THREAT-SPECIFIC BEHAVIORAL RESPONSES BY FORAGING ALLEGHENY MOUNTAIN DUSKY SALAMANDERS (*Desmognathus ochrophaeus*) TO CHEMICAL STIMULI ASSOCIATED WITH PREDATION

JOHNSON

THREAT-SPECIFIC BEHAVIORAL RESPONSES BY FORAGING ALLEGHENY MOUNTAIN DUSKY SALAMANDERS (*Desmognathus ochrophaeus*) TO CHEMICAL STIMULI ASSOCIATED WITH PREDATION

by
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Abstract

Prey species may reduce the likelihood of injury or death by engaging in antipredator behavior but often incur costs associated with foraging success or efficiency. One strategy used by prey to reduce such costs is to adjust the intensity of antipredator responses according to the level of threat perceived. We examined the propensity of *Desmognathus ochrophaeus* to engage in threat-specific responses to chemical stimuli associated with predation and the effect of food availability on salamander behavior. In our first experiment, we evaluated salamander chemosensory movements, activity, and edge behavior in response to chemical stimuli from distressed and damaged conspecifics as well as predatory *Gyrinophilus porphyriticus*. We also evaluated behavioral responses to the aforementioned cues while providing salamanders with prey (*Drosophila* sp.). We found that individual *D. ochrophaeus* differentially respond to the chemical stimuli associated with predation, showing increased chemosensory behavior when exposed to cues from distressed and damaged conspecifics, but a reduction in movement when exposed to cues from the predator. Furthermore, we found that salamanders exhibited a similar pattern of behavior in the presence of prey, showing increased chemosensory behavior when exposed to distressed conspecifics and reduced activity when exposed to the predator stimulus. In addition, foraging efficiency (estimated by the number of prey captured per strike), while only marginally significant, was lowest in salamanders exposed the predator treatment. In conclusion, our data suggest that *D. ochrophaeus* detect and differentially respond to chemical stimuli from conspecifics and predators,

even while foraging, which may allow them to reduce the costs associated with antipredator behavior.

Introduction

Prey species must balance antipredator responses with other activities related to fitness (Lima & Dill 1990; Brodie et al. 1991; Lima 1998; Richardson 2001). Although antipredator responses typically reduce the likelihood of injury or death during a predation event, they also may incur costs associated with a reduction in the time and energy devoted to mate search, courtship and foraging (Sih 1992; Candolin 1997; Polis et al. 1998). Depending on the species under consideration, these effects may have substantial fitness consequences. For instance, the effects of decreased foraging activity as a result of antipredator responses may result in the reduction of rates of growth and development (Skelly & Werner 1990; Scrimgeour & Culp 1994) or fecundity (Peckarsky et al. 1993) in both vertebrate and invertebrate taxa.

Because prey species must meet some minimum energetic requirement for growth and reproduction, responses to predator cues that simply involve lowering rates of foraging in favor of increasing vigilance behavior in the presence of potential predators may not be a viable strategy over long periods of time or where predators exist at relatively high densities (Sansom et al. 2009). This may be especially true in species that tend to respond to threat with a decrease in overall activity (Kohler & McPeek 1989; Madison et al. 1999), movement between foraging patches (Roberts & Liebgold 2008; Sansom et al. 2009), or delayed emergence from refugia (Sih 1992; Sullivan et al. 2002). Ultimately, prey that respond indiscriminately to every potential threat with antipredator behavior that limits foraging opportunities could greatly decrease energy available for other activities (Sih 1992).

Many prey species attempt to reduce the costs of antipredator behavior by adjusting the magnitude or intensity of their response according to the level of threat perceived. The moderation of prey responsiveness to varying levels of threat is the primary tenet of the threat-sensitivity hypothesis (Helfman 1989). The use of threat-sensitive antipredator behaviors is well documented in aquatic systems and may be manifest in a variety of ways. For example, the predator avoidance behavior of damselfish (*Stegastes planifrons*) exposed to models of trumpetfish predators (*Aulostomus maculatus*) is positively correlated with the size and proximity of the model (Helfman 1989). In addition, Puttlitz et al. (1999) demonstrated size-specific responses by Pacific treefrog tadpoles (*Hyla regilla*) exposed to predatory salamander larvae (*Ambystoma gracile*). In this instance, antipredator responses decreased as prey increased in size presumably because salamander larvae were no longer large enough to pose a significant risk. Additionally, Chivers et al. (2001) report the use of threat-sensitive defensive behavior in slimy sculpin (*Cottus cognatus*) exposed to predatory brook char (*Salvelinus fontinalis*) that varied according to size. In one of the relatively few studies to evaluate threat-sensitivity in terrestrial predator-prey contexts, Amo et al. (2004) show that the antipredator responses of wall lizards (*Podarcis muralis*) are greatest when lizards are exposed to both visual and chemical cues from smooth snakes (*Coronella austriaca*).

