

Predator Recognition and Learned Irrelevance in the Crayfish *Orconectes virilis*

Brian A. Hazlett

Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

Abstract

When individuals of the crayfish *Orconectes virilis* detect an unlearned danger cue (alarm odor) and a novel cue (goldfish odor) at the same time, they form a learned association and behave as if the novel cue is associated with increased predation risk (Hazlett et al. 2002). This study examined the potential for learned irrelevance in *O. virilis* and the circumstances under which blockage of the formation of a learned association could occur. If individuals experience a random pattern of alarm odor and goldfish odor over the days prior to the simultaneous detection of those two cues, no learned association is formed (= learned irrelevance). That is, there is no inhibition of responses to a food cue when goldfish odor is added if the crayfish has experienced a random pattern of the two cues. Learning was eliminated if the random pattern of cues was experienced before or after the simultaneous detection. To present the two cues (alarm and goldfish odors) to crayfish independently on separate days, the water containing goldfish odor had to be removed from the aquaria as the odor persisted at least 24 h. The importance of the learned irrelevance phenomenon on predator–prey interactions is discussed.

Corresponding author: Brian A. Hazlett, Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA. E-mail: bhazlett@umich.edu

Introduction

Prey animals can reduce the probability of predation by detecting cues that indicate increased predation risk and altering their behavior appropriately (Mathis & Smith 1993a; Wisenden et al. 1999; Downes 2002). In a number of cases, recognition of danger cues requires learning by prey individuals (Mathis & Smith 1993b; Chivers & Smith 1998; Dicke & Grostal 2001). That is, prey individuals must experience a predator cue (such as predator odor) and an unlearned danger cue (such as alarm odor or prey breakdown products)

simultaneously before the predator cue is treated as a danger signal (Chivers et al. 1996; Hazlett & Schoolmaster 1998; Grostal & Dicke 1999; Wisenden & Millard 2001). Prey can learn from exposure to a predator cue and the simultaneous observation of anti-predator behavior of other fish (Mathis et al. 1996), but as Chivers & Smith (1998) have pointed out, injury-released chemical cues are a reliable and imminent indication of elevated risk. Even a non-predator such as the herbivorous goldfish *Carassius auratus* will be treated as a predator following simultaneous detection by prey individuals of conspecific alarm odor and goldfish odor (Chivers & Smith 1994).

In a number of studies examining the formation of an association by prey animals between a predator cue and increased predation risk, the training schedule utilized has been unrealistically simple (Hazlett et al. 2002). The test animals are exposed simultaneously to the novel 'predator' odor and alarm odor on one occasion and the animals are subsequently tested for a response to the novel chemical cue. This simulation of a successful predation attempt by the novel 'predator' does lead to the formation of a learned association in crayfish (Hazlett et al. 2002), fish (Chivers et al. 1995), flatworms (Wisenden & Millard 2001) and damselflies (Chivers et al. 1996). However, in nature the presence of a predator (and its odor) will not always result in the release of alarm odor. Unsuccessful predation events would result in detection by prey animals of the predator odor without the detection of alarm odor released from a prey individual, that has been damaged during predation. In addition, prey are usually subject to risk from a number of predator species (Hobbs 1993) and thus alarm odor could be released and detected without simultaneous detection of the odor of a particular predator type. Thus, in nature, the temporal pattern of exposure and detection of two odors will involve complexities beyond the simple simultaneous detection of two odors.

The vast literature on learning includes recognition of two phenomena that are particularly relevant to the situations prey individuals probably actually experience in nature. Latent inhibition is said to occur when there is inhibition of the formation of a learned association by repeated exposure to one cue prior to the simultaneous detection of both cues (Ferguson et al. 2001). The 'latent' label refers to the fact that the effects of early exposure are not revealed (= are latent) without combining those experiences with later simultaneous exposure to two cues. When crayfish (*Orconectes virilis* and *O. rusticus*) were repeatedly exposed to goldfish odor prior to simultaneous exposure to goldfish odor and conspecific alarm odor, no learned association was formed (Acquistapace et al. 2003) although an association was formed following simple simultaneous exposure to the two cues (Hazlett et al. 2002).

A second mechanism that can block the formation of a learned association is learned irrelevance. When individuals are exposed to two cues at random prior to the simultaneous detection of the two cues, no association is formed (Bennet et al. 2000). The animals behave as if they have learned that the two cues are irrelevant to one another; the occurrence of one does not predict anything about the occurrence of the other. Having learned the non-association of the two cues, the formation of an association is blocked when they do occur together (i.e. when

successful predation by that type of predator does occur). While this mechanism has been well studied in the laboratory (Mackintosh 1974), and is known to occur in at least one invertebrate, the honeybee *Apis mellifera* (Cheng & Spetch 2001), its relevance to ecological processes such as predator-prey interactions has not been explored.

In the present study, individuals of the crayfish *O. virilis* were tested for the existence of the process of learned irrelevance. The effects of random exposures to two cues for different lengths of time on the formation of a learned association were examined as well as random association of cues both prior to and following simultaneous presentation of two odor cues. Experiments were first required to address the question of how long odors persist as testing for learned irrelevance requires separate exposure to two cues in addition to the simultaneous exposure to those cues.

