

Damselfly larvae learn to recognize predators from chemical cues in the predator's diet

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Abstract. Chemosensory recognition of predators by naive prey may be facilitated if the predator's diet chemically 'labels' the predator. In a laboratory experiment, behaviour patterns were quantified in individual damsselfly larvae, *Enallagma* spp., that had never been exposed to pike, *Esox lucius*, before and after exposing the damsselflies to one of three chemical stimuli: water from a tank that held pike fed a diet of (1) damsselfies, (2) fathead minnows, *Pimephales promelas*, or (3) mealworms, *Tenebrio molitor*. Damsselfies decreased their frequency of feeding bites, head bends and moves in response to stimuli from pike fed damsselfies and pike fed fathead minnows, but not to stimuli from pike fed mealworms. Damsselfies are sympatric with fathead minnows in the population tested, and probably have many of the same predators. A response to stimuli from pike fed fathead minnows indicates that damsselfies associate predation risk with stimuli from injured minnows. In a second experiment, responses of damsselfies previously exposed to stimuli from pike fed one of the three treatment diets (damsselfy, fathead minnow or mealworm) were tested for a response to stimuli from pike fed mealworms. Damsselfies that had been exposed to stimuli from pike fed damsselfies or fathead minnows in the first experiment responded to stimuli from pike fed mealworms in the second experiment, but damsselfies exposed to pike fed mealworms in the first experiment did not. Thus (1) pike-naive damsselfies may initially respond to chemical stimuli from pike based on stimuli of conspecifics or familiar hetero-specifics in the pike's diet, and (2) damsselfies can learn to recognize chemical stimuli of pike irrespective of the pike's recent feeding regime based on the initial association with damsselfies or minnows in the pike's diet.

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The ability of prey to distinguish predators from non-predators has important implications. Failing to recognize a predator is likely to increase the probability of capture during an encounter (e.g. Hirsch & Bolles 1980), and defensive responses to non-predators are likely to result in lost foraging and mating opportunities (Lima & Dill 1990). Recognition of predators through chemical stimuli occurs in a variety of vertebrate and invertebrate prey (Weldon 1990; Dodson et al. 1994). For aquatic prey, recognition of predators through chemical stimuli may be particularly important when vision is limited, such as at night, in densely vegetated habitats or in turbid water.

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Chemosensory predator recognition may also be essential when predators are cryptic or adopt an ambush foraging strategy. If a predator's diet chemically labels it, then naive prey may have an important cue available for predator recognition.

Diet-related chemical labelling of predators has been demonstrated in several studies. Crowl & Covich (1990) demonstrated that snails, *Physella virgata*, discriminate between crayfish, *Orconectes virilis*, foraging on conspecifics and those foraging on spinach. Brook trout, *Salvelinus fontinalis*, avoided water from an unfamiliar predator, Atlantic salmon, *Salmo salar*, that was fed goldfish, *Carassius auratus*, but not water from salmon fed mealworms, *Tenebrio molitor* (Keefe 1992). Red-legged frog tadpoles, *Rana aurora*, showed a stronger anti-predator response to predatory newts, *Taricha granulosa*, that were fed tadpoles than newts fed insect larvae, *Chironomus* spp. (Wilson & Lefcort 1993). Brook stickleback,

Culaea inconstans, showed an anti-predator response to chemical stimuli from pike, *Esox lucius*, if the pike had been fed stickleback but not if the pike had been fed swordtails, *Xiphophorus helleri* (Gelowitz et al. 1993).

A few studies have demonstrated a more specific diet-related predator-labelling effect than those described above. Howe & Harris (1978), for example, demonstrated that the sea anemone, *Anthopleura elegantissima*, responded to water containing a predatory nudibranch, *Aeolidia papillosa*, if the nudibranch had recently eaten anemones but not if the nudibranch had been starved for three days. They subsequently showed that the anemone alarm pheromone, anthopleurine, persisted in the tissues of a nudibranch for at least five days after an anemone was eaten, leading to the speculation that anemones responded to residual alarm pheromone being released by the nudibranch. Mathis & Smith (1993a) showed that pike-naive fathead minnows, *Pimephales promelas*, gave an anti-predator response to chemical stimuli from pike fed fathead minnows but not to pike fed swordtails. The results of Mathis & Smith are similar to those of Howe & Harris (1978), in that fathead minnows were subsequently shown to respond to residual alarm pheromone released by the predator (Mathis & Smith 1993b). Mathis & Smith (1993a) further demonstrated that pike-naive minnows exposed to chemical stimuli from pike fed a diet of minnows learned to recognize pike as a predator in future encounters regardless of the pike's recent diet.