The use of chemical cues to evaluate levels of predation risk may be especially advantageous given the variety of possible sources and information conveyed (Kats & Dill 1998). The range of chemical stimuli that may be used by prey includes predator kairomones (produced by the predators themselves), disturbance cues (secreted or excreted by distressed or startled individuals), and injury-released cues (from physical damaged or injured individuals) (Wisenden 2000; Ferrari et al. 2010; Crawford et al. 2011). These cues can relay information to prey regarding the proximity, intention and recent foraging activity of a predator (Ferrari et al. 2010). For example, Kusch et al. (2004) manipulated the concentrations of chemical stimuli associated with predation and discovered that prey response tended to be positively correlated with the concentration of the cue. The fathead minnows (*Pimephales promelas*) in this study displayed weaker responses to lower concentrations of the cue and stronger responses to higher concentrations. Zhao and Chivers (2005) identified a similar trend in juvenile goldfish (*Carassius auratus*) as individuals exposed to higher concentrations of conspecific injury-released cues increased the intensity of their avoidance behaviors as well. In general, studies that examined threat-sensitivity to chemical cues have documented similar behavioral patterns in range of (primarily aquatic) taxa including larval mosquitoes (Ferrari et al. 2008a), fish (Ferrari et al. 2005; Brown et al. 2006) and larval amphibians (Mirza et al. 2006; Ferrari et al. 2008b). However, graded responses to chemical stimuli associated with predation by terrestrial organisms are somewhat lacking.

Plethodontid salamanders are good candidates for the investigation of chemically-mediated antipredator behavior in terrestrial systems because they possess well-developed chemosensory organs (Dawley & Bass 1988) and utilize their chemosensory ability for foraging (Placyk & Graves 2002), territory maintenance (Wise et al. 2004), and mate recognition (Roudebush & Taylor 1987). Furthermore, some species respond to predator kairomones (Maerz et al. 2001; Sullivan et al. 2002; Sullivan et al. 2004; Sullivan et al. 2005), distress cues (Lutterschmidt et al. 1994), and injury-released cues from conspecifics as well as heterospecifics belonging to the same prey guild (Sullivan et al. 2003). Whether plethodontid salamanders respond to chemical cues from predation events using graded, or threat-sensitive, responses currently is unknown. The purpose of this study is to evaluate the potential for threat-sensitive responses by Allegheny mountain dusky salamanders (*Desmognathus ochrophaeus*) to chemical stimuli from disturbed and injured conspecifics as well as predatory northern spring salamanders (*Gyrinophilus porphyriticus*) and trade-offs between antipredator behavior and foraging. To this end, we attempted to answer three questions: 1) Do individual *D. ochrophaeus* respond to chemical cues associated with predation in a threat-sensitive manner? 2) Does the pattern of response exhibited by salamanders remain the same when exposed to a conflicting stimulus (e.g., the presence of potential prey)? and 3) Is foraging efficiency influenced by exposure to the chemical stimuli?

Methods and Materials

Collection and maintenance of salamanders

We collected 200 adult *D. ochrophaeus* along the streamside of Houghton Creek, in Houghton, New York, USA (42°25'N, 78°9'W), from 10 September – 03 October 2012 for use in behavioral trials. Each salamander was housed individually in 15 cm diameter × 1.5 cm height Petri dishes lined with acid-free paper towels saturated with distilled water. We maintained salamanders on a seasonal photoperiod (13:11 h light/dark, lights on at 0700 h EST) with temperatures ranging from 18.3°C (day) to 12.8°C (night). Paper towels were moistened as needed and changed weekly. Prior to use in behavioral trials, the salamanders were not fed, but were returned to the field within 10 d of capture. We collected four additional *D. ochrophaeus* (two males and two females) as well as three adult *G. porphyriticus* from our field site to serve as stimulus donor animals. The dusky salamanders were housed in the same manner as our test animals whereas spring salamanders were housed together in a clear plastic bin (31 × 18 × 11 cm) with acid-free paper towels for cover and 200 ml of distilled water. Spring salamanders were maintained in the laboratory at approximately 22°C and were not fed prior to the collection of the stimulus.

Preparation of chemical stimuli

To collect the distress and injury-released cue from the *D. ochrophaeus*, we transferred the four stimulus donors (3.7–4.6 cm SVL) to a clear plastic bin (31 × 18 × 11 cm) filled with 200 ml of distilled water. To prepare the distress cue, we used blunt forceps to repeatedly grasp each salamander for 1 min to simulate predator attack. After the four salamanders had been agitated, the solution was filtered through glass wool to remove debris (e.g., dirt, fecal pellets). Next, we prepared the injury-released cue by forcing the stimulus donor animals described above to autotomize their tails by grasping them with forceps 1 cm posterior to the cloacal vent. Previous studies suggest that the chemical stimulus from the autotomized tails of salamanders may indicate areas of elevated predation risk to conspecifics (Arnold 1982; Labanick 1984; Hucko & Cupp 2001). Each autotomized tail was weighed (0.7 g total tail tissue), homogenized in a blender with 200 ml of distilled water for five minutes, and filtered through glass wool to remove large particles of tissue. The distress and alarm solutions were then divided into equal aliquots in conical-bottom centrifuge tubes, rapidly frozen using liquid nitrogen, and stored in a freezer until use.

