Hydrobiologia (2015) 749:69–81
DOI 10.1007/s10750-014-2146-4

PRIMARY RESEARCH PAPER

Effects of newt chemical cues on the distribution and foraging behavior of stream macroinvertebrates

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Received: 26 September 2014 / Revised: 18 November 2014 / Accepted: 5 December 2014 / Published online: 19 December 2014

Abstract Many amphibians possess noxious or toxic substances for self defense. These compounds have been characterized largely as chemical defenses, but may promote ecological and evolutionary processes. The California newt, *Taricha torosa*, possesses a potent neurotoxin, tetrodotoxin (TTX), which serves as a chemical defense, chemical cue to conspecifics, and selection pressure that has selected for evolved resistance in a predator. However, the potential effects of TTX upon the broader community and on behavior, in general, have been overlooked. Field assays conducted during the newt breeding season indicate that the macroinvertebrate community responds to adult newt chemical cues by altering foraging behavior. In these assays, significantly fewer macroinvertebrates were found in experimental areas with enclosed newts relative to enclosures with a non-predatory amphibian. Laboratory bioassays showed that dragonfly nymphs (*Anax junius*) reduced predatory behavior and moved less in the presence of adult newt chemical cues. When exposed to TTX, nymph mean angular velocities were reduced four fold and mean velocity magnitude was reduced threefold relative to controls. Overall, these results support the hypothesis that chemical stimuli from predators, and TTX specifically, can shape species interactions at lower trophic levels and potentially affect community organization.

Keywords Chemical cues · Macroinvertebrates · Tetrodotoxin · Newts · *Taricha torosa* · *Anax* · Ephemeroptera · Santa Monica Mountains · Kairomone · Predator–prey

Introduction

Predation can have strong influences on prey populations, community organization, and ecosystem functions (Paine, 1966; Menge, 2000). Predators often affect communities directly via consumption (Sih et al., 1985), but they may also indirectly do so by inducing non-lethal effects (Lima & Dill, 1990) and alternative behavioral characteristics (Werner & Anholt, 1996; Kats & Dill, 1998). Such altered traits may include defensive posturing, initiating an evasive response, feigning death, hiding, and reducing activity (Endler, 1986; Lima & Dill, 1990; Scrimgeour & Culp, 1994; Relyea, 2000; Trussell et al., 2003; Ferrari et al., 2010). Modified traits may further affect the composition and structure of communities via

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Handling editor: Nicholas R. Bond

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changed species interactions (Abrams, 1983), resulting in significantly different feeding ecologies (Skelly, 1992; Wissinger & McGrady, 1993; Schmitz et al., 1997). Ultimately, ecologists have learned that direct and indirect effects of predator and prey interactions can profoundly influence community composition and diversity.

Historically, the effect that predators have upon communities has been assessed by quantifying the rates at which they consume prey. Though these studies have produced valuable concepts, such as trophic cascades (Carpenter et al., 1985), they have asymmetrically represented the predator perspective in predator–prey relationships. How prey respond to predators remains unclear in many systems. Furthermore, the indirect effects that predators may have upon the community remain vague, even though it is apparent that the indirect effects of some predators may exceed or match their direct effects (Trussell et al., 2003). The plasticity of prey phenotypes in response to predators, whether developmental, morphological, physiological, or behavioral, may have consequences upon species interactions at multiple trophic levels (Abrams, 1995; Werner & Peacor, 2003).

Chemical compounds released by predators can influence the behavior of prey species, as well as other organisms within the community (Petranka et al., 1987; Dahl, 1998; Turner et al., 2000; Hay & Kubanek, 2002; Pohnert et al., 2007; Hay, 2009), and facilitate numerous ecological processes (Pawlik, 1992; Hadfield & Paul, 2001; Roberts & Uetz, 2005; Chivers et al., 1996; Tamburri et al., 1996). Macroinvertebrates have demonstrated vast behavioral responses to predator chemical cues (Burks & Lodge, 2002; Dodson et al., 1994), whereby the indirect effects of these compounds elicit decreased browsing, foraging, and reproductive activity (Juliano & Gravel, 2002; Koch et al., 2007; Schneider et al., 2014), reduced predatory behavior (Wisenden et al., 1997), altered habitat use (Camacho & Thacker, 2013), and increased attachment strength to substrates and aggregation rate (Naddafi & Rudstam, 2013).

The unique chemical defenses that numerous taxa have evolved may elicit similar behavioral responses, potentially promoting ecological, as well as evolutionary processes beyond just the survival of those individuals (Elliott et al., 1993; Brodie et al., 2005). Larval Lepidopterans utilize pyrrolizidine alkaloids as a chemical defense, but it serves as a sexual attractant for adults (Weller et al., 1999). Amphibian noxious or toxic skin substances, which have long been viewed as poisons or feeding deterrents, may also facilitate intraspecific competitive interactions (Summers, 1999; Crossland et al., 2012) and influence the selection of larval rearing sites (Schulte et al., 2011). Given the strong role of these substances in predator–prey relationships (Daly, 1995), chemical defense molecules may not only affect prey directly, but may also broadly impact prey populations and community organization indirectly.

