LEARNED RECOGNITION OF HETEROSPECIFIC ALARM CUES ENHANCES SURVIVAL DURING ENCOUNTERS WITH PREDATORS

by

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Summary

Numerous species of aquatic animals release chemical cues when attacked by a predator. These chemicals serve to warn other conspecifics, and in some cases heterospecifics, of danger, and hence have been termed alarm cues. Responses of animals to alarm cues produced by other species often need to be learned, yet mechanisms of learned recognition of heterospecific cues are not well understood. In this study, we tested whether fathead minnows (*Pimephales promelas*) could learn to recognize a heterospecific alarm cue when it was combined with conspecific alarm cue in the diet of a predator. We exposed fathead minnows to chemical stimuli collected from rainbow trout, *Oncorhynchus mykiss*, fed a mixed diet of minnows and brook stickleback, *Culaea inconstans*, or trout fed a mixed diet of swordtails, *Xiphophorous helleri*, and stickleback. To test if the minnows had acquired recognition of the heterospecific alarm cues, we exposed them to stickleback alarm cues and introduced an unknown predator, yellow perch (*Perca flavescens*) or northern pike (*Esox lucius*). Both perch and pike took longer to initiate an attack on minnows that were previously exposed to trout fed minnows and stickleback than those previously exposed to trout fed swordtails and stickleback. These results demonstrate that minnows can learn to recognize heterospecific alarm cues based on detecting the heterospecific cue in combination with minnow alarm cues in the diet of the predator. Ours is the first study to demonstrate that behavioural responses to heterospecific chemical alarm cues decreases the probability that the prey will be attacked and captured during an encounter with a predator.

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Introduction

Many aquatic animals release chemical cues upon being attacked by a predator (Chivers & Smith, 1998). These cues function to 'warn' other nearby individuals of possible danger and hence have been termed alarm cues (Hews, 1988; Mathis & Smith, 1993a; Wisenden *et al*., 1999; Mirza & Chivers, 2001a, b). Different prey species often respond to each other's alarm cues (reviewed in Chivers & Smith, 1998). Such cross-species responses could reflect a close phylogeny between sender and receiver (Brown *et al*., 2000; Mirza & Chivers, 2001c; Mirza *et al*., 2001). In these cases the chemical structure of the alarm cues may be identical or nearly identical. Cross-species responses could also occur if prey species that co-occur together and are exposed to the same suite of predators, have learned to recognize each other's alarm cues (Mathis & Smith, 1993b; Chivers & Smith, 1994; Chivers *et al*., 1996).

Previous studies have shown that some cross-species responses to chemical alarm cues must be learned (Chivers *et al*., 1995; Pollock *et al*., in press). For example, Pollock *et al*. (in press) have shown that fathead minnows (*Pimephales promelas*) learn to recognize the alarm cues of brook stickleback (*Culaea inconstans*) following their introduction to a pond containing the minnows. Despite the studies that have shown that cross-species responses need to be learned, only 1 study has examined the way in which recognition may arise (Mirza & Chivers, 2001d).

Mirza & Chivers (2001d) exposed fathead minnows to chemical cues of a yellow perch (*Perca flavescens*) fed a mixed diet of both minnows and stickleback or perch fed a mixed diet of swordtails (*Xiphophorus helleri*) and stickleback and then subsequently tested the minnows for a response to stickleback alarm cues alone. Minnows previously exposed to perch fed minnows and stickleback subsequently exhibited anti-predator behaviour to stickleback cues alone. In contrast, minnows exposed to perch fed stickleback and swordtail did not subsequently respond to stickleback cues alone. These results indicate that minnows learn the identity of the stickleback alarm cues based on detecting them with conspecific alarm cues in the diet of the perch.

The current experiment follows from this work and asks the question: Can minnows learn to recognize stickleback alarm cues that are detected with minnow alarm cues in the diet of a rainbow trout, and if so, does this learned recognition provide a demonstratable survival advantage? Assessing whether the learning occurs when using a different predator will give us an indication of how common this type of learning may be. A critical element of the system requires that both the conspecific and heterospecific alarm cues need to be able to pass through the predator and remain recognizable to the prey.

