

## THE ROLE OF OLFACTION IN CHEMOSENSORY-BASED PREDATOR RECOGNITION IN THE FATHEAD MINNOW, *Pimephales promelas*

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**Abstract**—Solitary fathead minnows (*Pimephales promelas*) were rendered anosmic and exposed to chemical stimuli from a predatory northern pike (*Esox lucius*) to determine the role of olfaction in the minnow's ability to recognize predators on the basis of chemical stimuli. Anosmic fish did not respond to the pike stimuli with a typical fright reaction, while control fish, with intact olfactory receptors, did. These results demonstrate that the olfactory system is necessary for the ability of fathead minnows to recognize northern pike as a predator and that the gustatory and single-celled chemosensory systems are not sufficient for this recognition in the absence of olfactory input. Olfactory impairment was behaviorally confirmed by exposing minnows to alarm substance (Schreckstoff).

**Key Words**—Olfaction, chemoreception, solitary chemosensory cells, predator recognition, alarm pheromone, Schreckstoff, fathead minnow, *Pimephales promelas*, northern pike, *Esox lucius*.

### INTRODUCTION

The recognition of predators through chemoreception occurs in a variety of vertebrates (review: Weldon, 1990). For many prey species, including the fathead minnow (*Pimephales promelas*), the ability to chemically recognize a predator is an important component of an individual's antipredator arsenal, because it may result in a greater number of ecological situations in which predator recognition is possible. For example, prey fishes with chemical predator rec-

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ognition abilities may be able to utilize habitats with poor optic properties. As well, nonvisual detection of cryptic ambush predators, such as the northern pike (*Esox lucius*) (Savino and Stein, 1989), may be critical to the survival of prey fishes (Mathis et al., 1993).

In fishes, predator recognition through chemoreception could result from olfaction, gustation, or single-celled chemoreception (Kotrschal, 1991). Solitary chemosensory cells (SCCs), which are secondary epidermal sensory cells (Whitewar, 1971), may be used in predator recognition and avoidance, as they respond to mucus of heterospecific fishes (Baatrup and Doving, 1985; Kotrschal et al., 1989; Peters et al., 1987, 1990, 1991). Little information exists on the role of gustation in predator recognition. However, fishes possess numerous taste receptors (Hara, 1971) that could potentially be used in predator recognition. Olfaction has been demonstrated to be responsible for mediating predator recognition in coho salmon (*Oncorhynchus kisutch*) (Rehnberg et al., 1985), brook trout (*Salvelinus fontinalis*) (Keefe, 1992) and the bitterling (*Rhodeus sericesu amarus*) (Kasumyan and Pashchenko, 1985). Olfaction is based on nerve cell receptors contained within the olfactory pits (Kleerkloper, 1969; Hara, 1971). As these receptors are highly localized in the nares, this sensory system, unlike the gustatory or single-celled chemosensory system, can be blocked.

Mathis et al. (1993) demonstrated that fathead minnows from a population sympatric with northern pike are able to recognize pike as predators through chemoreceptive cues alone. They did not, however, determine the specific chemosensory system used by the minnows to recognize the chemical stimuli from the pike. The goal of this study was to determine the role of olfaction in the chemosensory-based predator recognition ability of fathead minnows. We tested the hypothesis that sham-operated control and olfactorily impaired minnows differed in their response to chemical stimuli from northern pike. If olfactorily blocked minnows exhibit a fright reaction [i.e., defensive behavior that occurs upon recognition of a predator (review: Smith, 1992)] to the chemical stimuli from a pike, then a chemosensory system other than olfaction must be used in predator recognition. This result would not, however, eliminate the possibility that olfaction can be used for predator recognition. In contrast, the lack of a fright reaction by olfactorily impaired minnows would indicate that olfaction is necessary for minnows to identify northern pike as a predator using chemical cues.

