

THE ROLE OF EXPERIENCE IN THE RESPONSE OF FATHEAD MINNOWS (*PIMEPHALES PROMELAS*) TO SKIN EXTRACT OF IOWA DARTERS (*ETHEOSTOMA EXILE*)

by

DOUGLAS P. CHIVERS, BRIAN D. WISENDEN and R. JAN F. SMITH¹)

(Department of Biology, 112 Science Place, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5E2)

(With 4 Figures)

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Summary

Detection of an alarm pheromone may allow conspecifics to lower their risk of predation if they avoid areas where alarm pheromones are detected. Similarly, individuals that learn to recognize and avoid alarm signals produced by heterospecifics of their prey guild (*i.e.* those with which they share common predators) will likely receive similar benefits. In two separate field studies we tested whether fathead minnows (*Pimephales promelas*) avoid areas labelled with Iowa darter (*Etheostoma exile*) skin extract (which contains an alarm pheromone). One population of minnows tested was sympatric with darters while the other was allopatric from darters. Swordtails (*Xiphophorus helleri*) are allopatric from fathead minnows in both of the populations tested. Swordtail skin lacks ostariophysan and darter alarm pheromones, and was used to control for a general response to damaged skin of an unfamiliar fish. In the darter-sympatric population we captured significantly fewer and smaller minnows in traps marked with darter skin extract (experimental traps) than traps marked with a control of swordtail skin extract. Given the correlation between age and size, and therefore experience, these data suggest that less experienced fish do not avoid darter skin extract to the same extent as do older more experienced fish. In the darter-allopatric population there was no difference in the number of minnows or the size of minnows captured in control *versus* experimental traps. Taken together, these results demonstrate that fathead minnows recognize and avoid areas where darter alarm pheromone is detected and that this is a learned response. Use of heterospecific alarm pheromones to assess predation risk may be a widespread phenomenon within communities of small fishes.

Introduction

Chemical alarm pheromones that are released by mechanical damage have been reported from a variety of taxa including gastropods (ATEMA & STENZLER, 1977; STENZLER & ATEMA, 1977), echinoderms (SNYDER &

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SNYDER, 1970), crayfishes (HAZLETT, 1994), amphibians (HEWS & BLAUSTEIN, 1985; HEWS, 1988) and fishes (review SMITH, 1992). Such alarm pheromones provide valuable information about predation threat to receivers of the signal. For prey fishes, several studies have shown that alarm signal receivers avoid areas where they detect conspecific alarm pheromones. VON FRISCH (1941), for example, showed that European minnows (*Phoxinus phoxinus*) abandon artificial feeding stations following exposure to conspecific alarm pheromone (Schreckstoff). Fathead minnows, *Pimephales promelas* (MATHIS & SMITH, 1993a) and brook sticklebacks, *Culaea inconstans* (CHIVERS & SMITH, 1994) also avoid traps marked with conspecific alarm pheromones. Such avoidance responses are likely adaptive because they remove fish from an area where a conspecific was recently attacked or captured by a predator.

Just as the detection of an alarm signal will benefit conspecifics, it will likely also benefit heterospecifics of the same prey guild if they can detect the alarm signal. Several studies have demonstrated that cross-species responses to alarm signals are common in closely related fishes. SMITH & SMITH (1989), for example, demonstrated that two goby species, *Asterropteryx semipunctatus* and *Gnatholepis anjerensis*, respond to visual alarm signals produced by each other. Another goby, *Brachygobius sabanus*, also responds to alarm pheromones of *A. semipunctatus* (SMITH *et al.*, 1991). Three darter species, *Percina nigrofasciata*, *Ammocrypta beani* and *Etheostoma swaini*, all respond to each other's alarm pheromones, with the exception that *A. beani* does not respond to pheromones produced by *E. swaini* (SMITH, 1982).

