Journal of Fish Biology (1994) **44**, 273–285

The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*

D. P. CHIVERS AND R. J. F. SMITH
Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

(*Received 8 December 1992, Accepted 21 March 1993*)

Young-of-the-year, predator-naive fathead minnows, *Pimephales promelas*, from a pike-sympatric population did not respond to chemical stimuli from northern pike, *Esox lucius*, while wild-caught fish of the same age and size did. These results suggest that chemical predator recognition is a result of previous experience and not genetic factors. Wild young-of-the-year minnows responded to pike odour with a response intensity that was similar to that of older fish, demonstrating that the ability to recognize predators is learned within the first year. The intensity of response of wild minnows which had been maintained in a predator free environment for 1 year was similar to that of recently caught minnows of the same age, suggesting that reinforcement was not required for predator recognition to be retained. Naive minnows that were exposed simultaneously to chemical stimuli from pike (a neutral stimulus) and minnow alarm substance exhibited a fright response upon subsequent exposure to the pike stimulus alone. Predator-naive minnows exposed simultaneously to chemical stimuli from pike and glass-distilled water did not exhibit a fright response to the pike stimulus alone. These results demonstrate that fathead minnows can acquire predator recognition through releaser-induced recognition learning, thus confirming a known mechanism through which alarm substance may benefit the receivers of an alarm signal.

Key words: predator recognition; alarm substance; Schreckstoff; releaser-induced recognition learning; fathead minnow; *Pimephales promelas*.

I. INTRODUCTION

THE ROLE OF EXPERIENCE IN PREDATOR RECOGNITION

It is somewhat difficult to differentiate the specific role of experience from that of genetic factors in the development of behaviour patterns (Huntingford, 1986). Several studies of predator avoidance (e.g. Seghers, 1974; Giles, 1984; Magurran, 1990*a*) address this issue with the view that if animals raised without exposure to predators exhibit the same predator evasion behaviour as is found in wild populations, then there is good evidence that the behaviour is inherited (Magurran, 1990*a*). By quantifying population differences between five predator-naive laboratory stocks of Trinidadian guppies, *Poecilia reticulata* Peters, Seghers (1974) demonstrated that differences in schooling tendency are retained in fish that have been bred in a predator-free environment for three or four generations. These results suggest that schooling, a known anti-predator behaviour (Magurran, 1990*b*), is controlled by genetic factors. Similarly, the overhead fright response of three-spined stickleback, *Gasterosteus aculeatus* L., appears to be under genetic control, as experience with predators is not a requisite for its expression (Giles, 1984). Magurran (1990*a*) demonstrated that population differences in anti-predator behaviour in European minnows, *Phoxinus phoxinus* (L.), are inherited. Furthermore, in European minnows anti-predator behaviour is modified by early experience with predators (Magurran, 1990*a*). Dill (1974) showed similarly that anti-predator behaviour in zebra danios, *Brachydanio rerio* (Hamilton-Buchanan), is modified by experience.

The above studies demonstrating inheritance of anti-predator responses have been based on visual stimuli. To identify the role of genetic factors, or alternatively experience, in the ability of fathead minnows, *Pimephales promelas* Rafinesque, to recognize chemical stimuli from pike, we compared the reaction of predator-naive minnows and wild-caught minnows. In our study we obtained predator-naive fathead minnows by raising minnows from eggs taken from a lake which contains northern pike, *Esox lucius* L. Adult minnows from this population recognize pike on the basis of either visual or olfactory cues (Mathis *et al.*, 1993; Chivers & Smith, 1993). To control for possible age/size effects, our experiment compared young-of-the-year predator-naive minnows with wild minnows of the same age and size. Our study also examined whether the responses of young-of-the-year wild-caught minnows exposed to chemical stimuli from pike differed from those of older wild minnows that we collected at the same time. Regardless of whether behaviour is inherited, learned, or some combination of the two, maintaining animals away from their natural environment for extended periods may, at least potentially, cause qualitative or quantitative differences in components of the animal's behavioural repertoire (Martin & Bateson, 1986). In our study, we tested for such an effect by quantifying the response of wild-caught fathead minnows to chemical stimuli from pike after the minnows had been maintained in a predator free environment for 1 year.

THE ROLE OF ALARM SUBSTANCE IN ACQUIRED PREDATOR RECOGNITION

Members of the superorder Ostariophysi possess an alarm substance, or Schreckstoff, within distinctive epidermal club cells (review: Smith, 1992). 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 ostariophysans which then perform a species-specific fright reaction.