The current study addresses whether the behavioural response of minnows to heterospecific alarm cues will provide the minnows with a survival advantage (*i.e.* will ‘warned’ minnows be attacked or captured less often than ‘unwarned’ minnows). We exposed minnows to stickleback alarm cues and then staged encounters between them and an unknown predator in order to assess whether the minnows that had the opportunity to learn the heterospecific alarm cue had a survival advantage over those that that did not have the opportunity to learn. Our experiment employed 2 different fish predators. Northern pike (*Esox lucius*) are specialized piscivores that are efficient at foraging on small prey fishes (Chivers *et al.*, 1996). Yellow perch are generalist aquatic predators (Scott & Crossman, 1979). They are less efficient foragers on prey fishes. Several authors have speculated that behavioural responses exhibited to heterospecific alarm cues should translate into a survival benefit for the prey, however, this assumption has not been empirically tested.

Methods

We collected fathead minnows from Briarwood pond in Saskatoon, Saskatchewan, in the fall and winter of 2001. The minnows were maintained in a 625-L holding tank at approximately 17°C and were fed a diet of commercial flake food. Briarwood pond does not contain stickleback or predatory fishes. Pollock *et al.* (unpub. data) showed that minnows from Briarwood Pond do not respond to stickleback alarm cues and Gazdewich & Chivers (2002) showed that these minnows do not recognize perch as a predator.

Brook stickleback were collected from Lakeview pond in Saskatoon in the fall and winter of 2001. They were maintained in 37-L aquaria and were fed previously frozen brine shrimp. Pike and adult perch were caught using seine nets and angling during the fall of 2001. Perch were collected from Blackstrap Lake and pike were collected from Brightwater reservoir, both of which are located in central Saskatchewan. Pike and perch were kept individually in separate compartments of a series of 150-L aquaria and were maintained on fathead minnows prior to the experiment. Juvenile perch, to be used as food for the pike and perch during the experiment, were also collected from Blackstrap Lake. Rainbow trout were acquired from the provincial hatchery at Fort Qu'Appelle, Saskatchewan. Trout were maintained in artificial stream tanks (350-L) and were fed commercial pellets and previously frozen brine shrimp prior to the experiments. Swordtails were obtained through a commercial supplier, and were kept in 150-L aquaria. Stickleback, perch, pike and trout were all maintained at approximately 15°C and swordtails were maintained at 23°C. All fishes were maintained on a 14 : 10 h light : dark cycle.

In order to collect the predator stimuli we placed four rainbow trout into separate flow through tanks measuring (54 × 32 × 36 cm). Two trout were fed a mixed diet of swordtail and stickleback and two trout were fed a mixed diet of fathead minnows and stickleback. Each predator was fed two prey (one of each appropriate species) every three days for a total of three feedings. Immediately after the last feeding the two trout that had been fed minnows and stickleback were transferred to a 37-L tank and the two trout that had been fed swordtails and stickleback were transferred to another 37-L tank. The tanks were aerated but not filtered. After 24 hours the trout were removed and the stimulus water was frozen in 50 ml units. The two types of trout stimuli were each prepared twice using the same protocol. One batch of trout stimuli was used for trials with perch and one was used for trials with pike.

We prepared two batches of stickleback skin extract using an identical protocol. One batch was used for trials with perch and the other was used for trials with pike. For each batch, the skin extract was prepared from 5 sticklebacks (mean ± SD, standard length = 6.08 ± 0.22 cm). Fish were sacrificed with a single blow to the head as outlined by the Canadian Council on Animal Care. A fillet of skin was removed from each side of each fish and placed into 100 ml of ice-chilled distilled water. A total of 22.8 cm^2 of skin was collected. Skin was homogenized and then filtered through filter floss to remove large particles. The mixture was then diluted with distilled water to make a final volume of 400 ml. Stimulus was separated in 20-ml allotments and then frozen.

We place a total of 60 groups of 4 fathead minnows (mean ± SD standard length = 52.4 ± 7.13 mm) into 10-L tanks and allowed them to acclimate for 24 hours. Half of the minnows were then exposed to the 50 ml of stimulus from trout fed minnow and stickleback (experimental treatment) and half were exposed to 50 ml of stimulus from trout fed swordtail and stickleback (control treatment). The stimulus was added to the tanks via plastic tubing. We predicted that the minnows should learn the identity of stickleback alarm cues when they detect the cues in combination with minnow alarm cues in the diet of the trout. We assessed whether the minnows learn to recognize stickleback alarm cues in test trials conducted the next day.