To collect the predator cue, we transferred all *G. porphyriticus* to a clear plastic bin (31 × 18 × 11 cm) containing 200 ml of distilled water for 28 h. At the end of this time period, we removed the salamanders and filtered the solution through glass wool, divided it into equal portions in conical bottom centrifuge tubes, and froze with liquid nitrogen and stored in a freezer until needed.

Experiment 1: Study of threat-sensitive responses to chemical stimuli

We examined the responses of dusky salamanders to a variety of chemical stimuli associated with predation in order to determine if this salamander species engage in threat-sensitive behavior. To begin, salamanders were transferred to the laboratory 30 min prior to the commencement of trials to allow acclimation to room temperature (~22°C) and ambient light levels (25 W red light). We conducted all trials between 1915–2230 h each evening because Maerz et al. (2001) suggest that another plethodontid salamander (*Plethodon cinereus*) tends to be most active near dusk and approximately 2–4 h after sunset. We tested each salamander once and observed 12–13 salamanders per night over seven consecutive nights. Treatments were stratified in an effort to test an equivalent number of salamanders to each treatment during each evening.

The bioassay used to evaluate salamander behavioral responses utilized 15 cm diameter Petri dishes lined with filter paper and saturated with 3 ml of one of our prepared cues or a distilled water control. We began trials by transferring one salamander from its home dish to the center of a test dish using a cotton swab. After a 2-min acclimation period, we recorded the following behaviors for 10 min: 1) nose-taps, 2) steps, and 3) edge behavior. Nose-tapping behavior among plethodontid salamanders serves to bring the nasolabial grooves into contact with the substrate, which then facilitates the transfer of chemicals from the substrate to the vomeronasal organ (Dawley & Bass 1989). For the purpose of our study, we defined a nose-tap as any time a stationary salamander quickly touched its cirri or the base of the nasolabial grooves to the substrate (Jaeger 1984). Steps, as a measure of activity, were counted as forward movements of each individual forelimb. Edge behavior included the amount of time that salamanders spent with their nose, face or body (excluding tail or legs) in contact with the side of the Petri dish. Lastly, a paper divider was placed between the observer and the test dish to reduce the likelihood that observer movements would influence salamander behavior. At the completion of each trial, salamanders were removed from the test dishes and placed back into its home dish until their release at the field site.

Experiment 2: Study of threat-sensitive responses in the presence of potential prey

In this experiment we examined the responses of foraging dusky salamanders to the chemical stimuli associated with predation and evaluated the effects of predation-related cues on foraging efficiency. To accomplish this, we utilized the same bioassay as described above with the following exception: after the 2-minute acclimation period, we added five wingless *Drosophilia* sp. to each test dish with the salamander and recorded the number of strikes and flies successfully captured in addition to the number of nose-taps, steps, and edge behavior. Strikes were defined by a rapid movement of the salamander in the direction of prey with its mouth open or tongue extended. A successful capture was recorded when a fly was ingested (Jaeger & Rubin 1982; Maerz et al. 2001).

Statistical analyses

We used single-factor between-subjects analysis of variance (ANOVA) to determine if the mean of each salamander response (nose-taps, movements, or edge time) and average foraging efficiency (i.e. number of prey successfully consumed per strike) differed among treatment groups. We performed pairwise comparisons, when the main effects were significant, using Tukey's HSD test (Sheskin 2007).

Salamander responses to chemical cues associated with predation

The chemical stimulus to which salamanders were exposed significantly influenced chemosensory behavior ($F_{3, 96}$ = 21.39, p <0.001) with salamanders performing more nose-taps in response to distressed and damaged conspecifics than to control and predator treatments (Tukey HSD: control vs. distress p < 0.001, control vs. damage p < 0.001, control vs. predator p = 0.368, distress vs. damage p = 0.023, distress vs. predator p = 0.024, damage vs. predator p < 0.001, Fig. 1). In addition, the chemical stimulus significantly affected activity ($F_{3, 96}$ = 7.87, p < 0.001) with salamanders reducing their movements when exposed to the predator cue (Tukey HSD: control vs. distress p = 0.692, control vs. damage p = 0.231, control vs. predator p = 0.047, distress vs. damage p = 0.848, distress vs. predator p = 0.002, damage vs. predator p < 0.001, Fig. 1). Lastly, the average duration of edge time by salamanders did not vary among the different treatment groups ($F_{3, 96}$ = 2.27, p = 0.085).

