Chemoecology 15:205–209 (2005)
0937–7409/05/040205–5

DOI 10.1007/s00049-005-0314-8

CHEMOECOLOGY

The avoidance response of fathead minnows to chemical alarm cues: understanding the effects of donor gender and breeding condition

M. S. Pollock, R. G. Friesen, R. J. Pollock, R. C. Kusch and D. P. Chivers

[1]Department of Biology, University of Saskatchewan 112 Science Place, Saskatoon, SK S7N 5E2, Canada

Summary. All animals are vulnerable to predation at some point in their lives and consequently prey organisms often develop effective risk assessment systems. For many aquatic species predation risk assessment occurs through the use of olfactory cues, including predator odours and alarm cues from damaged or disturbed conspecifics. When aquatic species encounter conspecific alarm cues they may respond, or not, based on specific information including cue concentration, health and size of the conspecific donor and potentially the gender and breeding condition of the donor. Previous laboratory studies have demonstrated that fathead minnows (*Pimephales promelas*) fail to respond to the skin extracts of breeding male minnows. The purpose of the current study was to verify these early laboratory findings in the field as well as to further investigate the effect of female reproductive state and donor gender on the response of minnows to damage-release alarm cues. Our results indicate that male breeding condition has a significant effect on how minnows will respond to conspecific cues. Minnows showed avoidance of cues of female minnows and male minnows not in breeding condition, in comparison to cues of breeding male minnows and cues of male and female swordtails. Neither the gender of non-breeding minnows nor the reproductive state of female minnows influenced the avoidance of minnows to alarm cues.

Keywords. Alarm cue – trap experiment – damage-release cue – *Pimephales promelas* – cyprinidae – Ostariophysan – actinopterygian.

Introduction

All animals at some time are potential prey for other species (Elton 1927). During these critical periods, prey must be able to accurately gauge their current risk of predation as it changes through space and time. Accurately assessing risk is important as antipredator behaviours often have associated fitness costs, such as lost foraging opportunities, decreased time defending territories, or decreased mating opportunities (Lima & Dill 1990, Lima 1998). In avoiding predation, and mediating fitness losses, a prey animal's first defenses are often its senses (Kats & Dill 1998).

Prey animals have been shown to use visual (Hartman & Abrahams 2000), mechanosensory (Shriner 1998), electrical and olfactory cues (Kats & Dill 1998, Chivers & Smith 1998) to assess predation risk. In aquatic systems the majority of research has focused on the use of chemical cues as an indication of predation risk. These cues most commonly include the odour of a predator, a damaged conspecific, or the odour of a conspecific in the diet of a predator (for review see Chivers & Smith 1998, Chivers & Mirza 2001). Cues of this type have been shown to invoke antipredator behaviours, changes in morphology and reproductive responses (Chivers & Smith 1998).

The superorder Ostariophysi, which includes minnows, characin, catfishes and suckers are the most studied group of fishes known to respond to the odour of damaged conspecifics. The chemical or chemicals thought to evoke the responses are stored in club cells within the epidermal layer (Pfeiffer 1974). This outermost layer is easily damaged during a predatory event at which times the contents are spilled warning nearby neighbors of predation risk (Chivers & Smith 1998). Numerous studies have shown analogous alarm systems in other groups of fishes including centrarchids, cichlids, salmonids and percids. Anti-predator responses to chemical alarm cues are often quite specific. In general fishes respond only to chemicals from their own species or from closely related species but do not show responses to any injured fish (review Smith 1992, Chivers & Smith 1998).

Mathis and Smith (1992) established a trapping technique to test the avoidance responses of fishes to chemical alarm cues released by injured prey fishes. They labeled Gee's Improved Minnow Traps with either skin extract of fathead minnows (*Pimephales promelas*) or distilled water and left the traps for 90 minutes. Results of this experiment appeared impressive; only 4 % of the minnows captured were in traps labeled with the conspecific skin extract. Several other experiments followed this initial design. For example, Chivers and Smith (1994) showed that brook stickleback (*Culaea inconstans*) avoid their own skin extract over a blank distilled water control. The original field studies offered the choice of skin extract versus a blank control; however, it is possible that many other substances in place of the skin extract would have produced the same results (Tremaine *et al*. in press).