General Methodology

The species studied was *O. virilis*. The individuals of *O. virilis* were collected from ponds at the DNR Fisheries Station in Saline, MI, USA. Tests were first carried out to examine the question of how long goldfish odor persisted as testing of learned irrelevance requires the animals experience the two stimuli separately prior to experiencing them together. The results of this first series of tests suggested the methodology that was then used to examine the basic phenomenon of learned irrelevance and to examine additional aspects of the phenomenon.

For all tests for both series of experiments, animals were placed in holding containers in the laboratory in Ann Arbor, MI, USA held at 20°C and a 12:12 h light cycle. The crayfish were fed pieces of codfish for at least 4 d before being placed in a training aquarium. Crayfish need experience with a food type before they recognize food odor cues (Hazlett 1994). Both holding aquaria and training aquaria were 120 l, supplied with constant aeration and filters, and a number of broken clay pots were provided as shelters for the crayfish.

Crayfish were trained over a period of days with sequences of exposures to a novel odor (the herbivorous goldfish, *C. auratus*), conspecific alarm odor (the unconditioned stimulus indicating elevated predation risk), or both odors presented at the same time. The particular sequence of exposures is described below for each of the separate experiments. Alarm odor was prepared in all cases by crushing an average size adult crayfish (25–30 mm cephalothorax length) in 200 ml of distilled water and filtering with coarse filter paper. In all experiments that involved the introduction of goldfish odor as part of the training treatment, four goldfish (average standard length 35 mm) were placed in an opaque plastic container (1000 ml) with many holes drilled in it and the container was placed in a training aquarium with 10 crayfish for 2 h. Goldfish were obtained from local pet stores as needed and fed flaked food for herbivorous fish.

Following training with different sequences of exposure to odors (see below), crayfish were transferred to individual 40 l aquaria for testing. Each visually isolated aquarium had a water depth of 10 cm, a clay pot for a burrow, and was

continually aerated. For all tests, animals were allowed 24 h acclimation in the aquaria before testing. In all tests, animals were observed for three 2-min periods after (a) introduction of 5 ml of distilled water, followed by (b) introduction of 5 ml of food odor, and then followed by (c) introduction of 5 ml of goldfish odor. The food odor cue was prepared by macerating 20 g of codfish in 150 ml of water and filtering with a coarse filter to remove particles. The goldfish odor used for testing responses to goldfish odor was prepared in all cases by placing four medium-size goldfish (average standard length 35 mm) in 2 l of aerated water for 24 h prior to use of the odor.

In all of the experiments, the observer observed behaviors and recorded their duration with an event program on a computer. The behaviors recorded were the same as in earlier studies (Hazlett 2000) and included: feeding movements of the chelipeds and walking legs, locomotion (movement of the whole animal as a result of ambulatory leg movement), and raised posture (raised posture = cephalothorax raised well off of the substrate and chelipeds held horizontal or higher). Although the crayfish could be in other postures, because the time spent in one posture affects the time spent in other postures they are not independent measures and only one posture was analyzed. The number of seconds spent executing each of the behaviors was recorded during the three treatment periods. Crayfish were used in only one experiment, and 20 individuals were tested in every case.

The results of all experiments in both series were analyzed in the same way. The number of seconds spent in the three behavioral categories (feeding, locomotion, raised posture) were analyzed for a treatment effect (control, food odor, food + goldfish odor) by repeated measures ANOVA. In every experiment, all three behaviors were significantly different among treatments (Table 1) and

Table 1: Results of ANOVAs comparing time spent by individuals of *Orconectes virilis* under the different odor treatments. F values (associated p values) are presented for three behaviors for all seven experiments. Degrees of freedom in all cases were 2, 57

	Feeding (p-value)	Raised posture (p-value)	Locomotion (p-value)
Day-old goldfish odor	32.3 (< 0.001)	14.5 (< 0.001)	12.1 (< 0.001)
Sequential exposure only	34.9 (< 0.001)	18.9 (< 0.001)	21.5 (< 0.001)
Sequential exposure with removal	15.7 (< 0.001)	5.6 (0.01)	9.2 (0.001)
Sequential (with removal) + simultaneous exposure	27.3 (< 0.001)	8.3 (0.001)	12.3 (< 0.001)
Sequential (without removal) + simultaneous exposure	139.8 (< 0.001)	53.7 (< 0.001)	81.0 (< 0.001)
Short sequential + simultaneous exposure	19.1 (< 0.001)	7.7 (0.001)	6.8 (0.002)
Simultaneous exposure only	125.0 (< 0.001)	77.5 (< 0.001)	103.3 (< 0.001)
Simultaneous followed by sequential exposure	67.9 (< 0.001)	22.6 (< 0.001)	41.4 (< 0.001)

thus Tukey tests were used to examine pairwise differences among treatments. In every experiment, these three measures were either all significantly different between treatment ($p < 0.001$ from Tukey tests) or none were different ($p > 0.20$ from Tukey tests).