Göz (1941) was the first to demonstrate that AS can be used to condition a fright response to formerly neutral stimuli. Blinded European minnows, which initially did not show a fright reaction to chemical stimuli from pike, would exhibit a fright reaction to such chemical stimuli after the pike attacked conspecifics in their presence. Göz suggested that AS released during the attack conditioned the blind minnows to exhibit a fright reaction upon subsequent exposures to the chemical stimuli from the pike. Magurran (1989) confirmed these findings. In her study, laboratory-reared European minnows, originating from a population that was allopatric from pike (A. Magurran, pers. comm.), were conditioned to respond to chemical stimuli from pike or tilapia, *Tilapia mariae* Boulenger. Chivers & Smith (1994) demonstrated that AS is also effective at conditioning predator-naive fathead minnows from a population allopatric from pike to the sight of pike and goldfish, *Carassius auratus* (L.).

This type of learning is known as releaser-induced recognition learning (Suboski, 1990, 1992*a*, *b*). Essentially, AS induces a transfer of control over the release of a fright response to a neutral stimulus.

We examined whether predator-naive minnows originating from a population in a lake containing pike, which we demonstrated do not respond to pike odour, can acquire predator recognition through releaser-induced recognition learning. We tested whether AS could induce a transfer of control over the release of a fright response to previously neutral chemical stimuli (i.e. pike odour). Acquisition of predator recognition through such a mechanism would have important implications for the receivers of an alarm signal.

II. MATERIALS AND METHODS

FISH COLLECTION AND MAINTENANCE

Northern pike were collected from Eagle Creek, a tributary of the North Saskatchewan River in south-central Saskatchewan, Canada, maintained at approximately 15° C on a 14 : 10 light : dark cycle in 150 l holding tanks, and fed one or two fathead minnows every 5 days.

In May 1992, artificial spawning surfaces (broken flower pots) were placed in Pike Lake, an oxbow lake of the South Saskatchewan River in south-central Saskatchewan. In June, the spawning surfaces with their attached fathead minnow eggs were transferred to separate aquaria in the laboratory, where the eggs were incubated at approximately 20° C. The eggs hatched in June and July, and the minnows were fed Tetramin commercial fish food supplemented with live food [including brine shrimp, *Artemia franciscana* (Kellogg), *Daphnia* spp. and other zooplankton]. These individuals are hereafter referred to as the ' predator-naive (age 0+) ' treatment group.

In September 1991, fathead minnows were collected from Pike Lake and maintained in large (approximately 18 000 l) outdoor pools at temperatures ranging from approximately 15° C (fall months) to 4° C (winter months). In the spring of 1992, the minnows were transferred to a 150 l holding tank in the laboratory, where they were maintained at approximately 20° C on a 14 : 10 L : D cycle and were fed daily with Tetramin flakes. These individuals were maintained in a predator-free environment for 1 yr prior to the beginning of this experiment and are hereafter referred to as the ' wild laboratory-held (age 1+) ' treatment group.

In September of 1992, fathead minnows were collected from Pike Lake and subsequently kept at approximately 15° C on a 14 : 10 L : D cycle in a 300 l holding tank and were fed daily with Tetramin flakes. Minnows in this group were categorized as either ' wild recently-caught (age 0+) ' or ' wild recently-caught (age 1+) '.

PIKE STIMULUS PREPARATION

Prior to the stimulus collection, two pike (F.L.=21 and 22 cm) were fed approximately equal volumes (range=4·0–5·0 ml, measured by volumetric displacement in water) of swordtails, *Xiphophorous helleri* Heckel, for each of three feedings (i.e. once every 5 days). A swordtail diet was used to eliminate secondary stimuli of fathead minnows from the pike's diet (Mathis & Smith, 1993). Approximately 1 h after their last feeding, the pike were rinsed with dechlorinated tap water to remove any swordtail residue from the pike's skin and then were returned to an identical holding tank that contained clean water. After approximately 16 h they were placed in separate clear plastic stimulus collection chambers (26 × 8 × 8 cm) that each contained 1200 ml of dechlorinated tap water, and were aerated but not filtered. This 16 h delay ensured that the pike did not regurgitate their stomach contents (A. Mathis, pers. comm.). After 3 days the pike were removed from the stimulus collection chambers and the pike stimulus water from the two collection chambers was combined before being pipetted into either

5 ml polypropylene containers or 20 ml plastic bags and frozen at approximately −20° C.

ALARM SUBSTANCE STIMULUS PREPARATION

Alarm substance stimulus (AS) was prepared from 15 female fathead minnows ($\bar{X}$ fork length ± 1 S.D. = 4·9 ± 0·35 cm). After killing the donors by a blow to the head, a skin fillet was removed from both sides of each fish (total area of skin collected = approximately 20 cm^2). Immediately upon removal, the skin samples were placed together in 100 ml of chilled glass-distilled water and homogenized with a polytron homogenizer. The homogenate was then filtered through glass wool and diluted with 300 ml of glass-distilled water before freezing it in separate 5 ml polypropylene containers. As a control stimulus separate 5 ml units of glass-distilled water were also frozen in polypropylene containers.