The California newt (*Taricha torosa*) and all congeners possess a potent neurotoxin, tetrodotoxin (TTX) that is considered to serve foremost as a chemical defense (Twitty, 1966). However, TTX also acts at other ecological and evolutionary scales. During aquatic life history stages, adult *T. torosa* are potentially cannibalistic. The neurotoxin, which is continuously given off by adults as a byproduct of chemical defense, elicits an antipredator behavior in *T. torosa* larvae (Elliott et al., 1993; Zimmer et al., 2006). For another *Taricha* species, the rough-skinned newt, *T. granulosa*, the toxin has acted as a selection pressure for resistance in predatory garter snakes (Brodie et al., 2002). These studies demonstrate the immense selective pressure TTX can exert within systems. However, what effect newt chemical cues, and specifically TTX, have upon organisms at lower trophic levels remains largely undetermined.

The hypothetical effects newt chemical cues and TTX may have on lower trophic levels can be investigated by testing whether sympatric macroinvertebrate species within lotic habitat respond to it. Macroinvertebrates are fully capable of perceiving and responding to chemical cues from predators (Krieger & Breer, 1999) and the modalities for such responses can be morphological, physiological, or behavioral (Tollrian & Harvell, 1999). Though the sclerotized cuticle of many macroinvertebrates traditionally is thought to function as an antipredator mechanism and physical barrier to mitigate external selective pressures (Wood et al., 2010; Castellanos et al., 2011), it also links external stimuli to internal neuronal pathways (Casas & Dangles, 2010). Newt chemical cues, and specifically the highly potent neurotoxin TTX that is found in these cues, may affect macroinvertebrates behavior, either as a result of initiating an antipredator response or by sub-lethal poisoning.

Stream habitats in southern California (USA) provide an ideal system to evaluate our hypotheses as macroinvertebrates and *T. torosa* co-occur during the breeding season of newts. As generalist consumers, adult *T. torosa* predominantly feed upon the macroinvertebrate community (Kerby & Kats, 1998), with the majority of their diet comprised of Ephemeroptera (Bucciarelli and Kats, unpublished data). In this study, we investigate if chemical cues from *T. torosa* affect the behavior of the macroinvertebrate community by altering foraging behavior and if TTX has any effect on invertebrate predator behavior. Our study is composed of three specific questions: (1) Does exposure to chemical cues from captive newts influence habitat selection by aquatic macroinvertebrates; (2) do newt chemical cues affect macroinvertebrate predator foraging behavior; and (3) does exposure to waterborne TTX affect macroinvertebrate locomotor activity.

Methods and materials

Field behavioral bioassay of newt chemical cues

Field behavioral bioassays of macroinvertebrate responses to newt chemical cues were conducted in Cold Creek Preserve (Los Angeles County, California, USA) using prefabricated transparent acrylic enclosures (18 cm long × 10 cm wide × 14 cm high). Each enclosure was ventilated by drilling 1-cm hole into the top. An acrylic grated bottom (18 cm long × 10 cm wide; grate size = 1.25 cm × 1.25 cm) allowed chemical cues from an animal to diffuse into pools.

Adult male *T. torosa* (mean $SVL \pm \mathrm{SD} = 77.5$ mm ± 0.14 mm, $n = 10$) and adult Pacific tree frogs, *Pseudacris regilla* (mean $SVL \pm SD = 31.1$ mm ± 0.88, $n = 10$) were collected by hand immediately before the start of a trial. This frog species was chosen as a biological control to provide an additional source of amphibian chemical cues because it is a habitat generalist that coexists in riparian habitats with *T. torosa,* is abundant, breeds in streams, and is often observed in stream pools. We also chose *P. regilla* because adults do not prey upon aquatic invertebrates. Enclosures were oriented with the long axis parallel to the direction of the stream current, grate side down, and approximately 12-cm deep in a pool. Each enclosure rested on a small frame to allow for approximately 3 cm between the bottom of the enclosure and the bed of the pool. An airspace of 2 cm remained at the top of the enclosure to allow animals to surface. Selected pools were devoid of newts and frogs, and no newts or frogs were observed 50 m upstream or downstream from experimental pools.

In total, we performed 10 replicates over a two-week period. We used 10 pools and no more than one trial was conducted in a pool at a time. A trial consisted of two paired enclosures, one housing an adult *T. torosa* and the other a single *P. regilla*. Each enclosure was separated by at least 80 cm. A distance of approximately 30 cm remained between each enclosure and the bank. The location of enclosures in a pool was varied for each trial. Enclosures were anchored and left until the following morning (~12 h). Upon returning, a 20-cm-diameter ring was placed around each enclosure to establish a search area. We chose a 20-cm diameter because it allowed for at least three diameters distance between each paired enclosure and because compounds released from newts in similar flow regimes (~1–2 cm^{-1}) are detectable at close proximity (1–9 cm from the animal, D. Schar, unpublished data). A distance of at least 100 m was maintained between experimental pools when multiple trials were conducted simultaneously.

At the end of trials, the location of each enclosed animal was noted. All animals were released by hand into the original pool they occupied. Rocks, vegetation, and cobble were collected to enumerate and identify macroinvertebrates within each search area. For each replicate, the number of macroinvertebrates was totaled by order for a frog area and a newt area. Count data from the ten frog areas were then merged and a final tally of macroinvertebrates for each order within all frog areas produced. This was repeated for newt areas. To test the effect of newt chemical cues on macroinvertebrate habitat selection, we compared tallies of macroinvertebrates by order between frog and newt areas using a Chi-square test. Thus, each row of the matrix in the 5 × 2 contingency table represented a macroinvertebrate order (Ephemeroptera, Trichoptera, Coleoptera, Plecoptera, and Odonata).