Cross-species responses also occur to alarm signals from phylogenetically distant groups of fishes. KRAUSE (1993) examined the possibility of cross-species alarm responses in three-spine stickleback (*Gasterosteus aculeatus*, Superorder Acanthopterygii) and chub (*Leuciscus cephalus*, Superorder Ostariophysi). His results showed that stickleback do not respond with anti-predator behaviour when exposed to the alarm pheromone of chub. However, when in mixed schools, the alarm response of chub to their conspecific alarm pheromone will induce an anti-predator response in the stickleback. MATHIS & SMITH (1993b) and WISENDEN *et al.* (1994; 1995) have shown that brook stickleback (Superorder Acanthopterygii) avoid areas labelled with fathead minnow alarm substance (Superorder Ostariophysi). Similarly, CHIVERS & SMITH (1994) demonstrated that finescale dace (*Phoxinus neogaeus*, Superorder Ostariophysi)

and fathead minnows avoid areas labelled with brook stickleback alarm pheromone. In this study we test for a cross-superorder response to an alarm pheromone. We test whether fathead minnows avoid areas labelled with Iowa darter (Superorder Acanthopterygii) alarm pheromone. Since both of these species are small prey fishes that likely experience predation by the same predators, it would benefit fathead minnows to respond to the alarm pheromone of Iowa darters.

Research with laboratory raised fishes has shown that cyprinids respond to conspecific alarm pheromones on the first exposure (*i.e.* with no prior experience). Nevertheless, physiological development and experience play a role in determining the strength of the response (PFEIFFER, 1963; WALDMAN, 1982; MAGURRAN, 1989). For other groups of fishes it is unknown whether experience plays a role in the response to conspecific alarm pheromones. However, experience is likely more important in mediating the response to heterospecific alarm pheromones than to conspecific alarm pheromones, especially if the species are phylogenetically distant. CHIVERS & SMITH (1994) provide indirect evidence that experience plays a role in the response of finescale dace and fathead minnows to heterospecific alarm pheromones. In their study, traps marked with brook stickleback skin extract caught significantly fewer dace and minnows than traps marked with a control substance. In addition, significantly more small and significantly fewer large fish were captured in experimental traps than in control traps. Given the correlation between size and age, and therefore experience, the results of CHIVERS & SMITH (1994) suggest that experience plays a role in avoidance of stickleback alarm pheromone by finescale dace and fathead minnows.

In this study we examine the response of fathead minnows to Iowa darter alarm pheromone, and the role of experience in mediating the development of this response. We test the response of two populations of minnows to traps labelled with either darter skin extract (experimental treatment) or swordtail (*Xiphophorus helleri*) skin extract. Swordtails are allopatric from fathead minnows in both of the populations tested. Swordtail skin lacks ostariophysan and darter alarm pheromones and was used to control for a general response to damaged skin of unfamiliar fish. One population of fathead minnows is sympatric with darters, while the other is allopatric from darters. If experience is responsible for mediating the responses of minnows to darter alarm pheromone, minnows from the darter-sympatric population should be caught significantly less often in

traps containing darter skin extract than in traps containing swordtail skin extract, while minnows from the darter-allopatric population should be caught in equal numbers in both types of traps. Further, if experience is responsible for mediating the response to darter skin extract, a significantly larger proportion of small fish and a significantly smaller proportion of large fish from the darter-sympatric population should be caught in experimental traps when compared to control traps. This would suggest that younger fish do not avoid darter alarm pheromone to the same extent as older, more experienced fish.

Experiment 1: Responses of darter-sympatric fathead minnows to darter skin extract

Methods.