In this study we investigated diet-related anti-predator responses in damselfly larvae, *Enallagma* spp. (Odonata, Zygoptera). *Enallagma* spp. collected from a pond population located on the University of Saskatchewan campus responded with an anti-predator response to chemical stimuli from injured conspecifics or injured fathead minnows, a prey species with which they co-occur and share predators (B. D. Wisenden, D. P. Chivers & R. J. F. Smith, unpublished data). *Enallagma* spp. do not, however, exhibit an anti-predator response to either chemical stimuli from injured swordtails, an allopatric fish species, or to chemical stimuli from pike fed a diet of swordtails. In the first experiment in the present study, we exposed individual pike-naive damselflies to chemical stimuli from pike fed a diet of damselflies, fathead minnows or mealworms. We quantified the frequency of feeding bites, head

bends and moves performed by damselflies before and after exposing them to one of the three pike diet treatments. We predicted that damselflies would show an anti-predator response (i.e. a reduction in feeding and movement) in response to chemical stimuli from pike fed a diet of damselflies or fathead minnows, but not to pike fed a diet of mealworms.

Because damselflies responded with an anti-predator response to chemical stimuli from pike fed a diet of damselflies or fathead minnows, but not to pike fed mealworms in the first experiment, in the second experiment we tested whether damselflies that were previously exposed to chemical stimuli from pike fed damselflies or fathead minnows subsequently responded to chemical stimuli from pike fed mealworms. If damselflies previously exposed to these chemical stimuli subsequently respond to stimuli from pike fed mealworms, this result would demonstrate learned recognition of predators through diet-related chemical-labelling of the predator.

GENERAL METHODS

Collection and Maintenance of Study Animals

In January 1995, we collected damselfly larvae from a pond located on the University of Saskatchewan campus, where they occur in sympatry with fathead minnows. This pond does not contain pike, but does contain other fish species including finescale dace, *Phoxinus neogoeus*, white suckers, *Catostomus commersoni*, and Iowa darters, *Etheostoma exile*. We maintained the damselflies in a 160 litre aquarium on a 12:12 h light:dark photoperiod at approximately 19°C, and fed them daily with brine shrimp nauplii, *Artemia franciscans*. All damselfly larvae were keyed to *Enallagma boreale* using Merritt & Cummins (1984) and Walker (1953). Species identification of *Enallagma* based on larval characteristics can be unreliable. Adult male *Enallagma* can be reliably identified based on the morphology of their genitalia. Of 22 male *Enallagma* collected from this pond in June 1995, 15 were identified as *E. boreale* and seven were identified as *E. cyathigerum*. Because larvae of *E. boreale* and *E. cyathigerum* are morphologically identical (McPeck 1990a), the animals tested could have been a mixture of the two species. We refer to the test animals as *Enallagma* spp.

We collected northern pike from Van Pattens Creek in south central Saskatchewan in the spring of 1994. The pike were maintained in 60-litre aquaria, on a 14:10 h L:D cycle, and were fed a diet of 1–2 fathead minnows every 3–5 days.

Pike Stimulus Preparation

We collected stimuli from pike that were fed on one of three different diets: (1) damselflies; (2) fathead minnows; or (3) mealworms. We used six different pike ($\bar{X} \pm SD$ standard length = 16.0 ± 3.3 cm) to prepare the stimuli. To ensure that we fed the pike a standard amount of food, we matched the meal sizes for each pike during each feeding. Each pike was fed approximately 0.60 g of prey, which consisted of either two fathead minnows, four damselflies or four mealworms. We fed the pike four times (once every 3 days) prior to collecting the stimuli. Approximately 6 h after the final feeding we rinsed each pike in dechlorinated tap water and placed the pike into a separate plastic stimulus collection chamber ($26 \times 8 \times 8$ cm) that contained 1200 ml of dechlorinated tap water. The chambers were aerated but not filtered. After 3 days we removed the pike, combined the stimulus water from the two pike fed on the same diet (individual pike identity does not affect predator recognition by prey species: Mathis & Smith 1993a, b; Mathis et al. 1993; Gelowitz et al. 1993; Brown et al. 1995) and froze the stimulus water at approximately -20°C until needed.

