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The role of experience in risk assessment: Avoidance of areas chemically labelled with fathead minnow alarm pheromone by conspecifics and heterospecifics¹

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Abstract: In two field experiments, we investigated risk avoidance behaviour by individual fathead minnows (*Pimephales promelas* Rafinesque) and brook stickleback (*Culaea inconstans* Kirtland) in response to release of fathead minnow alarm pheromone. There was an initial decrease in the number of fish caught in areas labelled with alarm pheromone (risky areas) relative to areas labelled with distilled water (safe areas), after the source of the pheromone was removed. Numbers of minnows or stickleback caught in risky and safe areas were no longer different 4 hours after the pheromone source was removed. For minnows, there was no significant difference in the immigration rates of individuals into risky and safe areas from neighbouring locations. For minnows, and probably stickleback, new individuals, naive to the association of an area with alarm pheromone, immigrated into risky areas before the return of experienced fish. Fish present at the time of pheromone release did not return for 7 or 8 hours after the source of the pheromone was gone. This raises the possibility that the chief beneficiaries of chemical alarm signals may be only those individuals present at the time of pheromone release.

Keywords: alarm pheromone, Schreckstoff, minnow, stickleback, predator-prey, area avoidance.

Résumé: Nous avons observé, lors de deux expériences sur le terrain, le comportement des têtes-de-boule (*Pimephales promelas* Rafinesque) et des épinoches à cinq épines (*Culaea inconstans* Kirtland) en réponse à l'émission de phéromones d'alarme par les têtes-de-boule. Après l'ajout de phéromones dans l'eau, on assiste à une diminution du nombre de poissons capturés. Les eaux avec phéromones représentent donc des eaux à risque pour les poissons. Dans les eaux témoins, caractérisées uniquement par l'ajout d'eau distillée, aucune baisse n'a été enregistrée dans le nombre de captures. Elles constituent donc pour les poissons des eaux sûres. Quatre heures après le retrait de la source de phéromone, le nombre de têtes-de-boule ou d'épinoches ne diffère pas entre les eaux où il y a eu ajout de phéromones et les eaux témoins. Les têtes-de-boule en provenance de milieux voisins pénètrent autant dans les eaux à risque que dans les eaux sûres. Les têtes-de-boule (et probablement aussi les épinoches) en provenance de milieux voisins, ne peuvent connaître l'existence de phéromones d'alarme dans une zone donnée et pénètrent dans les eaux à risque avant le retour de poissons en ayant déjà fait l'expérience. Les poissons qui étaient présents lors de la libération des phéromones ne sont pas retournés dans les sites pendant les 7 à 8 heures suivant le retrait de la source de phéromones. Cela suggère que les principaux bénéficiaires des signaux d'alarme d'origine chimique sont peut être seulement ceux qui étaient présents dans le milieu au moment de l'émission des phéromones.

Mots-clés: phéromone d'alarme, Schreckstoff, tête-de-boule, épinouche, prédateur-proie, évitement de site.

Introduction

In 1938, Karl Von Frisch reported "Schreckstoff", or alarm substance, located in the skin of the European minnow *Phoxinus phoxinus* L. It is now known that specialized epidermal cells which contain alarm substance are characteristic of the superorder Ostariophysi, approximately 72% of all freshwater fishes (Nelson, 1984; for reviews see Pfeiffer, 1977; 1982; Smith, 1992). Alarm substance can only be released if these epidermal cells are ruptured. Thus, alarm substance serves as a pheromone by communicating predation threat (Pfeiffer, 1963). Individuals that detect alarm pheromone exhibit a fright reaction consisting of anti-predator behaviour, such as increased shoaling, seeking refuge, dashing or 'skittering', freezing and area avoidance (Heczko & Seghers, 1981; Magurran, 1986; Fricke, 1987; Mathis & Smith, 1992; 1993a; Chivers & Smith, 1994a, b, in press a; Wisenden, Chivers & Smith, 1994). These

behaviours reduce the probability of being captured by a predator (Mathis & Smith, 1993b).

Cross-species reactions to alarm pheromones occur in a variety of fish species (Pfeiffer, 1963; Smith, 1982; Smith, Lawrence & Smith, 1991; Mathis & Smith, 1993a; Chivers & Smith, 1994a) suggesting that some fish associate heterospecific alarm pheromone with predation risk. Brook stickleback (*Culaea inconstans* Kirtland) are small, non-ostariophysan fish which share habitat and predators with fathead minnows (*Pimephales promelas* Rafinesque) (Scott & Crossman, 1973) and respond to the alarm pheromone of fathead minnows with anti-predator behaviour including area avoidance (Mathis & Smith, 1993a; Wisenden, Chivers & Smith, 1994; Chivers, Brown & Smith, in press).

