

RISK-SENSITIVE HABITAT USE BY BROOK STICKLEBACK (*Culaea inconstans*) IN AREAS ASSOCIATED WITH MINNOW ALARM PHEROMONE

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(Received March 30, 1994; accepted July 11, 1994)

Abstract—Brook stickleback (*Culaea inconstans*) share habitat and predators with cyprinid species, and they exploit the alarm pheromone of fathead minnows (*Pimephales promelas*) to avoid areas of high predation risk. In this field experiment, we measured the retention and duration of area avoidance by brook stickleback from areas marked with alarm pheromone of fathead minnows. Area avoidance was greatest during the first 2 hr after the source of the alarm pheromone was removed ($P < 0.05$), but after 4 hr, area use was not significantly different from premarking levels. This study shows that brook stickleback: (1) use the alarm pheromone of fathead minnows to avoid high risk areas, (2) continue to avoid locations associated with predation risk after the source of the pheromone has gone, and (3) avoid risky areas temporarily, and resume use of risky areas after 2–4 hr. This behavioral response by stickleback to minnow alarm pheromone could serve to minimize risk of predation.

Key Words—Predation risk, area avoidance, brook stickleback, *Culaea inconstans*, fathead minnow, *Pimephales promelas*, alarm pheromone, Schreckstoff.

INTRODUCTION

Fishes of the superorder Ostariophysi produce an alarm pheromone ("Schreckstoff") in specialized epidermal club cells. The alarm pheromone can only be released when these cells are ruptured (for review see Smith, 1992). This occurs

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when the skin is damaged during a predator attack (Pfeiffer, 1963). Nearby individuals that detect the pheromone respond with a fright reaction composed of species-specific antipredator behaviors (Frisch, 1941) and thereby reduce their probability of being captured by the predator (Mathis and Smith, 1993a). Fathead minnows avoid areas in the stream marked with cyprinid alarm pheromone (Mathis and Smith, 1992).

Brook stickleback have an alarm response to conspecific skin extract (Mathis and Smith, 1993b; Chivers and Smith, 1994b), even though they are non-ostariophysan fishes (Gasterosteiformes: Gasterosteidae) and lack the specialized epidermal cells which contain alarm pheromone in cyprinid fishes (Pfeiffer, 1960).

Cross-species responses to alarm signals occur in closely related (Pfeiffer, 1963; Smith, 1982; Smith et al., 1991) and distantly related (Mathis and Smith, 1993b; Chivers and Smith, 1994b) fish species. In many areas brook stickleback and fathead minnows occupy the same habitat and are vulnerable to the same predators, especially fish-eating birds (Scott and Crossman, 1973). Stickleback exploit the alarm system of minnows and thus reduce their own risk of predation. Brook stickleback avoid areas in a stream where the alarm pheromone of fathead minnows is present and wild-caught stickleback respond to this pheromone in the laboratory with a fright response (Mathis and Smith, 1993b). However, to date, field studies of this phenomenon (e.g. Mathis and Smith 1992, 1993b) have not monitored the retention and duration of antipredator behavior or area avoidance after exposure to minnow alarm pheromone.

Sensitivity to the alarm pheromone of cyprinids has obvious survival benefits. These benefits can accrue to the cyprinids producing the pheromone and other species, such as brook stickleback, that use this system to recognize and avoid predators and/or areas associated with high predation risk (Smith, 1992; Mathis and Smith, 1993a).

Minnows learn to recognize predators when the alarm pheromone is associated with the sight of a predator (Chivers and Smith, 1994c) or the odor of a predator (Göz, 1941; Magurran, 1989; Suboski et al., 1990; Chivers and Smith, 1993, 1994a). When a sample of stream water is presented simultaneously with alarm pheromone, fathead minnows (*Pimephales promelas*) can later distinguish between water from that water sample and water from another sample taken 15 m away in the same stream (Chivers and Smith, 1994d). Taken together, these experiments indicate that minnows can learn to recognize predators and risky habitats after a single pairing of the predator- or habitat-specific stimuli with alarm pheromone.