Persistence of Goldfish Odor

Learned irrelevance can occur only if the two stimuli that will be experienced simultaneously can also be experienced separately. In order to present the two odors separately, it was necessary to establish how long goldfish odor persisted. Three experiments were carried out to address this question.

Methods

Day-old goldfish odor

While earlier work had shown that crayfish alarm odor underwent degradation and was no longer effective after 6 h (P. Acquistapace, pers. comm.), it was necessary to determine if goldfish odor persisted for up to 24 h. To test the hypothesis that goldfish odor persisted for 24 h at room temperature, crayfish were exposed to goldfish odor and alarm odor simultaneously for 2 h and then placed in observation aquaria. Earlier work (Hazlett et al. 2002) showed that individuals of *O. virilis* form an association between goldfish odor and alarm odor following simultaneous presentation of the two odors and that without this experience crayfish show no responses to goldfish odor. At the same time a sample of water from a container holding goldfish was removed and set aside in a beaker. The next day the trained crayfish were tested with food odor and 24-h-old goldfish odor.

Sequential exposures only

Given the results of the tests with day-old goldfish odor, I tested the possibility that a learned association could be formed when odors were presented on separate days. On alternate days, crayfish were exposed to either goldfish odor alone (goldfish container in the training aquarium for 2 h) or alarm odor. Each type of odor was presented twice over a 4-day training period. Individuals received either alarm odor followed the next day by goldfish odor or the reverse. They were then placed in the observation aquaria and after 1 d were tested for an inhibitory effect of goldfish odor on the response to food odors.

Sequential exposures with removal

Given the results of the tests with sequential exposures alone, crayfish were exposed to the same training schedule but with the goldfish odor removed following 2 h of exposure. On alternate days goldfish were placed in a plastic

container and left in the training aquarium for 2 h. Immediately following removal of the goldfish themselves, I siphoned about 85% of the water from the training container and immediately replaced it with fresh water (at room temperature to avoid temperature shock). The water was removed, rather than moving the crayfish, to avoid disturbing the crayfish. This procedure was carried out on either days 1 and 3 or 2 and 4 and alarm odor was added on the alternate days. The crayfish were then placed in observation aquaria and after 1 d were tested for the inhibitory effects of goldfish odor on the responses to food odors.

Results

Day-old goldfish odor

Day-old goldfish odor was effective in inhibiting responses to food odor for crayfish that had experienced simultaneous exposure to goldfish and alarm odors (Table 1; Fig. 1). Clearly the aged solution still contained chemical cues that were detected by crayfish and treated as danger signals.

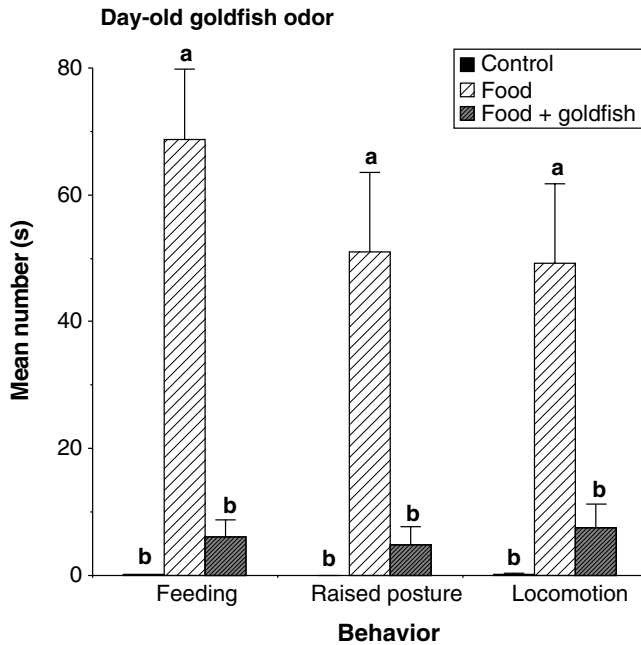


Fig. 1: Number of seconds ($\bar{x} + SE$) spent by individuals of *Orconectes virilis* in the raised posture, showing feeding behaviors and locomotion under three test conditions (control, food odor added, food odor and day-old goldfish odor added). Letters indicate significant differences between treatments by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor on the same day