Collection of egg masses and larval culture for laboratory assays

A total of five *T. torosa* egg masses were collected from Cold Creek Preserve and transported in stream water to the laboratory. Egg masses were selected based on embryo developmental stage to ensure larvae hatched at similar times. Egg masses were incubated in 5-μm-filtered, dechlorinated tap water in a walk-in cooler at stream temperature (11.1–12.6°C) and maintained on a 12:12 h light:dark cycle. Larvae were fed ad libitum crushed or whole black worms (*Lumbriculus variegates*). Approximately 30 days post-hatch, larvae were collected for use in macroinvertebrate behavioral bioassays.

Preparation of newt chemical cues

Adult male newts (mean $SVL \pm SD = 73.6$ mm $\pm$ 2.05 mm, $n = 15$) were collected by hand from Cold Creek Preserve. Newt chemical cue solutions were prepared in the field by placing five newts in ~1 l of 5 μm-filtered, dechlorinated tap water for 1 h. The dorsum of each newt was massaged for 1 min at the start of the hour to stimulate the release of skin secretions (Zimmer et al., 2006). At the end of the hour, newts were removed and released. Three batches were prepared, combined, and then transported back to the laboratory. The solution was brought to a final volume of 4 l, filtered to 0.45 μm, and then held at stream temperature before being used in laboratory behavioral bioassays. The entire solution was used within 3 h of being prepared. A 1-ml aliquot was taken from the final 4 l solution, and high-performance liquid chromatography coupled with fluorescence detection (HPLC–FLD) was used to confirm the presence and concentration of TTX. Calibration curves attained from analyzed standard TTX solutions were used to derive the concentration of TTX in our sample. Details of the chromatography methods are outlined in Bucciarelli et al. (2014).

Laboratory behavioral bioassay of newt chemical cues

Antipredator behavior in response to chemical compounds is observable in numerous taxa (Kats & Dill, 1998). However, it is unclear whether *T. torosa* chemical cues mediate similar behaviors in the macroinvertebrate community. One of the predatory odonate nymphs in this community, the green darner (Aeshnidae: *Anax junius*), was selected to test behavioral responses to *T. torosa* chemical cues and TTX. This odonate is an abundant and common predatory macroinvertebrate whose aquatic life history stages overlap *T. torosa* breeding and larval development periods. Additionally, green darners feed on *T. torosa* larvae and adult *T. torosa* feed on *A. junius* (Kerby & Kats, 1998). Nymphs were collected from Cold Creek Preserve 24 h before the start of experiments at sites upstream from our experimental pools. All nymphs were collected on the same day and transported in stream water to the laboratory. Individuals were housed in semi-opaque acrylic chambers (33 cm long × 20 cm wide × 12 cm high) filled with 250 l of 5-μm-filtered, dechlorinated tap water at stream temperature and maintained on a 12 h:12 h light:dark cycle.

Preliminary feeding experiments were conducted to determine *A. junius* feeding behavior. Nymphs were starved for 24 h and then fed ad libitum *L. variegates*. *Anax junius* was continuously fed and demonstrated predator behavior up to 140 min. Laboratory behavioral experiments were therefore terminated at 150 min.

Dragonfly nymph behavioral bioassays in response to newt solutions were performed in non flow-through semi-opaque acrylic chambers (33 cm long × 20 cm wide × 12 cm high). Dechlorinated, 5-μm-filtered, tap water at stream temperature (12–15°C) was used to bring each chamber to a volume of 750 ml. A semi-opaque acrylic glass plate (8 cm long × 8 cm wide) with four metal legs rested horizontally 2 cm from the bottom of each chamber to mimic stream refuge.

Before the start of trials, a nymph (mean length $\pm$ $SD = 37.9$ mm $\pm$ 3.8 mm, $n = 36$) was transferred to a chamber and allowed to acclimate for 5 min. At the start of each trial, three *T. torosa* larvae (mean length $\pm$ $SD = 18.68$ mm $\pm$ 0.41 mm, $n = 99$) were placed in a chamber with the nymph. A total of 250 ml of the newt solution was added to each of half of the chambers. Stream water (5-μm-filtered) was used as a control and added to the remaining chambers.

Nymph movements, use of refuge, and number of surviving larvae were noted every 10 min for 150 min. Nymphs were considered to move if they were observed crawling, swimming, striking at prey, or using limbs. The proportion of observations a nymph moved was calculated and percentages arcsine transformed. All data were normally distributed. A *t* test was used to detect if *T. torosa* chemical cues affected nymph movement.

The number of surviving larvae in each replicate at every 10 min interval was used to evaluate if newt chemical cues affected predatory behavior in *Anax junius*. Data were shaped to long format and treatment, replicate, time, and number of surviving larvae encoded as variables in STATA/SE (v 12.0). A Poisson regression tested the effect of time, treatment, and the interaction of both variables upon number of surviving larvae. Replicate was used as the random intercept variable to control for repeated measures across time.