A number of studies have documented permanent habitat shifts by prey species caused by the presence of predators (e.g., Seghers, 1974; Werner et al., 1983; Power et al., 1985; see Lima and Dill, 1990 for review). However, predation risk may vary seasonally, daily, or from minute to minute, depending on the ecological context of the predator-prey interaction. Over time, all loca-

tions in a stream may eventually be associated with the release of cyprinid alarm pheromone caused by predation events. Permanent avoidance of all stimuli associated with predation would incur unacceptably high costs in terms of loss of forage areas, foraging time, refuge, and spawning habitat (Lima and Dill, 1990; Milinski, 1993). Therefore, for the alarm system to be useful as a mechanism of predator avoidance, individuals should behave in a way that reflects a trade-off between the short-term benefits and costs of area avoidance. This may take the form of temporary avoidance of areas marked with alarm pheromone, followed by a gradual resumption of use of these areas as the threat of the predator's return decreases, i.e., when the predator has either moved to other areas or become satiated (Milinski, 1993).

In this field experiment we tested the retention and duration of area avoidance by brook stickleback to see if brook stickleback behavior would reflect this trade-off. We tested this by temporarily marking stream locations with alarm pheromone from the skin of fathead minnows (simulating a predation event). We predicted that brook stickleback would avoid marked areas because of a high risk of imminent predation, then gradually resume use of these areas.

METHODS AND MATERIALS

Preparation of Skin Extract. Ten adult fathead minnows (mean standard length \pm SE = 54.7 \pm 1.2 mm) were killed by a blow to the head. We then removed the skin from both sides of each minnow. We collected a total of 32.8 cm² of skin. We placed the skin in 100 ml of glass-distilled water and homogenized the sample using a polytron homogenizer. We filtered the homogenate through glass wool and then diluted the supernatant with 300 ml of glass-distilled water for a total volume of 400 ml. Fourteen blocks of synthetic sponge (5 \times 4 \times 2 cm) were soaked in this solution and frozen at -20°C for later use. Each sponge was weighted with a black rubber stopper attached to a piece of stainless steel wire. As a control, 14 additional sponge blocks were soaked with glass-distilled water and frozen at -20°C . Frozen skin extracts retain the capacity to elicit a fright response from stickleback (Mathis and Smith, 1993a).

Study Site and Sampling Schedule. Oscar Creek is a small body of water located 75 km northwest of Saskatoon, Saskatchewan (52 $^{\circ}$ 46'N, 107 $^{\circ}$ 07'W). At the study site, the creek ranges from about 1–10 m in width and contains populations of brook stickleback and fathead minnows. Current speed varied with basin morphology. Traps were placed in approximately 1-m-deep water, where the rate of flow was visibly present but relatively slow.

On October 7, 1993, 28 minnow traps (Gee's improved minnow traps: cylinders of 5 \times 5 mm wire mesh, measuring 43 cm long and 22 cm in diameter, with inverted cone entrances at each end), were placed along the edge of Oscar

Creek about 5–10 m apart. To ensure that trap location did not change from sample to sample, we attached each trap to a stake with cord. Each trapping period (one prestimulus and four poststimulus samples) lasted 2 hr. The sampling schedule was as follows. A prestimulus sample was collected from all trap locations. We counted the number of brook stickleback caught in each trap and then immediately released them at the same location. Next, we removed each trap from the stream and replaced it with a block of sponge. Traps were removed to prevent brook stickleback from associating the alarm pheromone with the traps. We attached each sponge to the appropriate stake on shore to ensure that sponges were in the same location previously occupied by each trap. Traps were set in pairs to maintain equal duration of trapping effort between treatments (alarm pheromone or distilled water control). Treatment was randomly assigned such that there was one trap per treatment within each consecutive pair of traps. Fourteen trap locations were marked with a block of sponge soaked in alarm pheromone, and the other 14 locations were marked with a block of sponge soaked in distilled water. Sponges soaked in distilled water allowed us to control for any effects of repeated trapping (i.e., either increased avoidance or habituation).

After 1 hr, we removed the sponges and immediately replaced the traps that had been used at each location for the prestimulus sample. This was the first poststimulus sample. After 2 hr we emptied the traps, counted and released the fish at the point of capture, and then reset the traps for another 2 hr. This procedure was repeated 2 hr later. These samples were the second and third poststimulus samples, respectively. The following day, 24 hr after the sponges were removed from the water, we collected a final 2-hr sample. This was the fourth poststimulus sample.

Statistical Analyses. For each trap location, we calculated the difference between the number of brook stickleback caught in each poststimulus sample and the prestimulus sample. The differences were ranked among the 28 traps, and a statistical comparison was made between locations marked with alarm pheromone and locations marked with distilled water using a Wilcoxon–Mann–Whitney test (Siegel and Castellan, 1988). This test was performed for each poststimulus sample to determine the statistical significance and duration of area avoidance by brook stickleback.

