

## Chemical alarm signaling by reticulate sculpins, *Cottus perplexus*

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### Synopsis

The distribution and extent of chemical alarm signaling systems among some families of fishes, including the Cottidae, remains unclear. In laboratory experiments, we tested whether reticulate sculpins, *Cottus perplexus*, respond to chemical alarm signals released by injured conspecifics. Sculpins decreased movement following exposure to skin extracts from conspecifics, but did not respond to cues of syntopic speckled dace, *Rhinichthys osculus*, or allotopic swordtails, *Xiphophorus helleri*. Additional tests demonstrated that the responses of sculpins to alarm cues were dependent on the hunger level of the test fish. Sculpins deprived of food for 2 days failed to respond to conspecific alarm cues, however, the same individuals fed to satiation did respond to alarm cues.

### Introduction

A wide diversity of fishes have been found to release chemical alarm signals upon detecting or being captured by a predator (reviews by Smith 1992, Chivers & Smith 1998). These signals function to 'warn' other members of the same species of the presence of the predator. For example, Mathis & Smith (1993a) showed that the behavioral responses of fathead minnows, *Pimephales promelas*, to alarm cues decrease the probability that they will be captured during interactions with northern pike, *Esox lucius*. Individuals exposed to alarm pheromones were significantly less likely to be captured than those individuals exposed to a control solution.

Most of the fishes that are known to possess chemical alarm signals belong to the superorder Ostariophysi, which includes minnows, characins, catfishes, loaches and suckers (Chivers & Smith 1998). Recent work has shown that chemical alarm signals may also

be common in other groups of fishes, including sticklebacks (Mathis & Smith 1993b, Chivers & Smith 1994, Brown & Godin 1997), gobies (Smith 1989, Smith & Lawrence 1992), darters (Smith 1979, Smith 1982), poeciliids (García et al. 1992, Nordell 1998, Brown & Godin 1999), cottids (Hugie et al. 1991, Houtman & Dill 1994), salmonids (Brown & Smith 1997) and cichlids (Wisenden & Seargent 1997).

The distribution and extent of chemical alarm signaling among the family Cottidae remains unknown. Studies have tested for the presence of chemical alarm signals in three of the approximately 300 species in this family. Schutz (1956) found no evidence that *Cottus gobio* exhibited anti-predator behavior in response to cues of injured conspecifics. Pfeiffer (1960) found similar results for *Myoxocephalus scorpius*. In contrast, Hugie et al. (1991) found that the tidepool sculpin, *Oligocottus maculosus*, reduced activity, moved to shelter and increased attachment to the substrate in response to cues of injured conspecifics, but not in

response to cues of uninjured conspecifics or cues of injured guppies, *Poecilia reticulata*. Houtman & Dill (1994) showed that alarm responses of tide-pool sculpins to alarm cues were influenced by substrate color. In this study, we tested for the presence of an alarm signal in reticulate sculpins, *Cottus perplexus*.

Cross-species responses to chemical alarm signals have commonly been reported in species that are closely related (e.g. Schutz 1956, Chivers & Smith 1998). Such responses suggest that closely related species release similar chemicals when they are captured by a predator. Several studies have also shown that cross-species responses may occur between distantly related species (e.g. Mathis & Smith 1993b, Chivers et al. 1995a,b, Wisenden et al. 1994, 1995a). In each of these cases the cross-species responses occur between syntopic prey species that belong to the same prey guild (i.e. those that co-occur spatially and temporally and are exposed to the same suite of predators). Chivers et al. (1995b) showed that cross-species alarm responses among distantly related species can be learned. In our study, we tested whether sculpins respond to cues of injured conspecifics, injured speckled dace, *Rhinichthys osculus*, and injured swordtails, *Xiphophorus helleri*. Speckled dace are syntopic with the sculpins used in our experiments. Dace are ostariophysan fishes that possess the Schreckstoff alarm system (Chivers & Smith 1998). Swordtails are allopatric from the sculpins tested and are not known to release chemical alarm cues (Chivers & Smith 1998).

In the second part of this study we examined whether short-term food deprivation influenced the alarm response of reticulate sculpins. Two previous studies have demonstrated that responses of prey fishes to chemical alarm cues are dependent on the hunger level of the test animals. For example, Smith (1981), found that Iowa darters, *Etheostoma exile*, deprived of food for 12–48 h exhibited a feeding response when presented with a combination of food odors and conspecific alarm cues. The same darters fed to satiation respond to the same mixture with an alarm response. In another study, Brown & Smith (1996) showed that fathead minnows deprived of food for 24 h exhibit a reduced alarm response compared to those fed ad libitum. Minnows deprived of food for 48 h show no alarm response when exposed to a conspecific alarm pheromone (Brown & Smith 1996).

