down the side of the container opposite the larva. We next conducted a 5-min post-stimulus period during which we recorded the time spent moving by the larva. After the trial, the larval newt was transferred back into its home container, the chamber was thoroughly rinsed, and the experimental procedure was repeated ($n = 44$; 14–16 per treatment). Larvae were never re-tested. We visually buffered concurrent trials with opaque partitions between each test chamber.

We subtracted the time spent moving in the pre-stimulus period from the time spent moving in the post-stimulus period to get the change in time moving by the larvae. We then compared the change in time moving between the 3 treatments using a one-way ANOVA followed by Tukey post-hoc comparisons. We used Sigmaplot 12.5 (Systat Software, Inc., San Jose, CA) to conduct all tests and analyses.

Survivorship trials

We conducted microcosm survivorship trials with larval newts (>30 mm) and dragonflies in 38-L aquaria between September and November 2017. Eight tanks (4 control and 4 experimental) were filled with 15 L of water, 450 g of dried vegetation (order: Poales) from the edge of a lake, and a tile (15 cm × 50 cm) angled such that half of the tile was submerged in the water providing a site for metamorphosing newts to leave the water. We placed sphagnum moss on the tile at the water's edge to provide a place for newts to stay moist and take cover after metamorphosis. The tanks were covered with plastic, and the water was aerated for the duration of the trial. We maintained the tanks at 18 °C in an environmental chamber on a 12-h light:12-h dark cycle. We randomly selected 5 newt larvae for placement in each tank. After a 24-hr acclimation period, we added a dragonfly to 4 of the tanks. We added 60 g of Blackworms daily during the first week, followed by every other day until the trial ended. We checked the tanks daily for metamorphosed newts. We moistened the sphagnum and replenished the water in each tank as needed. The trials were terminated when dragonflies metamorphosed (7 weeks; 1 trial) or after 9 weeks (6 trials). After a trial ended, the water and detritus were removed in small aliquots and placed in a white bin. We then carefully sorted the contents of the bin to find any remaining larvae.

We recorded the number of larvae that remained in the water at the completion of a trial. We used a chi-square test to compare the mean number of newts that successfully reached metamorphosis and left the water in the control and dragonfly treatments.

Results

Both small (<25 mm) and large (>30 mm) larval newts were palatable to dragonflies (Table 1). Recently metamorphosed newts were also palatable to dragonflies (Table 1). The entire newt was consumed in all trials except for 1 with a small larva in which all but the tip of the tail was consumed.

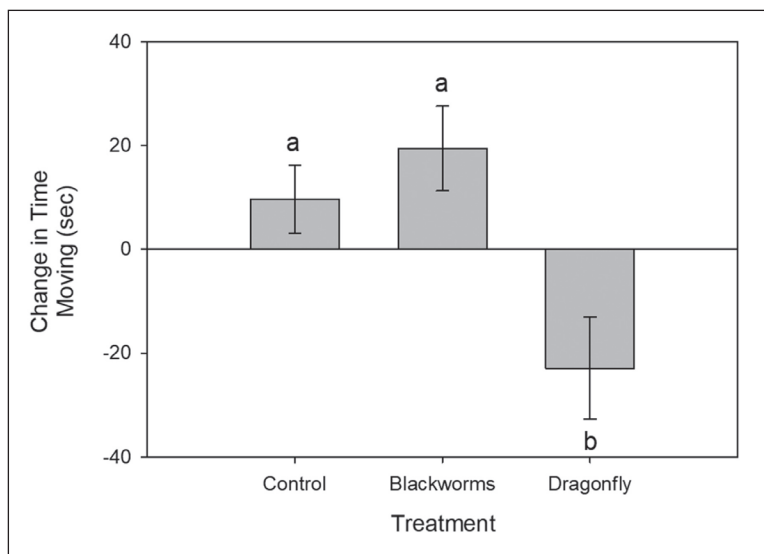
We found a significant difference in the time spent moving between newt larvae exposed to a control (water), a food stimulus (Blackworms), and stimulus from predatory dragonflies ($F_{[2,41]} = 7.2$, $P = 0.002$; Fig. 1). Newt larvae decreased movement when exposed to chemical stimuli from dragonflies, relative to both the control and food stimulus (Fig. 1). There was a slight but not significant ($P = 0.7$) increase in activity in the Blackworm treatment relative to the control.

Newts in the control treatment were more likely to successfully reach metamorphosis and leave the water relative to newts exposed to predatory dragonflies ($\chi^2 = 4.5$, $df = 1$, $P < 0.033$, Table 2). Ninety-five percent (19) of newts in the control treatment completed transformation, whereas only 5% (1) of newts in the dragonfly treatment reached metamorphosis. Survival of newts in the control treatment was 100% (20), while only 5% (1) of newts survived in the dragonfly treatment.

Table 1. Developmental stages of larval *Notophthalmus viridescens* (Eastern Newt) exposed to nymphs of the dragonfly *Anax junius* (Common Green Darner) during palatability trials. All developmental stages were completely consumed by the predatory dragonflies.

Developmental stage	<i>n</i>	Failed attacks	Successful attacks	Consumed
Small larvae (25 mm)	2	1	2	2
Large larvae (35 mm \pm 0.5)	2	1	2	2
Transformed	2	2	2	2

Figure 1. Mean (\pm SE) change in time spent moving by larval *Notophthalmus viridescens* (Eastern Newt) when exposed to a control (water) and chemical stimuli from a food source (Blackworms) and predator (*Anax junius* [Common Green Darner]). Newts significantly decreased activity when exposed to chemical cues from predatory dragonflies ($F_{[2,41]} = 7.2$, $P = 0.002$). Different letters indicate significantly different means (Tukey's post-hoc comparisons: $P < 0.05$).