In order to test whether the avoidance of fishes to conspecific alarm cues represents avoidance of alarm cues and not a generalized response to any fish cue or anything that smells, we need to conduct field experiments testing the response of fishes to the skin extracts from an unknown and distantly related species. Several authors (Chivers and Smith 1998, Kats and Dill 1998) have stressed the importance of

Correspondence to: M.S. Pollock e-mail: mike.pollock@sasktel.net

confirming laboratory results in the field whenever possible; inconsistent data such as those above, underscore the importance of field verification.

Studies by Smith (1973, 1974) found that male fathead minnows lost their club cells during the mating season. Smith hypothesized that the loss of the club cells thought to contain the "alarm substance" was due to high androgen levels during the breeding season. Smith (1973, 1974) further speculated that the vigorous breeding behaviours undertaken by the male minnows during this time would undoubtedly release the "alarm substance" from the club cells if they were present, thereby scaring away potential female mates and attracting predators (Mathis *et al*. 1996). In subsequent studies Smith (1976) noted club cell loss in seven other cyprinid species, and confirmed complete loss of the club cells in breeding male minnows.

In a laboratory study, Smith (1973) showed that minnows do not exhibit antipredator behaviours when exposed to the skin extract of breeding male minnows, but these findings have never been tested in the field. Likewise, no studies have been conducted to determine whether the breeding status of female donors influences the avoidance responses of minnows to conspecific alarm cues. In a field experiment we address these questions, as well as whether the gender of non-breeding donors influences the avoidance response of minnows to alarm cues.

Materials and Methods

We conducted a large scale field trapping experiment to test the area avoidance of fathead minnows to the skin extract of breeding and non-breeding conspecifics of both genders and an unknown heterospecific (swordtails, *Xiphophorus helleri*). The study was conducted in late summer at a pond located in Saskatoon, Saskatchewan between the dates of August 6-12 2003. The pond is part of a man made storm drainage system spanning several kilometers and contains only fathead minnows (a small prey fish common to most lakes, streams and ponds of northern North America). The pond is relatively homogenous and stagnant, and is surrounded by vegetation consisting mainly of common cattails (*Typha latipolia*). The experiment was conducted at the end of the breeding season of fathead minnows.

Experimental Design

A total of 240 Gee's Improved Minnow traps were labeled with one of six skin extract treatments (n = 40). The study was conducted on four separate days with 60 traps used each day for a sample size of ten per treatment each day. Treatments included the skin extracts of male and female fathead minnows, in breeding condition and not, as well as male and female swordtail skin extracts to control for a response to an unknown fish of a particular gender.

All skin extracts were created in a similar fashion. The donor fish were killed with a blow to the head (University of Saskatchewan Committee on Animal Care and Supply, Protocol Number 19970077) and skin fillets were removed from both sides of the fish. The fillets were then placed in enough distilled water to produce a concentration of 1cm^2 of skin per 10 ml of distilled water. The solution was then homogenized with a polytron homogenizer and the homogenate was filtered with glass wool. The solutions for each of the treatments were then frozen in 60 ml syringes until needed.

To produce fathead minnow skin extract the following number and sizes of fish were used: five non-breeding males (mean standard length 5.74 cm $\pm$ 0.42 cm) yielding 42.86 cm^2 of skin; four breeding males (mean standard length 6.13 cm $\pm$ 0.62 cm) yielding 48.2 cm^2 of skin, seven non-breeding females (mean standard length 5.23 cm $\pm$ 0.69 cm) and eight breeding females (mean standard length 4.81cm $\pm$ 0.44 cm) yielding 45.71 cm^2 and 47.55 cm^2 of skin respectively. Male minnows were considered breeding when breeding colours and tubercles were present (Smith 1973, 1974, 1976). The breeding condition of females was based on Gonadoso-matic Index (GSI – gonad weight/body weight expressed as a percent). Fifteen females were randomly chosen, and their GSI calculated. The mean GSI for the seven females in low breeding condition was 5.0 $\pm$ 2.6 %, while that of females in high breeding condition was 14.0 $\pm$ 4.3 %.