Laboratory behavioral bioassay of TTX

Bioassays were performed in flow-through transparent acrylic chambers (7 cm long × 3 cm wide) with a 1-cm water depth to test the behavioral response of nymphs to TTX. Nymphs were collected and housed as previously described. Dechlorinated, 5-μm-filtered, tap water at stream temperature (12–15°C) was pulled into chambers using gravity lines (internal diameter = 0.16 cm). A foam diffuser (pore diameter = 100 μm) was mounted at the input of the chamber to create a uniform distribution of TTX as it moved toward nymphs. The velocity of water in chambers was similar to observed stream velocities when nymphs were collected ($\sim$1–2 cm^{-1}). Outputs on the flow-through chambers prevented overfilling and maintained a consistent 1-cm water depth. The test solution of TTX was prepared at a concentration of 1.0×10^{-8} mol l^{-1} using dechlorinated, 5-μm-filtered tap water and TTX powder (Sigma Aldrich). This concentration was chosen based on HPLC–FLD analysis of amounts of TTX present in the prepared newt solution. Dechlorinated, 5-μm-filtered, tap water was used as the control.

All bioassays were recorded at 30 frames s^{-1} using a digital camera with video recording capabilities (Nikon, D90, 18–200 mm lens). The camera was mounted 50 cm directly above a chamber to record nymph behavioral responses. The flow-through chamber and camera were placed within a blind to minimize visual disturbance. White parchment was placed underneath chambers to create contrast. Each sheet had a 5-cm marker placed on it to create calibration points for video analyses. Chambers were rinsed for 20 min with a 1:10 solution of hydrochloric acid and dechlorinated, 5-μm-filtered, tap water at the end of each trial to remove any remaining compounds.

For each experiment, a nymph (mean length $\pm SD =$ 192 mm ± 21 mm) was placed in a chamber and oriented toward the input. Nymphs were allowed to acclimate for 5 min, during which filtered tap water passed through the chamber. At the start of a trial, the input for the chamber was either switched to the control ($n = 3$) or TTX solution ($n = 5$). Approximately 1 min was provided for the control or TTX solution to reach the nymph before offering a prey item (*L. variegates*). Prey were placed at the top of the chamber, directly in front of the diffuser, approximately 7 cm from the nymph. Behavioral reactions were recorded from the start of the 5 min acclimation period to 5 min post consumption of the prey item and ranged from 63 to 509 s.

Recorded video of the bioassays was categorized into three segments: (1) the acclimation period, (2) introduction of prey to first strike, and (3) post consumption of prey. Video of nymph bioassays was then transferred to a computer, played back, and movement noted during each second of each segment. We used the same methods outlined in the behavioral bioassay of newt chemical cues to quantify nymph movement. Data were arcsine transformed and normally distributed. An effect of TTX upon percentage of observations animals moved, latency to strike at prey, and time required to consume prey was evaluated using *t* tests.

Both angular velocity and velocity magnitude were analyzed in bioassays to determine the dynamics of nymph predatory behavior and locomotor activity in the presence of TTX. We considered angular velocity to be an indicator of the speed at which nymphs changed directions when stalking prey and velocity magnitude a measurement of the speed at which nymphs strike at prey. Vector quantities derived from Tracker software (version 4.8) were used to calculate mean angular and mean velocity magnitudes for each nymph. We used Tracker data from when a nymph began stalking prey up to the start of a strike to produce individual mean angular velocity, and the data from the duration of each strike to derive individual mean velocity magnitude values. For each vector quantity, a *t* test was used to statistically compare individual means between treatments.

Results

Field behavioral bioassay of amphibian chemical cues

A significantly lower number of macroinvertebrates were found within newt areas relative to frog areas

($\chi^2 = 24.14$, $P < 0.001$, $df = 4$, Fig. 1). Collected macroinvertebrates consisted of six orders: Ephemeroptera, Trichoptera, Coleoptera, Plecoptera, Odonata, and Megaloptera, listed from most abundant to least. Because only two Megaloptera specimens were found within all search areas, they were excluded from our analysis. Prior to removing enclosures, we noted the location of individual frogs and newts. Frogs were always found at the water surface, while newts were either at the bottom or at the water surface of an enclosure.

Laboratory behavioral bioassay of newt chemical cues

Nymph behavior was affected by chemical cues released from newts. We found that nymphs moved significantly less when exposed to newt chemical cues relative to control solutions (t test, $t = 5.3204$, $P < 0.001$, $df = 31$, Fig. 2). Poisson regression indicated that the percentage of surviving larvae was affected by the presence of newt chemical cues (Fig. 3). The interaction term between time and treatment in the Poisson regression was significant ($P = 0.049$), indicating a time effect across treatments. Because the interaction term was significant, we tested if the effect of time was significant in each treatment using predicted values from the previously fit model. The effect of time was significant ($P = 0.040$) and it was twice as strong in the control treatment. Additionally, the number of replicates with all larvae surviving by the end of the experiment totaled 7 of 16 in the newt chemical cue treatment relative to 2 of 17 in the control.

Percentage of instances nymphs used refuge did not differ between treatments (mean percentage of time in refuge ± *SD*: cue $= 33.69 \pm 7.14$, $n = 16$; control $= 32.06 \pm 7.36$, $n = 17$; t test, $t = 0.1585$, $P = 0.8751$, $df = 31$). Nymphs were observed under and on the side of refuge in both treatments.

HPLC–FLD was used to determine if newt solutions contained TTX. Chromatograms indicated the presence of TTX in newt solutions at concentrations comparable to amounts observed in previous studies (Zimmer et al., 2006; Bucciarelli et al., 2014). The actual amount of TTX detected from the aliquot of the 4 l newt chemical cue solution was approximately 1×10^{-9} mol l^{-1} of TTX. Because the analyzed solution was diluted when it was brought to a final volume of 4 l, behavioral bioassays of TTX were conducted using a concentration of TTX a magnitude of order greater than what was measured with HPLC–FLD.