## Methods

### Experiment #1

Reticulate sculpins and speckled dace were collected in the spring of 1997 from the Corvallis Watershed Reservoir in the Oregon Coast Range (44°00'N, 123°30'W elevation 183 m) and transported to Oregon State University. Prior to testing, sculpins were maintained for a minimum of 2 weeks in 371 glass aquaria between 15–20°C on a 14 L : 10 D photoperiod with a single airstone in each tank. They were fed ad libitum daily a diet of live zooplankton and tubifex worms. Swordtails were obtained from a commercial supplier. Both speckled dace and swordtails, to be used for chemical cue donors, were maintained under the same conditions as the sculpins.

Three days prior to the start of testing, 10 sculpins (mean  $\pm$  SD standard length =  $5.11 \pm 0.77$  cm) were placed into individual 371 glass aquaria, each of which had a fine sand substrate. Tanks were filled with dechlorinated tap water and aerated with a single airstone. A piece of plastic tubing, to be used for injection of the chemical stimuli, was tied to the airstone tubing. Sculpins were fed ad libitum daily with live zooplankton. Each of the 10 fish was tested once in each of three treatments. Treatments included chemical cues from: (1) injured conspecifics, (2) injured swordtails and (3) injured dace. Trials were performed every three days, during daylight hours (8:30 and 15:00 h). The order of presentation of the three stimuli to each fish was determined randomly.

Skin extracts were prepared fresh daily. We used two donor fish to prepare each stimulus each day. The donor fish were first killed with a quick blow to the head and then 50 equal length vertical cuts were made on each side of each fish. The two fish were then rinsed with 40 ml of dechlorinated tap water. Ten ml of the resulting stimulus was used in each trial. The mean ( $\pm$ SD) standard length of donor sculpins, swordtails and dace was  $4.91 \pm 0.10$ ,  $3.81 \pm 0.27$  and  $5.33 \pm 0.10$  cm, respectively.

Each trial consisted of two 10-minute phases, a pre-stimulus and a post-stimulus phase. Between each phase there was a 30-second interval at which time 10 ml of the appropriate stimulus was injected via the plastic tubing. During both the pre- and post-stimulus phases, we recorded the time each fish spent swimming. A reduction in movement is considered an anti-predator response for sculpins (e.g. Hugie et al. 1991,

Houtman & Dill 1994). After each day of trials, we transferred the fish to a holding tank and then drained and cleaned each tank and its associated tubing. The test tanks were refilled with clean dechlorinated tap water and the fish were returned for subsequent tests.

We calculated the difference in the time spent swimming before versus after injection of each chemical stimulus and compared the response of the sculpins to each of the three chemical stimuli using a Friedman two-way analysis of variance by ranks (Siegal & Castellan 1988). This was followed by post-hoc non-parametric multiple comparisons (with the alpha level adjusted for non-independent pairwise comparisons) to identify which of the three treatments differed.

#### *Experiment #2*

The purpose of this experiment was to test the effects of short-term food deprivation on the alarm response of reticulate sculpins. Fish used in experiment 2 were not the same ones used in experiment 1. On day 1 of the experiment, a single sculpin was placed into each of ten test tanks that were identical to those used in experiment 1. All sculpins were fed ad libitum with live zooplankton. Two days later (day 3), we removed the fish from each tank, cleaned the tanks (ensuring that all food was removed from each tank) and then refilled the tanks with dechlorinated tap water and returned the fish. We immediately began to feed five randomly selected fish ad libitum with live zooplankton while depriving the other five fish of food. Two days later (day 5) we tested both the satiated and food deprived fish for a response to chemical cues of injured sculpins that was mixed with a solution of live zooplankton. The testing protocol was identical to that used experiment 1. The mean ( $\pm$ SD) standard length of the donor sculpins was  $5.10 \pm 0.76$  cm.

Immediately after completing the trials on day 5, we removed the fish from each tank, cleaned the tanks (ensuring that all food was removed from the tanks) and then refilled the tanks with dechlorinated tap water and returned the fish. We immediately began to feed the five fish that were previously food deprived, while food depriving the ones that were previously fed to satiation. Two days later (day 7) we tested both the satiated and food deprived fish for a response to chemical cues of injured sculpins that was mixed with a solution of live zooplankton.

