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Free-living fathead minnows rapidly learn to recognize pike as predators

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Individuals from a natural population of approximately 20 000 fathead minnows from a pike-free pond did not respond with appropriate anti-predator behaviour upon encountering pike odour in laboratory tests. However, 14 days after 10 pike were stocked into the pond, minnows had acquired recognition of pike odour. Laboratory studies have indicated several possible mechanisms for acquiring predator recognition in fathead minnows. This study indicates that these, or similar processes, can produce major changes in predator recognition in the wild.

Key words: fish behaviour; predator recognition; alarm substance; Schreckstoff; fathead minnow; northern pike.

INTRODUCTION

Acquisition of predator recognition has important implications for prey fishes. Failure to recognize a predator has the obvious consequence of an increased risk of capture. In addition, defensive responses to non-predators conflict with activities such as foraging and reproduction (Lima & Dill, 1990; Milinski, 1993). For members of the superorder Ostariophysi (which includes fathead minnows, *Pimephales promelas* Rafinesque) several mechanisms of the acquisition of predator recognition have been documented in laboratory studies.

Ostariophysan fishes possess an alarm substance, or Schreckstoff, in distinctive epidermal club cells. Mechanical damage to the club cells, as would occur during a predatory attack, releases the alarm substance (AS). Once released, the AS can be detected by other Ostariophysan fishes which then perform anti-predator behaviours (i.e. a species-specific fright reaction, see review by Smith, 1992). Laboratory studies have shown that both European minnows, *Phoxinus phoxinus* (L.) (Göz, 1941; Magurran, 1989) and fathead minnows (Chivers & Smith, 1994*a,b*) learn to recognize potential predators when AS is presented in conjunction with the predator. AS ingested by predators also acts to chemically label the predator as dangerous to naive minnows, thereby resulting in learned predator recognition by the minnows (Mathis & Smith, 1993*a,b*).

With the exception of a portion of Mathis and Smith's (1993*b*) experiment, all studies of acquired predator recognition by fathead minnows have thus far been completed under laboratory conditions. In this study we identify and test a natural population of pike-naive minnows. We then expose the minnows to pike predation in the natural habitat and subsequently test for the acquisition of predator recognition. Such a field demonstration would provide external

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validation for laboratory studies of acquired predator recognition by fathead minnows and may provide an indication of the speed and scope of learned predator recognition under natural conditions.

METHODS

PIKE STIMULUS PREPARATION

One northern pike, *Esox lucius* L. (fork length = 18 cm) was fed a volume of 3·0–4·0 ml (measured by volumetric displacement in water) of swordtails, *Xiphophorus helleri* Heckel, for three consecutive feedings (once every 5 days) before collecting the stimulus. Approximately 1 h after the last feeding, the pike was rinsed with dechlorinated tap water to remove any swordtail residue from the pike's skin and returned to a holding tank that contained clean water. After approximately 16 h, the pike was placed into an aerated but unfiltered clear plastic stimulus collection chamber (26 × 8 × 8 cm) that contained 1200 ml of dechlorinated tap water. After 3 days the pike was removed from the chamber and samples of the stimulus water pipetted into 20 ml plastic bags and frozen at approximately −20° C. As a control stimulus, 20 ml units of dechlorinated tap water were also frozen.

COLLECTION AND MAINTENANCE OF PIKE-NAIVE FATHEAD MINNOWS

In early May 1993, fathead minnows were collected from a pond (approximate area = 1 ha) on the University of Saskatchewan campus and transferred to the laboratory where they were maintained in a 300-l stream tank on a 14 L : 10 D cycle, and fed daily with Nutrafin Goldfish Food. This population of minnows originated from the South Saskatchewan River when the pond was filled in 1959 to provide water for agricultural purposes. Other fish species present in the pond included finescale dace, *Phoxinus neogaeus* Cope, lowa darters, *Etheostoma exile* (Girard), and white suckers, *Catostomus commersoni* (Lacépède). Unlike the South Saskatchewan River, which contains many predatory fish, including pike, walleye, *Stizostedion vitreum* (Mitchill), sauger, *Stizostedion canadense* (Smith), perch, *Perca flavescens* (Mitchill), and burbot, *Lota lota* (L.), the experimental pond was free of predatory fish species.

