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INTRA- AND INTERSPECIFIC AVOIDANCE OF AREAS MARKED WITH SKIN EXTRACT FROM BROOK STICKLEBACKS (*Culaea inconstans*) IN A NATURAL HABITAT

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Abstract—The detection of a chemical alarm pheromone may allow receivers to avoid areas where a predator has captured the prey's conspecifics. We marked minnow traps with either brook stickleback (*Culaea inconstans*) skin extract or a control of distilled water and tested whether sticklebacks avoided the skin extract marked traps in a natural habitat. Significantly more sticklebacks were captured in traps marked with control water, thereby demonstrating avoidance of conspecific skin extract. The stickleback captured in traps marked with conspecific extract were significantly smaller than those captured in traps marked with control water, implicating ontogenetic factors (i.e., experience or physiological development) in the development of the response. We also captured significantly fewer finescale dace (*Chrosomus neogaeus*) and fathead minnows (*Pimephales promelas*) in traps marked with skin extract. These data suggest that dace and minnows may benefit by avoiding areas where predators have recently captured sticklebacks.

Key Words—Alarm signaling, antipredator behavior, Schreckstoff, brook stickleback, *Culaea inconstans*, finescale dace, *Chrosomus neogaeus*, fathead minnow, *Pimephales promelas*.

INTRODUCTION

An effective means for prey to reduce the risk of capture is to avoid areas where predation occurs. For many prey fishes this avoidance may be facilitated by

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some type of alarm pheromone system. In the typical Schreckstoff alarm system of cyprinid fishes, for example, exposure to alarm substance results in area avoidance. Fricke (1987) demonstrated that blind cave fish (*Astyanax fasciatus*) avoid the area of a tank where alarm substance is introduced. In field experiments, Von Frisch (1941), Smith (1976), and Mathis and Smith (1993) have demonstrated avoidance of areas marked with the cyprinid alarm pheromone. European minnows (*Phoxinus phoxinus*) abandon artificial feeding stations (Von Frisch, 1941) and breeding male fathead minnows (*Pimephales promelas*) temporarily abandon breeding territories (Smith, 1976) following exposure to conspecific alarm substance. Fathead minnows also avoid traps marked with conspecific alarm substance (Mathis and Smith, 1993). As the Schreckstoff is released only by mechanical damage to the skin, receivers that avoid an area that contains alarm substance effectively avoid sites where a predator has recently captured or injured a conspecific.

In a laboratory experiment, Mathis and Smith (1994) reported that brook stickleback increase shoaling in response to skin extract from conspecifics, but not to a control of swordtail (*Xiphophorus helleri*) skin extract or distilled water. This was the first report of a chemical alarm signal for fishes in the Order Gasterosteiformes. Chemical alarm signals have been reported from a variety of other non-ostariophysan fishes, including darters (Perciformes), gobies (Perciformes), and sculpins (Scorpaeniformes) (reviewed in Smith, 1992), as well as from a variety of other taxa including gastropods (Stenzler and Atema, 1977; Atema and Stenzler, 1977), echinoderms (Snyder and Snyder, 1970), and amphibians (Hews and Blaustein, 1985; Hews, 1988). To our knowledge, however, there have not been any field studies examining the effectiveness of chemical alarm signals in promoting area avoidance in non-ostariophysan fishes. In this study we examine whether brook stickleback avoid areas in a natural habitat that are marked with stickleback skin extract. Specifically, we test the hypothesis that brook sticklebacks will be captured significantly less often in traps marked with conspecific skin extract than in traps marked with a control substance.

In this study we also examine the influence of ontogenetic factors (experience or physiological development) in affecting the ability of fish to respond to skin extract. Given that small fish are generally less developed and less experienced, we would expect a greater proportion of small fish to be caught in skin extract marked traps than in control traps if experienced or more developed fish are more likely to avoid skin extract.

Finally, we examine the possibility that heterospecific prey fishes [finescale dace (*Chrosomus neogaeus*) and fathead minnows] will avoid areas marked with stickleback skin extract. Since all three of these species are small prey fishes that share common predators, individuals should benefit by detecting alarm signals produced by heterospecifics. Cross-species responses to alarm signals are common for closely related fishes (e.g., Pfeiffer, 1963; Smith, 1982; Smith et al., 1991); however, only one study has demonstrated cross-species reactions by fishes from distantly related taxa. Mathis and Smith (1994) demonstrated that brook sticklebacks respond with antipredator behavior upon exposure to alarm substance from fathead minnows. In this study we test whether finescale dace and fathead minnows (Superorder Ostariophysi) will avoid skin extract from distantly related brook sticklebacks (Superorder Acanthopterygii).

METHODS AND MATERIALS

Stimulus Preparation. We prepared the experimental stimulus from 13 male and 12 female sticklebacks ($\bar{X} \pm$ SD fork length = 5.71 ± 0.58 cm). We killed the donors by a blow to the head and removed a skin fillet from both sides of each fish. The total area of skin collected was approximately 90 cm^2. Immediately upon removal, we placed the skin samples together in 100 ml of chilled glass-distilled water. We homogenized the skin samples with a polytron homogenizer and filtered the homogenate through glass wool to remove any solid particles. We diluted the skin extract with an additional 300 ml of glass-distilled water (total volume = 400 ml).