Effect of salamander foraging on responses to chemical cues associated with predation

In the presence of potential prey, salamander chemosensory behavior significantly differed among treatment groups ($F_{3, 96}$ = 3.84, p = 0.012) with salamanders performing more nose-taps in response to damaged conspecifics compared to the control (Tukey HSD: control vs. distress p = 0.110, control vs. damage p = 0.017, control vs. predator p

Results

Salamander responses to chemical cues associated with predation

The chemical stimulus to which salamanders were exposed significantly influenced chemosensory behavior ($F_{3, 96} = 21.39$, $p < 0.001$) with salamanders performing more nose-taps in response to distressed and damaged conspecifics than to control and predator treatments (Tukey HSD: control vs. distress $p < 0.001$, control vs. damage $p < 0.001$, control vs. predator $p = 0.368$, distress vs. damage $p = 0.023$, distress vs. predator $p = 0.024$, damage vs. predator $p < 0.001$, Fig. 1). In addition, the chemical stimulus significantly affected activity ($F_{3, 96} = 7.87$, $p < 0.001$) with salamanders reducing their movements when exposed to the predator cue (Tukey HSD: control vs. distress $p = 0.692$, control vs. damage $p = 0.231$, control vs. predator $p = 0.047$, distress vs. damage $p = 0.848$, distress vs. predator $p = 0.002$, damage vs. predator $p < 0.001$, Fig. 1). Lastly, the average duration of edge time by salamanders did not vary among the different treatment groups ($F_{3, 96} = 2.27$, $p = 0.085$).

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= 0.878, distress vs. damage p = 0.878, distress vs. predator p = 0.422, damage vs. predator p = 0.110, Fig. 2). Likewise, the chemical stimulus significantly affected activity ($F_{3, 96} = 3.67$, p = 0.015) with salamanders reducing their movements when exposed to the predator cue compared to the distress conspecific (Tukey HSD: control vs. distress p = 0.947, control vs. damage p = 0.999, control vs. predator p = 0.064, distress vs. damage p = 0.899, distress vs. predator p = 0.015, damage vs. predator p = 0.091, Fig. 2). Lastly, the average duration of edge time by salamanders did not vary according to chemical treatment ($F_{3, 96} = 0.783$, p = 0.506).

Salamander foraging efficiency, as defined by the number of prey successfully captured per strike, did not significantly differ according to the different treatment groups ($F_{3, 96} = 2.39$, p = 0.073, Fig. 3).

Discussion

Our results suggest that individual Allegheny mountain dusky salamanders exhibit threat-specific responses to different chemical stimuli associated with predation. Salamanders graded the intensity of their chemosensory behavior by significantly increasing the number of nose-taps when exposed to distressed and injured conspecifics relative to the control and predator treatment. However, their response to chemical cues from predatory *Gyrinophilus* involved a significant reduction in movement relative to the other chemical treatments. Furthermore, our results demonstrate that salamanders respond to chemical stimuli in a similar manner when provided with *Drosophila* prey, although the magnitude of the differences between treatments decreased. In this trial, the foraging dusky salamanders increased chemosensory behaviors when exposed to injured conspecifics, and reduced their activity levels when exposed to the predator stimulus. Lastly, our results regarding foraging behavior suggest that the behavioral responses to the chemical stimuli associated with predation may incur a cost related to foraging as salamanders exposed to the predator exhibited marginally lower efficiency (i.e. *Drosophila* successfully captured per strike).

Many prey species adjust their behavioral response to cues associated with predation based on the type of threat perceived (e.g., Puttlitz et al. 1999; Mirza et al. 2006; Ferrari et al. 2008b; Crawford et al. 2011). The variety of responses observed is likely due to the complex nature of the interactions with different predators and the conflicting demands that such exposure may place on prey (Kotler et al., 1992; Matsuda et al., 1993). In the case of our study, the predator stimulus elicited a significant decrease in activity which is a common predator avoidance mechanism in response to visually-oriented predators (Brodie et al., 1974; Chelini et al., 2009). Conversely, chemical stimuli from injured and distressed conspecifics caused an increase in chemosensory behavior, which may indicate an increase in vigilance as these cues may convey information about a recent predation event in the vicinity (Kiesecker et al., 1999). The flexibility to respond differentially to different sources of threat may allow individual *D. ochrophaeus* to balance the costs and benefits of defensive behavior.

The capacity to engage predators in a threat-sensitive or threat-specific manner while foraging may be particularly advantageous for prey species. Responding to relatively low levels of threat or 'false-alarms' with an unnecessary decrease in foraging activity will likely have substantial fitness consequences. This may be especially true among salamanders that tend to reduce rates of food consumption or decrease attempts to capture prey when exposed to stimuli from predators (Whitham & Mathis 2000; Maerz et al., 2001). Our data suggest that dusky salamanders adjust the type and intensity of their behavioral responses, based on the stimulus to which they are exposed, during foraging periods. This ability to differentially respond to cues related to predation will, presumably, allow individuals to maintain relatively high levels of food intake when the level of perceived threat is relatively low. Our data, although marginally significant ($p = 0.073$) support this hypothesis as foraging efficiency gets progressively lower for salamanders exposed to stimuli from distressed and injured conspecifics, and predators, respectively. These data suggest, therefore, that certain behavioral responses to chemical stimuli carry with them a higher cost with regards to foraging ability or potential.

In conclusion, the utilizing threat-sensitive or threat-specific responses may be particularly advantageous for organisms in both a non-foraging and foraging context. Prey capable of distinguishing among a variety of environmental stimuli and differentially responding may offset potential costs associated with antipredator behavior. This may allow for the conservation of energy and time to be utilized in other fitness-related activities. Lastly, the use of threat-specific behaviors has implications regarding foraging behavior in terrestrial salamanders as well as community interactions within a variety of habitats.