Our design allows us to test the response to chemical cues of injured conspecifics while both satiated and

food deprived. We used Wilcoxon signed rank tests to determine whether satiated and food deprived fish showed a significant decrease in time spent moving prior to versus after exposure to the injured sculpin stimulus (Siegel & Castellan 1988).

## **Results**

### *Experiment #1*

We observed qualitative differences in the responses of sculpins to the three different chemical cues. During pre-stimulus trials of all treatments and the post-stimulus trials of the dace and swordtail treatments, sculpins moved about the bottom of the tank and often swam up into the water column, sometimes capturing prey, and then glided back to the bottom of the tank. In contrast, fish that were exposed to injured sculpin cues rarely swam into the water column. The fish changed position in the tank by making short rapid hops that appeared to end in a rigid, alert posture. This behavior is similar to that described by Wisenden et al. (1995b) for Iowa darter exposed to conspecific chemical alarm cues. The hops were separated by long intervals of motionlessness. A Friedman two-way analysis of variance by ranks revealed that there was a significant difference in the response of the sculpins to the three chemical stimuli ( $F = 9.6$ ,  $K = 3$ ,  $N = 10$ ,  $p < 0.01$ , Figure 1). Post hoc comparisons revealed that sculpins spent significantly less time moving following detection of chemical cues of injured sculpins compared to injured dace or swordtails ( $p < 0.05$  for both comparisons). There was no difference in the response of sculpins to cues of injured dace versus injured swordtails ( $p > 0.30$ ).

### *Experiment #2*

We observed qualitative differences in the responses of sculpins when they were satiated versus food deprived. Fish fed to satiation responded to cues of injured conspecifics by reducing the time they spend in the water column. The fish typically moved around the tank by making short rapid hops that appeared to end in a rigid, alert posture. The hops were separated by long intervals of motionlessness. These observations are reflected in a significant reduction in time spent moving following detection of the stimulus ( $T = 39$ ,  $N = 9$ ,  $p = 0.027$ , Figure 2). This was the same response we observed in

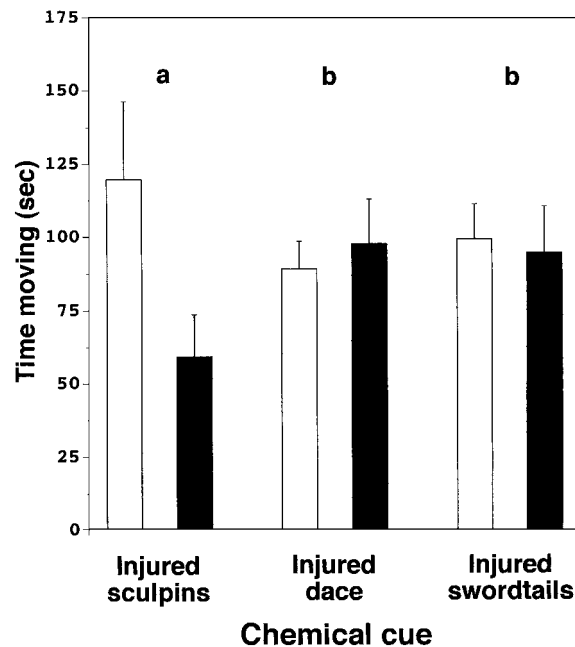


Figure 1. Mean ( $\pm$ SE) time (sec) reticulate sculpins spent moving prior to (open bars) versus after (solid bars) exposure to chemical cues of injured sculpins, injured speckled dace or injured swordtails. Different letters over bars indicate significant differences among treatments. Statistics are based on comparisons of the changes in time sculpins spent moving prior to versus after exposure to chemical cues (see text for details).

experiment 1. In contrast, fish that were food deprived did not respond as strongly to alarm cues. These fish tended to spend more time swimming up into the water column capturing prey and then returning to the substrate. There was no significant change in activity following detection of alarm cues by food deprived fishes ( $T = 23$ ,  $N = 9$ ,  $p = 0.50$ , Figure 2).

## Discussion

The results of our study demonstrate that reticulate sculpins exhibit an anti-predator response to cues released from injured conspecifics. The sculpins decreased the time they spent moving upon detecting chemical alarm cues. Moreover, the sculpins spent less time swimming in the water column after detecting the cues of injured conspecifics. By decreasing movement, and in particular movement in the water column, sculpins likely decrease their probability of being captured by a predator.

