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# FIELD VERIFICATION OF PREDATOR ATTRACTION TO MINNOW ALARM SUBSTANCE

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**Abstract**—Fishes such as minnows in the superorder Ostariophysi possess specialized alarm substance cells (ASC) that contain an alarm cue. Alarm substance can only be released by damage to the epidermis; thus, the release of alarm substance is a reliable indicator of predation risk. When nearby minnows detect the cue, they adopt a range of antipredator behaviors that reduce their probability of predation. Predator–predator interactions afford prey an opportunity to escape and, thus, a fitness benefit that maintains alarm substance calls over evolutionary time. Here, we present data from a simple field experiment verifying that nearby predators are attracted to minnow alarm substance because it signals an opportunity to pirate a meal. Fishing lures were baited with sponge blocks scented with either (1) water (control for sponge odor and appearance), (2) skin extract from non-ostariophysan convict cichlids (superorder Acanthopterygii, *Archocentrus* "*Cichlasoma*" *nigrofasciatus*) to control for general injury-released cues from fish, or (3) skin extract from fathead minnows (superorder Ostariophysi, *Pimephales promelas*). Predator strike frequency on each sponge type was 1,1, and 7 for water, cichlid, and minnow cues, respectively. These data provide the first field test using fish predators of the predator-attraction hypothesis for the evolution of Ostariophysan alarm substance cells.

**Key Words**—*Schreckstoff*, alarm substance, alarm pheromone, minnow, predator attraction, evolution, chemical signal, predator–prey, Ostariophysi.

## INTRODUCTION

Predation, and risk of predation, influences the evolution of behavior and morphology of virtually all animals because predation is the final arbiter of natural

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*To whom correspondence should be addressed. E-mail: wisenden@mnstate.edu selection (Lima and Dill, 1990). Chemical cues mediate predator–prey interactions in aquatic habitats for organisms ranging from ciliates to mammals (Wisenden, 2001). Chemical cues released by predators, or from conspecifics that have been injured or frightened, are used by prey to detect and evade predators (Smith, 1992, 1999; Kats and Dill, 1998; Chivers and Smith, 1998; Wisenden, 2000).

Predators cause physical damage and injury to their prey in the process of capture, handling, and ingestion. Consequently, chemical cues from injured prey are often released during a predator attack, and these cues serve as public information about predation risk. Most aquatic taxa, from ciliates to amphibians, exhibit antipredator behavior in response to injury-released chemical cues from conspecifics (Chivers and Smith, 1998).

Fishes in the superorder Ostariophysi, i.e., minnows, catfish, characins, and suckers, represent about 64% of all freshwater fish species (Nelson, 1994). One character that unites this group is the presence of specialized epidermal cells that contain an alarm substance; hence, the cells are referred to as alarm substance cells (ASCs). These cells lack an external duct. The cell contents are released only when the skin is damaged. Predator attacks on Ostariophysans release alarm substance into the surrounding water and warn nearby prey of predation risk. Alarm substance is potent. Minnow skin elicits overt antipredator behavior when diluted to 1 $cm^2$ skin per 58,000 liters (Lawrence and Smith, 1989), or the equivalent volume of a sphere with a radius of 3.7 m. The behavioral response to this cue significantly reduces the probability of predation risk (Mathis and Smith, 1993).

ASCs have caught the attention of evolutionary ecologists. It is not readily apparent how the alarm signaling function of ASCs provides a selfish benefit to the signal sender, and thereby provides a mechanism for these cells to be maintained by natural selection (Smith, 1992; Williams, 1992). The only evolutionary mechanism for ASCs for which there is experimental support is the predator-attraction hypothesis (Smith, 1992). In a laboratory experiment, northern pike *Esox lucius* were more attracted to ASC–minnow skin extract than skin extract without ASCs (Mathis et al., 1995). Similarly, in a field experiment, dytiscid predaceous diving beetles were more attracted to traps scented with minnow skin with ASCs than traps scented with minnow skin that lacked ASCs (Mathis et al., 1995). Chivers et al. (1996) showed in staged predation events in laboratory aquaria that fathead minnows in the grasp of a small pike have a significant chance of escape if a second, slightly larger, pike is allowed to interfere. Fathead minnows receive a selfish fitness benefit by investing in ASCs because ASCs attract secondary predators, not because they warn conspecifics of predation risk. The studies by Mathis et al. (1995) and Chivers et al. (1996) make important contributions to the understanding of the evolution of chemical alarm signaling in this ecologically important group of fishes. No other experiments have been conducted to verify these findings, and importantly, they have not been verified with fish predators in the field. In this study, we report the results of a simple field experiment that provides further support for the secondary predator-attraction hypothesis.