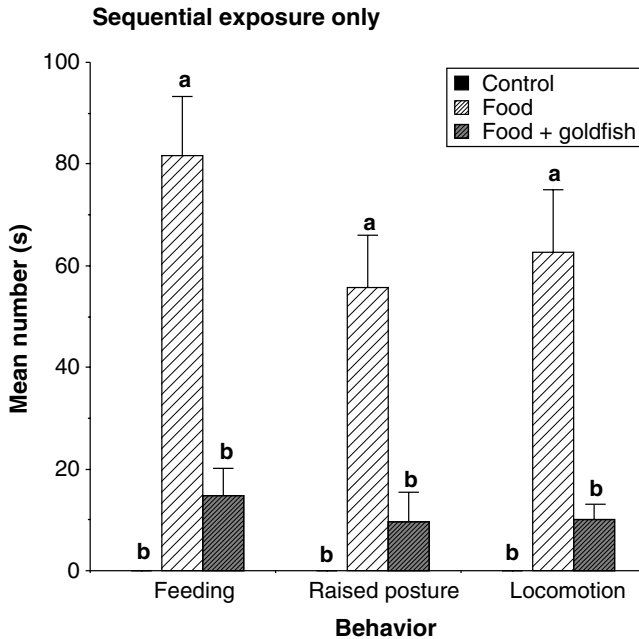


Fig. 2: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions (control, food odor added, food odor and goldfish odor added). Letters indicate significant differences by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor on alternate days

Sequential exposure only

The training period exposure to the two odors over a 4-day period resulted in a strong inhibition of responses to food odor by the introduction of goldfish odor (Table 1; Fig. 2). In fact, the responses to food odor were decreased to control levels by the introduction of goldfish odor. The training period exposures alone were sufficient to allow the formation of a learned association. These results are consistent with the possibility that the goldfish odor was persisting from 1 d to the next.

Sequential exposure with removal

Crayfish exposed to random presentations of the two odors, with the goldfish odor being removed physically, did not appear to form an association between the two odors as had occurred in the previous experiment (Table 1; Fig. 3). There was no inhibitory effect of goldfish odor on the responses to food odor for these individuals when goldfish odor had been removed and the crayfish did not experience the two odors simultaneously.

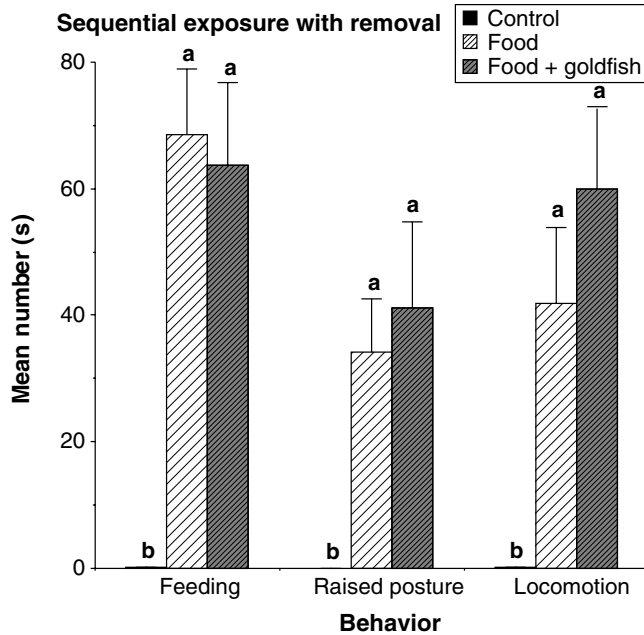


Fig. 3: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions. Letters indicate significant differences by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor on alternate days and the goldfish odor was removed by draining the water from the training aquaria

Learned Irrelevance

Methods

Sequential (with removal) + simultaneous exposure

Crayfish were trained with presentation of goldfish odor and alarm odor on alternate days for 4 d. On alternate days goldfish were placed in a plastic container and left in the training aquarium for 2 h. Following removal of the goldfish themselves, I siphoned about 85% of the water from the training container and immediately replaced it with fresh water (at room temperature to avoid temperature shock). This was performed on either days 1 and 3 or 2 and 4 and alarm odor was added on the alternate days. On day 5, the crayfish were exposed to goldfish in the plastic container and to alarm odor for 2 h and then the crayfish were placed in the observation aquaria. On day 6, they were tested with food and then goldfish odor introductions.

Sequential (without removal) + simultaneous exposure

Given the results of the previous experiment and of the tests for the persistence of goldfish odors, it was of interest to see if sequential presentation

of the two odors without physical removal of the goldfish odor still could result in blockage of the formation of a learned association. Each type of odor was presented to crayfish twice over a 4-day training period. Individuals received either alarm odor followed (the next day) by goldfish odor or the reverse. On the day 5, the crayfish were exposed to simultaneous presentation of goldfish odor and alarm odor for 2 h. They were then placed in the observation aquaria and on day 6 were tested for an inhibitory effect of goldfish odor on the response to food odors.

Short sequential + simultaneous exposure

In the first experiment in this series, crayfish were exposed to two cycles of one odor followed by the other odor. In this experiment, crayfish experienced only one cycle of odor presentations prior to the simultaneous exposure to the two odors. Individuals were exposed to either alarm odor on 1 d and goldfish odor the next day (with draining to physically remove the goldfish odor after 2 h) or the reverse order. On the day 3, they were exposed to both odors simultaneously and then placed in individual observation aquaria. Tests on responses to food and goldfish odor introductions were performed on day 4.