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Fig. 1: The mean ± SEM (a) number of nose-taps, (b) number of forward movements, and (c) duration of edge behavior exhibited by non-foraging *Desmognathus ochrophaeus* exposed to four chemical stimuli: distilled water (Control), chemical secretions from distressed conspecifics (Disturbance), a rinse prepared from the damaged skin tissue from conspecifics (Injured), and an aqueous rinse from *Gyrinophilus porphyriticus* (Predator). Bars capped with different letters indicate significant differences.

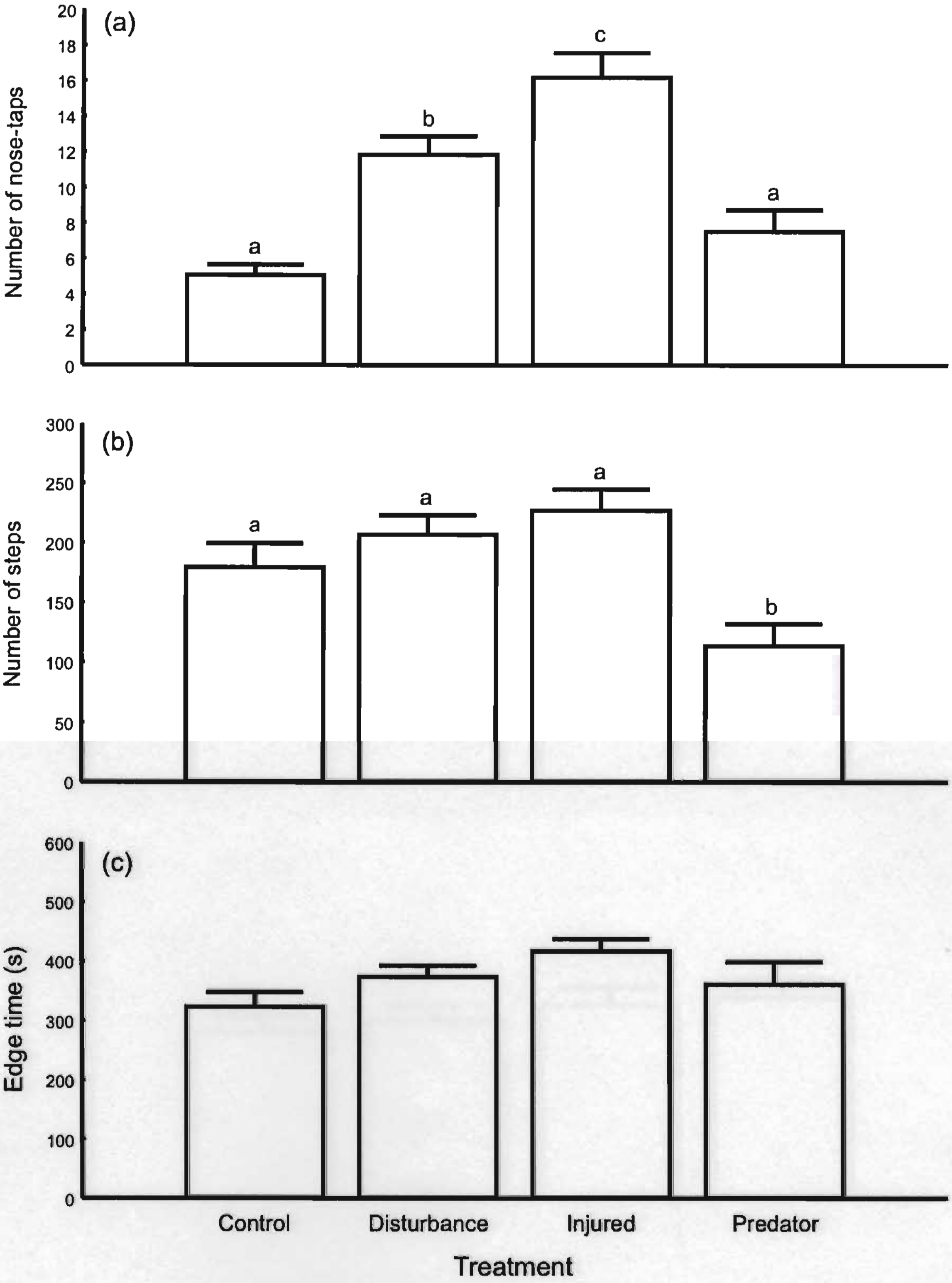
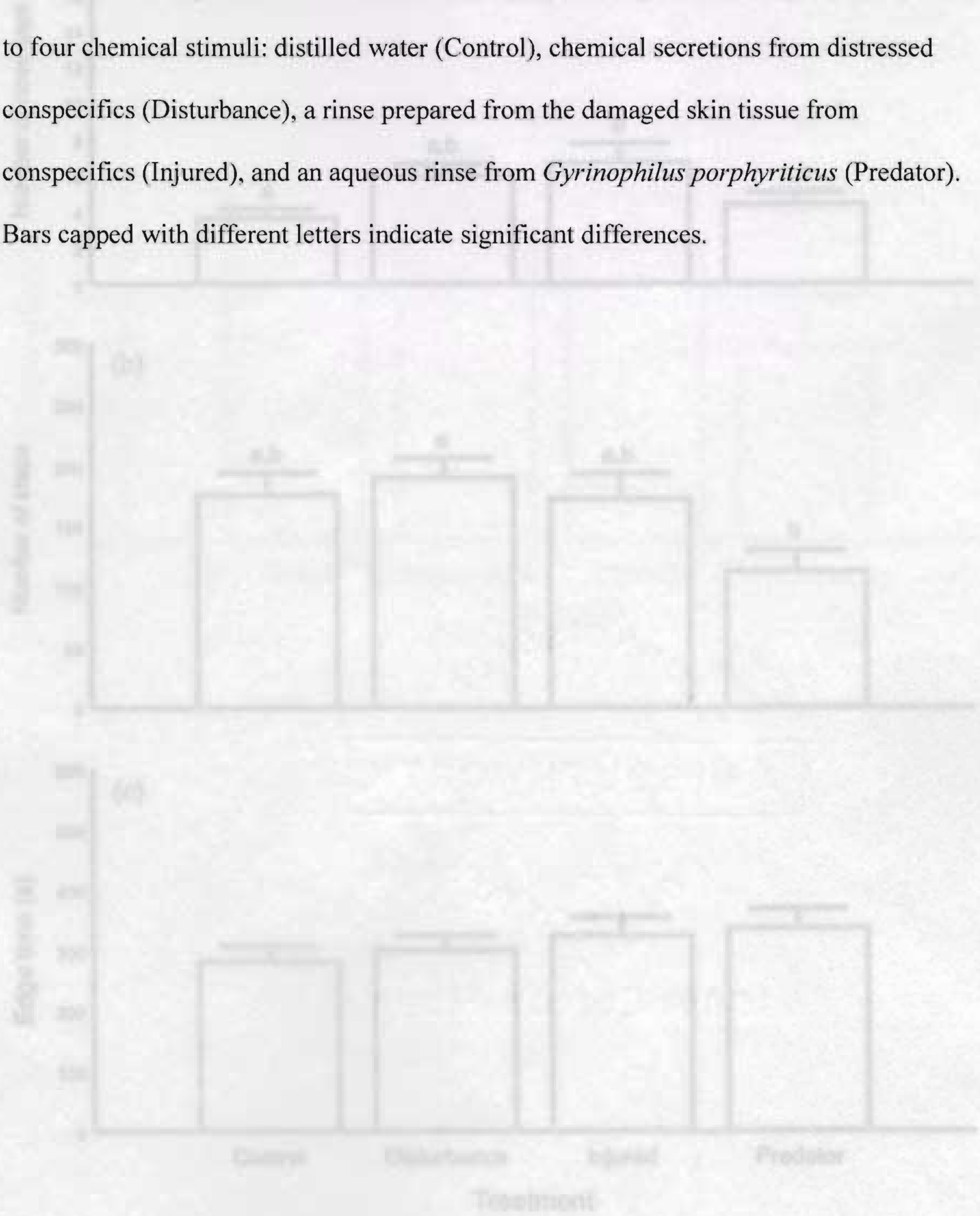

