

Anti-predator behaviour in response to conspecific chemical alarm cues in an esociform fish, *Umbra limi* (Kirtland 1840)

Brian D. Wisenden · Justin Karst ·
Jeffrey Miller · Stacey Miller · Linda Fuselier

Received: 4 November 2006 / Accepted: 27 March 2007 / Published online: 15 May 2007
© Springer Science+Business Media B.V. 2007

Abstract When a predators attack prey, damaged prey tissue releases chemical information that reliably indicates an actively foraging predator. Prey use these semiochemicals to cue anti-predator behaviour and reduce their probability of predation. Here, we test central mudminnows, *Umbra limi* (Kirtland 1840), for anti-predator behavioural responses to chemical cues in conspecific skin extract. In a field experiment, traps scented with mudminnow skin extract (alarm cue) caught fewer mudminnows than traps scented with water (control). Under controlled laboratory conditions, mudminnows showed a significant reduction in activity and movement to the bottom in response to alarm cues relative to water controls. Reduced activity and increased time on the bottom of the tank are both known components of an anti-predator response. Thus, based on field and lab data, mudminnows exhibited anti-predator behavioural responses to chemical alarm cues released by damaged epidermal tissue. Histological preparations of epidermal tissue did not reveal the presence of specialised “alarm substance” cells for the production of chemical alarm cues. This is the first report of an alarm reaction in an esociform, an order with a long evolutionary history of piscivory.

Keywords Chemical alarm cue · Mudminnow · *Umbra* · Anti-predator behaviour · Field study

Introduction

Public information guides many aspects of behavioural decision-making (Danchin et al. 2004). Public information about predation risk in aquatic habitats often comes in the form of chemical cues released passively as a normal consequence of predator–prey interactions (Chivers and Smith 1998; Wisenden and Stacey 2005; Wisenden and Chivers 2006). To detect predation risk, prey fish use chemical cues that emanate from predators (kairomones and dietary cues), from disturbed but uninjured prey, or injured prey. Chemical alarm cues of prey damaged by predatory attack are released only in the context of predation and thus, reliably inform nearby prey of the presence of an actively foraging predator.

Behavioural responses of fish to chemical alarm cues present in skin have been studied primarily in species in the superorder Ostariophysi [minnows, tetras, catfish, et al., comprising ~64% of all freshwater fish species (Nelson 1994)]. Some of the reasons for why this has been the case include (1) the first observation of fright reactions to skin extract was on a minnow (von Frisch 1938), (2) ostariophysans possess specialised skin cells that produce the active ingredient in alarm cue (Pfeiffer 1977; Smith 1992), (3) ostariophysans are small in size and therefore fall

B. D. Wisenden (✉) · J. Karst · J. Miller ·
S. Miller · L. Fuselier
Biosciences Department, Minnesota State University,
Moorhead, MN 56563, USA
e-mail: wisenden@mnstate.edu

prey to predators even as adults and (4) adapt well to laboratory aquaria. Non-ostariophysan fishes have received some attention and they too show anti-predator behaviour in response to conspecific skin extract (Chivers and Smith 1998; Wisenden 2003) thus, specialised epidermal cells are not always required for a species to produce and detect chemical alarm cues. As evidence accumulates, it seems increasingly clear that injury-released chemical cues are a general form of public information about predation risk perceived by all aquatic taxa, from protists to amphibians, as an indicator of predation risk (Wisenden 2003; Wisenden and Stacey 2005; Wisenden and Chivers 2006).

Alarm reactions among fishes in the order esociformes have received little attention. Early work on esociforms (Pfeiffer 1960) indicates that they do not have epidermal club cells that may contribute olfactorally conspicuous components to skin extract. The one esociform species that has been tested, *Esox lucius*, did not show any alarm reaction (Schutz 1956). Unlike other taxa for which an alarm reaction to conspecific skin extract has been demonstrated (Chivers and Smith 1998), there are two aspects of the esociformes that make a study of alarm reaction in this group more than a stamp-collecting exercise of “one more species” to add to a long list of fishes with alarm reactions to skin extract. First, there may be a phylogenetic constraint on anti-predator behaviour in response to injury-released chemical cues because fishes in the esociformes (superorder Protacanthopterygii) are typically large predatory species that are dominant predators in many ecosystems. A long evolutionary history as specialist piscivores may have selected for indifference to alarm cues. Second, esociform species are well known for cannibalism. For example, northern pike, *E. lucius* L. become cannibalistic at 60 mm in total length (TL) and by 100 mm TL cannibals can represent as much as 41%

filleted to produce a total mass of 9.2 g of skin. For each day, the skin solution was homogenised with a blender, filtered through polyester wool to remove connective tissue and diluted to a total volume of 750 ml with dechlorinated tap water. Cellulose sponge blocks ($5.5 \times 5.5 \times 5.5 \text{ cm}^3$) each received 50 ml of this solution (equivalent to about 640 mg of skin per sponge) and were frozen at -20°C . Control sponges of the same size received 50 ml each of dechlorinated tap water, and were frozen at -20°C . Skin collection methods were approved by the Minnesota State University Moorhead Institutional Animal Care and Use Committee (protocol number 05-R-Biol-015-N-R-1).