EXPERIMENT 1—THE ROLE OF EXPERIENCE IN PREDATOR RECOGNITION

In September and October 1992, the reaction of minnows from four treatment conditions to chemical stimuli from pike was compared: (1) predator-naive minnows (age 0+), (2) wild recently-caught minnows (age 0+), (3) wild recently-caught minnows (age 1+), and (4) wild laboratory-held minnows (age 1+). Minnows were placed individually into a Plexiglas acclimation tank (45 × 45 × 20 cm), to which clean water was supplied constantly at a rate of approximately 250 ml min^{-1}, maintaining a constant water depth of 4 to 5 cm. Feeding and lighting conditions were as described earlier. After 2 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^{-1}. Testing occurred the following day (i.e. after the minnows had remained in the test tanks for 24 h).

Each test tank was surrounded by an Opto-Varimex Aqua tracking meter (Columbus Instruments), described in detail by Lemly & Smith (1986, 1987), which lays down a grid of light beams across the tank. An interfaced microcomputer detected changes in the fish's movement by determining, at intervals of 0·125 s, the number and location of light beams that were broken between the light source and photocell. Our system differed from that of Lemly & Smith (1986, 1987) only in that outflowing water was discarded rather than recirculated. Two measures of activity were quantified by the computer: (1) total distance travelled (cm) and (2) number of stereotypic movements (i.e. activity in which a fish breaks light beams without moving outside of one grid square). Exposure of solitary fathead minnows to AS in this apparatus has led Lawrence & Smith (1989) to interpret a decrease in distance travelled and number of stereotypic movements as a fright reaction. A reduction in activity may result in the fish being less conspicuous to visually hunting predators.

An observer in an adjacent room utilized video equipment to view the experimental tanks without disturbing the minnows and injected the stimulus water into inflowing water lines that passed through the observation room before entering the test tanks. Experimental observations were conducted between 07.30 and 11.30 hours. Each trial lasted 16 min, with 5·0 ml of the pike stimulus being injected at the end of 8 min. After testing, scale samples were removed from each fish and aged as 0+ if no annuli had formed and 1+ if one or more annuli had formed.

The responses of 20 'predator-naive (age 0+)' minnows ($\bar{X}$ F.L. ± 1 S.D. = 4·8 ± 0·30 cm) from five different broods (i.e. four from each brood) were compared with 20 'wild recently-caught (age 0+)' minnows ($\bar{X}$ F.L. ± 1 S.D. = 4·6 ± 0·36 cm) to determine if the recognition of pike odour was a result of previous experience with pike. The responses of the 20 'wild recently-caught (age 0+)' minnows were compared with those of 12 'wild recently-caught (age 1+)' minnows ($\bar{X}$ F.L. ± 1 S.D. = 4·7 ± 0·38 cm) to determine if recognition of pike odour differed with age. Finally, the responses of the 12 'wild recently-caught (age 1+)' minnows were compared with those of 20 'laboratory-held (age 1+)' minnows ($\bar{X}$ F.L. ± 1 S.D. = 4·9 ± 0·44 cm) to determine if reinforcement was required to maintain the ability to recognize pike odour. Minnows from each of the four test groups were tested only once. A Kruskal–Wallis one-way analysis of variance with non-parametric multiple comparisons (Siegel & Castellan, 1988) was used to compare the significance of the minnows' response in terms of distance travelled and number of stereotypic movements in the above analyses. The minnow's response to the stimulus was defined as post-stimulus activity minus pre-stimulus activity and was assessed using two-tailed statistical tests with $a=0{\cdot}05$.

EXPERIMENT 2—CONDITIONING OF A FRIGHT REACTION TO CHEMICAL STIMULI

Day 1: conditioning trials, initial response to AS

Experimental observation tanks (37 l : 50 × 30 × 25 cm) were filled with dechlorinated tap water and aerated with single airstones located at the back of each tank. Each tank contained a centrally located shelter consisting of a ceramic tile (9·8 × 20·0 cm) mounted on three cylindrical glass legs (5·5 cm long). A plastic tube, for introduction of chemical stimuli was attached to the airline.

Thirty 'predator-naive' minnows ($\bar{X}$ F.L. ± 1 S.D. = 4·8 ± 0·37) (i.e. six fish from five different broods) were selected arbitrarily and placed individually into the experimental tanks. Half of the minnows were assigned randomly to be conditioned with AS+pike odour while the other half were conditioned with water+pike odour. Observations were conducted at a mean water temperature of 20° C (range 18–22° C) on a 14 : 10 L : D cycle, after the minnows had remained in the observation tanks undisturbed, except for daily feedings, for 3 days.

As a standard testing protocol, the amount of time that a minnow spent beneath the shelter was recorded for 8 min prior to and 8 min after introduction of the chemical stimulus (i.e. either 20 ml of pike stimulus+5·0 ml of AS or 20 ml of pike stimulus+5·0 ml of control water). During the post-stimulus period the presence or absence of two additional behaviour patterns which have been interpreted as components of a fright reaction in fathead minnows (e.g. Lawrence & Smith, 1989; Mathis *et al.*, 1993) were also recorded. 'Dashing' was a sudden burst of very rapid, apparently disoriented swimming. 'Freezing' occurred when a minnow sank to the substrate and remained immobile for a minimum of 30 sec.