Simultaneous followed by sequential exposure

In nature, a series of independent exposures to two cues could occur either before or after the simultaneous detection of those cues. In this experiment, crayfish were exposed simultaneously to goldfish odor and alarm odor for 2 h (with draining to remove the goldfish odor) on day 1. The control animals remained in their training aquaria for the next 4 d and were then placed in individual aquaria on day 5. The test animals were exposed to goldfish odor (with draining) on days 2 and 4 or 3 and 5 and alarm odor on alternate days. Test animals were placed in individual aquaria on day 5 and all crayfish were tested with food and goldfish odors on day 6.

Results

Sequential (with removal) + simultaneous exposure

Crayfish exposed to independent presentation of the two odors, with the goldfish odor being physically removed, did not appear to form an association between the two odors even though they were exposed to both odors at the same time on day 5 (Table 1; Fig. 4). There was no inhibitory effect of goldfish odor when tested on day 6. Thus, individuals of *O. virilis* exposed to the two odors independently during the training period did not form a learned association and they show the effects of learned irrelevance.

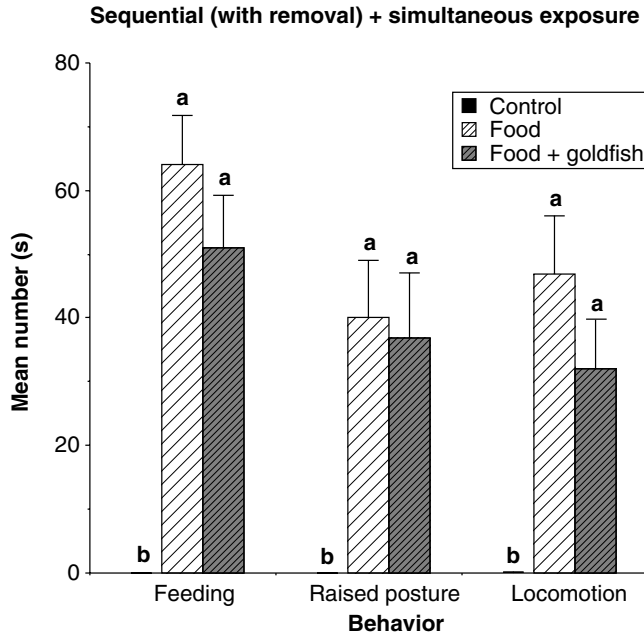


Fig. 4: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions. Letters indicate significant differences between by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor on alternate days and the goldfish odor was removed by draining the training aquaria. Following the alternate day training the crayfish were exposed to alarm odor and goldfish odor on the same day

Sequential (without removal) + simultaneous exposure

The introduction of goldfish odor on the test day very significantly reduced the responses to food odor (Table 1, Fig. 5). The strength of the inhibition was even stronger than that originally reported for this species (Hazlett et al. 2002). Clearly the experiences the crayfish had during the training period did not have an inhibitory effect on the formation of a learned association, thus reinforcement rather than learned irrelevance occurred with this training methodology. In light of the results of the tests on the persistence of goldfish odor, one possibility for this result is that the goldfish odor persisted in the training aquarium after removal of the goldfish and when alarm odor was added the next day, the crayfish were actually being exposed simultaneously to the two odors on repeated occasions.

Short training + simultaneous exposure

Individuals exposed to just the one sequence of independent exposures prior to simultaneous exposure showed no evidence of the formation of a learned

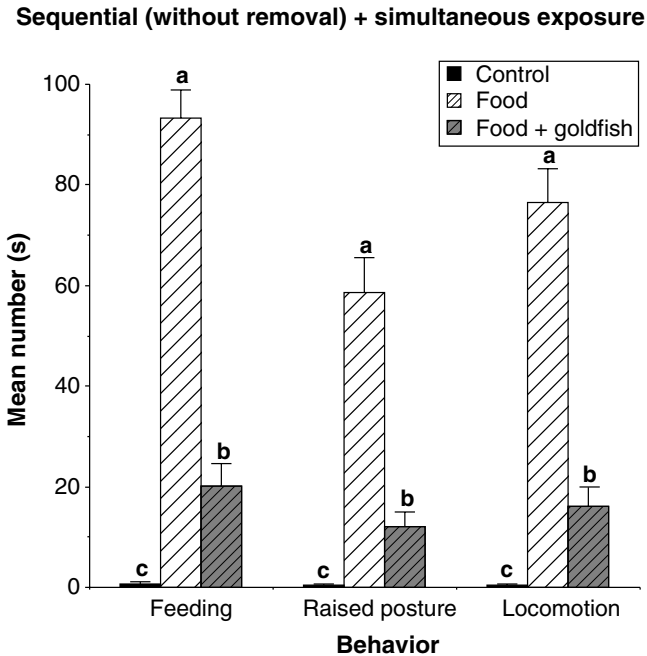


Fig. 5: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions. Letters indicate significant differences by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor (without removal of the goldfish odor) on alternate days and then alarm odor and goldfish odor on the same day

association (Table 1; Fig. 6). None of the behaviors recorded showed a significant difference between exposure to food odor and to the combination of food and goldfish odor although there appeared to be a weak tendency towards a reduction of responses. The crayfish did not appear to treat the goldfish odor as a cue associated with elevated predation risk.