Swordtail skin extracts were produced in a similar fashion using 13 males (mean standard length 3.92 cm $\pm$ 0.59 cm) and six females (mean standard length 4.47 cm $\pm$ 0.38 cm) yielding 44.20 cm^2 and 47.86 cm^2 respectively. As swordtails are a tropical fish in a perpetual state of reproduction, we did not differentiate between breeders and non-breeders.

Gee's Improved Minnow Traps containing one of the six treatments were placed randomly around the pond approximately 10 m apart, with the condition that no more than two traps of the same treatment were in succession. The large distance between traps reduced the possibility of cross-contamination between sites (Wisenden *et al*. 1995). Testing was conducted every second day at which time the traps were shifted 5 m to the left to ensure that the same place was not tested twice within 48 hours. Wisenden (1995) showed that minnows present in an area at the time of cue release retuned to the affected area within seven or eight hours after the source of cues were removed. Our 48 hour time period between testing eliminated the possibility that testing on multiple days influenced the avoidance responses. Traps were equipped with two sponges (2 cm^3) fastened approximately 2 cm from each opening using a safety pin. The traps' sponges were injected with the thawed stimulus and introduced into the pond at two minute and thirty second intervals. Traps were collected after two hours and thirty minutes in the same order in which they were thrown. Any fish caught in a trap were anesthetized using MS-222 and stored in appropriately labeled bags containing ethyl alcohol (95 %).

All fish collected were brought back to the laboratory, counted, weighed and measured for standard length. Using the length and weight (weight/length^3), Body Condition Index was also calculated. This calculation gave us a relative index of the energy reserves available to each individual. If the trap contained more than ten fish the weight and standard length were attained by calculating the mean of ten randomly chosen fish from each trap. If the trap contained fewer than ten fish, the mean of all fish present was calculated.

Eleven paired comparisons were made (see Table 1) using Mann-Whitney U tests with the alpha value adjusted to 0.023 using the modified Bonferroni procedure [(alpha * treatments-1)/number of comparisions] (Keppel 1982). All calculations were conducted using SPSS v. 12.

Results

Area Avoidance

Results of the Mann-Whitney U tests revealed several significant differences and trends in regard to area avoidance (see Table 1, Post-hoc adjusted p-value = 0.023). As predicted, more minnows were captured in traps containing male swordtail skin extract when compared with non-breeding male minnow extracts ($Z = -2.349$, $p = 0.019$) (Fig. 1, Tab. 1). Likewise, more minnows were captured in traps containing female swordtail skin extract when compared to non-breeding female minnow extract ($Z = -2.368$, $p = 0.017$) (Fig. 1, Tab. 1). However, minnows did not differentiate between male swordtails and breeding male minnows ($Z = -1.064$, $p = 0.287$) which lack alarm cells (Fig. 1, Tab. 1). Minnows showed a strong trend towards avoidance of breeding female minnows over female swordtail skin extract ($Z = -2.154$, $p = 0.031$) (Fig. 1, Tab. 1). Minnows also showed a strong trend in avoiding non-breeding male minnow extract over breeding male minnow extract ($Z = 1.602$,