A Wilcoxon–Mann–Whitney test (Siegel & Castellan, 1988) was used to compare mean change in time spent under cover by AS- and water-conditioned minnows and a Fisher Exact probability test (Siegel & Castellan, 1988) was used to compare the frequency of dashing and freezing by AS- and water-conditioned minnows.

Day 5: conditioned responses to pike odour?

Mathis & Smith (1993) have demonstrated that, for fathead minnows, a fright response may be elicited by a neutral chemical stimulus (i.e. water) that is presented in the same manner as a previous fright stimulus. In their study, moving the test fish to a different experimental situation eliminated the fright response to the neutral stimulus. To control for possible conditioning to test conditions in our study, the experimental situation in which the fish were tested was also changed. The day after the initial conditioning, the minnows from the 37 l aquaria were transferred to the individual Plexiglas acclimation tanks (45 × 45 × 20 cm) used in the first experiment. After 2 days, the minnows were transferred into the Opto-Varimex testing tanks and the individuals were tested the following day. Four days elapsed between initial conditioning and subsequent testing. Fish were fed daily as before and were kept on a 14 : 10 L : D cycle.

Between 07.30 and 11.30 hours, each minnow was tested with 5·0 ml of control water. This treatment controlled for possible differences in the response of water- and AS-conditioned minnows to a neutral chemical stimulus. Subsequent observations of these minnows were completed later the same day between 10.30 and 16.30 hours using 5·0 ml of the pike stimulus. Testing protocol was as in experiment 1.

Within each conditioning group (i.e. the AS- and water-conditioned groups), a Wilcoxon-signed rank test (Siegel & Castellan, 1988) was used to compare the minnows'

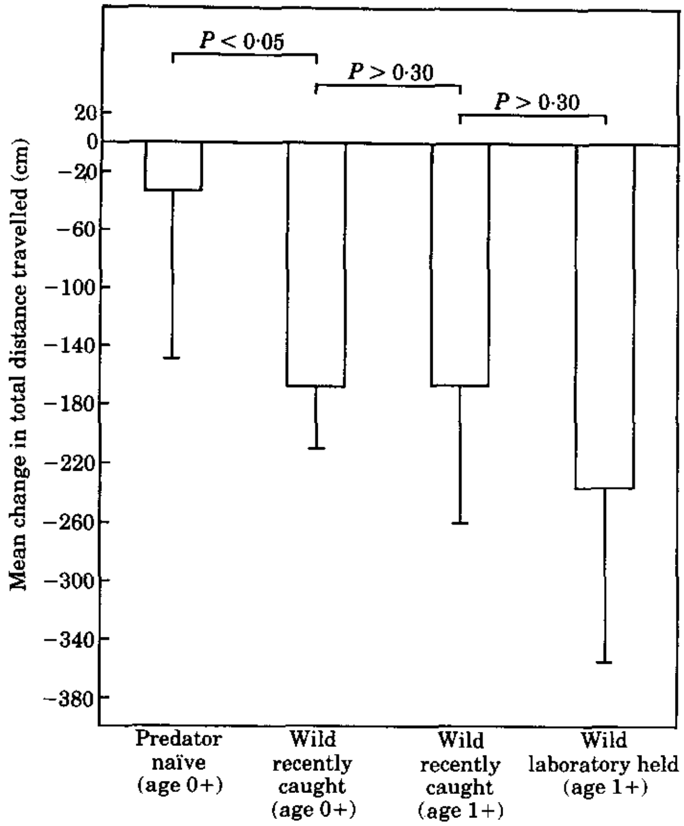

FIG. 1. Mean (± 1 S.E.) change in total distance travelled (cm) by 'predator-naive (age 0+)', 'wild recently-caught (age 0+)', 'wild recently-caught (age 1+)', and 'wild laboratory-held (age 1+)' minnows following exposure to chemical stimuli from northern pike.

response to control water *v*. pike stimulus. A Wilcoxon–Mann–Whitney test (Siegel & Castellan, 1988) was used between conditioning groups to compare the minnows' response to control water and pike stimulus.