Avoidance of areas labelled with alarm pheromone presents some ecological problems. How long should these areas be avoided? Is information about predation risk com-

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municated to individuals that did not, themselves, encounter the pheromone? In two field experiments, we investigated the duration of area avoidance in response to alarm pheromone from the skin of fathead minnows. One experiment tested the avoidance response by conspecifics, the other tested the avoidance response by a sympatric heterospecific fish belonging to a different superorder, the brook stickleback.

Fish were trapped and marked at several locations. As soon as traps were removed from the water, a sponge saturated with alarm pheromone or control stimulus was placed at each trap site. Fish were marked by clipping a fin and immediately returned to the location of capture. Thus individuals marked and released in risky areas experienced pheromone release and had the opportunity to associate the location with predation risk. Areas labelled with distilled water controlled for area avoidance resulting from any effect of handling and marking. Sponges were removed after a standardized time interval and the traps were set again in the same locations, about an hour after the initial release of the fish. The traps were pulled again after two or three hours and the fish counted and marked fish recorded. The fish were immediately returned to the water.

This procedure provided an initial estimate of fish abundance in each location and a means of recognizing some of the fish that were present before pheromone release. We could then use these data to compare (1) the number of returning fish between the pheromone and control sites and (2) the rates of immigration into pheromone and control sites of fish marked in adjacent areas.

Information about predation risk associated with a certain area may be socially transmitted from experienced individuals (those that experienced the presence of alarm pheromone) to naive individuals that were elsewhere at the time of pheromone release (Chivers & Smith, in press b). In laboratory studies, cyprinid fright reactions induce nearby conspecifics and heterospecifics to adopt similar anti-predator behaviour (Verheijen, 1956; Magurran & Higham, 1988; Chivers & Smith, in press a; Krause, 1994) and result in transmission of predator recognition (Suboski *et al.*, 1990).

We use data from these experiments to look for evidence of information transfer by comparing rates of immigration of marked individuals into risky areas and safe areas. If the proportion of marked fish recaptured in post-stimulus samples remains lower in pheromone sites even after the total number of fish caught no longer differs between treatments, this would indicate that new fish immigrated into risky areas before the return of experienced fish. If predation risk is socially transmitted, we would expect the proportion of marked fish that move from the location where they were marked into risky areas, should be less than the proportion that move into safe areas.

Materials and methods

Male fathead minnows and brook stickleback defend nests in fixed territories from early to mid-summer. To prevent this behaviour from confounding the response to alarm pheromone, we collected data outside of the breeding season of both species when they are not territorial.

EXPERIMENT 1. AREA AVOIDANCE BY FATHEAD MINNOWS

To prepare the alarm pheromone stimulus, 8 adult fathead minnows (mean fork length \pm SE = 52.0 \pm 1.1 mm) were killed by a blow to the head. Skin fillets were removed from both sides of each fish for a total of 28.79 cm² of skin. We homogenized the skin in 100 mL of glass distilled water using a polytron homogenizer. We filtered the homogenate through glass wool and added distilled water for a total volume of 240 mL. Twelve cellulose sponges (5 cm \times 4 cm \times 2 cm) were infused with 20 mL of skin extract solution. Thus, each sponge contained an amount of alarm pheromone equivalent to that contained in 2.4 cm² of minnow skin. We estimated that this amount of skin is approximately the area of skin that may be damaged and release alarm pheromone during a predatory attack. The active space of alarm pheromone created by 2.4 cm² of minnow skin is approximately equivalent to a sphere with a radius of 3.2 m (Lawrence & Smith, 1989). Twelve additional sponges (5 cm \times 4 cm \times 2 cm) were infused with 20 mL of distilled water to serve as a control stimulus. Each sponge was attached to a #9 rubber stopper with a piece of stainless steel wire that supported the sponge about 12 cm above the stopper. All 24 sponges were chilled at 0°C until needed.