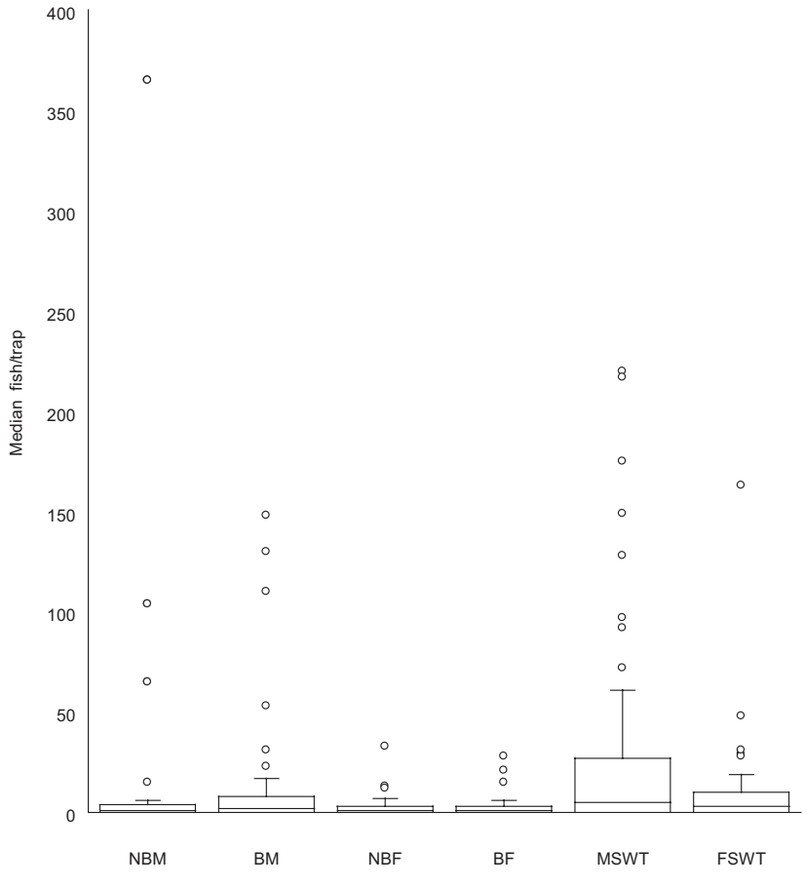

Fig. 1 Median number of minnows captured in traps labeled with one of six skin extracts [female swordtail (FSWT), male swordtail (MSWT), breeding male minnow (BM), non-breeding male minnow (NBM), breeding female minnow (BF) and non-breeding female minnow (NBF), circles are outliers greater or less than the difference between upper and lower quartiles multiplied by 1.5]

Table 1 Results for paired comparisons (Mann-Whitney U-tests) of minnows exposed to female swordtail (FSWT), male swordtail (MSWT), breeding male minnow (BM), non-breeding male minnow (NBM), breeding female minnow (BF) and non-breeding female minnow (NBF) skin extracts (α = 0.023, n = 40/treatment).

	Avoidance		**Standard Length**		**Body Condition Index**	
Comparison	Z-Value	P-Value	Z-Value	P-Value	Z-Value	P-Value
NBM-BM	–1.602	**0.055** (1 tailed)	–0.085	0.47 (1 tailed)	–1.173	0.12 (1 tailed)
NBM-NBF	0.351	0.726	–0.510	0.610	–1.263	0.206
NBM-BF	–0.287	0.774	–1.614	0.106	–0.878	0.380
NBM-MSWT	–2.349	**0.019**	0.836	0.403	–2.111	**0.035**
BM-NBF	–1.959	**0.025** (1 tailed)	–0.948	0.172 (1 tailed)	–0.099	0.461 (1 tailed)
BM-BF	–1.811	**0.035** (1 tailed)	–1.986	**0.024** (1 tailed)	–0.961	0.169 (1 tailed)
BM-MSWT	–1.064	0.287	–0.810	0.418	–1.000	0.317
NBF-BF	–0.054	0.957	–0.440	0.660	–0.631	0.528
NBF-FSWT	–2.368	**0.017**	–1.354	0.176	–0.660	0.509
BF-FSWT	–2.154	**0.031**	–2.117	**0.034**	–1.297	0.195
MSWT-FSWT	–0.780	0.436	–0.392	0.695	–0.577	0.564

p = 0.055), as well as trends in avoiding both non-breeding females and breeding female extract over breeding male skin extract (Z = –1.959, p = 0.025; Z = –1.811, p = 0.035) (Fig. 1, Tab. 1).