(a)
Number of nose-taps
20
18
16
14
12
10
8
6
4
2
0
a
b
c
a
(b)
Number of steps
300
250
200
150
100
50
0
a
a
a
b
(c)
Edge time (s)
600
500
400
300
200
100
0
Control
Disturbance
Injured
Predator
Treatment

Fig. 2: The mean ± SEM (a) number of nose-taps, (b) number of forward movements, and (c) duration of edge behavior exhibited by foraging *Desmognathus ochrophaeus* exposed to four chemical stimuli: distilled water (Control), chemical secretions from distressed conspecifics (Disturbance), a rinse prepared from the damaged skin tissue from conspecifics (Injured), and an aqueous rinse from *Gyrinophilus porphyriticus* (Predator). Bars capped with different letters indicate significant differences.



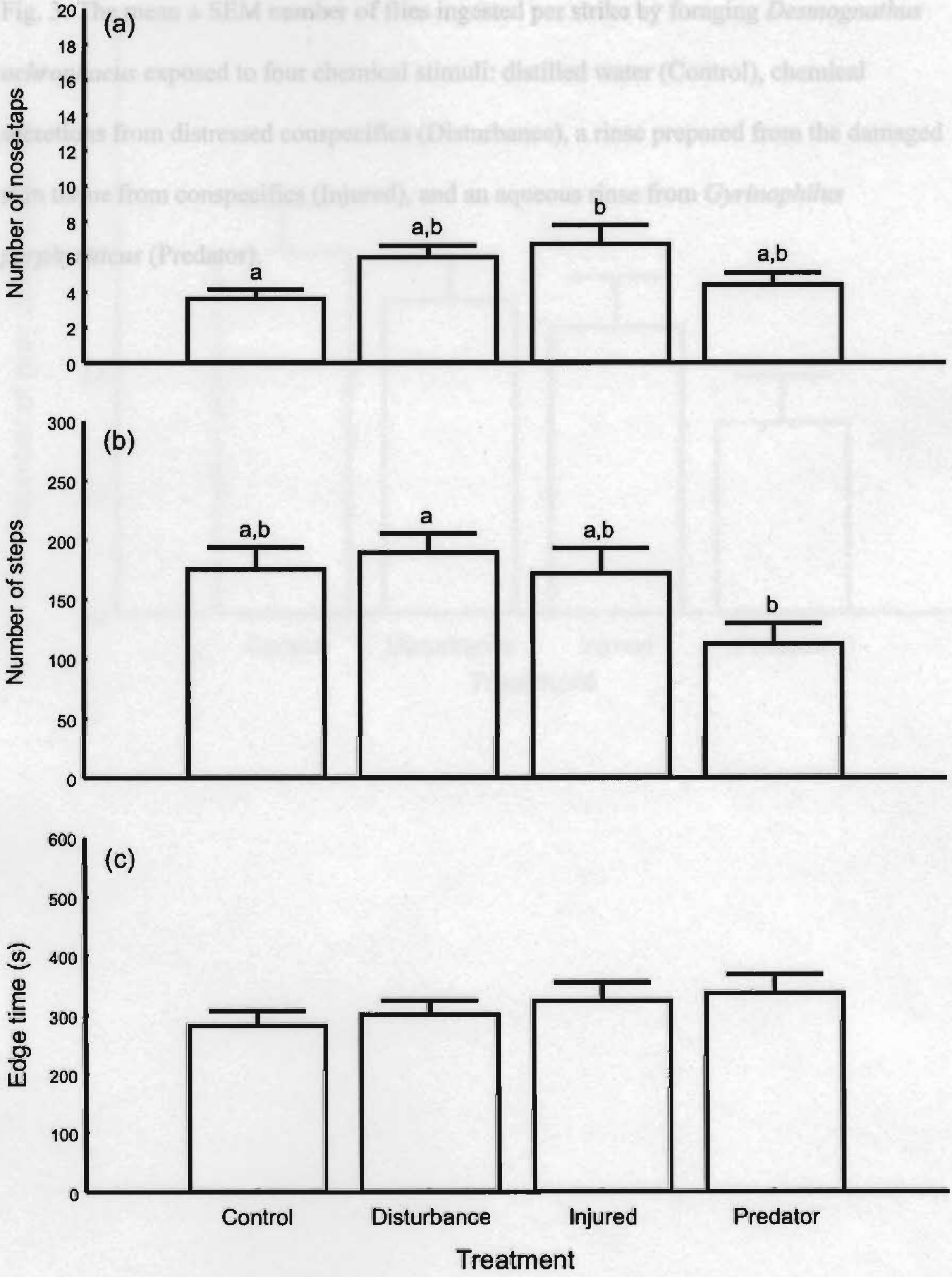