The study site, Lakeview Pond, is a man-made pond located in Saskatoon, Saskatchewan (52° 07' N, 106° 40' W). The fathead minnow was the only fish species caught during extensive trapping in the pond during the summer of 1994 (Mathis, Chivers & Smith in press, Chivers, Wisenden & Smith in press, this study). The experimental method used here is similar to that of Wisenden, Chivers & Smith (1994). On 3 August, 1994, we placed 24 Gee's Improved Minnow Traps (see Wisenden, Chivers & Smith, 1994 for description), separated by approximately 15 m, along the southern shore of Lakeview Pond, about 2 to 5 m from shore. Each trap was attached with a piece of rope to a labelled stake on shore to ensure that trap locations did not change between samples. Six traps (3 experimental and 3 control) were placed simultaneously in the pond every 15 minutes. After an average of 2.7 hours, we removed each set of 6 traps (3 experimental and 3 control traps) and immediately attached the rope from the stake to a sponge and placed the sponge (weighted with the rubber stopper) in the same location as each trap. We termed this the pre-stimulus sample. We recorded the number of minnows captured at each location. All minnows in the pre-stimulus sample were given one of 6 fin clips. Clip and sponge treatments were assigned in a regular pattern, alternating treatments for each consecutive trap location (Table I). To minimize any effect of damaged minnow skin on area avoidance, clipped fish from each trap location were retained in a separate pail until all fish were clipped. Then, clipped fish were transferred to the site of capture using a hand net. The water in the pail was discarded on shore. Any alarm pheromone released as a result of fin clipping would make our results conservative because it would serve to delay the return of control fish relative to experimental fish.

Sponges were left in place for an average of 2.6 hours (matched between treatments) and then removed from the water. We immediately reset the traps in the same locations. Two post-stimulus samples were taken from each trap location

TABLE I. Fin clips and sponge treatments assigned to consecutive trap locations for experiments 1 and 2

Trap locations	Fin clipped	Solution in sponge
Experiment 1: Brook stickleback in Marshy Creek		
1, 5, 9, 13, 17, 21	left pectoral	distilled water
2, 6, 10, 14, 18, 22	right pectoral	alarm pheromone
3, 7, 11, 15, 19, 23	caudal, upper lobe	distilled water
4, 8, 12, 16, 20, 24	caudal, lower lobe	alarm pheromone
Experiment 2: Fathead minnows in Lakeview Pond		
1, 7, 13, 19	caudal, upper lobe	distilled water
2, 8, 14, 20	caudal, lower lobe	alarm pheromone
3, 9, 15, 21	left pectoral	distilled water
4, 10, 16, 22	right pectoral	alarm pheromone
5, 11, 17, 23	left pelvic	distilled water
6, 12, 18, 24	right pelvic	alarm pheromone

before dusk. Mean duration of each post-stimulus sample was 3.6 and 3.3 hours. The total number of minnows captured and clips, if any, were noted. All captured fish were immediately released at the site of capture.

We used two statistical procedures. The first tested for the significance and duration of numerical changes in the number of fish caught. For each trap location, we calculated the difference in the number of minnows caught between the pre-stimulus sample and each of the post-stimulus samples. Using a Wilcoxon-Mann-Whitney test (Siegel & Castellan, 1988), we compared the change in the number of fish caught at locations previously labelled with alarm pheromone with the change in the number of fish caught at locations labelled with distilled water.

We tested for treatment differences in the return rates of marked fish, measured by the percent of fish marked in the pre-stimulus sample that were recaptured in post-stimulus samples, using a Wilcoxon-Mann-Whitney test (Siegel & Castellan, 1988). We predicted that (1) fish marked at control sites should return significantly sooner than fish marked at pheromone sites and (2) fish that are recaptured in a location other than the one where they were marked should immigrate into locations labelled with distilled water significantly more frequently than into locations labelled with alarm pheromone.

EXPERIMENT 2. AREA AVOIDANCE BY BROOK STICKLEBACK

Skin extract was prepared using the same method as described for experiment #1. Eleven adult fathead minnows (mean fork length ± SE = 47.6 ± 1.6 mm) were killed and filleted to collect a total skin area of 28.87 cm² which we homogenized in 240 mL of distilled water. Twenty mL of the skin solution were infused into each of 12 experimental sponges and 20 mL of distilled water were added to 12 control sponges. The sponges were frozen at -20°C until needed.