EXPERIMENT 1: RESPONSE OF PIKE-NAIVE MINNOWS TO CHEMICAL STIMULI FROM PIKE

To test the response of pike-naive minnows to chemical stimuli from pike, 12 minnows ($\bar{X} \pm$ S.D. fork length = 4·72 ± 0·52 cm) were randomly selected and one minnow was placed into each experimental observation tank (37 l: 50 × 30 × 25 cm). The remaining pike-naive minnows were maintained as control fish for later use. Each tank was aerated with a single airstone located at the back of the tank and contained a centrally located shelter consisting of a ceramic tile (9·8 × 20·0 cm) mounted on three cylindrical glass legs (5·5 cm high). The tanks contained no filtration system but the bottom of each was covered with a shallow layer of sodium zeolite chips, which remove ammonia from the water. A plastic tube to introduce either water or the pike stimulus was attached to the airline. Observations were conducted at approximately 20° C on a 14 L : 10 D cycle, after the minnows had been introduced to the observation tanks 3 days earlier.

As a standard testing protocol, the amount of time a minnow spent beneath the shelter for 8 min prior to and after the introduction of a chemical stimulus into the tank was recorded. During the post-stimulus period the presence or absence of dashing (very rapid, apparently disoriented swimming) was also recorded. Several authors including Lawrence & Smith (1989), Mathis & Smith (1993*c*) and Mathis *et al.* (1993), have interpreted an increase in cover use and the presence of dashing as components of a fright reaction in fathead minnows. Increased shelter use likely makes the fish less conspicuous to visually hunting predators, while dashing may prevent a predator from locking its sights onto the minnow, and may take the minnow away from the immediate vicinity of the predator.

Between 07.30 and 11.30 hours, each of the 12 minnows was tested for a response to 20 ml of dechlorinated tap water. Later the same day between 12.30 and 15.30 hours we tested the response of the same minnows to 20 ml of pike stimuli. The change in time spent under cover by the minnows in response to water and pike stimuli was compared using a Wilcoxon–Mann–Whitney test (Siegel & Castellan, 1988), while the frequency of dashing between the water and pike treatments was compared using a Fisher's Exact probability test (Siegel & Castellan, 1988).

Minnow population estimation and pike stocking

A 'mark and recapture' study was conducted to determine an appropriate pike stocking rate. In late May 1993, 2000 minnows were trapped from the experimental pond, clipped on the dorsal lobe of their caudal fins and then released. Three days later, 404 (20·2%) of the marked fish were recaptured in a total catch of 3935 minnows. Using the Schnabel method (Smith, 1980) the minnow population was estimated at 19 480 (95% C.L. = 17 840–21 120). This estimate is likely quite conservative given that some minnows would be small enough to swim through the mesh of the minnow traps.

Two weeks was arbitrarily selected as the time period to elapse before acquisition of the ability to recognize pike as predators was tested. Laboratory observations indicated that small pike (14 to 22 cm) eat between two and five minnows per day. By releasing 10 pike ($\bar{X} \pm$ S.D. fork length = 17·4 ± 2·75 cm) into the pond approximately 1·4 to 3·6% of the minnow population would be eaten before testing for the acquisition of predator recognition.