EXPERIMENT 1: EFFECTS OF PREDATOR DIET ON ANTI-PREDATOR RESPONSES

Methods

We placed damselflies ($\bar{X} \pm SD$ length = 16.9 ± 1.4 mm, measured from the tip of the head to the tip of the abdomen) into individual 5-litre round plastic containers (diameter = 18 cm) that each contained 2 litres of dechlorinated tap water. The damselflies remained in the experimental containers for 3 days before the experiment began. During each trial we injected 5 ml of a standard concentration of brine shrimp culture (approximately 1200 shrimp nauplii) into each container

and waited 5 min before conducting observations. During the observations we recorded the frequency of three behaviour patterns (feeding bites, head bends and moves) performed by the damselflies for 5 min before and 5 min after injecting one of the three treatment stimuli (water from pike fed damselflies, fathead minnows or mealworms). We tested 10 different damselflies in each of the three diet treatments.

A feeding bite occurred when a damselfly quickly extended the prementum of its labium towards a prey item. Head bending occurred when a damselfly changed the orientation of its head horizontally or vertically at least 22.5° from its stationary position in line with the main body axis. Head bending served to orient the damselfly towards prey that were not directly in line with the main body axis. A feeding bite typically occurred within 2 s of the damselfly orienting towards the prey with a head bend. A move occurred when a damselfly changed its position in the chamber by walking. We defined a move as a short walk of less than one body length. Walking a distance of greater than one body length was not observed.

For each trial we calculated the change in the frequency of feeding bites, head bends and moves by subtracting the number performed before the stimulus exposure from the number performed after stimulus exposure. We used separate Kruskal–Wallis one-way analysis of variance tests with non-parametric multiple comparisons (Siegel & Castellan 1988) to compare the significance in the change in the frequency of feeding bites, head bends and moves in response to the three diet treatments. Larval damselflies, including *Enallagma* spp., decrease conspicuous behaviour in response to a predation threat (Heads 1985; Dixon & Baker 1988; McPeck 1990b; B. D. Wisenden, D. P. Chivers & R. J. F. Smith, unpublished data). Because we predicted that damselflies would decrease conspicuous behaviour in response to pike fed damselflies and fathead minnows, but not pike fed mealworms, we used one-tailed statistical tests.

Results

There was a significant overall effect of the diet treatment condition for each of the three behaviour patterns (feeding bites: $H=11.23$, $P<0.01$; head bends: $H=15.70$, $P<0.001$; moves: $H=14.91$, $P<0.001$). The frequency of feeding bites, head

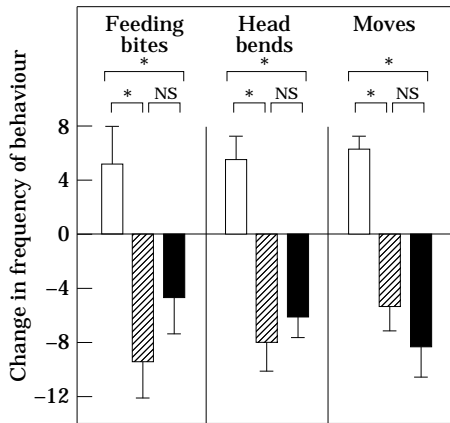


Figure 1. Mean (+SE) change in frequency of feeding bites, head bends and moves by damselflies exposed to chemical stimuli from pike fed either mealworms (□), fathead minnows (▨) or damselflies (■) (* denotes $P < 0.05$, NS denotes $P > 0.15$).

bends and moves by damselflies typically increased in response to stimuli from pike fed mealworms, but decreased in response to pike fed damselflies and pike fed fathead minnows. A priori multiple comparisons revealed that there was a significantly greater reduction in the frequency of feeding bites, head bends and moves in response to pike fed damselflies and fathead minnows compared with pike fed mealworms (all P s < 0.05 ; Fig. 1). There was no significant difference between the responses of damselflies to pike fed damselflies or fathead minnows (all P s > 0.15).

EXPERIMENT 2: THE EFFECT OF PREVIOUS EXPOSURE

Methods

Within 5 h of completing trials in experiment 1, we transferred the damselflies to identical testing chambers that contained fresh dechlorinated tap water. After the damselflies had remained in the testing chambers for an additional 2 days, we conducted experiment 2. The testing protocol and statistical comparisons were identical to those used in experiment 1, except that all damselflies were exposed to stimuli from pike fed mealworms. We predicted that damselflies that had been previously exposed to stimuli from pike fed a damselfly or minnow diet would respond with an anti-predator response to the stimuli of pike fed a mealworm diet.