III. RESULTS

EXPERIMENT 1

There was an overall significant difference between the treatment groups in both distance travelled (Kruskal–Wallis=13·48, $P<0·01$) and number of stereotypic movements (Kruskal–Wallis=13·60, $P<0·01$). The response of the 'predator-naive (age 0+)' minnows was significantly different from that of the 'wild recently-caught (age 0+)' minnows in both distance travelled ($P<0·05$) (Fig. 1) and number of stereotypic movements ($P<0·05$) (Fig. 2), indicating that experience was required for fathead minnows to recognize pike odour. The response of 'wild recently-caught (age 0+)' minnows was not significantly different from that of 'wild recently-caught (age 1+)' minnows in either distance travelled ($P>0·30$) (Fig. 1), or number of stereotypic movements ($P>0·30$) (Fig. 2), demonstrating that minnows acquired predator recognition within their first year. The response of 'wild recently-caught (age 1+)' minnows also did not

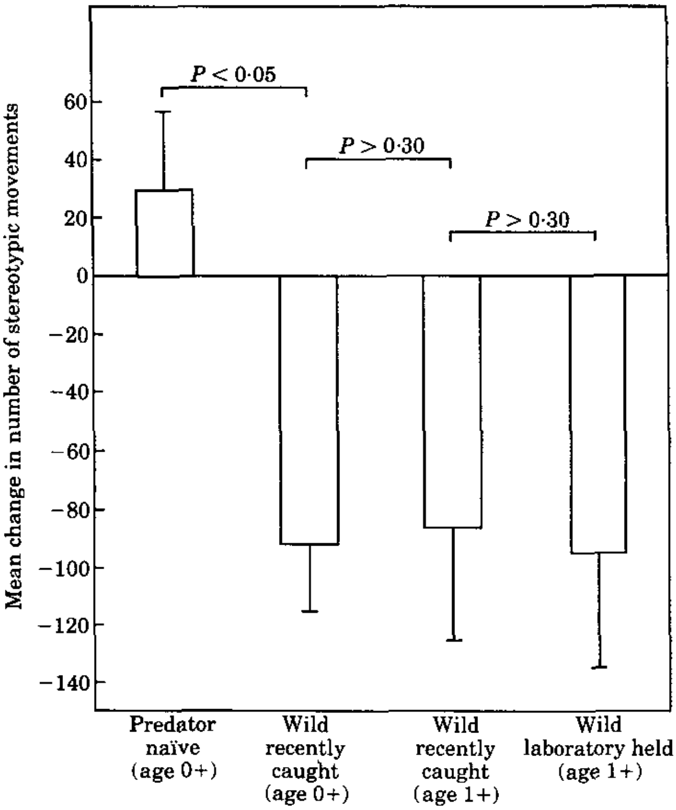

FIG. 2. Mean (± 1 S.E.) change in number of stereotypic movements by 'predator-naive (age 0+)', 'wild recently-caught (age 0+)', 'wild recently-caught (age 1+)', and 'wild laboratory-held (age 1+)' minnows following exposure to chemical stimuli from northern pike.

differ significantly from that of 'laboratory-held (age 1+)' minnows in either distance travelled ($P>0{\cdot}30$) or number of stereotypic movements ($P>0{\cdot}30$), indicating that reinforcement was not required to maintain the ability to recognize pike odour.

EXPERIMENT 2

Day 1: conditioning trials, initial response to AS

In direct comparisons of AS- and water-conditioned minnows, AS-conditioned minnows exhibited a significantly greater increase in time spent under cover ($Wx=151$, $P<0{\cdot}001$) (Fig. 3) and a significantly greater frequency of dashing and freezing (Table I) than did water-conditioned minnows. These results demonstrated that minnows exposed to AS+pike exhibited a fright response, while those exposed to water+pike did not.

Day 5: conditioned responses to pike odour

The responses of water-conditioned minnows to pike stimulus were not significantly different from the responses of the same individuals to control water in either distance travelled ($T=66$, $P=0{\cdot}762$) (Fig. 4) or number of stereotypic movements ($T=76$, $P=0{\cdot}389$) (Fig. 5). In contrast, AS-conditioned minnows

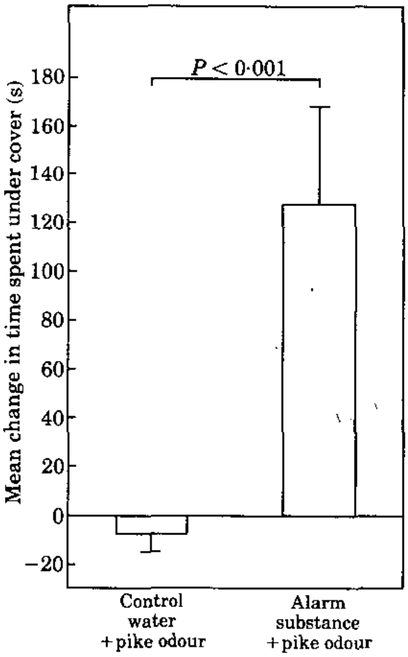

FIG. 3. Mean (± 1 S.E.) change in time spent under cover (sec) during initial conditioning trials [i.e. by minnows exposed to alarm substance (AS)+pike or water+pike].

TABLE I. Number of fathead minnows exhibiting dashing and freezing behaviour during initial conditioning trials

Trial	Yes	No	P
1. Dashing			
AS+pike	10	5	<0·001
Water+pike	1	14	
2. Freezing			
AS+pike	6	9	=0·040
Water+pike	1	14	

AS, Alarm substance.

exposed to pike stimulus responded with a significantly greater decrease in distance travelled (T=115, P<0·001) (Fig. 4) and number of stereotypic movements (T=114, P<0·001) (Fig. 5) in comparison with their responses to distilled water.