Simultaneous followed by sequential exposure

The control animals showed evidence of the persistence of a learned association between goldfish odor and elevated predation risk by the significant reduction in responses to food odor upon the introduction of goldfish odor (Table 1; Fig. 7a). For crayfish exposed to both cues on just day 1, the learned association persisted for at least 5 d. The test animals, with the intervening independent exposure to the two odors, showed no evidence of a learned association (Table 1; Fig. 7b). The independent exposures apparently eliminated the learned association. Thus the sequence of when simultaneous and independent exposures to two stimuli occurs is not critical in the blockage of a learned association via learned irrelevance (see Fig. 4 for comparison).

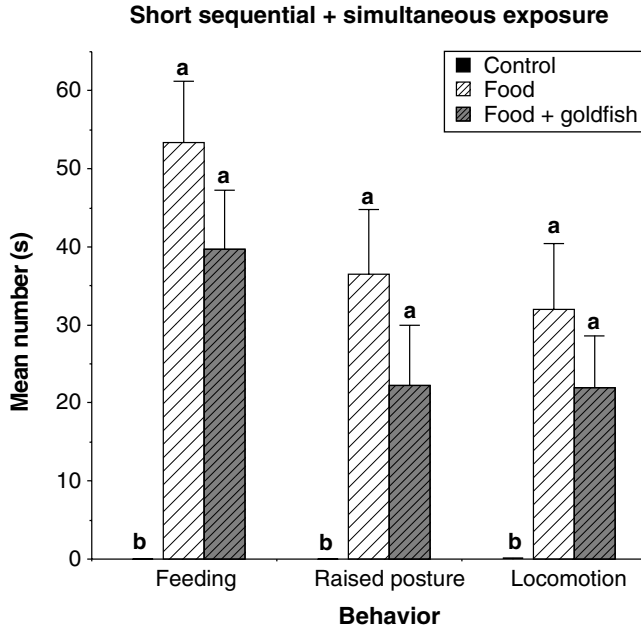


Fig. 6: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions. Letters indicate significant differences by Tukey tests. Prior to testing, individuals were exposed to just one cycle (rather than two) of alarm odor and goldfish odor on alternate days and the goldfish odor was removed by draining the training aquaria. Following the alternate day training the crayfish were exposed to alarm odor and goldfish odor on the same day

Discussion

These experiments clearly demonstrate that two factors need to be considered in the role of past experience in affecting the behaviors shown in ecological interactions such as predator detection and avoidance by prey animals. First, the temporal pattern of exposures to cues completely determines whether a prey organism will form a learned association when inputs from a successful predation event are detected. Secondly, cues from predators that could be used by prey in recognizing an elevated predator risk situation differ in their persistence.

The role of learning in predator recognition has been demonstrated in a wide variety of taxa (Chivers & Smith 1998; Brown & Godin 1999). In almost every case, the methods used to establish the formation of a learned association between stimuli have followed a similar protocol. Test animals are presented simultaneously with an unconditioned cue such as conspecific alarm odor and a conditioned stimulus, a novel odor associated with a potential 'predator'. The duration of the simultaneous odor presentations varies among studies but the presentation is viewed as one exposure by all the experimenters (including Hazlett et al. 2002). Korpi & Wisenden (2001) reported the formation of a learned association in zebra danios, *Danio rerio*, when the unconditioned stimulus (alarm