RESULTS

Brook stickleback avoided areas that had previously been marked with cyprinid alarm pheromone. The strength of this avoidance decreased over time (Table 1; Figure 1). There was a steady return to prestimulus levels in the number of brook stickleback caught in traps at locations previously marked with

TABLE 1. MEAN (\pm SE) NUMBER OF BROOK STICKLEBACK CAUGHT PER TRAP BEFORE (PRE) THE ADDITION OF STIMULUS SPONGES, AND 2, 4, 6, AND 24 HOURS AFTER (POST) THEIR REMOVAL

Sample	Alarm pheromone (<i>N</i> = 14)	Control (<i>N</i> = 14)
Pre-Stim	6.21 \pm 1.75	4.00 \pm 1.32
Post 2 hr	3.14 \pm 1.04	4.79 \pm 1.29
Post 4 hr	4.21 \pm 1.30	4.71 \pm 1.45
Post 6 hr	5.21 \pm 1.01	5.71 \pm 1.16
Post 24 hr	2.50 \pm 0.97	1.36 \pm 0.68

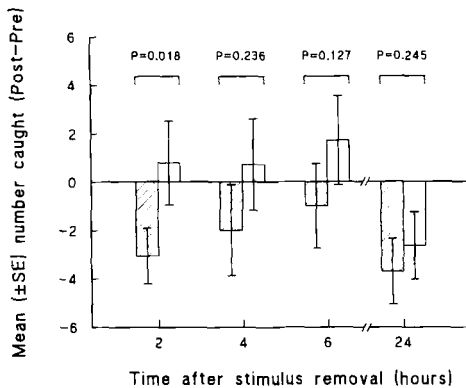


FIG. 1. The mean (\pm SE) difference in the number of brook stickleback caught per trap before stimulus sponges were placed in the stream and 2, 4, 6, and 24 hr after stimulus removal. Hatched bars, locations marked with cyprinid alarm pheromone; open bars, locations marked with distilled water. Means and SE's are used to illustrate trends in the data only. The data were analyzed using nonparametric statistics (see text).

alarm pheromone for samples taken 2, 4, and 6 hr after stimulus removal. Two hours after the removal of the sponges (first poststimulus sample), the number of brook stickleback caught relative to prestimulus catches was significantly less at trap locations marked with alarm pheromone than trap locations marked with distilled water ($W_x = 130$, $P = 0.018$, one-tailed). Stickleback avoidance of these areas was not statistically significant for any of the subsequent poststimulus samples ($P > 0.127$). The number of brook stickleback caught in traps at control locations increased slightly, but not significantly, for the remainder of the day (Table 1). The number of brook stickleback caught in the fourth poststimulus sample, 24 hr after the removal of the sponges, were equally low relative to the prestimulus sample for both treatments groups (Table 1; Figure 1).

DISCUSSION

These data demonstrate that brook stickleback avoided areas marked by cyprinid alarm pheromone after the stimulus had been removed. This behavior is consistent with brook stickleback recognizing cyprinid alarm pheromone as a signal that the marked area carried a high risk of predation (Mathis and Smith, 1993b). A shift in area use by brook stickleback away from locations associated with minnow alarm pheromone may have caused the slight increase in the number of brook stickleback caught in traps at control locations (Table 1). Alternatively, the increase in stickleback catches in control traps may reflect a diurnal pattern.

The duration of avoidance suggests that brook stickleback significantly avoided high-risk areas for 2–4 hr after the source of the stimulus was gone. There is an indication (based on small sample sizes) that the number of brook stickleback caught in traps marked with alarm pheromone continued to converge with prestimulus numbers for the 4- and 6-hr poststimulus samples. This pattern was not evident in locations marked with control sponges. We do not know whether any alarm pheromone remained in the sponges after 1-hr exposure in the stream. It is possible that some alarm pheromone lingered in the area for a short time after the removal of the sponge. However, there was sufficient current to dilute and disperse residual alarm pheromone within a short time relative to the duration of the first 2-hr trapping effort.

Catches after 24 hr were low for both treatment groups. This could have resulted if stickleback were less active on the second day than on the first for reasons not related to the experiment. These low catches do not represent area avoidance, because alarm and control traps caught equally low numbers of brook stickleback.