Minnows failed to differentiate between non-breeding male and female skin extracts (Z = 0.351, p = 0.726), or between the skin extracts made from females in high versus low breeding condition (Z = –0.054, p = 0.957) and

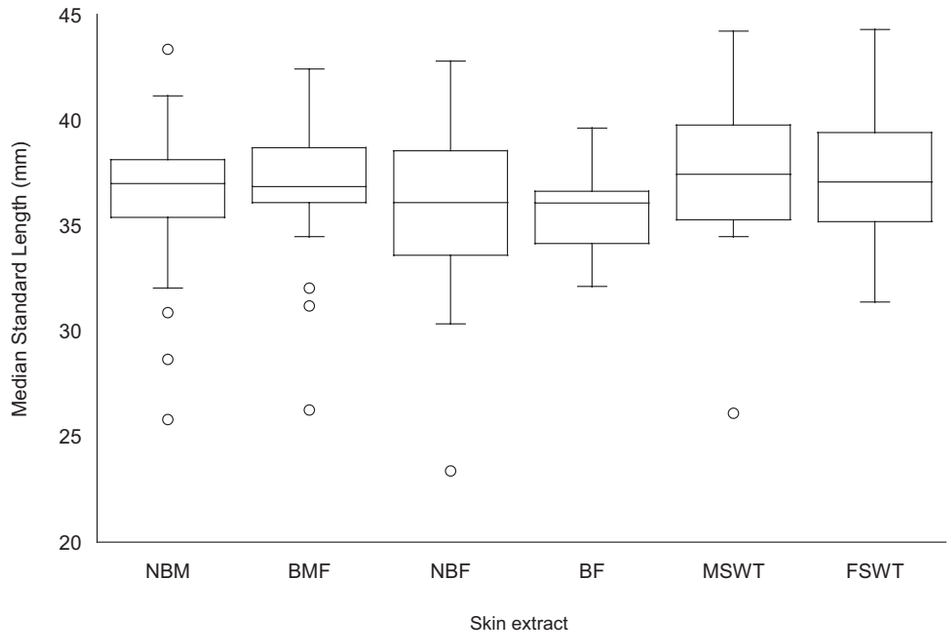

Fig. 2 Median standard length (SL) of minnows captured in traps labeled with one of six skin extracts [female swordtail (FSWT), male swordtail (MSWT), breeding male minnow (BM), non-breeding male minnow (NBM), breeding female minnow (BF) and non-breeding female minnow (NBF), circles are outliers greater or less than the difference between upper and lower quartiles multiplied by 1.5]