Discussion

Dragonfly nymphs are considered top predators in fishless ponds and prey on many organisms (Crumrine 2006). In palatability trials in this study, larval newts at all sizes and developmental stages were consumed by dragonflies, indicating that dragonflies are important predators of Eastern Newt larvae in aquatic systems. Previous studies have shown that newt larvae are palatable to some other invertebrate predators such as the predaceous diving beetle *Dytiscus verticalis* Say (Vertical Diving Beetle; Formanowicz and Brodie 1982). For these larvae, the timing of palatability likely depends upon their age and the amount of maternally invested chemical defense. Adult newts deposit TTX in the yolk of their eggs, which provides a period of protection for both the developing embryo and recently hatched larvae (Gall et al. 2011, Hanifin et al. 2003). For example, research by Marion and Hay (2011) found that 2-day-old Eastern Newt larvae were unpalatable to fish. This maternally derived protection probably extends for several weeks after hatching until the TTX is metabolized. A study examining interactions between dragonflies and *Taricha granulose* (Skilton) (Rough-skinned Newt) found that TTX concentrations greater than 0.78 ng/mg body mass provided protection against these predators. While we did not test the TTX concentration of the newts in our study, Eastern Newt larvae from a population in Pennsylvania had ~1.2 ng TTX per mg body mass roughly 15 days after hatching (Brossman et al. 2014), which would likely make them unpalatable to many predators. However, we believe the larvae used in our study were greater than 10 weeks post-hatching and therefore beyond the chemically defended period afforded by their mothers (Gall et al. 2011).

While we expected older larval newts to be palatable, our finding that recently metamorphosed newts were also palatable to dragonflies was surprising. The metamorphic newts tested in our study had fully transformed, possessing the rough skin of the toxic eft stage. During metamorphosis, granular glands arise in the developing skin of the juvenile (Duellman and Trueb 1994, Formanowicz and Brodie 1982). These glands quickly develop noxious secretions (including TTX) that can make newly metamorphosed newts unpalatable to multiple predators, including diving beetles and crayfish (Formanowicz and Brodie 1982). While these predators rejected some recently metamorphosed newts, ~40% of the juveniles tested by Formanowicz and Brodie (1982) were actually palatable (Formanowicz and Brodie 1982). While the palatability of our juveniles relative to other studies could be due to population-level variation in toxicity (Yotsu-Yamashita et al. 2012), it is more

Table 2. Results from experiment in which larval *Notophthalmus viridescens* (Eastern Newt) were placed in a microcosm with a single nymph of the predatory dragonfly *Anax junius* (Common Green Darner) or no predator ($n = 4$ per treatment). Values indicate number of larvae that successfully metamorphosed, remained untransformed, or are presumed eaten by dragonflies during the 9-week trial.

Treatment	Number metamorphosed	Untransformed larvae	Number dead	Total
Control	19	1	0	20
Dragonfly	1	0	19	20

likely that our small sample size ($n = 2$) for this developmental stage obscures the fact that some individuals are actually unpalatable.

Dragonfly nymphs are sit-and-wait predators that rely on visual cues to detect prey (Chovanec 1992a). When prey are within range, they strike using palpal lobes located on the labium to grasp the victim, which is then slowly consumed (Corbet 1999). The ponds we sampled contain very thick aquatic vegetation, and predators are likely well hidden in these habitats. In such visually obstructed environments, chemical communication is often an important channel by which prey can avoid predation (Dodson et al. 1994, Ferrari et al. 2010). In our trials exposing larval newts to chemical stimuli from dragonflies, larval newts responded by reducing activity. Other studies have shown adult newts also decrease movement in the presence of predators including Common Snapping Turtles and predatory *Ambystoma tigrinum* (Green) (Tiger Salamander) larvae (Chapman et al. 2015, Mathis and Vincent 2000). Because dragonfly nymphs have been documented to prey more heavily on species that are more active (Chovanec 1992b), mediating activity patterns may lead to higher survivorship in Eastern Newt larvae.

Despite reducing activity in experimental trials, these behavioral changes were not sufficient to protect larval newts from predation during the microcosm survivorship trials. In these trials, virtually all newts were consumed, reaffirming that dragonflies are successful predators of larval newts. While decreasing movement should be an effective response to avoid detection of dragonflies, our experimental microcosm had homogenous plant cover and limited prey diversity relative to a natural pond setting. In addition, behavioral adjustments in response to predation often conflict with behaviors facilitating growth. If larval newts continuously limited mobility in the microcosm, foraging would be reduced leading to limited nutrient consumption, a decrease in growth, and an ultimate cost to fitness (Dixon and Baker 1988, Lima and Dill 1990). Eventually, larval newts would likely be forced to increase activity to find food, thus increasing their exposure to the dragonfly.

Our study suggests that dragonfly nymphs find larval and recently metamorphosed newts palatable. In a microcosm experiment, dragonflies dramatically reduced newt survivorship and the likelihood of reaching metamorphosis. In response, larval newts appear to chemically detect dragonflies and exhibit predator-avoidance mechanisms to reduce the chance of a predation event. In a natural setting, these behavioral adjustments in response to dragonflies likely help to increase the probability of surviving and reaching metamorphosis for the newt life-history stages that possess little chemical defense.

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