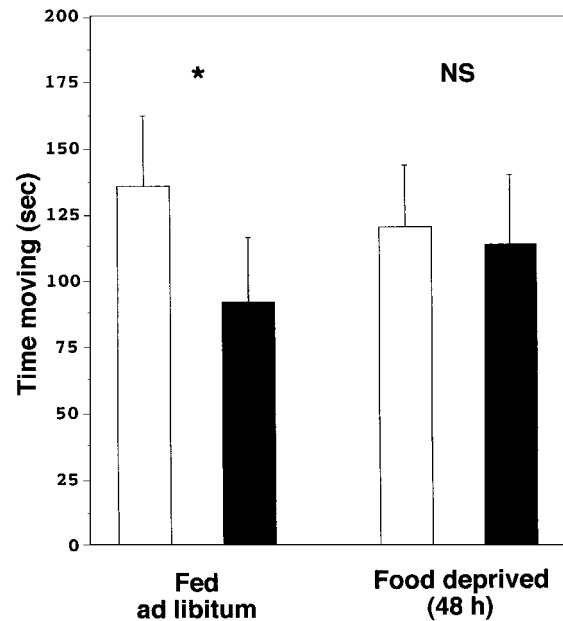


Figure 2. Mean ( $\pm$ SE) time (sec) reticulate sculpins spent moving prior to (open bars) versus after (solid bars) exposure to chemical cues of injured sculpins. \* over bars indicate significant change in time spent moving before versus after exposure to chemical cues of injured sculpins (see text for details).

In our experiment the response of the sculpins to the cues of injured conspecifics was not a generalized response to cues of injured fish. Sculpins did not respond to chemical cues of injured dace or injured swordtails. Dace are ostariophysan fishes that possess the Schreckstoff alarm system characteristic of the superorder (Chivers & Smith 1998). Other experiments have shown that it is common to observe cross-species responses to alarm cues by sympatric species that are members of the same prey guild (e.g. Mathis & Smith 1993b, Chivers et al. 1995a,b, Wisenden et al. 1995a). Specifically, cross-species responses are common between species that co-occur in the same micro-habitat and are exposed to the same suit of predators. In these cases the species may learn to associate heterospecific alarm cues with predation risk. The lack of a response by sculpins to cues of injured dace may indicate that sculpins do not learn to respond to heterospecific alarm cues like other prey fishes (e.g. Chivers et al. 1995b). Alternatively, the lack of a response may indicate that dace and sculpins do not belong to the same prey guild. Sculpins are primarily benthic fishes, while dace are more often located in open water (Scott & Crossman 1979). There may

be minimal overlap in habitat and/or predators, and consequently a limited opportunity for acquiring cross-species responses.

Future studies examining the extent and distribution of chemical alarm signals among the Cottidae are warranted. There are approximately 300 species in the family Cottidae, of which only 4 species have been tested for chemical alarm signals. Neither *Cottus gobio* (Schutz 1956) nor *Myoxocephalus scorpius* (Pfeiffer 1960) are known to respond to conspecific alarm pheromones. In contrast, *Oligocottus maculosus* (Hugie et al. 1991, Houtman & Dill 1994) and *Cottus perplexus* (this study) both respond to cues of injured conspecifics. We stress that future studies must be cautious when concluding that a species does not respond to cues of injured conspecifics, especially in the laboratory. Care must be taken to ensure that the animals are tested under appropriate conditions to observe an alarm response. Houtman & Dill (1994), for example, documented that tidepool sculpins respond to alarm cues by decreasing movement if they are on a substrate that they match. The sculpins failed to exhibit a reduction in movement if they were on a substrate that they did not match. Remaining motionless on a matching background facilitates crypsis, while remaining motionless on a non-matching background does not facilitate crypsis.

In laboratory studies care should be given to ensure that test animals are fed adequately prior to being tested. In our study, sculpins that were satiated responded to chemical alarm cues while those that were food deprived for 48 h showed a reduced alarm response. Similar results are known in other alarm systems (e.g. darters: Smith 1981, minnows: Brown & Smith 1996). Brown & Smith (op. cit.) documented that the absence of an alarm response to cues of injured conspecifics by food deprived fishes does not indicate that an alarm cue was ignored. In their study, predator-naïve minnows that were deprived of food did not respond to alarm cues that were mixed with chemical cues of northern pike, an unknown predator. However, in subsequent tests the minnows responded to pike cues alone with an anti-predator response. Their results show that minnows acquire recognition of predators by detecting alarm cues associated with the cues of the unknown predator, and that this learning occurs even in the absence of an overt response to the alarm cue. Additional studies designed at examining alarm responses should pay attention to these types of subtle, yet important, responses.

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