We conducted this trapping experiment in an approximately 1 ha pond located on the University of Saskatchewan campus, where fathead minnows occur in sympatry with Iowa darters. The fish in this assemblage originated from the South Saskatchewan River drainage basin when the pond was filled in 1959. We marked 15 traps with sponges containing skin extract from Iowa darters and 15 traps with sponges containing skin extract from swordtails. Extracts were prepared using one donor fish per trap location. The donors were killed by a blow to the head and a razor blade was used to make 20 vertical cuts along both sides of each fish. The fish were then rinsed with 12 ml of pond water. This stimulus water was transferred onto two cellulose sponges ($2 \times 2 \times 2$ cm) that were threaded onto a stainless steel wire. The wires containing the sponges were then attached to the inside of a Gee's Improved Minnow trap (a roughly cylindrical wire enclosure, 43 cm length \times 22 cm diameter, with a funnel located at each end leading into the trap) such that a sponge was approximately 4 cm from each trap entrance. Pairs of traps (consisting of one control and one experimental trap) were placed into the water along the edge of the pond approximately 10 m apart. After setting one pair of traps, we prepared the stimulus for the next pair of traps. Skin extracts were prepared just prior to use because darter alarm pheromone loses its efficacy within 1 hr (R.J.F. SMITH, personal observations). Pairs of traps were placed into the water at four min intervals until all 15 pairs were set. The order of placement of the control and experimental traps along the pond edge was determined randomly within each pair.

Trap pairs were removed from the water at 4 min intervals, starting one hour after the first pair of traps was set. All fish were removed, preserved in 10% formalin and their total length was measured. We compared the number of minnows captured in traps marked with darter skin extract with the number of minnows captured in traps marked with swordtail skin extract using a Wilcoxon-Mann-Whitney test (SIEGEL & CASTELLAN, 1988). A one-tailed statistical test was used because we predicted that darter-sympatric fathead minnows would be captured significantly less often in experimental traps than control traps. We divided the range of the length distribution into three equal size intervals (38-50, 51-62, and 63-75 mm total length) and termed them small, medium and large fish. We compared the proportion of small and large fish captured in control *versus* experimental traps. When comparing the proportion of fish from the different size categories, we used one-tailed statistical tests because we predicted that the experimental traps should catch a higher proportion of small fish and a lower proportion of large fish than control traps.

Results.

A total of 3041 fathead minnows were captured in this experiment, 981 in experimental traps and 2060 in control traps. Significantly fewer fathead minnows were captured in traps marked with darter skin extract than in traps marked swordtail extract ($W_x = 184$, $m = 15$, $n = 15$, $p = 0.023$, Fig. 1). One Iowa darter was captured in a control trap during this experiment. Iowa darters are not readily captured in Gee's Improved Minnow traps.

There was a significantly greater proportion of small fathead minnows ($W_x = 116.5$, $m = 12$, $n = 12$, $p = 0.029$, Fig. 2) and a significantly lower proportion of large minnows captured in experimental traps ($W_x = 106.5$, $m = 12$, $n = 12$, $p = 0.007$, Fig. 2) than in control traps.

Experiment 2: Responses of darter-allopatric fathead minnows to darter skin extract

Methods.

The purpose of this experiment was to determine (1) whether darter-allopatric minnows avoid areas labelled with darter skin extract, and (2) whether small and large minnows in the darter-allopatric population respond to darter skin extract in the same way as minnows in the darter-sympatric population. We conducted the trapping experiment at Lakeview Pond, an approximately 2 ha man-made pond located in Saskatoon, Saskatchewan. Several censuses in 1993 and 1994 indicated that Iowa darters are absent from the pond. Minnows in this population originated from the same drainage basin as those in the previous experiment, and have been isolated from other populations for a similar time period. Therefore, any population differences in the responses of minnows to darter alarm pheromone are most likely a result of experience and not differences in genetic origin. Experimental protocol was the same as in Experiment 1 except that we set 16 pairs of traps instead of 15 pairs. Statistical analyses were the same as in Experiment 1.

Results.

A total of 1721 fathead minnows were captured in this experiment, 1117 in experimental traps and 604 in control traps. There was no significant difference in the number of fathead minnows captured in traps marked with darter skin extract than traps marked with swordtail extract ($W_x = 302.5$, $m = 16$, $n = 16$, $p = 0.924$, Fig. 3).