The response to distilled water was not significantly different for minnows conditioned with AS *v.* those conditioned with water in distance travelled Wx=231, P=0·968) (Fig. 4) or number of stereotypic movements (Wx=218·5, P=0·575) (Fig. 5). In contrast, during exposure to pike, AS-conditioned minnows exhibited a significantly greater decrease in distance travelled (Wx=161, P=0·003) (Fig. 4) and number of stereotypic movements (Wx=169·5, P<0·010) (Fig. 5), than water-conditioned minnows.

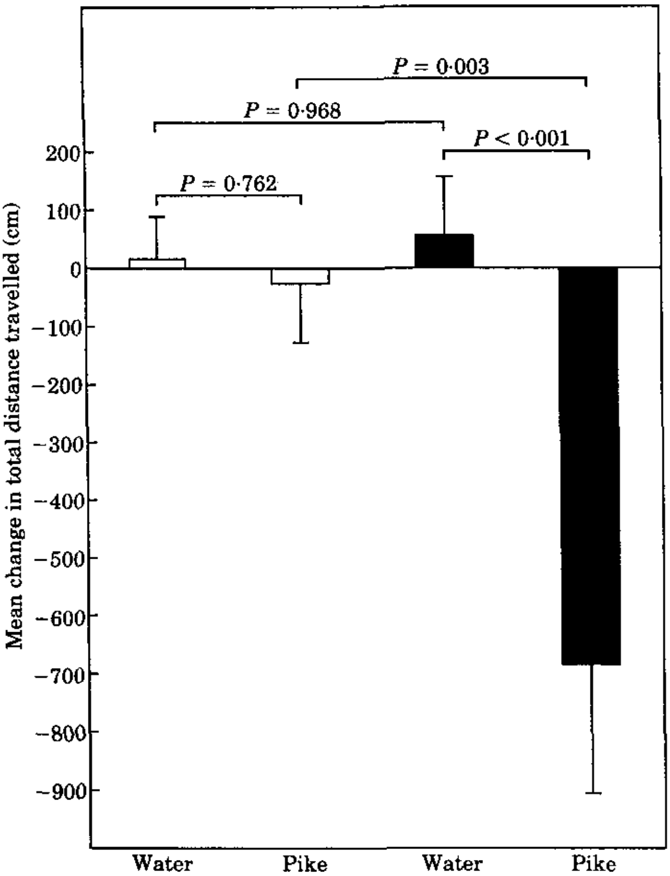

FIG. 4. Mean (± 1 S.E.) change in total distance travelled (cm) by alarm substance (■) and water-conditioned (□) minnows following exposure to glass-distilled water and chemical stimuli from pike.

IV. DISCUSSION

The results of this study demonstrate that young-of-the-year predator-naive fathead minnows from a pike-sympatric population do not recognize chemical stimuli from pike, while wild-caught minnows of the same age and size do, indicating that experience is more important than genetic factors in mediating olfactory recognition of pike. These results contrast with findings from guppies (Breden *et al.*, 1987), three-spined stickleback (Giles, 1984), European minnows (Magurran, 1990*a*), coho salmon, *Oncorhynchus kisutch* (Walbaum) (Patten, 1977), and Atlantic salmon, *Salmo salar* (L.) (Jakobsson & Järvi, 1976) which demonstrated that anti-predator responses occur independently of experience, even though the form of the response can be readily modified by experience with predators. An important difference between our study and other studies of the role of inheritance in predator recognition is that we utilized only olfactory stimuli. Other studies have demonstrated inheritance of anti-predator responses by chasing the prey fish with a net (Breden *et al.*, 1987), by exposing them to a model fish (Magurran, 1990*a*) or a model bird (Giles, 1984) predator, or by