In the test trials we staged encounters between predatory adult perch (mean ± SD standard length = 20.3 ± 1.6 cm) or predatory pike (17.6 ± 0.9 cm) and minnows that were previously exposed to 1) cues of trout fed minnows and stickleback or 2) cues of trout fed swordtail and stickleback. Each perch ($N = 15$) was tested twice, once with minnows from the experimental treatment and once with minnows from the control treatment. Similarly, each pike ($N = 15$) was tested twice, once with minnows from the experimental treatment and once with minnows from the control treatment. The order of presentation of the control and experimental treatments for each perch and each pike was randomized. Trials using perch as a predator were conducted in the winter of 2001 and trials using the pike as a predator were completed in the fall of 2001.

Prey animals may recognize any unknown predator that has recently been fed conspecifics of the prey (Reviewed by Kats & Dill, 1998; Chivers & Mirza, 2001). Consequently, prior to each test the adult perch and pike were fed juvenile perch (mean ± SD standard length $= 6.0 \pm 0.4$ cm) every other day for two feedings and then starved for two days.

Test trials were conducted in light coloured plastic tubs ($100 \times 47 \times 40$ cm) that were covered with a dark gravel substrate. Each tub contained a pail (29 cm diameter) at each end. The pails had their bottoms removed and were perforated with many small holes. For each test trial we placed the groups of four minnows into one pail and the perch or pike into the other pail. The fishes were allowed to acclimate for six hours prior to the trials.

At the start of each trial we used a syringe to add 5 ml of stickleback skin extract to the pail containing the minnows. Two minutes later the pails were removed and the minnows and perch or minnows and pike were allowed to interact. We recorded the time the predator took to initiate a strike and whether the strike was successful. A strike involved a rapid burst towards the prey. Trials were terminated after 15 minutes or after a predator strike was initiated. We stopped the trials after the first predator strike because a strike should indicate to minnows in both treatments that the predator is a threat. We used a series of Wilcoxon Signed Rank tests to compare the time to first strike (Siegel & Castellan, 1998). We used one-tailed statistics because we predicted that minnows that recognize the predator should have higher survival (greater latency to attack and capture) than those that do not recognize the predator.

Results

One of the perch did not strike during either the control or experimental trials and hence was dropped from the analyses. Perch were inefficient predators on minnows. Only 1 strike resulted in a successful capture. Nevertheless, the minnows that were trained to recognize the stickleback cues (*i.e.* those previously exposed to trout fed minnows and stickleback, experimental treatment) were not attacked as quickly as those that were not trained to recognize the stickleback cues (*i.e.* those previously exposed to trout fed swordtails and stickleback, control treatment) ($z = -1.664$, $N = 14$, $p = 0.043$ one-tailed test; Fig. 1).

Pike were highly efficient predators. They were always successful when they struck at prey. Pike took significantly longer to strike at minnows that were previously exposed to trout fed minnows and stickleback (experimental treatment) than those that were previously exposed to trout fed swordtails and stickleback (control treatment) ($z = -1.76$, $N = 15$, $p = 0.039$; Fig. 2). Given that the first strike was always successful for pike, our data demonstrates that minnows that had the opportunity to learn the heterospecific alarm cue survived longer during an encounter when they were 'warned' with the cue than naïve (control) minnows that did not have the opportunity to learn the heterospecific cue.

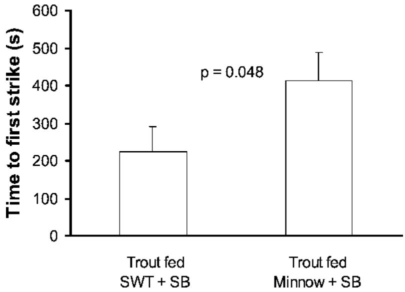

Fig. 1. Mean (+SE) time taken for perch to strike minnows that were exposed to stickleback alarm cues. Minnows were previously exposed to chemical stimuli from trout fed swordtails (SWT) and stickleback (SB) (control treatment) or exposed to chemical stimuli from trout fed minnows and stickleback (SB) (experimental treatment).