EXPERIMENT 2: RESPONSE OF PIKE-EXPERIENCED MINNOWS TO CHEMICAL STIMULI FROM PIKE

Fourteen days after pike were stocked into the experimental pond a further set of minnows (hereafter referred to as pike-experienced minnows) were collected and maintained in identical conditions as the pike-naive minnows. Using the same testing protocol and statistical analyses as above, the response of 12 pike-experienced minnows ($\bar{X} \pm$ S.D. fork length = 5·73 ± 0·38 cm) was tested to both dechlorinated tap water and pike stimuli. To control for possible seasonal effects in predator recognition, another 12 pike-naive minnows ($\bar{X} \pm$ S.D. fork length = 5·57 ± 0·63 cm) that had been held in the laboratory since the testing of the initial pike-naive minnows were also tested.

RESULTS

EXPERIMENT 1: RESPONSE OF PIKE-NAIVE MINNOWS TO CHEMICAL STIMULI FROM PIKE

The minnows showed an increase in their use of cover following exposure to both distilled water (mean increase = 55·3%) and chemical stimuli from pike (mean increase = 120·9%). Comparing the responses between the pike stimulus and the control water stimulus revealed no significant difference in response [$W_x = 145·5$, $m = 12$, $n = 12$, $P = 0·818$, Fig. 1(a)] in terms of time spent under cover. Similarly, there was no difference in the frequency of dashing between the pike and water treatments [Table I(a)]. These results clearly demonstrate that the pike-naive fathead minnows from the experimental pond do not respond with a fright response upon exposure to chemical stimuli from pike. These minnows, therefore, provide an excellent opportunity for studying acquisition of predator recognition.