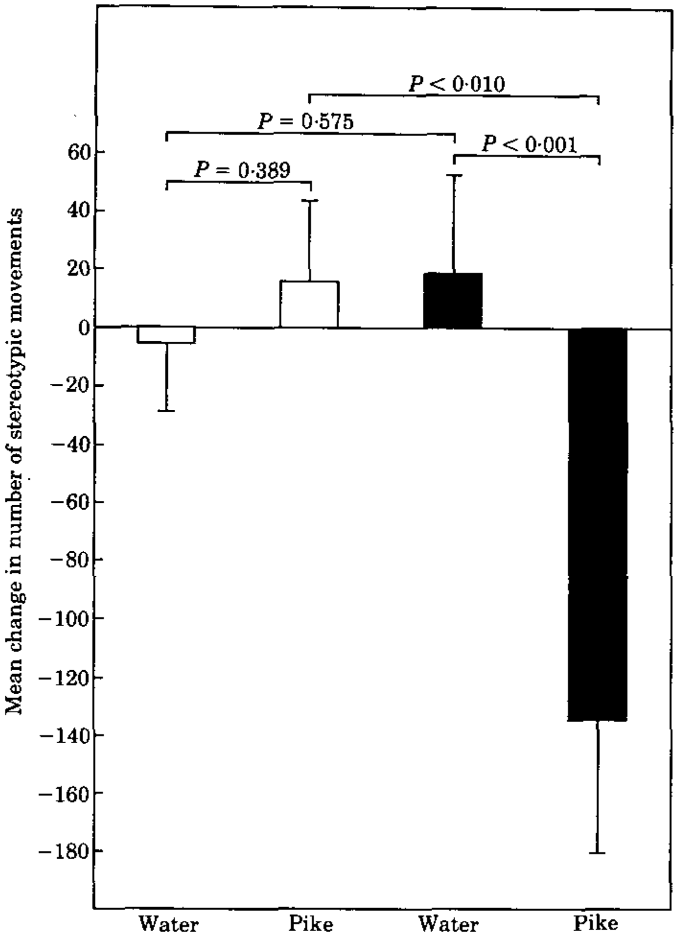

FIG. 5. Mean (± 1 S.E.) change in number of stereotypic movements by alarm substance (■) and water-conditioned (□) minnows following exposure to glass-distilled water and chemical stimuli from pike.

exposing them to a live fish predator (Jakobsson & Järvi, 1976; Patten, 1977). Unlike our study, which utilized only chemical stimuli, visual stimuli were presented in these other studies. Although predator-naive fathead minnows do not inherit the ability to recognize pike chemically, genetic factors may be involved in visual recognition of pike.

Giles (1984) showed that the overhead fright response of three-spined stickleback heightens with age and/or body size. Our study compared the response of young-of-the-year (i.e. age 0+) wild-caught minnows with older wild-caught minnows (i.e. age 1+). Although the fish differed in age, both groups of minnows were of approximately equal size and, presumably, equal development. Our results do not demonstrate a quantitative difference in the intensity of the anti-predator behaviour based on age.

The similarity of the responses to pike odour by laboratory-held minnows and wild recently-caught minnows indicates that, in the absence of reinforcement for 1 yr, minnows retain the ability to recognize chemical stimuli from pike. This length of time is substantial considering the lifespan of a fathead minnow is rarely over 2 or 3 yr (Scott & Crossman, 1979). Little is known concerning the retention of learned responses in fishes. Hasler & Wisby (1951) demonstrated that bluntnose minnows, *Pimephales notatus* Rafinesque, can retain a conditioned attraction or avoidance response to stream odours for 6 weeks, even with weekly extinction testing. Fathead minnows retain a conditioned visual avoidance of pike or goldfish for at least 2 months (Chivers & Smith, 1994). As well, paradise fish, *Macropodus opercularis* (L.), can recognize heterospecific fishes (goldfish) for a time period of at least 3 months (Csányi *et al.*, 1989).

In this study a single exposure to AS conditioned predator-naive fathead minnows (which originated from a pike-sympatric population) to respond to previously neutral chemical stimuli from pike with an appropriate anti-predator response. Previous conditioning studies, which have utilized either chemical (Magurran, 1989) or visual stimuli (Chivers & Smith, 1994) have reported similar findings even though the source of the minnows in the previous studies have been from populations that were allopatric from the predators. Our study indicates that fathead minnows learn to recognize pike as predators through releaser-induced recognition learning. Such a mechanism for acquiring predator recognition also occurs in a variety of other vertebrates and invertebrates (Suboski, 1990, 1992*a*, *b*).

By demonstrating that AS can condition a fright response to previously neutral stimuli, our study confirms a known mechanism through which AS may benefit the receivers of an alarm signal. A fright response given during subsequent encounters with the predator has important implications for the survival of the individual. The individual's fright response may also act to warn other prey in the vicinity (Verheijen, 1956), and thereby increase the inclusive fitness of the individual through the process of warning close relatives (Sherman, 1977). Warning other conspecifics may also 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 response of the individual may act to inform the predator that its presence has been detected and that an attack is unlikely to succeed (Högstedt, 1983).

We thank Dr Alicia Mathis and Jeff Matity for assisting in the collection of fish and in preparation of the alarm substance stimulus. Dr Mathis provided helpful suggestions concerning experimental design and preparation of the manuscript. Dr Anne Magurran, Dr Ian Winfield and an anonymous reviewer provided suggestions on an earlier version of the manuscript. This study was supported by the Natural Sciences and Engineering Research Council of Canada and the University of Saskatchewan.

References

Breden, F., Scott, M. & Michel, E. (1987). Genetic differentiation for anti-predator behaviour in the Trinidad guppy, *Poecilia reticulata*. *Animal Behaviour* **35,** 618–620.

Chivers, D. P. & Smith, R. J. F. (1993). The role of olfaction in chemosensory-based predator recognition in the fathead minnow, *Pimephales promelas*. *Journal of Chemical Ecology* **19,** 623–633.