Laboratory behavioral bioassay of TTX

Behavioral responses of nymphs significantly differed between treatments in only one segment of the

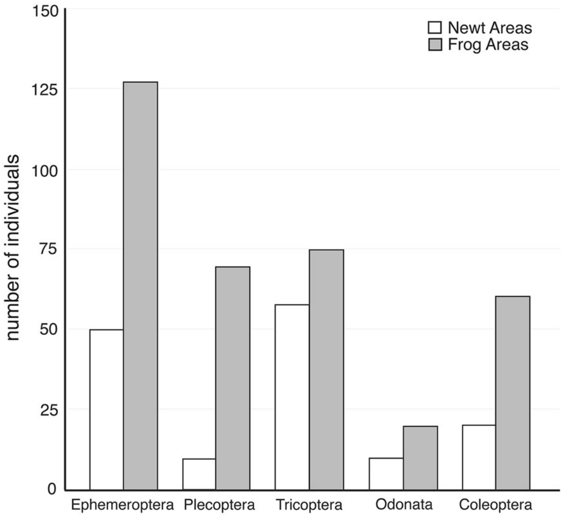

Fig. 1 Cumulative number of macroinvertebrates by order from either newt or frog areas in field behavioral bioassays. Significantly fewer macroinvertebrates were found in newt areas relative to frog areas

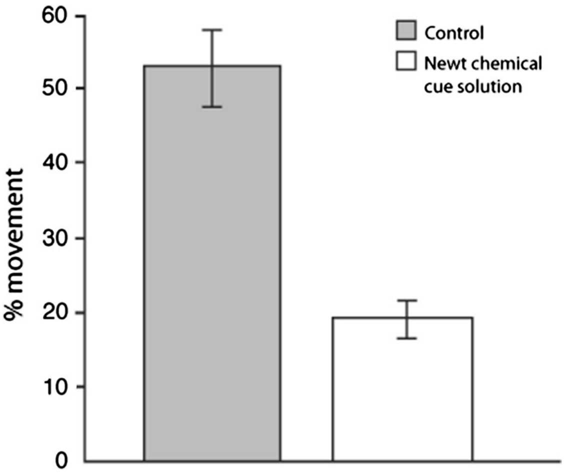

Fig. 2 Behavioral response of *Anax junius* nymphs to prepared newt chemical cue solutions in laboratory behavioral bioassays. The percentage of instances nymphs moved over the 150-min period was significantly less in the presence of newt chemical cue solutions

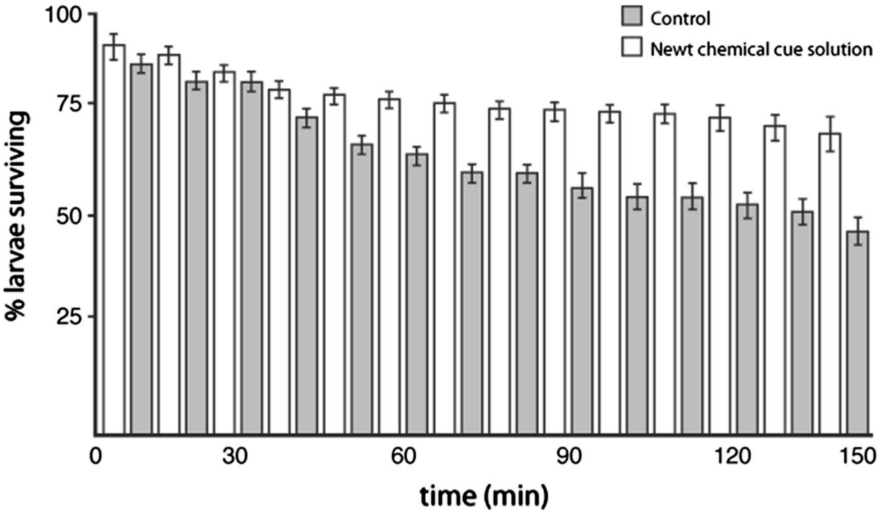

Fig. 3 Percentage of surviving *T. torosa* larvae through time in the presence of predatory *Anax junius* nymphs. *Open bars* indicate overall percentage of surviving larvae in experimental chambers where *A. junius* was exposed to newt chemical cue solutions. *Solid bars* show the same data for controls

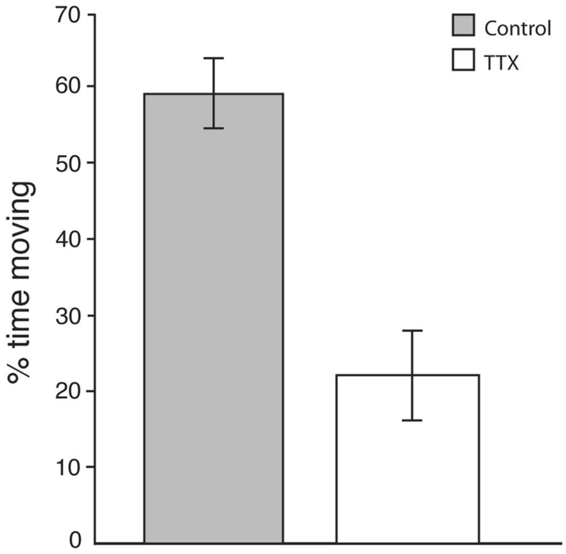

Fig. 4 Behavioral response of *Anax junius* nymphs to TTX in laboratory behavioral bioassays. The mean percentage of time nymphs moved, from when prey was introduced to the first strike, was significantly less when nymphs were exposed to TTX