The study site, Marshy Creek, is a small tributary of Redberry Lake, located about 75 km northwest of Saskatoon, Saskatchewan (52° 40' N, 107° 20' W). It contains brook stickleback, fathead minnows, and other cyprinids; pearl dace (*Margariscus margarita* Cope) and finescale dace (*Phoxinus neogaeus* Cope). On 12 July, 1994, we placed 24 traps, spaced approximately 10 m apart, along the length of the creek within a few metres from shore. Each trap was attached to a stake as described for experiment 1.

Four traps were placed simultaneously into the stream every 10 minutes. After a 2 hour set duration, each group of 4 traps (2 experimental and 2 control traps) was removed at 10 minute intervals and replaced with sponges as described for experiment 1. Each trap location was assigned a fin clip and sponge treatment (Table I). Fish were clipped, held in pails and returned as described for experiment 1. Sponges were left in place for 35 to 60 minutes and then removed from the water. We immediately reset the traps in the same locations. Four post-stimulus samples were taken from each trap location at 2 hour intervals. The number of stickleback and clips, if any, were noted. Captured fish were always immediately released at the site of capture.

In both experiments, experimental and control traps were always paired and checked simultaneously so that there were never any differences in set durations between treatments.

Numerical changes in area use were determined by the same procedure described for experiment 1. Because the number of fish marked in the pre-stimulus sample were few, we compared the cumulative proportion of trap locations which recaptured at least one fish in the post-stimulus sample bearing the mark issued at that location in the pre-stimulus sample. We used a Fisher's Exact Probability test (Siegel & Castellan, 1988) to compare treatments.

Results

EXPERIMENT 1. AREA AVOIDANCE BY FATHEAD MINNOWS

There was no significant difference in the change in number of fish caught between locations previously labelled with alarm pheromone and locations previously labelled with distilled water after 3.6 hours (first post-stimulus sample: $W_x = 126, P = 0.087, 1$ -tailed) or 6.9 hours (second post-stimulus sample: $W_x = 129, P = 0.154, 1$ -tailed; Figure 1).

In the pre-stimulus sample we captured, clipped and released 4366 fathead minnows (mean ± SE: 160.6 ± 36.8

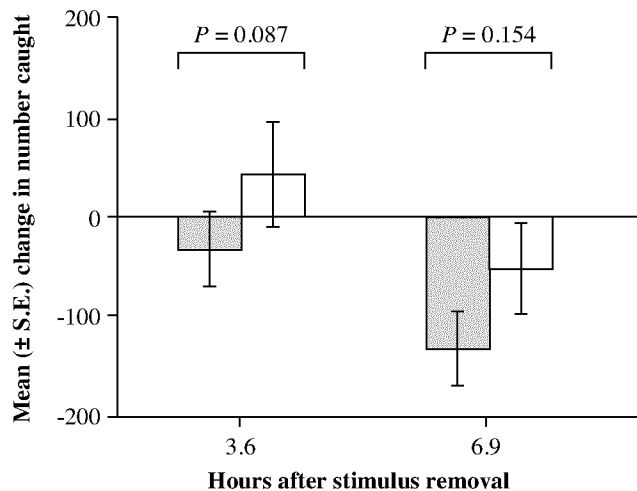


FIGURE 1. Mean ± SE change in number of fathead minnows caught in locations labelled with either minnow alarm pheromone (hatched bars) or distilled water (open bars). Samples were collected 3.6 and 6.9 hours after the stimulus was removed. Means and SE's are used to illustrate trends in the data only. The data were analyzed using Wilcoxon-Mann-Whitney tests.

per control trap and 203.3 ± 30.7 per experimental trap, $W_x = 134.5$, $P = 0.384$, 2-tailed). The proportion of fish captured and marked at locations labelled with minnow alarm pheromone that later returned to the same location was significantly lower in the first post-stimulus sample (3.6 hours after stimulus removal) than for areas previously labelled with distilled water ($W_x = 101.5$, $P = 0.008$, 1-tailed; Figure 2). This difference was no longer significant for the second post-stimulus sample, 6.9 hours after stimulus removal ($W_x = 143$, $P = 0.352$, 1-tailed).