Risk-sensitive foraging is an active field of research. Numerous studies indicate that the trade-off between predation risk and foraging benefits is a dynamic process (Milinski and Heller, 1978; Milinski, 1985) where foraging individuals frequently sample their surroundings to assess predation risk and foraging potential (e.g., Clark and Mangel, 1984) and facultatively adjust their behavior in response to changes in these two opposing variables (Lima and Dill, 1990). For example, juvenile coho salmon reduce foraging rates after detection of chemical stimuli from predation events (Martel and Dill, 1993). After the disappearance of a predation threat, juvenile Atlantic salmon (*Salmo salar*) gradually resume foraging activity over a 2-hr period (Metcalf et al., 1987). Elective shoal size of European minnows did not return to normal (undisturbed) levels one day after exposure to a northern pike (*Esox lucius*), a predator of minnows (Pitcher et al., 1983).

Food benefit may be independent of predation risk (Cerri and Fraser, 1983). However, in European minnows (*Phoxinus phoxinus*), avoidance of open areas

vulnerable to aerial attack by a model kingfisher continued for at least 6 hr when food levels were held constant, but returned to normal within 6 hr when food reward in open areas was high (Pitcher et al., 1988). Furthermore, the rate at which parasitized three-spine stickleback resumed use of foraging patches following attacks by a model heron increased with food availability (Godin and Sproul, 1988).

Oscar Creek is a small body of water with no large piscivorous fish (i.e., esocids, centrarchids, percids). Important predators of small fish, such as minnows and brook stickleback, in this type of habitat are fish-eating birds (Scott and Crossman, 1973; Krause, 1994). Great blue herons (*Ardea herodias*) frequent the study site where these data were collected. Herons are sit-and-wait predators that remain in the same location for long periods of time. Kingfishers swoop down from high above the water surface to capture prey, and often initiate diving attacks from a favored perch. If predators tend to remain in the same area while fishing, then stickleback can assess the expected probability of encounter with a predator as unacceptably high in areas where cyprinid alarm pheromone is detected (Lima and Dill, 1990). When this occurs, avoidance of an area for two or more hours, followed by a gradual, cautious resumption of use of the area should be a useful tactic for brook stickleback that should reduce predation risk from aerial predators. Additional field experiments are needed to test the universality of this phenomenon in other species sympatric with cyprinids and in other habitats with different species of predators.

Acknowledgments—Dr. Grant Brown provided helpful suggestions on the logistics of this project, and his comments on earlier drafts improved the quality of this manuscript. Funding was provided by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

- CERRI, R.D., and FRASER, D.F. 1983. Predation and risk in foraging minnows: Balancing conflicting demands. *Am. Nat.* 121:552–561.
- CHIVERS, D.P., and SMITH, R.J.F. 1993. The role of olfaction in chemosensory-based predator recognition in the fathead minnow, *Pimephales promelas*. *J. Chem. Ecol.* 19:623–633.
- CHIVERS, D.P., and SMITH, R.J.F. 1994a. The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. *J. Fish Biol.* 44:273–285.
- CHIVERS, D.P., and SMITH, R.J.F. 1994b. Intra- and interspecific avoidance of areas marked with skin extract from brook sticklebacks (*Culaea inconstans*) in a natural habitat. *J. Chem. Ecol.* 20:1517–1524.
- CHIVERS, D.P., and SMITH, R.J.F. 1994c. Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Anim. Behav.* 48:597–605.
- CHIVERS, D.P., and SMITH, R.J.F. 1994d. Fathead minnows (*Pimephales promelas*) learn to recognize chemical stimuli from high risk habitats by the presence of alarm substance. *Behav. Ecol.* In press.