bioassay, which was during the introduction of prey to first strike segment. Nymphs exposed to TTX moved on an average nearly three times less during the time leading up to first strike relative to nymphs in control chambers (t test, $t = 4.11$, $P = 0.006$, $df = 6$, Fig. 4). Nymph movement did not differ between treatments during the 5-min acclimation segment or the 5-min post-feeding period (acclimation: t test, $t = 0.75$, $P = 0.48$, $df = 6$; post-feeding: t test, $t = 1.07$, $P = 0.32$, $df = 6$). No significant difference in time to strike at food (t test, $t = 1.09$, $P = 0.316$; mean: TTX = 69.6 s; control = 24.0 s) or time to consume food (t test, $t = 2.18$, $P = 0.072$; mean: TTX = 229.0 s; control = 38.3 s) was detected.

Mean velocity magnitude and angular velocities significantly differed between treatments (Fig. 5). Nymph mean velocity magnitude was significantly reduced in TTX treatments (t test, $t = 2.33$, $P = 0.049$, $df = 6$). Similarly, nymph mean angular velocity was significantly reduced when nymphs were exposed to TTX (t test, $t = 2.51$, $P = 0.045$, $df = 6$; Fig. 6).

Discussion

Chemical compounds from animals are capable of altering predator and prey behavior (Stowe et al., 1987; Hare & Eisner, 1993; Flowers & Graves, 1997; Nyström & Åbjörnsson, 2000; Turner et al., 2000), deter or poison predators (Kats et al., 1988; Daly, 1995), indicate reproductive status (Roelofs et al., 2002), aid in foraging (Weissburg & Zimmer-Faust, 1993), or signal an impending threat (Sullivan et al., 2011). It is also clear that chemical cues from predators affect foraging behavior and trophic relationships (Abrams, 1983; Juliano & Gravel, 2002; Koch et al., 2007; Camacho & Thacker, 2013; Naddafi & Rudstam, 2013; Schneider et al., 2014). The idea that defensive compounds can affect community organization, trophic webs, and biogeochemical processes has been proposed (McClintock & Baker, 2001; Pohnert et al., 2007; Hay, 2009), and in marine

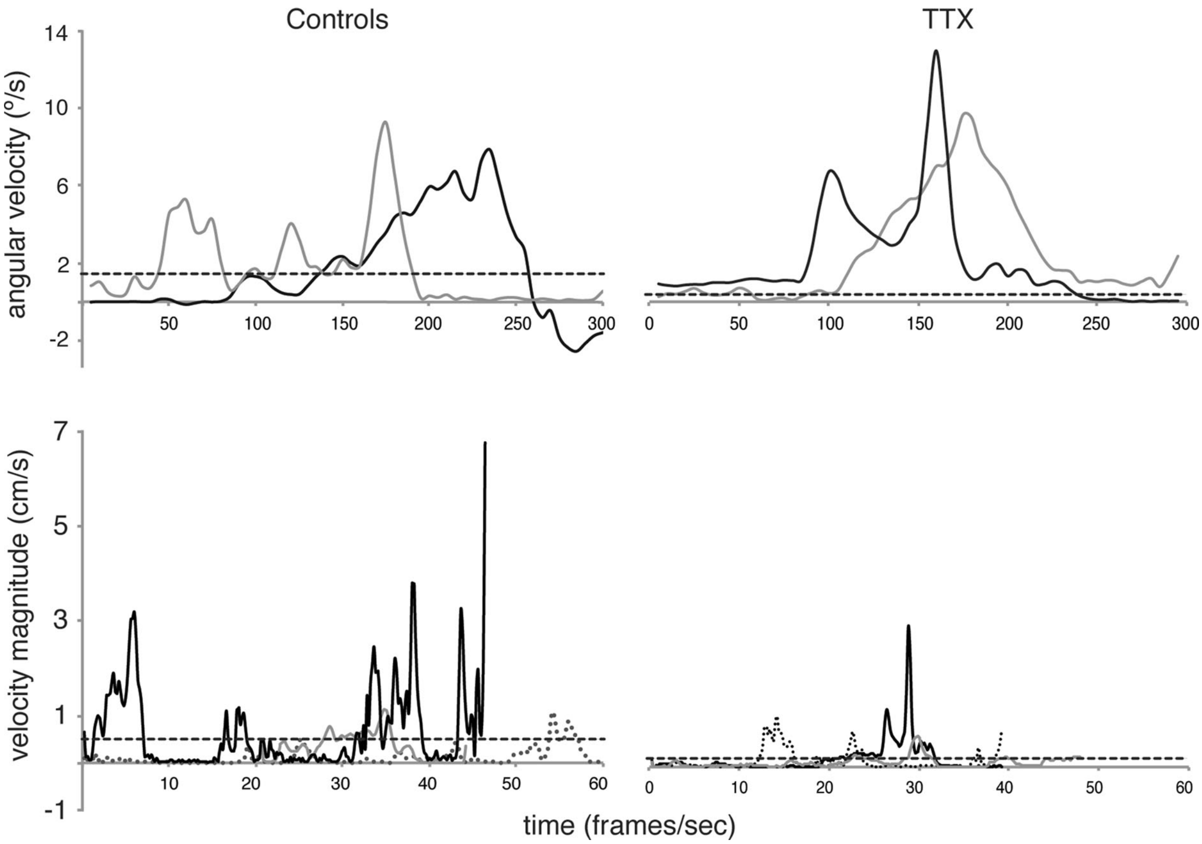

Fig. 5 Examples of angular velocities and velocity magnitudes of *Anax junius* nymphs recorded in laboratory behavioral bioassays. Each line represents an example of *A. junius* angular velocities or velocity magnitudes from TTX or control bioassays. *Solid* and *gray lines* show the angular and magnitude velocities of the same nymph in a replicate. The velocity magnitude of additional nymphs is represented by *dotted lines*. *Horizontal dashed lines* show mean values