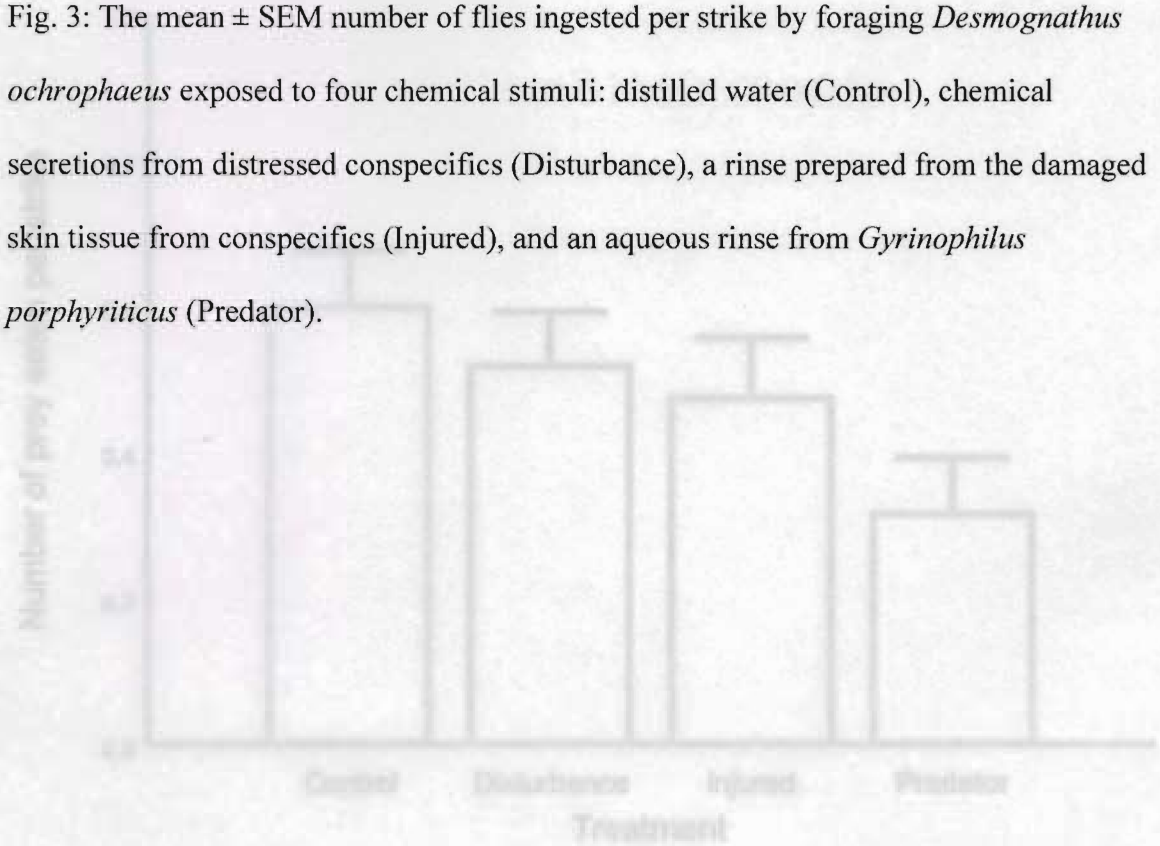
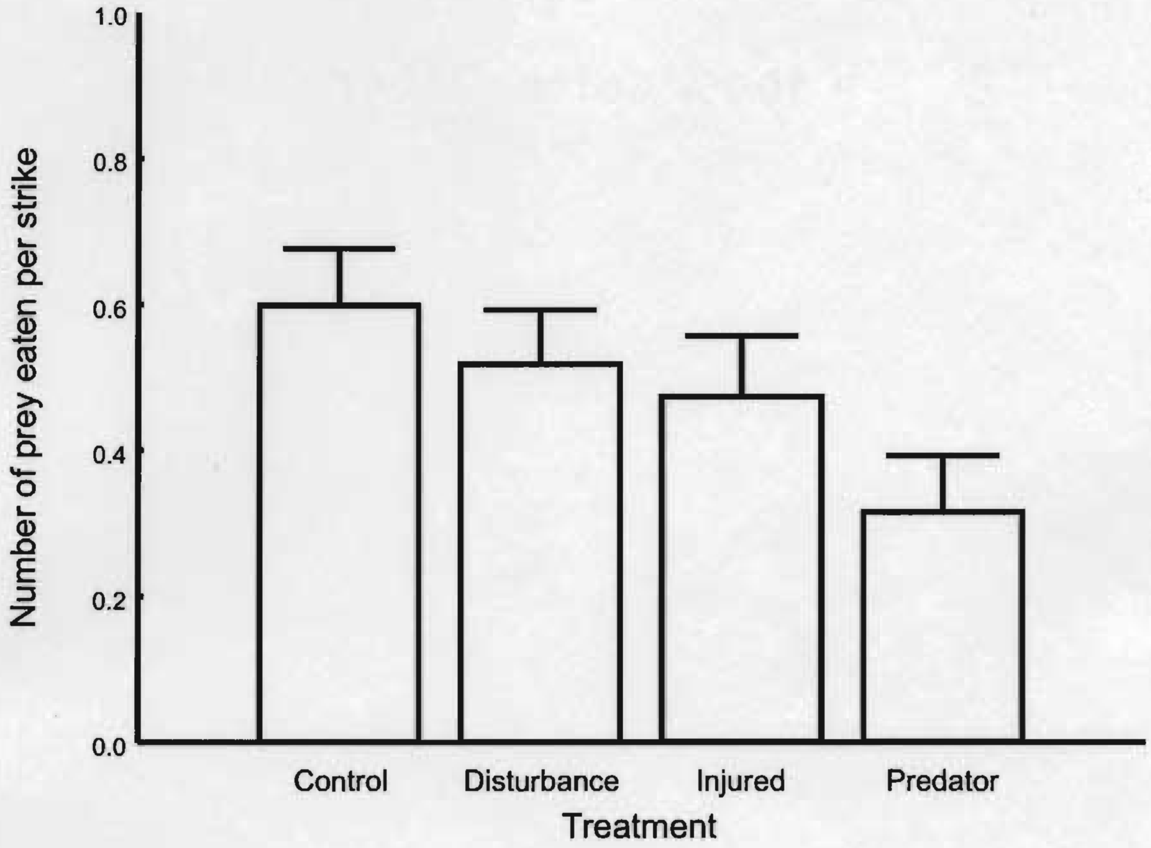

(a)
Number of nose-taps
0
2
4
6
8
10
12
14
16
18
20
a
a,b
b
a,b
(b)
Number of steps
0
50
100
150
200
250
300
a,b
a
a,b
b
(c)
Edge time (s)
0
100
200
300
400
500
600
Control
Disturbance
Injured
Predator
Treatment

Fig. 3: The mean ± SEM number of flies ingested per strike by foraging *Desmognathus ochrophaeus* exposed to four chemical stimuli: distilled water (Control), chemical secretions from distressed conspecifics (Disturbance), a rinse prepared from the damaged skin tissue from conspecifics (Injured), and an aqueous rinse from *Gyrinophilus porphyriticus* (Predator).




1.0
0.8
0.6
0.4
0.2
0.0
Number of prey eaten per strike
Control
Disturbance
Injured
Predator
Treatment