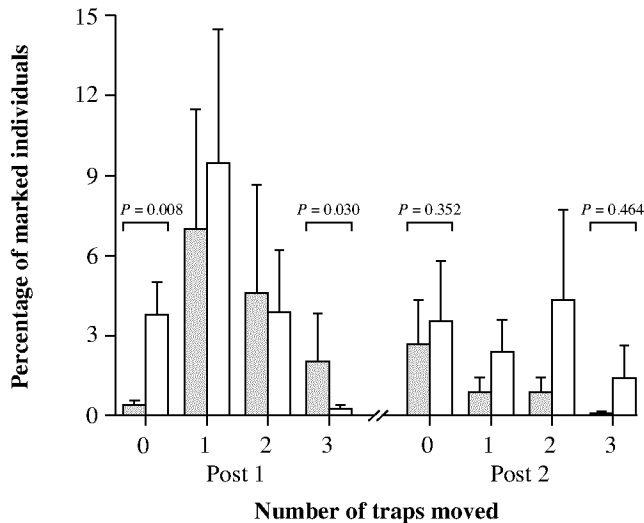


FIGURE 2. Percentage of marked fathead minnows which were recaptured at their location of origin in the pre-stimulus sample (0 trap distances), or 1, 2 or 3 traps away in the first and second post-stimulus samples. Hatched bars, locations previously labelled with minnow alarm pheromone; open bars, locations previously labelled with distilled water.

These data suggest that new, unmarked fish restored the numbers of fish using risky areas within 3.6 hours, but that individual minnows present at the time of pheromone release did not resume use of risky areas to the same extent as individual minnows marked at control areas, until some time between 3.6 and 6.9 hours.

In calculating inter-trap movements, we assumed, conservatively, that recaptured fish bearing marks other than the one issued at the location of recapture, came from the nearest trap location where the mark of the captured fish was issued. In the minnow experiment, the greatest detectable inter-trap distance was 3 traps, or about 45 m (*i.e.* fish that were considered to have moved from 2 traps away may have immigrated from 4 traps away, etc.). After 3.6 hours, a significantly greater proportion of fish initially present in areas labelled with alarm pheromone, than fish initially present in areas labelled with distilled water ($W_x = 117$, $P = 0.030$, 1-tailed; Figure 2) moved three trap locations. In the second post-stimulus sample there was no significant difference between treatments in the proportion of fish that moved 3 traps away from their location of capture in the pre-stimulus sample ($W_x = 148$, $P = 0.464$, 1-tailed).

If fish present in risky areas at the time of pheromone release socially transfer information about predation risk to nearby minnows, fish bearing clips from other trap locations should represent a lower percentage of the fish caught at locations labelled with alarm pheromone than at locations

labelled with distilled water. However, the proportion of the total catch represented by fish bearing clips other than the one issued at the site of recapture did not differ between risky areas and control areas in the post 1 ($W_x = 134$, $P = 0.187$, 1-tailed) or the post 2 samples ($W_x = 141$, $P = 0.312$, 1-tailed) (Table II). These data provide further evidence that naive fish immigrated into risky areas before the return of experienced fish.

TABLE II. The number of traps in each treatment group ($n = 12$ per group) in the first and second post-stimulus samples which contained 0, 1, 2, 3, 4, or 5 different fin clips. AP, alarm pheromone treatment; Con, distilled water control. Bottom two rows: mean and SE percentage of samples of each treatment group represented by marked immigrant fish

Number of new clips in sample	Post 1		Post 2	
	AP	Con	AP	Con
0	1	2	4	3
1	2	2	3	1
2	5	1	2	3
3	2	3	1	2
4	2	4	2	2
5	0	0	0	1
Percent of samples that were marked immigrants				
Mean	3.23	1.70	3.62	2.92
SE	1.03	0.33	1.45	0.71

EXPERIMENT 2. AREA AVOIDANCE BY BROOK STICKLEBACK

In the pre-stimulus sample, 63 brook stickleback were captured and marked at locations assigned to alarm pheromone (mean \pm SE = 2.5 ± 1.0 , $n = 30$) and distilled water sponges (mean \pm SE = 2.75 ± 0.9 , $n = 33$; $W_x = 149$, $P = 0.976$, 2-tailed). The total number of stickleback caught at locations marked with alarm pheromone was significantly lower than control locations 2 hours after the source of the pheromone was removed ($W_x = 117$, $P = 0.031$, 1-tailed; Figure 3). The changes in number caught, no longer differed for the sample taken 4 hours post-stimulus ($W_x = 126.5$, $P = 0.092$, 1-tailed). An increase in the number of stickleback caught at control locations reflects an increase in stickleback activity throughout the day. The significant difference