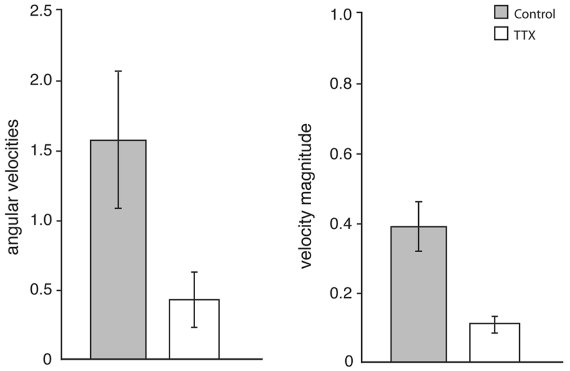

Fig. 6 Mean angular velocities and velocity magnitudes of *Anax junius* nymphs in laboratory behavioral bioassays. Relative to nymphs in the control, nymphs exposed to TTX in flow-through chambers showed significantly decreased mean angular velocities in the period leading up to the first strike at prey. When striking at prey, nymph velocities were significantly less when TTX was present

systems these effects can reverberate throughout ecosystems, ultimately affecting structure and function (Hay & Kubanek, 2002). However, the capacity of chemical defense compounds to alter communities, affect trophic relationships, and prey populations in freshwater systems remains understudied.

Our results lend support to the idea that amphibian toxins may have ecological significance at lower trophic levels. In-stream behavioral bioassays showed that a greater number of individuals in the macroinvertebrate community foraged away from point sources of *T. torosa* chemical compounds (Fig. 1). This implies that newt chemical cues elicit a behavioral response similar to those observed in other macroinvertebrates (Peckarsky, 1980; Petranka & Fakhoury, 1991; Kiesecker et al., 1996; Wisenden et al., 1997; Jacobsen & Stabell, 2004; Gall & Brodie, 2009). Remaining in close proximity to newts would likely be maladaptive since adult *T. torosa* are opportunistic consumers, feeding preferentially on macroinvertebrates (Kerby & Kats, 1998), and primarily on Ephemeroptera. It is possible that macroinvertebrates were attracted to frog chemical cues. However, over the course of our field experiment, we sampled three random areas in pools, each 20 cm in diameter and found that the mean cumulative number of macroinvertebrates in these areas (mean $= 36.67 \pm 6.33$ SE, $n = 3$) did not differ from frog areas (mean $=$ 35.40 ± 4.86 SE, $n = 10$). Given the feeding ecology of *T. torosa*, compounds from newts seem likely to influence the macroinvertebrate community, whereas macroinvertebrates would likely forego responding to *P. regilla* chemical cues since they pose no threat (Sih, 1987; Chivers & Smith, 1998). Ultimately, when *T. torosa* breed in streams, TTX as a byproduct of chemical defense may indirectly affect trophic relationships and community organization.

Macroinvertebrate behavioral responses to *T. torosa* chemical cues appear to be an antipredator response to a waterborne signal. Particularly interesting in our study is the behavioral response of Ephemeroptera, which seem to be highly responsive to newt chemical cues (Fig. 1). Aquatic mayflies have demonstrated a remarkable ability to detect chemical stimuli and modify behavior in order to avoid predation (McIntosh & Peckarsky, 1996; Scrimgeour et al., 1994). Scrimgeour et al., (1994) found that chemical cues alone will increase drift rates and reduce the use of upper substratum surfaces. Similar antipredator responses to *T. torosa* compounds would seem adaptive given that Ephemeroptera larvae compose approximately 50 % of the aquatic newt diet (Bucciarelli, unpublished data) and that densities are highest in March through June, considerably overlapping the *T. torosa* breeding season (R. Ferrer, unpublished data).

In our laboratory bioassays, dragonfly nymphs exposed to newt solutions on average consumed fewer *T. torosa* larvae than controls (Fig. 3). The results of Poisson regression showed that the effect of time was twice as strong in the control group, indicating that larvae in the presence of nymphs without TTX were much more likely to be consumed as time progressed. Ultimately, this may be due to the significantly reduced percentage of time nymphs spent moving. An underlying cause of reduced nymph activity could be the synergistic effect of exposure to waterborne chemical stimuli and TTX in larvae, whereby cues from newt solutions induced antipredator behavior and TTX from ingested larvae sub-lethally poisoned nymphs. However, nymphs observed in bioassays using newt solutions and pure TTX spent relatively similar amounts of time moving (Figs. 2, 4). Although time scales varied, the pattern suggests that TTX in larvae does not poison nymphs. While no larvae were observed to be unpalatable, we cannot conclude if larvae were devoid of TTX due to ontogeny (Mathis & Vincent, 2000; Zimmer et al., 2006) or if nymphs are resistant to TTX, as observed in other macroinvertebrate orders (Gall et al., 2011). Attempts to understand community resistance and *Taricha* larval TTX levels are ongoing.