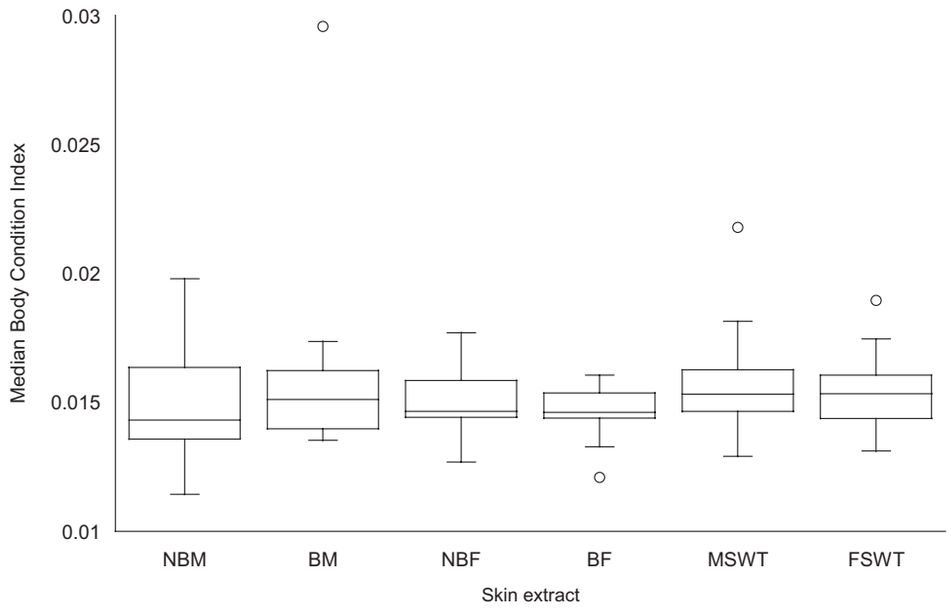

Fig. 3 Median body condition index (BCI) of minnows captured in traps labeled with one of six skin extracts [female swordtail (FSWT), male swordtail (MSWT), breeding male minnow (BM), non-breeding male minnow (NBM), breeding female minnow (BF) and non-breeding female minnow (NBF), circles are outliers greater or less than the difference between upper and lower quartiles multiplied by 1.5]

finally between non-breeding males and breeding females ($Z = -0.287$, $p = 0.774$) (Fig. 1, Tab. 1).

Taken together these data support Smith's (1973, 1974, 1976) laboratory studies verifying that minnows treat the genders equally when not in breeding condition but fail to avoid skin extracts composed of male breeding minnows, treating such cues no differently than the unknown control.

Standard Length

Results of the data regarding length produced two strong trends. Minnows captured in traps containing breeding male minnow extracts, which lack alarm cells, tended to be larger than fish captured in traps labeled with breeding female extracts ($Z = -1.986$, $P = 0.024$) (Fig. 2, Tab. 1). Similarly, minnows captured in traps containing breeding female extract were smaller than minnows captured in traps labeled with female swordtail extract ($Z = -2.117$, $P = 0.034$) (Fig. 2, Tab. 1). As length has been used as an indication of age and experience (Chivers & Smith 1998) we can assume that older and more experienced fish in both cases avoided what was predicted to be the "dangerous" cue.

Body Condition Index

Results pertaining to body condition are similar to those concerning length. Although no significant results were found, one trend indicated that fish in poor body condition

are more likely to enter a trap containing dangerous cues (Fig. 3, Tab. 1). Minnows entering traps containing non-breeding male minnow stimulus tended to be in poorer body condition than minnows entering traps containing male swordtail stimulus ($Z = -2.111$, $p = 0.035$) (Fig. 3, Tab. 1).

Discussion

The findings of our study provided the first field confirmation that minnows fail to avoid skin extracts from breeding male conspecifics, as well as revealing that minnows do not differentiate between skin extracts of female fish in different reproductive states (Fig. 1). Moreover, we demonstrated that the gender of non-breeding fishes used to produce skin extracts does not influence the avoidance response of minnows. This data is especially important as most researchers do not often control for the ratio of females to males when producing skin extracts, nor is the breeding condition of donor fishes commonly reported (review Chivers & Smith 1998).

We caution researchers that these questions need to be addressed in other alarm systems. There are often differences in predation rates associated with size, gender and body condition, consequently it would be most advantageous for fishes to respond most strongly to alarm cues of individuals that are of similar status. Mirza and Chivers (2002) showed that brook char (*Salvelinus fontinalis*) showed stronger anti-predator responses to chemical alarm cues of similar sized conspecifics than from alarm cues of different sized conspecifics. Likewise, Brown *et al*. (2004) showed that cichlids showed stronger anti-predator responses to individuals in better body condition. Many fishes are sexually dimorphic and subject to differential predation. Testing the effect of donor gender and reproductive status on alarm responses would be most fruitful.

We concur with Smith's hypothesis that the lack of "alarm substance" in breeding male minnows was selected for due to the vigorous rubbing behaviours which occur during the breeding season (Smith 1973). If the "alarm substance" was present and released at these times they would not only scare away potential conspecific mates (Smith 1973), but may also attract predators to the area (Chivers *et al*. 1996).