There was not a significantly greater proportion of small fathead minnows ($W_x = 148.5$, $m = 11$, $n = 14$, $p = 0.371$, Fig. 4) or a significantly lower proportion of large minnows captured in experimental traps ($W_x = 136$, $m = 11$, $n = 14$, $p = 0.644$, Fig. 4) compared to control traps.

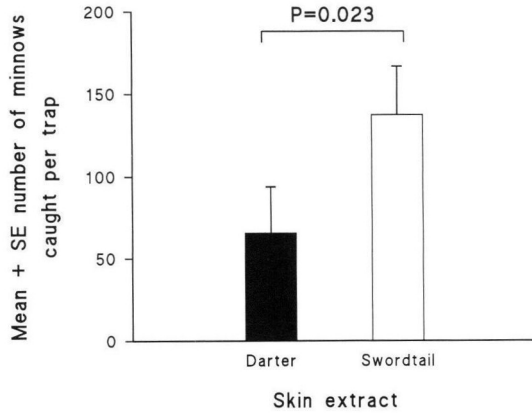


Fig. 1. Mean (+ SE) number of minnows captured per trap in the darter-sympatric population.

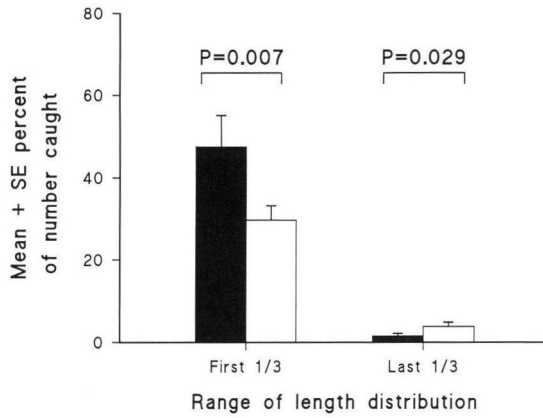


Fig. 2. Mean (+ SE) percent of number of small (first third of length distribution) and large (last third of length distribution) minnows captured in control (open bars) and experimental traps (closed bars) in the darter-sympatric population.

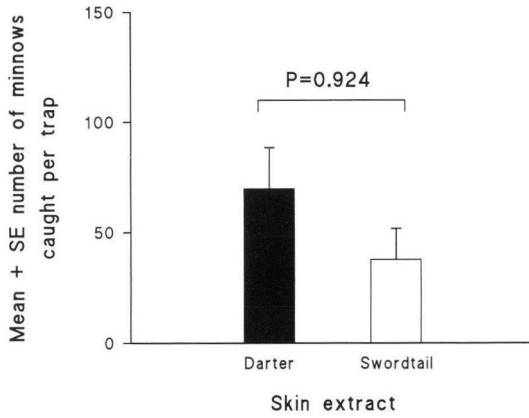


Fig. 3. Mean (+ SE) number of minnows captured per trap in the darter-allopatric population.

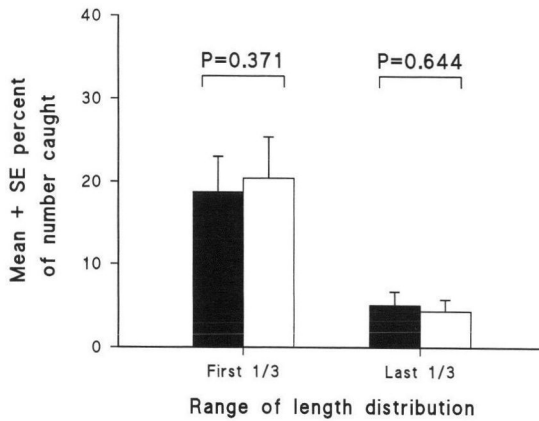


Fig. 4. Mean (+ SE) percent of number of small (first third of length distribution) and large (last third of length distribution) minnows captured in control (open bars) and experimental traps (closed bars) in the darter-allopatric population.