- CLARK, C.W., and MANGEL, M. 1984. Foraging and flocking: Information in an uncertain environment. *Am. Nat.* 123:626-641.
- FRISCH, K. VON 1941. Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Z. Vergl. Physiol.* 29:46-145.
- GODIN, J.-G.J., and SPROUL, C.D. 1988. Risk taking in parasitized sticklebacks under threat of predation: effects of energetic need and food availability. *Can. J. Zool.* 66:2360-2367.
- GÖZ, H. 1941. Über den Art- und Individualgeruch bei Fischen. *Z. Vergl. Physiol.* 29:1-45.
- KRAUSE, J. 1994. Transmission of fright reaction between different species of fish. *Behaviour* 127:37-48.
- LIMA, S.L., and DILL, L.M. 1990. Behavioral decisions made under the risk of predation: A review and prospectus. *Can. J. Zool.* 68:619-648.
- MAGURRAN, A. E. 1989. Acquired recognition of predator odour in the European minnow (*Phoxinus phoxinus*). *Ethology* 82:216-233.
- MARTEL, G., and DILL, L.M. 1993. Feeding and aggressive behaviors in juvenile coho salmon (*Oncorhynchus kisutch*) under chemically-mediated risk of predation. *Behav. Ecol. Sociobiol.* 32:365-370.
- MATHIS, A., and SMITH, R.J.F. 1992. Avoidance of areas marked with a chemical alarm substance by fathead minnows (*Pimephales promelas*) in a natural habitat. *Can. J. Zool.* 70:1473-1476.
- MATHIS, A., and SMITH, R.J.F. 1993a. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). *Behav. Ecol.* 4:260-265.
- MATHIS, A., and SMITH, R.J.F. 1993b. Intraspecific and cross-superorder responses to alarm signals by brook stickleback. *Ecology* 74:2395-2404.
- METCALFE, N.B., HUNTINGFORD, F.A., and THORPE, J.E. 1987. The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Anim. Behav.* 35:901-911.
- MILINSKI, M. 1985. Risk of predation of parasitized sticklebacks (*Gasterosteus aculeatus* L.) under competition for food. *Behaviour* 93:203-216.
- MILINSKI, M. 1993. Predation risk and feeding behavior, pp. 285-305, in T.J. Pitcher (ed.). *The Behaviour of Teleost Fishes*, 2nd ed. Chapman and Hall, London.
- MILINSKI, M., and HELLER, R. 1978. Influence of a predator on the optimal foraging behavior of sticklebacks (*Gasterosteus aculeatus* L.). *Nature* 275:642-644.
- PFEIFFER, W. 1960. Über die Schreckreaktion bei Fischen und die Herkunft des Schreckstoffes. *Z. Vergl. Physiol.* 43:578-614.
- PFEIFFER, W. 1963. The fright reaction in North American fish. *Can. J. Zool.* 41:69-77.
- PITCHER, T.J., MAGURRAN, A.E. and ALLAN, J.R. 1983. Shifts of behaviour with shoal size in cyprinids. Proceedings, 3rd British Freshwater Fisheries Conference pp. 220-228.
- PITCHER, T.J., LANG, S.H., and TURNER, J.A. 1988. A risk-balancing trade off between foraging rewards and predation in a shoaling fish. *Behav. Ecol. Sociobiol.* 22:225-228.
- POWER, M.E., MATTHEWS, W.J. and STEWART, A.J. 1985. Grazing minnows, piscivorous bass and stream algae: Dynamics of a strong interaction. *Ecology* 66:1448-1456.
- SCOTT, W.B., and CROSSMAN, E.J. 1973. *Freshwater Fishes of Canada*. Fisheries Research Board of Canada, Bulletin 184. 966 pp.
- SEGHERS, B.H. 1974. Geographic variation in the responses of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia* 14:93-98.
- SIEGEL, S., and CASTELLAN, N.J., JR. 1988. *Nonparametric Statistics for the Behavioral Sciences*, 2nd ed. McGraw-Hill, New York.
- SMITH, R.J.F. 1982. The adaptive significance of the alarm substance—fright reaction system, pp. 327-342, in T.J. Hara (ed.). *Chemoreception in Fishes*. Elsevier, Scientific, Amsterdam.
- SMITH, R.J.F. 1992. Alarm signals in fishes. *Rev. Fish Biol. Fish.* 2:33-63.

- SMITH, R.J.F., LAWRENCE, B.J., and SMITH, M.J. 1991. Cross-reactions to skin extract between two gobies, *Asterropteryx semipunctatus* and *Brachygobius sabanus*. *J. Chem. Ecol.* 17:2253-2259.
- SUBOSKI, M.D., BAIN, S., CARTY, A.E., MCQUOID, L.M., SEELEN, M.I., and SEIFERT, M. 1990. Alarm reaction in acquisition and social transmission of simulated predator recognition by zebra danio fish (*Brachydanio rerio*). *J. Comp. Psychol.* 104:101-112.
- WERNER, E.E., GILLIAM, J.F., HALL, D.J., and MITTELBACH, G.G. 1983. An experimental test of the effects of predation risk on habitat use in fish. *Ecology* 64:1540-1548.