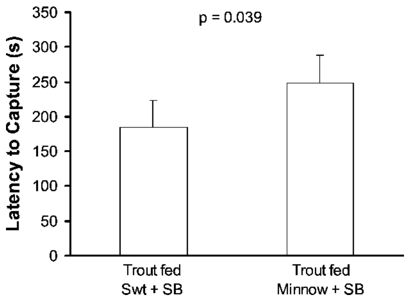

Fig. 2. Mean (+SE) time taken for pike to capture minnows that were exposed to stickleback alarm cues. Minnows were previously exposed to chemical stimuli from trout fed swordtails (SWT) and stickleback (SB) (control treatment) or exposed to chemical stimuli from trout fed minnows and stickleback (SB) (experimental treatment). Data for latency to capture is the same as time to first strike (see text for details).

Discussion

The results of our study show that fathead minnows can learn to recognize stickleback alarm cues when they detect those cues combined with minnow alarm cues in the diet of a predator. Moreover, behavioural responses of minnows exhibited to stickleback alarm cues can decrease the probability that the prey will be attacked or captured during an encounter with a predator.

Cross-species responses to chemical alarm cues are commonly reported in a variety of aquatic organisms (review Chivers & Smith, 1998). In many cases the responses are learned yet we know little about the way in which learning occurs. The results of our current study confirm the results of Mirza

& Chivers (2001d). Both of these studies show that minnows can learn the identity of stickleback alarm cues based on detecting it in combination with conspecific alarm cues in the diet of a predator. Our experimental design does not allow us to differentiate whether the acquired recognition of heterospecific cues is a result of associative learning or is a result of sensitization. Future research will be needed to differentiate these alternatives. Work with other fishes have shown that they can learn to recognize unknown predators that are associated with conspecific alarm cues, and that this is a result of associative learning not sensitization (Mirza & Chivers, 2000).

A prerequisite for the ability of prey to learn to recognize heterospecific cues through a mixed diet effect requires that both the conspecific and heterospecific alarm cues need to be able to pass through the diet of the predator and remain recognizable to the prey. It is well established that the alarm cue of fathead minnows and other Ostariophysan fishes can pass through the digestive system of fishes and can still be recognized (Mathis & Smith, 1993b, c; Brown *et al.*, 1995a, b). Mirza & Chivers (2001d) had similarly demonstrated that stickleback alarm cues could pass through the digestive system of perch and remain recognizable to the prey. The results of these studies show that stickleback alarm cues can pass through the digestive system of two different fish predators and remain recognizable. Consequently, learned recognition of heterospecific alarm cues through a mixed predator diet may be a widespread phenomenon, but additional studies in different predator/prey systems are needed.

Numerous researchers have speculated that behavioural responses exhibited by prey animals that are 'warned' with heterospecific alarm cues should increase the probability that the prey will escape an encounter with a predator. The benefits to receivers of heterospecific alarm cues have generally been inferred from the observation that the responses to the cues are consistent with known anti-predator responses. However, the assumption that cue receivers benefit from detection of heterospecific alarm cues has not been empirically tested. Our study is the first to document that behavioural responses to heterospecific cues will decrease the probability that the prey will be attacked or captured during an encounter with a predator.

Behavioural responses to heterospecific alarm cues could influence several aspects of the predation sequence. For example, a decrease in movement by the prey could lower the probability of detection by the predator and hence we may expect a decrease in the probability of attack. In addition, prey that are warned by an alarm cue may increase their group cohesion, increase their vigilance and maintain a greater distance from the predator. As such we would expect that warned prey might be less likely to be attacked or less likely to be captured during an attack (Lima & Dill, 1990). Results of the experiments with perch showed that the time taken to initiate an attack was longer for experienced prey than for naïve (control) prey. A difference in time to initiate an attack provides indirect evidence of a survival advantage of learned recognition of the heterospecific cue. However, when using perch as a predator we do not have a direct test of survival differences between control and experimental treatment because the predator was inefficient at capturing the prey. Results of trials with pike showed that the time taken to initiate an attack on experienced prey was longer than on naïve (control) prey. Because pike were always successful when attempting to capture prey we can conclude that experienced prey have increased survival over naïve prey. It is important to note that if the interaction had occurred outside the confined tank, we may also have observed that the prey could maintain a larger distance from the predator and hence avoid an encounter altogether. Our results provide direct empirical evidence of a survival benefit to receivers of a heterospecific alarm cue.

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