In recent years several authors have expressed the importance of field validations of laboratory findings (Chivers & Smith 1998, Kats & Dill 1998). There are many reasons to predict that responses under field conditions may be different than those under laboratory conditions. For example, in the laboratory fishes are fed to satiation and tested in water that is free of chemical signals from competitors, mates, etc. Field validations are especially important as a few laboratory and field studies have produced inconsistent results (Magurran *et al*. 1996; Pollock *et al*. unpub. data; Tremaine *et al*. in press). We believe that laboratory studies are a necessary and crucial first step in many investigations but that researchers should strive to conduct innovative field experiments.

References

Brown GE, Foam PE, Cowell HE, Fiore PG, Chivers DP (2004) Production of chemical alarm cues in covict cichlids: the effects of diet, body condition and ontogeny. Ann. Zool. Fennici. 41: 487–499

Chivers DP, Brown GE, Smith RJF (1996) The evolution of chemical signals: attracting predators benefits alarm signal senders. Am. Nat. 148: 649–659

Chivers DP, Mirza RS (2001) Predator diet cues and the assessment of predation risk by aquatic vertebrates: a review and prospectus. Chem. Sig. Vert. 9. edited by Marchlewska-Koj et al. Kluwer Academic/Plenum publishers, New York. 277–284

Chivers DP, Smith RJF (1994) Intra- and interspecific avoidance of areas marked with skin extract from brook sticklebacks in a natural habitat. J. Chem. Ecol 20: 1517–1524

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Écoscience 5: 338–352

Elton C (1927) Animal Ecology. University of Chicago Press

Hartman EJ, Abrahams MV (2000) Sensory compensation and the detection of predators: the interaction between chemical and visual information. Proc. Roy. Soc. Lon. B. 267: 571–575

Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Écoscience 5: 361–394

Keppel G (1982) Design and analysis: a researchers handbook. Prentice hall, Englewood Cliffs, New Jersey

Lima SL (1998) Stress and decision making under the risk of predation: recent developments from behavioral, reproductive and ecological perspectives. Advan. Stud. Behav. 27: 215–290

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68: 619–640

Magurran AE, Irving PW, Henderson PA (1996) Is there a fish pheromone? A wild study and critique. Proc. Roy. Soc. Lon. 1551–1556

Mathis A, Chivers DP, Smith RJF (1996) Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. Anim. Behav. 51: 185–201

Mathis A, Smith RJF (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

Mirza RS, Chivers DP (2002) Brook char (*Salvelinus fontinalis*) can differentiate chemical alarm cues produced by different age/size classes of conspecifics. J. Chem. Ecol. 28: 555–564

Pfeiffer W (1974) Pheromones in fish and amphibians. In Pheromones. Edited by Martin C. Birch. Frontiers of Biology. Vol. 3

Shriner WM (1998) Yellow-bellied marmot and golden-mantled ground squirrel responses to heterospecific alarm calls. Anim. Behav. 55: 529–536

Smith RJF (1973) Testosterone eliminates alarm substance in male fathead minnows. Can. J. Zool. 51: 875–876

Smith RJF (1974) Effects of 17 a-methyltestosterone on the dorsal pad and tubercles of fathead minnows (*Pimephales promelas*). Can. J. Zool. 52: 1031–1038

Smith RJF (1976) Seasonal loss of alarm substance cells in North American cyprinid fishes and its relation to abrasive spawning behaviour. Can. J. Zool. 54: 1172–1182

Smith RJF (1992) Alarm signals in fishes. Review. Fish Biol. Fish. 2: 33–63

Tremaine RJ, Pollock MS, Friesen RG, Kusch RC, Chivers DP (In Press) The response of prey fishes to chemical alarm cues: What recent field experiments reveal about the old testing paradigm. Chem. Sig. Vert. 10. Kluwer Academic/Plenum publishers, New York

Wisenden BD, Chivers DP, Brown, GE. & Smith, RJF (1995) The role of experience in risk assessment: Avoidance of areas chemically labelled with fathead minnow alarm pheromone by conspecifics and heterospecifics. Écoscience. 2: 116–122

Received 12 November 2004; accepted 6 June 2005.
Published Online First 17 August 2005.