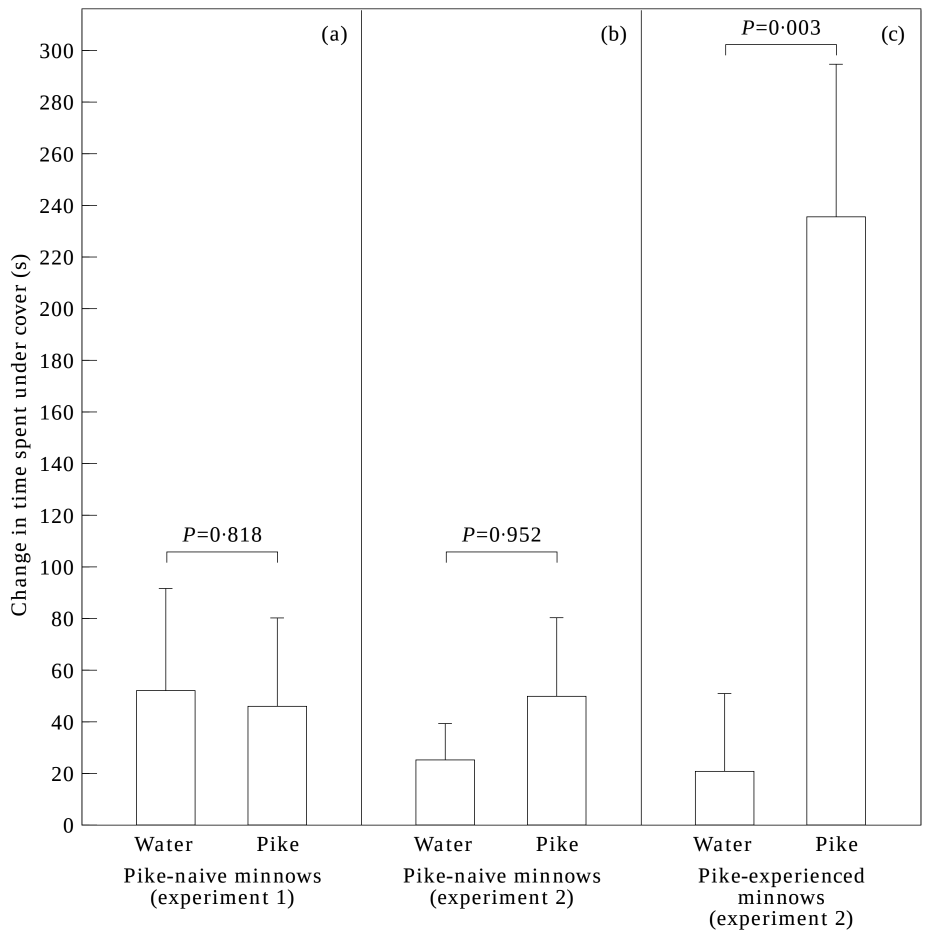

FIG. 1. Change (± S.E.) in time spent under cover following exposure to water and pike stimuli by (a) pike-naive minnows that were collected prior to the pike stocking (minnows tested in experiment 1), (b) pike-naive minnows that were collected prior to the pike stocking but held in the laboratory until use in experiment 2, and, (c) pike-experienced minnows that were collected 14 days after the pike stocking (minnows tested in experiment 2).

EXPERIMENT 2: RESPONSE OF PIKE-EXPERIENCED MINNOWS TO CHEMICAL STIMULI FROM PIKE

As in the previous experiment there was no significant difference in the change in cover use in response to water or pike stimuli [$W_x = 148{\cdot}5$, $m = 12$, $n = 12$, $P = 0{\cdot}952$, Fig. 1(b)] by pike-naive minnows (mean increase to water = 12·6%, mean increase to pike = 31·8%). For pike-naive minnows there was also no significant difference in the frequency of dashing between the water and pike treatments [Table I(b)]. In contrast, pike-experienced minnows increased their use of cover significantly more [$W_x = 97{\cdot}5$, $m = 12$, $n = 12$, $P < 0{\cdot}003$, Fig. 1(c)] following exposure to pike stimuli than to water stimuli (mean increase to water = 14·7%, mean increase to pike = 220·3%) and exhibited significantly more dashing in response to the pike stimuli than to the water stimuli [Table I(c)].

TABLE I. Number of minnows exhibiting dashing behaviour following exposure to water and pike stimuli

		Dashing	Not dashing	P
(a)	Pike-naive minnows collected prior to the pike stocking (experiment 1)			
	Water	2	10	0·240
	Pike	0	12	
(b)	Pike-naive minnows collected prior to the pike stocking but held in the laboratory until use in experiment 2			
	Water	0	12	1·0
	Pike	0	12	
(c)	Pike-experienced minnows collected 14 days after the pike stocking (experiment 2)			
	Water	0	12	0·047
	Pike	4	8	

DISCUSSION

The results of this study clearly demonstrate the ability of fathead minnows to acquire predator recognition under natural field conditions, and therefore validate the previous laboratory studies (Mathis & Smith, 1993*a*,*b*; Chivers & Smith, 1994*a*,*b*) of the acquisition of predator recognition. The observed change in predator recognition behaviour in this population of approximately 20 000 minnows was achieved after only a 2-week exposure to 10 pike. Such results are consistent with the presence of multiple effective mechanisms for acquiring predator recognition. Nevertheless, we do not have any indication of which mechanism(s) are most responsible for this learned ability. To our knowledge this is the first study to document learned recognition of an introduced predator by a free-living population of fish.

Pitcher (1980) has suggested that roach, *Rutilus rutilus* L., European minnows and schooling prey fishes in general may rarely be outside the attack range of many predators, including pike. Given the potential for such frequent encounters with predators, the acquisition of predator recognition has important implications for the survival of fathead minnows. Upon encountering a predator an individual that shows a fright response may increase its probability of surviving the encounter (Mathis & Smith, 1993*c*). The fright response of an individual may also warn other prey in the vicinity (Verheijen, 1956; Smith & Smith, 1989), thereby increasing the inclusive fitness of the individual through the process of warning close relatives (Sherman, 1977). Warning other conspecifics may protect valuable neighbours or group members (Smith, 1986) and may benefit the signaller or its kin through reducing predator success, and thus discouraging future hunting in the signaller's home range (Trivers, 1971). Finally the fright response of the individual may act directly upon the predator to inform it that its presence has been detected, making it unlikely that an attack will be successful (Högstedt, 1983).

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