To confirm olfactory impairment behaviorally, we also tested control and olfactorily impaired minnows for a fright reaction to minnow alarm substance. The alarm substance, or Schreckstoff, probably hypoxanthine-3-(*N*)-oxide (Pfeiffer et al., 1985), is detected through olfaction (Frisch, 1941; Pfeiffer et al., 1984). Therefore, we tested the hypothesis that control and olfactorily impaired minnows differed in their response to alarm substance.

## METHODS AND MATERIALS

*Collection and Maintenance.* In September 1990, northern pike were collected from Eagle Creek, a tributary of the North Saskatchewan River in south-central Saskatchewan. They were maintained in a 300-liter tank at approximately 15°C on a 14:10 light-dark photoperiod and were fed once every five days with fathead minnows.

Fathead minnows were collected in the fall of 1990 from Pike Lake, an oxbow lake of the South Saskatchewan River in south-central Saskatchewan where they occurred in sympatry with northern pike. The minnows were maintained in outdoor pools (approximately 18,000 liters) at temperatures ranging from 15°C (fall months) to 4°C (winter months). In the spring of 1991, the minnows were placed in the laboratory in a 600-liter holding tank at approximately 15–18°C on a 14:10 light-dark photoperiod and were fed daily with commercial fish food.

*Pike Stimulus Preparation.* Prior to the stimulus collection, three pike [mean fork length = 19.2 cm  $\pm$  3.55 (1 SD)] were fed approximately equal volumes (range = 3–4 ml, measured by volumetric displacement in water) of swordtails (*Xiphophorus helleri*) for each of three feedings (i.e., once every five days). A swordtail diet was used on the last three pike feedings to eliminate secondary stimuli from the pike's diet of fathead minnows (Mathis and Smith, 1993). Mathis et al. (1993) demonstrated that this stimulus evokes a fright reaction from Pike Lake minnows. Just prior to the last feeding, the pike were moved from their 300-liter holding tank to a separate holding tank (150 liters). Approximately 1 hr later they were removed from the tank and rinsed with dechlorinated tap water to remove any swordtail residue from the pike's skin. The pike were then returned to an identical holding tank (150 liters) that contained clean water. Approximately 16 hr after the last feeding, the pike were placed in separate clear plastic stimulus collection chambers (26  $\times$  8  $\times$  8 cm) that contained 1200 ml of dechlorinated tap water. This 16-hr delay ensured that the pike did not regurgitate their stomach contents (Mathis, personal communication). The stimulus collection chambers were aerated but contained no filtration system. After three days, the pike were removed from the stimulus collection chambers. The stimulus water was pipetted into separate 5-ml polypropylene containers and was frozen at approximately -20°C.

*Alarm Substance Stimulus Preparation.* The alarm substance stimulus was prepared from 15 donor fathead minnows. The twelve male and three female donors [mean fork length = 5.10  $\pm$  .382 (1 SD)] were killed and a skin filet was taken from both sides of each fish. Total area of skin collected from all donors was approximately 29.0 cm<sup>2</sup>. Immediately upon removal, the skin samples were placed in 100 ml of chilled glass-distilled water. The skin samples

were then homogenized with a polytron homogenizer and the homogenate was filtered through glass wool to remove any solid particles. The homogenate was diluted with 100 ml of glass-distilled water, resulting in a total volume of 200 ml that was frozen in separate 5-ml polypropylene containers.

*Olfaction Impairment.* Olfactory impairment was induced in 15 experimental minnows [mean fork length =  $6.17 \text{ cm} \pm 0.623$  (1 SD)] by injecting hot petroleum jelly ( $> 150^\circ\text{C}$ ) into the olfactory pit of minnows anesthetized with 140 mg/liter of MS 222 (Tricaine methanesulfonate). The petroleum jelly solidified immediately to block the olfactory pit. This technique was successfully used to induce anosmia in coho salmon (Rehnberg et al., 1985) and brook trout (Keefe, 1992). Upon completion of testing, each minnow was examined under a dissecting microscope to ensure that the plug remained intact.