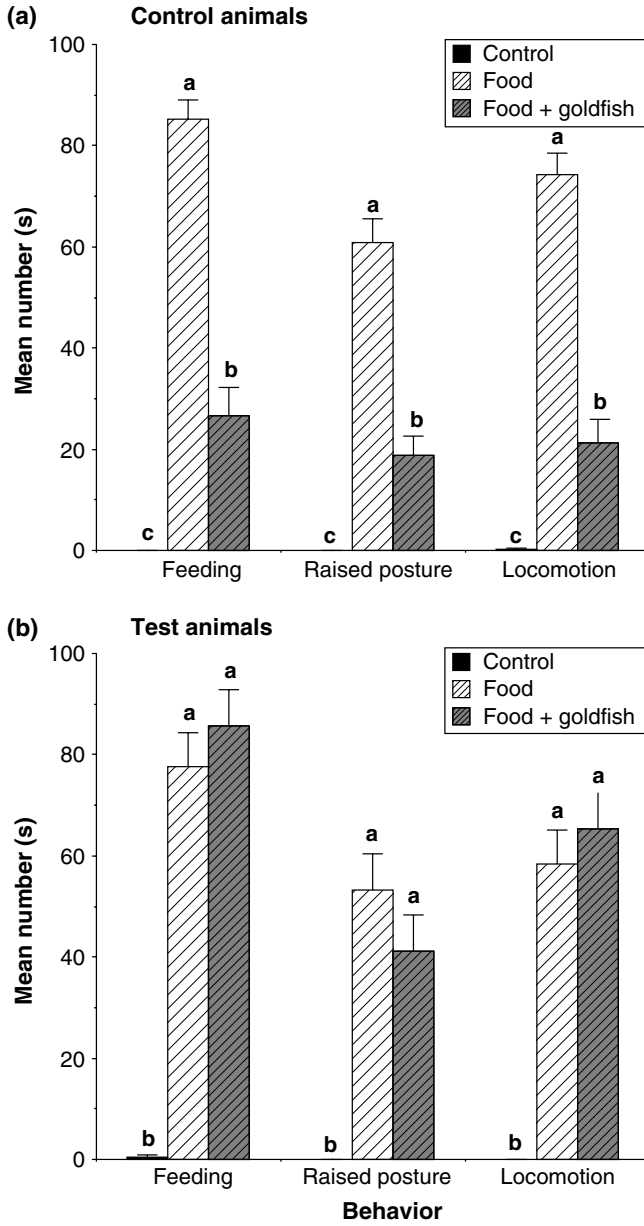


Fig. 7: Number of seconds ($\bar{x} + SE$) spent by *Orconectes virilis* in the three behaviors under three test conditions. Letters indicate significant differences by Tukey tests. Prior to testing, individuals were exposed to alarm odor and goldfish odor on the same day and either received no further treatment (control animals) (Fig. 7a) or on subsequent days were exposed to either alarm odor alone or goldfish odor (with draining) alone (Fig. 7b)

odor) was introduced 5 min prior to the unconditioned stimulus (pike odor) but as the authors point out, the potential persistence of the alarm odor would mean both cues were experienced at the same time.

The actual pattern of exposure to cues associated with elevated predation risk that a prey organism experiences in nature is almost certainly more complex than a 'single' period of detection of two (chemical) stimuli. Prey must frequently detect odors of potential predators without the simultaneous detection of an unlearned cue such as alarm odor, e.g. during a period without successful predation by that type of predator. Conversely, prey must also frequently detect alarm odor without the simultaneous detection of odors (or other cues) associated with a particular potential predator. If prey are exposed to some sequence of an unlearned danger signal and the odors of a novel predator prior to or after a successful predation event by that type of predator, exposures could block or reduce the strength of the formation of a learned association.

In this study, individuals of the crayfish *O. virilis* failed to form an association between an unconditioned stimulus and a novel odor when they either previously had been or subsequently were exposed to the two cues separately. Thus the process of learned irrelevance was demonstrated for these animals. Learned irrelevance is thought to occur because the random exposure to the two cues during the training period teaches the animal that the two cues are not causally connected. Thus, when they do experience a temporal correlation, no association is formed because of the effects of the learning of irrelevance. This pattern is in contrast to the formation of an association that lasts for a number of days by individuals of *O. virilis* when a 'single' period of simultaneous detection of an unlearned cue and novel odor is presented (Hazlett et al. 2002).

The experiment with just one cycle of separate presentation of the two cues prior to the simultaneous presentation indicates that at least for this species, the formation of a learned association from a 'single' simultaneous exposure is rather easily disrupted. Earlier work on *O. virilis* (Acquistapace et al. 2003) showed that individuals are also susceptible to the blocking of the formation of an association simply by the repeated exposure of the crayfish to the novel stimulus prior to the simultaneous exposure period.

The first three experiments reported here demonstrate that some types of odors can persist for a considerable number of hours. It would appear that goldfish odors have not broken down in aerated water at room temperature in 24 h. This odor persistence is in contrast to other odors such as snapping turtle odor that lasts less than 2 h (Hazlett 1999) or crayfish alarm odors, which last less than 6 h (P. Acquistapace, pers. comm.). If we can extrapolate from one species of fish to real potential predators of crayfish, this means that in a pond situation (relatively still water, odor not quickly physically removed), crayfish would learn differently if a predator was a fish vs. a turtle. For an association to be formed, the temporal proximity to the detection of alarm odor would have to be closer for a turtle predator than a fish predator. It is clearly open to speculation why taxonomic groups would differ in the duration of 'body odors', but the abundant mucopolysaccharides and the disaccharides which result from polysaccharide

degradation in fish (Forward & Rittschof 1999) may be much slower to break down or be degraded than classes of chemicals more common in other organisms.

The demonstration of the process of learned irrelevance presented in this study required the physical removal of the persistent novel fish odor to train the crayfish with separate presentations of the two cues. Draining of the water from an aquarium is functionally similar to living in a stream or river where the odors present at one point in time are removed by water flow. This means that the process of learning about predator recognition will be different in lentic and lotic environments. The same sequence of novel odor presence and alarm odor presence will block the formation of a learned association in a stream situation but reinforce the formation of a learned association in a pond situation. But this would be the case only for predator odors that persist (as demonstrated with goldfish odor used in this study). For odors that degrade faster, learned irrelevance would affect the formation of learned associations in more similar ways in lentic and lotic habitats.