Chivers, D. P. & Smith, R. J. F. (1994). Fathead minnows (*Pimephales promelas*) acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Animal Behaviour* in press.

Csányi, V., Csizmadia, G. & Miklosi, A. (1989). Long-term memory and recognition of another species in the paradise fish. *Animal Behaviour* **37**, 908–911.

Dill, L. M. (1974). The escape response of the zebra danio (*Brachydanio rerio*): the effects of experience. *Animal Behaviour* **22**, 723–730.

Giles, N. (1984). Development of the overhead fright response in wild and predator-naive three-spined sticklebacks, *Gasterosteus aculeatus* L. *Animal Behaviour* **32**, 276–279.

Göz, H. (1941). Über den Art-und Individualgeruch bei Fischen. *Zeitschrift für Vergleichende Physiologie* **29**, 1–45.

Hasler, A. D. & Wisby, W. J. (1951). Discrimination of stream odors by fishes and its relation to parent stream behavior. *American Naturalist* **85**, 223–238.

Högstedt, G. (1983). Adaptation unto death: function of fear screams. *American Naturalist* **121**, 562–570.

Huntingford, F. A. (1986). The development of behaviour in fishes. In *The Behaviour of Teleost Fishes* (Pitcher, T. J., ed.), pp. 47–68. London: Croom Helm.

Jakobsson, S. & Järvi, T. (1976). Anti-predator behaviour of two-year-old hatchery-reared Atlantic salmon, *Salmo salar*, and a description of the predatory behaviour of burbot, *Lota lota*. *Zoologisk Revy* **38**, 57–70.

Lawrence, B. J. & Smith, R. J. F. (1989). Behavioural response of solitary fathead minnows, *Pimephales promelas*, to alarm substance. *Journal of Chemical Ecology* **15**, 209–219.

Lemly, A. D. & Smith, R. J. F. (1986). A behavioural assay for assessing effects of pollutants of fish chemoreception. *Ecotoxicology and Environmental Safety* **11**, 210–218.

Lemly, A. D. & Smith, R. J. F. (1987). Effects of chronic exposure to acidified water on chemoreception of feeding stimuli in fathead minnows (*Pimephales promelas*): mechanisms and ecological implications. *Environmental Toxicology and Chemistry* **6**, 225–258.

Magurran, A. E. (1989). Acquired recognition of predator odour in the European minnow (*Phoxinus phoxinus*). *Ethology* **82**, 216–223.

Magurran, A. E. (1990*a*). The inheritance and development of minnow anti-predator behaviour. *Animal Behaviour* **39**, 834–842.

Magurran, A. E. (1990*b*). The adaptive significance of schooling as an anti-predator defence in fish. *Annales Zoologici Fennici* **27**, 51–66.

Martin, P. & Bateson, P. (1986). *Measuring Behaviour: An Introductory Guide.* Cambridge: Cambridge University Press.

Mathis, A., Chivers, D. P. & Smith, R. J. F. (1993). Population differences in responses of fathead minnows (*Pimephales promelas*) to visual and chemical stimuli from predators. *Ethology* **93**, 31–40.

Mathis, A. & Smith, R. J. F. (1994). Fathead minnows (*Pimephales promelas*) learn to recognize pike (*Esox lucius*) as predators on the basis of chemical stimuli from minnows in the pike's diet. *Animal Behaviour* **46**, 645–656.

Patten, B. G. (1977). Body size and learned avoidance factors affecting predation on coho salmon, *Onchorhyncus kisutch*, fry by torrent sculpin, *Cottus rhotheus*. *U.S. National Marine Fishery Service, Fishery Bulletin* **75**, 457–459.

Scott, W. B. & Crossman, E. J. (1979). *Freshwater Fishes of Canada.* Fisheries Research Board of Canada, Bulletin 184.

Seghers, B. H. (1974). Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary approach to predation. *Evolution* **28**, 486–489.

Siegel, S. & Castellan, N. J. (1988). *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill.

Sherman, P. W. (1977). Nepotism and the evolution of alarm calls. *Science* **197**, 1246–1253.

Smith, R. J. F. (1992). Alarm signals in fishes. *Reviews in Fish Biology and Fisheries* **2,** 33–63.

Suboski, M. D. (1990). Releaser-induced recognition learning. *Psychological Review* **97,** 271–284.

Suboski, M. D. (1992*a*). Releaser-induced recognition learning by amphibians and reptiles. *Animal Learning and Behaviour* **20,** 63–82.

Suboski, M. D. (1992*b*). Releaser-induced recognition learning by gastropod molluscs. *Behavioural Processes* **27,** 1–26.

Trivers, R. L. (1971). The evolution of reciprocal altruism. *Quarterly Review of Biology* **46,** 35–57.

Verheijen, F. J. (1956). Transmission of a fright reaction amongst a school of fish and the underlying sensory mechanisms. *Experientia* **12,** 202–204.