Fifteen control minnows (mean fork length =  $6.23 \pm 0.477$ ) were similarly anesthetized and subjected to a sham nasal blocking treatment in which two drops of hot petroleum jelly were deposited just ventral to the nares. Impairment of the olfactory system of the experimental minnows and the sham operation of the control minnows took place three days prior to testing these individuals.

*Testing Protocol.* Immediately following the olfactory impairment or the sham operation, the minnows were individually placed into separate Plexiglas acclimation tanks ( $45 \times 45 \times 20 \text{ cm}$ ). Clean water was constantly supplied to the acclimation tanks at a rate of approximately 250 ml/min, maintaining a constant water depth of 4–5 cm. The fish were fed daily with commercial fish food and were kept under a 14:10 light–dark photoperiod. After two days, the minnows were transferred into separate testing tanks that were identical to the acclimation tanks except that the flow rate was increased to 500 ml/min. The holding and testing tanks were the same as those used by Mathis et al. (1993). Acclimation and testing occurred at a mean water temperature of  $16.7^\circ\text{C}$  (range  $16\text{--}18^\circ\text{C}$ ).

The test tanks were surrounded by an Opto-Varimex-Aqua tracking meter (Columbus Instruments), which lays down a grid of light beams across the tanks. A microcomputer that was interfaced with the tracking meter detected changes in the fish's movement by determining the number and location of light beams that were broken between a light source and photocell. Broken light beams were detected at intervals of 0.125 sec. Lemly and Smith (1986, 1987) described this testing apparatus in detail. Our system differed from that of Lemly and Smith (1986, 1987) only in that outflowing water was discarded rather than recirculated. Three measures of activity that typically decrease during a fright reaction in fathead minnows (Lawrence and Smith, 1989) were quantified by the computer: (1) total distance traveled (centimeters), (2) number of stereotypic movements (i.e., activity in which the fish breaks light beams without moving outside one grid square), and (3) total time active (seconds).

Utilizing video equipment, an observer in an adjacent room was able to

view the experimental tanks without disturbing the minnows. The observer was also able to inject the stimulus water into inflowing water lines that passed through the observation room before entering the test tanks. Experimental observations were conducted using the pike stimulus water between 0700 and 1130 hr. These trials lasted a total of 16 min, with 5.0 ml of the pike stimulus being injected at the end of 8 min. Injection of the stimulus water took approximately 5 sec. Subsequent observations of these same minnows were completed later that day between 1200 and 1730 hr using 5.0 ml of the alarm substance stimuli. Injection of the alarm substance stimulus was done in the same fashion as injection of the pike water stimulus. The mean time between tests with the same individuals was 255 min.

The minnows' response was based on three different parameters (total distance traveled, number of stereotypic movements, and total time active), each of which was tested independently. To test for differences in prestimulus activity that may have resulted from the olfactory impairment procedure, prestimulus activities of control and olfactorily impaired minnows were compared using a Wilcoxon-Mann-Whitney test (Siegel and Castellan, 1988) ( $W_x$  = Mann-Whitney U; Siegel, 1956) prior to exposure to the first stimulus (i.e., the pike stimulus). The significance of the response of the minnows to both pike stimuli and alarm substance stimuli was determined by the Wilcoxon signed rank test (Siegel and Castellan, 1988) (i.e., prestimulus versus poststimulus activity). For each stimulus, statistical comparisons of control and olfactorily impaired minnows were made using the Wilcoxon-Mann-Whitney test (Siegel and Castellan, 1988). In all cases two-tailed statistical tests were employed with  $\alpha = 0.05$  and  $N = 15$ .

## RESULTS

*Prestimulus Activity: Control vs. Anosmic Fish.* Prior to presentation of the first stimulus (i.e., the pike stimulus), the activity levels of the control and olfactorily impaired minnows were not significantly different, in terms of either distance traveled ( $W_x = 221$ ,  $P = 0.646$ ), number of stereotypic movements ( $W_x = 209.5$ ,  $P = 0.352$ ), or time active ( $W_x = 209$ ,  $P = 0.342$ ). These comparisons demonstrate that the normal activity levels of anosmic minnows were not altered by the olfactory impairment procedure.