We threaded two rectangular cellulose sponges (2.0 × 2.0 × 5.0 cm) onto each of 13 stainless steel wires and saturated the sponges with the skin extract. Since sticklebacks respond with antipredator behavior to stickleback extract but not to swordtail extract or distilled water (Mathis and Smith, 1994), as a control stimulus in this study we saturated identical wire-threaded sponges with glass-distilled water. All sponges were frozen at approximately −20°C for three days. They were removed from the freezer on the day of the experiment and were kept on ice until the beginning of the experiment.

Experiment Protocol. We conducted the trapping experiment in Marshy Creek in south-central Saskatchewan in September 1993. At this site the creek is 1–2 m deep and several meters wide. Previous censuses have indicated that brook stickleback, finescale dace, and fathead minnows occur in large numbers at this site.

We assigned 13 minnow traps to each of the experimental and control treatments. The traps (Gee's Improved Minnow Traps) consisted of roughly cylindrical wire enclosures (43 cm length × 22 cm diameter) with a funnel located at each end leading into the trap. The funnel entrances were approximately 2.5 cm in diameter. We attached the wires to the inside of each trap with the sponges located approximately 4 cm in front of each trap entrance.

We placed control and experimental traps into the water along the north and south shores of the creek, with approximately 5 m between traps. The order of placement of the experimental and control traps was determined randomly with the proviso that no more than two traps in a row could be of the same treatment condition. Trap pairs (consisting of one control and one experimental trap) were placed into the water at the same time, with the first pair being set at 1030 hr. It took approximately 1 hr to set all the traps. Each trap remained in the water for 3.5 hr, with each pair of traps being removed simultaneously. All fish from each trap were removed and preserved in 10% formalin. The fish were identified to species and their length was measured: fork length for finescale dace and fathead minnows and total length for brook sticklebacks (stickleback tails are rounded not forked).

RESULTS

A total of 1156 fish were captured in the 26 traps. Of these, 822 (71.1%) were brook sticklebacks, 257 (22.2%) were finescale dace, and 77 (6.7%) were fathead minnows. A Wilcoxon–Mann–Whitney test (Siegel and Castellan, 1988) revealed that for each of the three species, there were significantly more fish caught in control traps than experimental traps (brook sticklebacks, $W_x = 139$, $m = 13$, $N = 13$, $P = 0.032$, one-tailed, Figure 1a; finescale dace, $W_x = 140$, $m = 13$, $N = 13$, $P = 0.037$, one-tailed, Figure 1b; fathead minnows, $W_x = 135.5$, $m = 13$, $N = 13$, $P = 0.021$, one-tailed, Figure 1c).

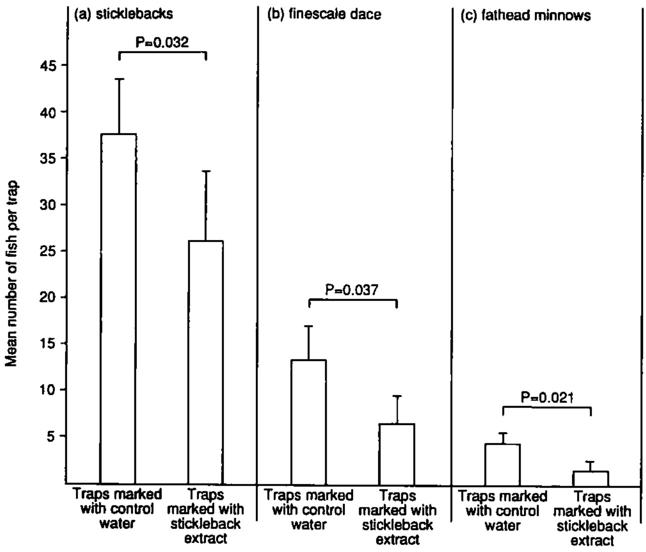

FIG. 1. Mean (+SE) number of: (a) sticklebacks, (b) finescale dace, and (c) fathead minnows captured in traps marked with either brook stickleback skin extract or control water.

One explanation for the difference in the number of finescale dace and fathead minnows caught in control and experimental traps may be interspecific social facilitation. The dace and minnows may be attracted to control traps that contain sticklebacks rather than avoiding the skin extract in experimental traps. To test for social facilitation, we used a Spearman rank-order correlation to test the relationship between the number of sticklebacks and both the number of dace and the number of minnows in the control traps. There was no correlation between the number of sticklebacks and the number of finescale dace in the control traps ($R_s = 0.332$, $N = 13$, $P > 0.10$, one-tailed). However, there was a significant correlation between the number of sticklebacks and the number of fathead minnows in control traps ($R_s = 0.075$, $N = 13$, $P < 0.005$, one-tailed).