## METHODS AND MATERIALS

We conducted our experiment on Lake Ida, approximately 25 km north of Alexandria, Minnesota, USA (46°00′ N, 95°25′ W). Potential minnow predators in Lake Ida are: northern pike, *Esox lucius*; walleye, *Stizostedion vitreum vitreum*; largemouth bass, *Micropterus salmoides*; yellow perch, *Perca flavescens*; black crappie, *Poxomis nigromaculatus*; several types of bullhead catfish, *Ameiurus melas, A. nebulosus, A. natalis*; and perhaps bluegill sunfish, *Lepomis machrochirus*.

We fished for 4 hr on January 27 and 4 hr on February 11, 2001. Ice cover at the time of data collection was about 80 cm thick. We drilled six holes about 25 cm in diameter with a gas-powered auger and spaced the holes about 8 m apart. We used a depth sonar to standardize water depth below all holes at about 3 m. We fitted each hole with an ice-fishing tip-up. A tip-up has a small platform and an upright stand connected to an arm that extends over the hole. The arm was equipped with a reel of anti-freezing line followed by a 1.1 m length of transparent monofilament line "leader." A lure was attached to the end of the leader. At the other end of the arm there was a piece of springy metal attached to a fluorescent orange flag. The flag was flexed against the reel of line in such a way that when a fish tugs on the line the flag is released, signaling a strike. In addition, a broad metal paddle was attached to the arm designed to catch any wind movement, causing the arm to bob up and down and jig the line. Jerky vertical motion on the line attracts fish to the lure. All holes were about 300 m from shore and equally exposed to wind. By using wind-powered tip-ups, we standardized the visual presentation of test stimuli among treatments.

Each tip-up was fitted with a fluorescent green, 1/32 oz (0.9 g) teardrop style jig and a small block of cellulose sponge ($1 \times 1 \times 0.7$ cm). The sponge block was soaked with one of three test solutions: (1) dechlorinated tap water to control for the odor and appearance of the sponge and background attractiveness of the jig lure, (2) skin extract of a tropical fish (convict cichlid, *Archocentrus "Cichlasoma" nigrofasciatus*) to control for general chemical cues released from injured fish, or (3) skin extract of fathead minnows, *Pimephales promelas*. Convict cichlids are not in the Ostariophysi (superorder Acanthopterygii) and, therefore, do not possess Ostariophysan ASCs. Skin extracts for each species were prepared using the same protocol. We used five cichlids (mean ± SE $= 6.8 \pm 0.8$ cm, $N = 5$)

and 11 minnows (mean ± SE $= 6.5 \pm 0.17$ cm, $N = 11$) to make the skin extract solutions. We killed each fish by a blow to the head, then carefully filleted the skin from each flank. Skin fillets were measured, then placed in chilled dechlorinated tap water. The total area of skin collected was 68.44 cm$^2$ and 66.97 cm$^2$ for cichlids and minnows, respectively. To simulate a predator attack we macerated the skin fillets with a conventional hand-held blender. The solution was filtered through polyester wool to remove scales, connective tissue, and suspended tissue particles. Dechlorinated water was added to bring the total volume of each skin extract to 32 ml. We made 64 sponge blocks of each odor type. Each sponge block was infused with 0.5 ml of dechlorinated water, cichlid skin extract, or minnow skin extract. Therefore, cichlid and minnow cues were of equivalent strength, based on 1.0 cm$^2$ of skin extract per sponge for each species cue. We froze the sponges at $-20^\circ$C until needed. Conspecifics exhibit an alarm reaction to skin extract that has been frozen for both fish species used in this experiment (Lawrence and Smith, 1989; Wisenden and Sargent, 1997).