*Prestimulus vs. Poststimulus Activity.* Control minnows significantly decreased their activity following exposure to chemical stimuli from northern pike in terms of distance traveled (mean decrease = 57.3%, Wilcoxon  $T = 5.5$ ,  $P < 0.001$ ), number of stereotypic movements (mean decrease = 40.0%,  $T = 9$ ,  $P = 0.002$ ), and time active (mean decrease = 39.0%,  $T = 13$ ,  $P < 0.006$ ). Control minnows also significantly decreased their activity following

exposure to alarm substance stimuli in terms of distance traveled (mean decrease = 69.8%,  $T = 0$ ,  $P < 0.001$ ), number of stereotypic movements (mean decrease = 49.4%,  $T = 1$ ,  $P < 0.001$ ), and time active (mean decrease = 52.7%,  $T = 0$ ,  $P < 0.001$ ). In contrast, olfactorily impaired minnows exhibited no significant change in distance traveled (mean increase = 0.6%,  $T = 51$ ,  $P = 0.639$ ), number of stereotypic movements (mean increase = 2.5%,  $T = 51$ ,  $P = 0.639$ ), or time active (mean increase = 5.0%,  $T = 50.5$ ,  $P = 0.619$ ) following exposure to chemical stimuli from northern pike. They also did not significantly alter their activity in terms of distance traveled (mean increase = 4.5%,  $T = 42$ ,  $P = 0.359$ ), number of stereotypic movements (mean decrease = 24.0%,  $T = 27$ ,  $P = 0.064$ ), or time active (mean decrease = 14.6%,  $T = 29$ ,  $P = 0.083$ ) following exposure to alarm substance stimuli.

*Control vs. Experimental Conditions.* In direct comparisons of control and experimental minnows, control minnows exhibited a significantly greater decrease in distance traveled (Wilcoxon-Mann-Whitney test,  $Wx = 165.5$ ,  $P = 0.006$ ) (Figure 1), number of stereotypic movements ( $Wx = 167$ ,  $P = 0.007$ ) (Figure 2), and time active ( $Wx = 171$ ,  $P = 0.011$ ) (Figure 3) than did olfactorily blocked minnows that were exposed to the chemical stimuli from pike. Similarly, control minnows exhibited a significantly greater decrease in distance traveled ( $Wx = 155.5$ ,  $P = 0.002$ ) (Figure 1), number of stereotypic movements ( $Wx = 175$ ,  $P = 0.018$ ) (Figure 2), and time active ( $Wx = 175$ ,  $P = 0.018$ ) (Figure 3) than did olfactorily blocked minnows that were exposed to alarm substance stimuli.

## DISCUSSION

The results of this study demonstrate that chemical cues are necessary for fathead minnows to recognize northern pike as a predator. The role of olfaction in predator recognition has been documented in several vertebrates. Kats (1988), for example, demonstrated that larval small-mouthed salamanders (*Ambystoma texanum*) detected fish predators through olfaction. Individual salamanders with plugged nares failed to increase their use of refuges when exposed to chemicals from green sunfish (*Lepomis cyanellus*), while control individuals increased their use of refuges. Webster (1973) reported that kangaroo rats (*Dipodomys merriami*) with olfactory bulbectomies did not avoid sidewinders (*Crotalus cerastes*), while intact individuals did. Olfaction has also been demonstrated to be responsible for the ability of brook trout to recognize chemical stimuli from red fin pickeral (*Esox americanus*) (Keefe, 1992) and for bitterlings to recognize chemical stimuli from northern pike (Kasumyan and Pashchenko, 1984). Furthermore, olfaction mediates the avoidance response of juvenile coho salmon to L-serine (Rehnberg et al., 1985), an active salmon repellent in mammalian skin extract (Idler et al., 1956).