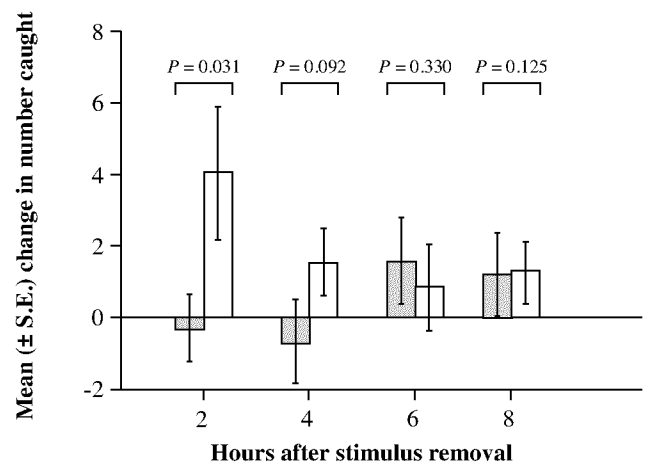


FIGURE 3. Mean \pm SE change in number of brook stickleback caught in locations labelled with either minnow alarm pheromone (hatched bars) or distilled water (open bars). Samples were collected 2, 4, 6, and 8 hours after the stimulus was removed. *P*-values are from Wilcoxon-Mann-Whitney tests.

between treatments at 2 hours post-stimulus resulted from the absence of a similar increase at locations labelled with alarm pheromone.

In the pre-stimulus sample, 9 experimental traps and 7 control traps caught stickleback. Within the first 2 hours post-stimulus, 4 of the 7 locations labelled with distilled water, but none of the experimental locations recaptured marked fish from their pre-stimulus sample (Fisher's Exact Probability test, $P = 0.019$, Figure 4). By 4 hours post-stimulus, 6 of 7 control trap locations had recaptured marked fish. Marked fish were not recaptured in locations labelled with minnow alarm pheromone until 4 to 6 hours post-stimulus. It was not until 6 to 8 hours after stimulus removal that the proportion of trap locations, which recaptured marked fish that originated at that location in the pre-stimulus sample, no longer differed between the treatments (Fisher's Exact Probability test, $P = 0.059$). These data are consistent with the hypothesis that new fish immigrated and occupied the risky areas vacated by experienced fish.

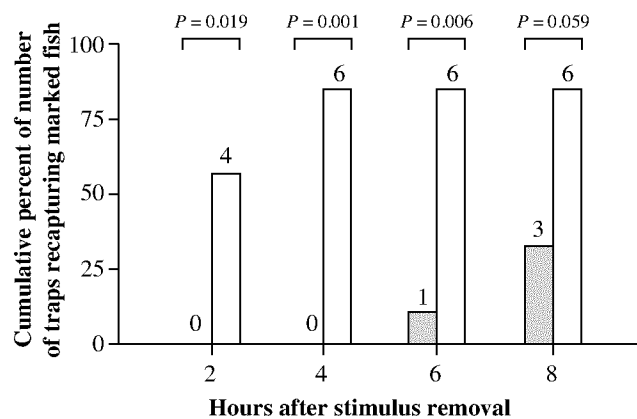


FIGURE 4. Cumulative percent of traps with brook stickleback containing marked individuals returning to their location of capture in the pre-stimulus sample. Hatched bars, trap locations previously labelled with alarm pheromone ($n = 7$), open bars, trap locations previously labelled with distilled water ($n = 9$). Numbers above bars, number of traps which recaptured marked fish.

In this experiment, there were only 5 instances of recaptured fish bearing a different fin clip than the one issued at the location of capture. Only one such individual was ever recaptured per location per sample and in each case the marked individual moved to an adjacent location from where it had been marked in the pre-stimulus sample. Four individuals moved from locations labelled with alarm pheromone to an adjacent control location and one individual moved from a control location to an adjacent location labelled with alarm pheromone (Binomial test $P = 0.188$).

Discussion

Individuals that experienced the alarm pheromone left the area and stayed away, even when individuals marked in other locations immigrated to the area. Minnows and stickleback continued to avoid labelled areas after the concentration of alarm pheromone was no longer sufficient to affect the behaviour of naive individuals. Further, continued avoidance of areas labelled with alarm pheromone did not result from residual pheromone lingering in the area after

the sponges were removed. Minnows from risky areas responded to alarm pheromone with an increase in dispersal distance. Significantly more marked minnows from areas labelled with alarm pheromone were recaptured at locations a distance of 3 traps away (*ca* 45 m) than marked minnows from areas labelled with distilled water (Figure 4). Minnow dispersal does not preclude an initial response of freezing or cover seeking as important components of anti-predator behaviour. The immediate response to the detection of alarm pheromone may be to seek cover and freeze, assess risk, then flee the area.