Discussion

Our results demonstrate that fathead minnows can recognize and avoid areas marked with Iowa darter skin extract. This is probably not a general response to damaged fish skin because minnows did not avoid areas marked with swordtail skin. To our knowledge, this is the first study to demonstrate that cyprinid fishes recognize and avoid areas marked with darter alarm pheromone. These results thereby demonstrate a cross-superorder response to a chemical alarm signal. Fathead minnows overlap the entire geographic range of Iowa darters (approximately one third of North America, SCOTT & CROSSMAN, 1979). Therefore, this cross-species response may influence predator-prey interactions within many freshwater systems. By avoiding areas where darter alarm pheromone is detected, and hence an area where a predator has recently attacked or captured another small prey fish, minnows likely lower their risk of predation.

In our study, two lines of evidence suggest that the avoidance response of fathead minnows to Iowa darter alarm pheromone results from experience. First, only minnows from the darter-sympatric population avoided the darter skin extract. Second, fewer large and more small minnows were captured in experimental traps than in control traps in the darter-sympatric population. Given the correlation between size and age, and therefore experience, our results indicate that the probability of an avoidance response by fathead minnows increases with experience. We can rule out the possibility that other ontogenetic factors, such as physiological development, are solely responsible for the differential responses of large *versus* small fathead minnows in the darter-sympatric population because the same response pattern was not seen in the darter-allopatric population.

Interspecific responses to distress and alarm calls have been studied extensively in birds and mammals (*e.g.* STEFANSKI & FALLS, 1972; HAUSER, 1988; AUBIN, 1991; SEYFARTH & CHENEY, 1990). Some mammals detect and respond to bird alarm calls. HAUSER (1988), for example, showed that vervet monkeys (*Cercopithecus aethiops*) learn to respond to alarm calls of superb starlings (*Spreo superbus*).

Several recent studies (KRAUSE, 1993; MATHIS & SMITH, 1993b; CHIVERS & SMITH, 1994; WISENDEN *et al.*, 1994, 1995; this paper) provide evidence that cross-species responses to alarm signals are common not only between closely related fishes, but also between phylogenetically

distant species that are members of the same prey guild. Responses to heterospecific alarm signals may therefore be a common phenomenon among fishes, and could potentially influence predator-prey relationships in many different fish assemblages. For example, if it is a widespread phenomenon that prey fish can learn to respond to ostariophysan alarm pheromones (which includes approximately 70% of all freshwater fish species, NELSON, 1984) in the same manner as brook sticklebacks (MATHIS & SMITH, 1993; WISENDEN *et al.*, 1994), this would suggest predator-prey interactions in the majority of freshwater systems are influenced by cross-species responses to chemical alarm signals. The number of fish assemblages influenced by cross-species responses to alarm signals may be higher still if it is a general phenomenon that cyprinids respond to stickleback alarm pheromones (as demonstrated by CHIVERS & SMITH, 1994) and to darter alarm pheromones (as demonstrated in this study).

A variety of factors may influence the ability of different prey species to recognize and avoid heterospecific alarm pheromones. One of these factors could be the frequency of predator attacks on co-habiting heterospecifics. A high attack frequency would provide many opportunities to associate the heterospecific cue with predation risk. In addition, if different heterospecific species release the same pheromone (as in Ostariophysan fishes), there would again be more opportunities to learn to respond to the particular alarm pheromone, regardless of which heterospecific produced the signal. The degree to which different heterospecifics share predators may also affect the responses to heterospecific pheromones. The greater the overlap the prey species have in the diet of particular predators, the greater the selective pressure favouring individuals that recognize and learn to respond to heterospecific alarm pheromones. The degree of overlap in the diet of a particular predator will likely change with ontogenetic shifts in the niche and relative growth rates of the prey species.

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