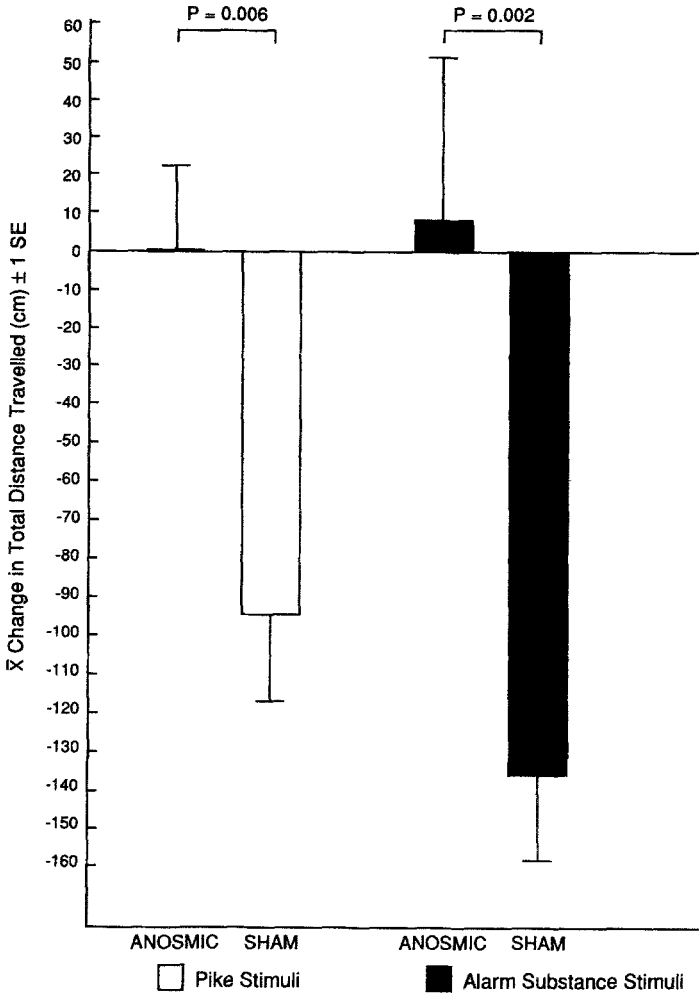


FIG. 1. Mean change in total distance traveled (cm) by sham operated (control) and anosmic fathead minnows following exposure to chemical stimuli from pike and to alarm substance stimuli.

Three lines of evidence confirm that the olfactory system of the experimental fish was impaired. Microscopic analysis upon completion of the tests revealed that the olfactory plugs remained intact in all of the experimental minnows. Control minnows significantly altered their activity following exposure to pike stimuli while olfactorily impaired minnows did not. Furthermore, control minnows significantly altered their activity following exposure to alarm substance stimuli while olfactorily impaired minnows did not.