Based on the number of fish caught, area avoidance by the brook stickleback population in Marshy Creek was of similar duration to a population of stickleback in Oscar Creek (Wisenden, Chivers & Smith, 1994). For the first 2 hours after pheromone removal, the number of fish caught in locations previously labelled with minnow alarm pheromone was significantly lower than the number of fish caught in locations previously labelled with distilled water. In the present study we followed marked fish and showed that the numerical method underestimated the true duration of area avoidance by 4 to 6 hours. Stickleback avoid areas for at least 6 to 8 hours if they are present when minnow alarm pheromone is released.

Fish marked at control locations returned to their site of origin in the pre-stimulus sample significantly sooner than fish marked at pheromone locations returned to their site of origin. In the minnow experiment there was no significant difference in immigration rates by marked fish between risky and safe areas. Therefore, new individuals immigrated into the risky areas and masked the numerical absence of the fish originally present at the time of pheromone release. The same pattern seems to hold true for stickleback. However, an insufficient number of marked individuals immigrated to permit a statistical test of this hypothesis.

Return rates of marked fish indicate that the duration of area avoidance in minnows is between 3.6 and 6.9 hours, approximately the same as the duration of area avoidance by stickleback populations. This suggests that area avoidance may be an adaptive response to predation risk that is approximately the same for each species. This response duration agrees with that for the European minnow (6 hours after attack by a model kingfisher; Pitcher, Lang & Turner, 1988), but is longer than the response duration in juvenile Atlantic salmon, *Salmo salar* L. (2 hours after the disappearance of a predation threat; Metcalfe, Huntingford & Thorpe, 1987) and is much shorter than response duration shown by three-spined stickleback, *Gasterosteus aculeatus* L. (more than one day after attack by a model heron; Godin & Sproul, 1988) and cave characins, *Astyanax fasciatus* Cuvier (3 to 6 days after exposure to conspecific alarm pheromone; Fricke, 1987). Variation in response duration may reflect adjustments to the relative costs and benefits of area avoidance in different species and habitats.

Decreased philopatry and greater dispersal distance by fish from areas where alarm substance was released did not result in a detectable level of social transmission from these experienced fish to naive marked fish of the risk associated with areas labelled with minnow alarm pheromone (Table II). This suggests that information about predation risk was

retained by the few individuals which experienced the pheromone release, and was not communicated to all others. It was not possible for us to determine whether unmarked fish captured in post-stimulus samples experienced alarm pheromone and subsequently associated risk with use of the area. However, statistical comparisons were conducted on data from marked fish only. Marked fish released at one site may have visited a risky site before sponges were removed and detected residual alarm pheromone in the area, thereby becoming 'experienced'. This should have resulted in a lower rate of immigration of marked individuals into risky areas compared to safe areas. Since that did not happen, the main beneficiaries of the alarm signal may be those individuals present when the alarm pheromone was released. Opportunities for social transmission of information relating to predation risk may be limited to immediate neighbours and take place over a relatively short period.

Kin selection is the basis of a promising hypothesis for the evolution and maintenance of alarm pheromone in minnows. The benefit of inclusive fitness to an individual may outweigh the cost of producing alarm substance cells if kin are among the receivers (*i.e.* beneficiaries) of the signal (Hamilton, 1964; Smith, 1992; Naish, Carvalho & Pitcher, 1993). Fathead minnows separated by at least 30 m in the morning frequently shoaled together later the same day, and vice versa. This high degree of mixing indicates that large groups of adult minnows may not necessarily maintain close or consistent associations with each other. This appears to be consistent with studies of the genotypic structure of natural minnow shoals which have thus far shown little (Ferguson & Noakes, 1981) or no evidence (Naish, Carvalho & Pitcher, 1993) of genetic variation within or among geographically separate shoals. However, neither the present study, nor those discussed above preclude the possibility of the occurrence of kin groups within larger shoals. Further, Brown & Smith (1994) showed that individual fathead minnows can recognize former shoal mates after extended separation, which may allow for mixing and later association. The role of kin selection in minnow shoals remains untested.

Acknowledgements

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