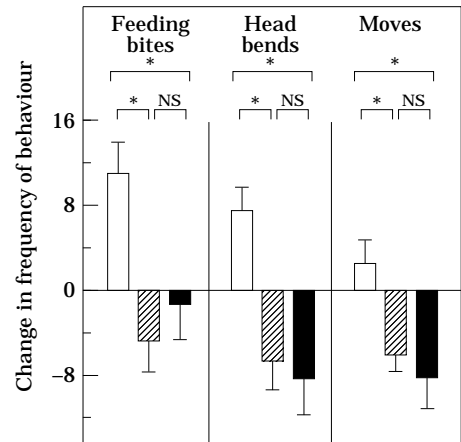


Figure 2. Mean (+SE) change in frequency of feeding bites, head bends and moves by damselflies exposed to chemical stimuli from pike fed mealworms in experiment 2. Damselflies exposed to chemical stimuli from pike fed mealworms, fathead minnows or damselflies in the previous experiment are denoted by □, ▨ and ■ bars, respectively (* denotes $P < 0.05$, NS denotes $P > 0.15$).

Results

The response of damselflies to stimuli from pike fed mealworms was significantly affected by the stimulus treatment the damselflies had received in experiment 1 (feeding bites: $H = 11.07$, $P < 0.01$; head bends: $H = 12.72$, $P < 0.01$; moves: $H = 7.95$, $P < 0.02$). When exposed to stimuli from pike fed mealworms, damselflies previously exposed to stimuli from pike fed damselflies or fathead minnows showed a significantly greater reduction in their frequency of feeding bites, head bends and moves than damselflies previously exposed to stimuli from pike fed mealworms (all P s < 0.05 ; Fig. 2). There was no significant difference between the responses of damselflies previously exposed to stimuli of pike fed damselflies and those of damselflies previously exposed to stimuli of pike fed fathead minnows (all P s > 0.15).

DISCUSSION

The results of this study show that damselflies respond differentially to predatory northern pike based on diet-related cues in the pike's diet. Pike-naive damselflies recognize and respond to chemical stimuli from pike that have recently fed on damselflies or fathead minnows, but not to stimuli

from pike that have fed on mealworms. This is the first study to demonstrate diet-related predator-labelling effects in insects.

By decreasing foraging and movement in response to stimuli from conspecifics in the diet of pike, damselflies should lower their risk of predation. Many predators that feed on fathead minnows are also likely to feed on damselflies. By responding to chemical stimuli from pike that have fed on fathead minnows, damselflies will similarly lower their risk of predation. Anti-predator responses to distantly related hetero-specifics probably result from experience (Chivers & Smith 1994a; Chivers et al. 1995a), therefore, such responses should be limited to damselfly populations that occur in sympatry with minnows. In a similar diet-related predator-labelling experiment, Mathis & Smith (1993b) showed that pike-naive minnows respond specifically to the alarm pheromone of fathead minnows released by pike fed fathead minnows. We do not know whether damselflies similarly respond specifically to the minnow alarm pheromone released from the pike.

Prey species learn to recognize predators when stimuli from injured conspecifics are paired with stimuli from predators (Magurran 1989; Chivers & Smith 1994b, c; Chivers et al. 1995a; B. D. Wisenden, D. P. Chivers, R. J. F. Smith, unpublished data). One study has shown that prey can learn to recognize predators through stimuli released from the diet of the predator. Mathis & Smith (1993a) showed that pike-naive fathead minnows can learn to recognize pike as predators by being exposed to chemical stimuli from pike that have been fed fathead minnows. Our results show a similar learning phenomenon. Damselflies exposed to chemical stimuli from pike fed damselflies or fathead minnows (but not mealworms) subsequently responded to stimuli from pike fed mealworms. This result shows that damselflies learn to recognize predators through diet-related stimuli in the predator's diet. Furthermore, this learned recognition results from a single exposure to chemical stimuli from the predator.

Diet-related predator-labelling effects may influence many predator/prey systems. The ability of naive prey to recognize predators from diet-related chemical cues may also be widespread. Researchers reporting the responses of prey to chemical stimuli from predators should report

the experience of the prey and the diet of the predators.

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