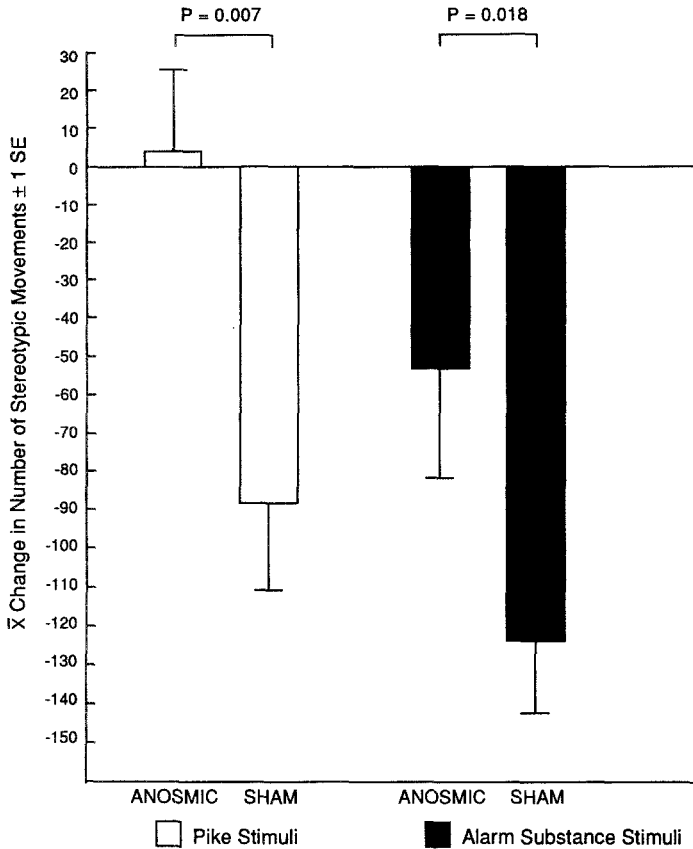


FIG. 2. Mean change in number of stereotypic movements by sham operated (control) and anosmic fathead minnows following exposure to chemical stimuli from pike and to alarm substance stimuli.

There is some controversy as to whether SCCs function in mediating recognition of chemical stimuli from predators or whether their function is related to feeding (Kotrschal et al., 1991). In our study the lack of a defensive response by anosmic minnows suggests that SCCs are not sufficient for recognition of chemical stimuli from pike. Physiological evidence from rocklings (*Ciliata mustela*) suggests that SCCs may be used in predator avoidance, as they appear tuned to the body mucus of heterospecific fishes (Peters et al., 1989, 1991; Kotrschal et al., 1989). Furthermore, oligovillous cells of lampreys, which are structurally comparable to SCCs (Whitear and Lane, 1983), have been shown

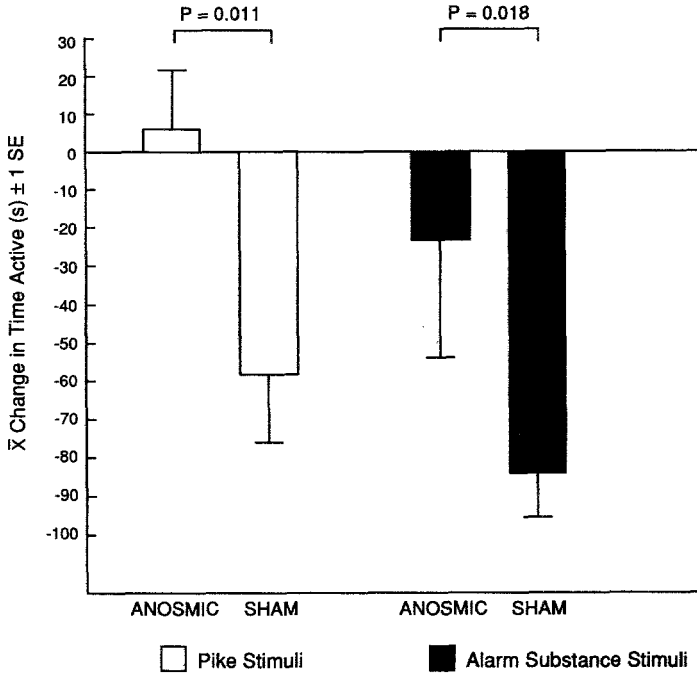


FIG. 3. Mean change in total time active (sec) by sham operated (control) and anosmic fathead minnows following exposure to chemical stimuli from pike and to alarm substance stimuli.

to respond best to mucoid substances (Baatrup and Doving, 1985). In contrast, electrophysiological evidence from the sea robin (*Prionotus carolinus*) suggests that SCCs on the free pectoral fin rays respond best to amino acids, including betaine (trimethylglycine), thereby implicating a function related to feeding and not predator avoidance (Silver and Finger, 1984). The SCCs of fathead minnows, like those of rocklings, may still respond electrophysiologically to chemical stimuli from the pike; however, we have demonstrated that olfaction is necessary for mediating the ability of fathead minnows to recognize chemical stimuli from pike.

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