Of the six holes fished each day, two were assigned water sponges, two were assigned cichlid sponges, and two were assigned minnow sponges. We replaced the sponges with fresh ones every 30 min. We also rotated locations so that each sponge type was used at every location during each test day. We recorded the number of times that the tip-up arms showed a clear tip, i.e., the tip of the arm pulled into the hole, and the number of times the indicator flags were released.

We analyzed the data by using a chi-square goodness of fit test. Conventional wisdom maintains that a chi-square test cannot be used if more than 25% of the cells contain frequencies less than 5. However, this oft-repeated rule of thumb is overly conservative and unnecessary (Haldane, 1943; Snedecor and Cochran, 1980, p. 77).

## RESULTS

Over the course of 48 hole-hr of fishing effort, holes with the minnow-sponges received seven predator strikes, of which six released the flag, while holes with the cichlid-sponges and water-sponges received one strike (and flag release) each ($\chi^2 = 8.0$, $P < 0.02$). Tip-ups baited with the scent of injured minnows were more attractive to predators than general chemical cues released by an injured non-Ostariophysan fish ($\chi^2 = 4.5$, $P < 0.05$). Predator attraction to the cichlid cue was not different from water ($P \cong 1$).

The time between replacement of the sponge and strike on the lure varied and did not seem related to treatment type. Lures scented with minnow skin were struck 6–24 min (mean ± SE $= 14 \pm 6$ min, $N = 4$) after sponge replacement. The single strike on the cichlid-scented lure occurred 4 min after sponge replacement. Strike time was not recorded for the water-scented strike.

## DISCUSSION

The first conclusion from this experiment is that chemosensory cues are important in eliciting strike behavior. Because minnows are small fishes and fall prey to a wide variety of predators, it is reasonable to infer that fish large enough to trip the tip-up flag were minnow predators. The second conclusion is that not all scents are equally effective. The strike frequency on the cichlid cue was the same as for the water cue, indicating that general substances released from injured fish (amino acids, proteins, lipids, etc.) do not automatically elicit a strike response from predators. Minnow scent was clearly preferred by predators, consistent with predictions from chemical alarm signaling theory (Smith, 1992; Mathis et al., 1995; Chivers et al., 1996). Therefore, this study provides the first field verification from fish predators of the predator-attraction hypothesis for the evolution of ASCs. The predator species that struck the scented lures could not be observed through the ice; however, Lake Ida is best known for its pike fishery during the ice-fishing season. None of the fish striking the scented lures were landed because the sponge block was on the jig hook. Nevertheless, fish large enough to release the tip-up flag would be large enough to eat a fathead minnow or interrupt a predation event by attempts of piracy or cannibalism.

The precise chemistry of the alarm cue contained in minnow skin cells is not completely understood. A nucleotide, hypoxanthine-3(N)-oxide, has long been thought to be the active ingredient in the alarm cue/predator attractant (unpublished PhD thesis by Argentini in 1976, after Smith 1999). Laboratory (Pfeiffer et al., 1985; Mathis et al., 1995) and field (Brown et al., 2000) tests confirm the biological activity of hypoxanthine-3(N)-oxide.

An alternative explanation for the differences in strike frequency is that predators may have preferred minnow cue because minnow cue is familier to them. Cichlids are tropical fish and allopatric to the fish species in Lake Ida. However, there is no evidence to suggest that the chemical cues released by cichlids should differ qualitatively from those typical of general non-Ostariophysan fishes. Cichlids are in the same order, Perciformes, and ecologically similar to centrarchids (sunfish, bass), which are abundant in Lake Ida. The most likely explanation of the differences in strike frequency in the current experiment is that skin from minnows produces a more potent cue, with perhaps better dispersing properties than an equivalent area of cichlid skin. The presence of ASCs in minnow skin is the most likely cause of this.

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