A Wilcoxon–Mann–Whitney test revealed that for all three fish species, individuals caught in experimental traps were significantly smaller than individuals in control traps (brook sticklebacks, $W_x = 121$, $m = 12$, $N = 14$, $P = 0.027$, one-tailed, Figure 2a; finescale dace, $W_x = 59$, $m = 8$, $N = 11$, $P = 0.038$, one-tailed, Figure 2b; fathead minnows, $W_x = 13.5$, $m = 4$, $N = 10$, $P = 0.008$, one-tailed, Figure 2c). The observed size differences may be due

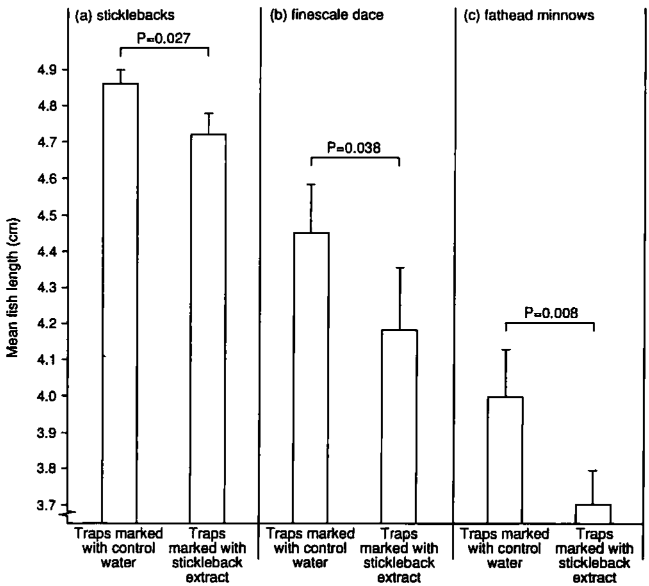

FIG. 2. Mean (+SE) length (cm) of: (a) sticklebacks, (b) finescale dace, and (c) fathead minnows captured in traps marked with either brook stickleback skin extract or control water (length measurements are based on total length for sticklebacks and fork length for finescale dace and fathead minnows).

to either significantly more small sized fishes caught in skin extract marked traps or significantly more large sized fishes caught in control traps or both. For each control and experimental trap, we determined the proportion of sticklebacks belonging to each of three equal size categories (3.2–4.3, 4.4–5.5, and 5.6–6.7 cm total length). A Wilcoxon–Mann–Whitney test shows that there were both significantly more small stickleback caught in experimental traps ($W_x = 139$, $m = 13$, $N = 13$, $P = 0.0495$) and significantly more large stickleback caught in control traps ($W_x = 110$, $m = 13$, $N = 13$, $P < 0.007$). Insufficient numbers of captured fish precluded a similar analysis for finescale dace and fathead minnows.

DISCUSSION

The results of this study demonstrate that brook sticklebacks avoid areas marked with conspecific skin extract in a natural habitat, and thereby provide a field confirmation of chemical alarm signaling by brook sticklebacks. By avoiding conspecific skin extract, sticklebacks likely avoid high risk areas where predators have been successful at capturing conspecifics. In this study 40.75% of the sticklebacks were caught in traps marked with stickleback extract. Despite the fact that the concentration of skin extract, in terms of skin area per trap, in our study was approximately 4.3 times greater than that used in a similar trapping study by Mathis and Smith (1993), they reported that less than 4% of fathead minnows were captured in traps marked with conspecific extract. This apparent difference in the effectiveness of skin extract in promoting area avoidance is consistent with Mathis and Smith's (1994) suggestion that the antipredator behavior associated with skin extract exposure in sticklebacks is weaker than the response of fathead minnows to their conspecific skin extract.

For laboratory-reared cyprinid fishes, responses to alarm substance occur when the fish have no prior experience. Nevertheless, in cyprinids physiological development and experience play a role in determining the strength of the response (Pfeiffer, 1963; Waldman, 1982; Magurran, 1989). In sticklebacks it is unknown if naive fish show antipredator behaviors upon first encountering conspecific extract; however, as we demonstrated that small (and therefore less developed or experienced) fish were more likely to be caught in skin extract-marked traps, experience or physiological development likely plays a role in determining the strength of the response in sticklebacks.

In this study we have demonstrated a cross-superorder response to an alarm signal as finescale dace significantly avoided traps marked with stickleback skin extract. Interpreting the response of fathead minnows to stickleback extract is somewhat more complicated. Minnows were caught significantly less often in traps marked with stickleback skin extract; however, the number of fathead minnows is positively correlated with the number of sticklebacks in the control traps. Therefore, it is possible that social facilitation is occurring and that the minnows simply are being attracted by the stickleback in the control traps as opposed to avoiding the stickleback extract in the experimental traps. In a laboratory study, Mathis and Smith (1994) failed to detect a fright response by fathead minnows exposed to skin extract from brook stickleback. Nevertheless, for both finescale dace and fathead minnows, it appears that the response to stickleback extract is influenced by experience or development as smaller (younger) fish were more often captured in skin extract-marked traps.

By avoiding areas marked with conspecific skin extract, brook sticklebacks likely are able to lower their risk of capture. By detecting interspecific alarm signals, similar benefits may also extend to finescale dace and fathead minnows that are sympatric with sticklebacks.

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