Collectively, our laboratory bioassays show that compounds from *T. torosa* affect *A. junius* nymphs. In our control treatments, nymph mean angular velocities were nearly four times greater in control bioassays compared to TTX bioassays, while mean striking velocities in controls were more than three times greater relative to mean velocities of nymphs exposed to TTX (Fig. 6). In other studies, quantitative estimates of velocities have successfully been used to understand escape behavior of shrimp (*Crangon crangon*) and how varied kinematics may affect predation risk (Arnott et al., 1998). Other researchers have determined that slugs (*Deroceras reticulatum*) alter velocities and turning rates in response to predator chemical cues (Armsworth et al., 2005). Moreover, a recent study of grasshopper (*Melanoplus femurrubrum*) jumping biomechanics measured angular and takeoff velocities and found that escape performance and endurance are positively associated with spider predators (Hawlena et al., 2011). What effect these modified locomotor behaviors have on fitness is undetermined, but for *A. junius* nymphs such changes may likely be an additional component to effective antipredator behavior (Hopkins et al., 2011).

Although the mechanism is unidentified, the observed reductions in nymph movement and velocity when exposed to TTX suggest that it can elicit behavioral responses, which may not be akin to the antipredatory behavioral response observed in other community members (Zimmer et al., 2006). In previous studies of odonates (Wisenden et al., 1997; Baker et al., 1999; Elkin & Baker, 2000) and other macroinvertebrates (Juliano & Gravel, 2002), Zygopterans responded to predator chemical stimuli by reducing the frequency of feeding strikes, head turns, and walking movements (Wisenden et al., 1997). Additionally, Juliano & Gravel (2002) observed that larval mosquitoes reduce browsing activity when waterborne chemical stimuli from predatory macroinvertebrates are present. Thus, it is likely that costs and benefits of behavioral and physiological strategies are balanced by natural selection. Specifically, TTX may simultaneously deter predators and warn prey.

It is also possible that TTX physiologically affects nymphs, and as a result inhibits locomotor activity. Voltage-gated sodium channels, which are the target of TTX, control action potentials (Catterall, 1980). These channels are also the targets of numerous poisons known to affect macroinvertebrate physiology (Catterall, 1980; Catterall et al., 1981; Gusovsky et al., 1988; Olivera et al., 1990; Narahashi et al., 1992; Song et al., 1996; Cestele et al., 1998; Dechraoui et al., 1999; Li et al., 2001; Yotsu-Yamashita et al., 2004; Du et al., 2013). Some taxa buffer the effects of such poisons by investing energetic resources into cuticle deposition, thereby decreasing the need to upregulate transcription of more enzymes to catalyze the metabolism of poisons (Wood et al., 2010). Potentially, *A. junius* buffers the effects of waterborne TTX by both cuticle deposition and enzyme activity, although the amounts of TTX entering their system may still have consequences, especially when ingested. One such effect may include reduced locomotor activity. In behavioral bioassays to TTX, time to consume prey did not significantly differ between nymphs exposed to waterborne TTX and controls. As such, it is not likely that waterborne TTX poisons nymphs. However, the observed behavioral responses of odonate nymphs to waterborne TTX suggest that interacting with this compound is costly. How exactly other macroinvertebrates in this study system behaviorally respond to TTX is not known.

Unlike *Taricha* in more northern localities, newts in the Santa Monica Mountains occupy streams with low volumes of water and slow flow (G. Bucciarelli & L. Kats, personal observations). Contrary to conventional wisdom (e.g., Stebbins & McGinnis, 2013), a small number of adult newts stay in these streams far after the breeding period has ended. Given the unique environmental conditions in this part of their distribution, and the existing roles of TTX in mediating ecological and evolutionary processes, it appears that community members at lower trophic levels detect TTX-laden chemical cues from *T. torosa* and that these chemical cues can affect macroinvertebrate behavior. Selection for a response to newt chemical stimuli could have evolved because of the slow flowing and low volume pools found throughout the Santa Monica Mountains relative to breeding localities with greater volumes of water and faster flow regimes. Given the community response observed in field behavioral bioassays (Fig. 1), TTX given off by *T. torosa* likely functions as a chemical cue and not a poison that induces paralysis or sub-lethal affects. If the results of our field and laboratory bioassays are an accurate indication of the ways in which TTX affects foraging and predatory behavior of the macroinvertebrate community, as well as the stream community at a broader scale (Elliott et al., 1993), then we speculate that the loss of *T. torosa* could impact trophic relationships at multiple scales. Future studies to assess the indirect role of *T. torosa*, as well as TTX in other systems, are of great promise considering the diverse roles of chemical signals and the ubiquity of the neurotoxin TTX.

Acknowledgments We appreciate the support of R. Honeycutt, Chair of the Natural Science Division, Pepperdine University. We thank Debbie Sharpton and Jo Kitz with Mountains Restoration Trust for their enthusiasm and permission to conduct our research in the Cold Creek Preserve. Wendy Willis assisted with identification of macroinvertebrates and we appreciate her help. Many thanks to Ryan Ferrer for providing experimental materials. Comments from three anonymous reviewers helped to improve this manuscript.

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