Acknowledgements

I wish to thank Catherine Bach, Keith Pecor, and Dan Rittschof for helpful comments on the manuscript. Thanks also to Gappy and the staff at the Saline DNR Fisheries Facility for providing the crayfish used in this study.

Literature Cited

- Acquistapace, P., Hazlett, B. A. & Gherardi, F. 2003: Unsuccessful predation and learning of predator cues by crayfish. *J. Crust. Biol.* **23**, 364–370.
- Bennet, C. H., Wills, S. J., Oaakeshott, S. M. & Mackintosh, N. J. 2000: Is the context specificity of latent inhibition a sufficient explanation of learned irrelevance?. *Quart. J. Exper. Psych. B, Comp. Physiol. Psychol.* **53B**, 239–253.
- Brown, G. E. & Godin, J. G. J. 1999: Who dares, learns: chemical inspection behavior and acquired predator recognition in a characin fish. *Anim. Behav.* **57**, 475–481.
- Cheng, K. & Spetch, M. L. 2001: Blocking in landmark-based search in honeybees. *Anim. Learn. Behav.* **29**, 1–9.
- Chivers, D. P. & Smith, R. J. F. 1994: Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Anim. Behav.* **48**, 597–605.
- Chivers, D. P. & Smith, R. J. F. 1998: Chemical alarm signaling in aquatic predator–prey systems: a review and prospectus. *Ecoscience* **5**, 338–352.
- Chivers, D. P., Brown, G. E. & Smith, R. J. F. 1995: Acquired recognition of chemical stimuli from pike, *Esox lucius*, by brook stickleback, *Culaea inconstans* (Osteichthyes, Gasteroisteidae). *Ethology* **99**, 234–242.
- Chivers, D. P., Wisenden, B. D. & Smith, R. J. F. 1996: Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Anim. Behav.* **52**, 315–320.
- Dicke, M. & Grostal, P. 2001: Chemical detection of natural enemies by Arthropods: an ecological perspective. *Ann. Rev. Ecol. Syst.* **32**, 1–24.
- Downes, S. J. 2002: Does responsiveness to predator scents affect lizard survivorship? *Behav. Ecol. Sociobiol.* **52**, 38–42.
- Ferguson, H. J., Cobey, S. & Smith, B. H. 2001: Sensitivity to change in reward is heritable in the honeybee, *Apis mellifera*. *Anim. Behav.* **61**, 527–534.
- Forward, R. B., Jr. & Rittschof D. 1999: Brine shrimp larval photoresponses involved in diel vertical migration: activation by fish mucus and modified amino sugars. *Limnol. Oceanog.* **44**, 1904–1916.

- Grostal, P. & Dicke, M. 1999: Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behav. Ecol.* **10**, 422–427.
- Hazlett, B. A. 1994: Crayfish feeding responses to zebra mussels depend on microorganisms and learning. *J. Chem. Ecol.* **20**, 1525–1535.
- Hazlett, B. A. 1999: Responses to multiple chemical cues by the crayfish *Orconectes virilis*. *Behaviour* **136**, 161–177.
- Hazlett, B. A. 2000: Information use by an invading species: do invaders respond more to alarm odors than native species? *Biol. Invasions* **2**, 289–294.
- Hazlett, B. A. & Schoolmaster, D. R. 1998: Responses of cambarid crayfish to predator odor. *J. Chem. Ecol.* **24**, 1757–1770.
- Hazlett, B. A., Acquistapace, P. & Gherardi, F. 2002: Differences in memory capabilities in invasive and native crayfish. *J. Crust. Biol.* **22**, 439–448.
- Hobbs, H. H. III 1993: Trophic relationships of North American freshwater crayfishes and shrimps. *Milwaukee Public Museum Contrib. Biol. Geol. No. 85*, 1–110.
- Korpi, N. L. & Wisenden, B. D. 2001: Learned recognition of novel predator odour by zebra danios, *Danio rerio*, following time-shifted presentation of alarm cue and predator odour. *Environmental Biol. Fishes* **61**, 205–211.
- Mackintosh, N. J. 1974: *The Psychology of Animal Learning*. Academic Press, London.
- Mathis, A. & Smith, R. J. F. 1993a: Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). *Behav. Ecol.* **4**, 260–265.
- Mathis, A. & Smith, R. J. F. 1993b: Fathead minnows, *Pimephales promelas*, learn to recognize northern pike, *Esox lucius*, as predators on the basis of chemical stimuli from minnows in the pike's diet. *Anim. Behav.* **46**, 645–656.
- Mathis, A., Chivers, D. P. & Wmih, R. J. F. 1996: Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. *Anim. Behav.* **51**, 185–201.
- Wisenden, B. D. & Millard, M. C. 2001: Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. *Anim. Behav.* **62**, 761–766.
- Wisenden, B. D., Cline, A. & Sparkes, T. C. 1999: Survival benefit to antipredator behavior in the amphipod *Gammarus minus* (Crustacea: amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. *Ethology* **105**, 407–414.

Received: March 11, 2003

Initial acceptance: May 8, 2003

Final acceptance: May 15, 2003 (S. A. Foster)