Sponges were transported to the field on ice to keep them frozen. Each sponge block was affixed centrally inside a Gee's Improved minnow trap (a cylinder of wire mesh 23 cm in diameter, 44.5 cm in length, with an inverted funnel entrance at each end) to chemically label the trap with either skin extract (alarm cues) or water (control). Thirty traps were set ($n = 15$ labelled with skin extract and $n = 15$ labelled with water) around the perimeter of the oxbow on 4 September, and again on 18 September 2006. After 2.5 h, the number of fish caught in each trap was counted. Fish were returned immediately to the oxbow at the location of capture. The number of mudminnows captured in each trap type was used as the measure of the behavioural response to the chemical cue treatments. A sum total of 19 mudminnows were caught on 4 September and 55 mudminnows on 18 September 2006, from a population known to number in the many hundreds (unpublished data). Thus, catch data from the 2 days were considered independent tests of the effect of the test cues and data for the 2 days were pooled for analysis. Natural populations of free-swimming fish avoid areas where skin extract has been released (von Frisch 1941; Wisenden et al. 2004a; Wisenden and Barbour 2005; Friesen and Chivers 2006). Based on many previous studies (e.g. Mathis and Smith 1992; see Chivers and Smith 1998, for review) traps labelled with alarm cues were predicted to catch fewer fish than traps labelled with water. Thus, a one-tailed Mann–Whitney *U*-test was used to compare the number of fish caught in traps of each type of chemical cue.

Laboratory experiment

Juvenile mudminnows were captured from the Buffalo River oxbow using minnow traps set over night. The fish were transferred to the aquatic research facility at MSUM, placed in 190 l stock tanks and fed thawed brine shrimp once daily. Mudminnows were held in captivity for 2 weeks and adapted to a diet of thawed brine shrimp before testing began. Test aquaria were 18 l in volume with a thin layer of naturally coloured gravel and a sponge filter operated by compressed air. A second line of airline hosing wedged into the lift tube of the filter was used to surreptitiously inject test stimuli. Three mudminnows were placed in each test aquarium. Three fish were used per trial because mudminnows are not active when kept singly but move often when in a group, thus, a group of fish is a prerequisite for detecting a reduction in activity. No fish was used more than once in this experiment.

Skin extract stimulus was prepared from the skin fillets of 15 juveniles ($TL = 45.0 \pm 0.9 \text{ mm}$) to produce a mass of 1.2 g of skin. This was aliquoted into 17, 10-ml doses of alarm cue (each containing about 70 mg of skin). Alarm cue was frozen at -20°C until needed. A similar 17 doses of dechlorinated water was prepared and frozen to be used as the control stimulus.

Behavioural observations were recorded live (i.e. not videotaped) while observers stood quietly $\sim 2 \text{ m}$ away. Activity and vertical distribution were recorded for 8 min before and after the introduction of test stimuli. Activity was measured by the total number of times any of the three fish crossed a line of a grid of $5 \times 5 \text{ cm}^2$ cells drawn on the front pane of each tank. Vertical distribution was scored every 15 s by recording which of the four horizontal rows of the grid each fish occurred. The surface row was scored as a "1" and the bottom row as a "4". Thus, the vertical distribution score for three fish ranged from 3 to 12. We conducted 15 trials in which mudminnow skin extract (alarm cue) was injected, and 15 trials in which water (control) was injected. We predicted that an anti-predator response to skin extract (relative to controls) would be manifested as a reduction in activity and an increase in time spent near the bottom, based on many previous studies (Chivers and Smith 1998).

Histological tissue collection

As a subquestion of our investigation of mudminnow chemical ecology, we examined histological preparations of epidermal tissue taken from the dorsal region between the head and the dorsal fin from eight mudminnows. Four individuals were juveniles and four individuals were adults. This region was chosen because research on perch and darters (Wisenden et al. unpublished data) show that this region has the greatest density of club cells. Tissue samples were preserved in 10% buffered formalin and stained with periodic acid Schiff's reagent and counterstained with Lyllie's haematoxylin. Carbohydrates (such as mucus) sequester this stain and become purple. Club cells in ostariophyans and percids do not sequester this stain (e.g. Wisenden and Smith 1997). For comparison purposes, histological sections of epidermal tissue of a redbelly dace and a 15 cm walleye, *Sander vitreum*, previously published in Wisenden (2003) are presented. The same histological methods as described above were used to prepare epidermal tissue for the dace and walleye.

Results

Field experiment

On 4 September 2006 we caught 19 mudminnows (6 in traps labelled with mudminnow skin extract and 13 in traps labelled with water) and 4 redbelly dace (3 in mudminnow traps and 1 in a water trap). On 18 September 2006, we caught 55 mudminnows (21 in mudminnow traps and 34 in water traps) and 2 redbelly dace (0 in mudminnow traps and 2 in water traps). Too few redbelly dace were captured to be useful for analysis. Traps chemically labelled with conspecific skin extract caught a sum total of 27 mudminnows over the two sampling days, significantly fewer than the 47 mudminnows that were caught in traps labelled with water (Mann–Whitney U -test, $U = 328.5$ and $P_{(\text{one-tailed})} = 0.030$; Fig. 1). The median TL of fish caught in each trap type did not differ significantly (Mann–Whitney U -test, $U = 559$ and $P_{(\text{two-tailed})} = 0.140$, Fig. 2).

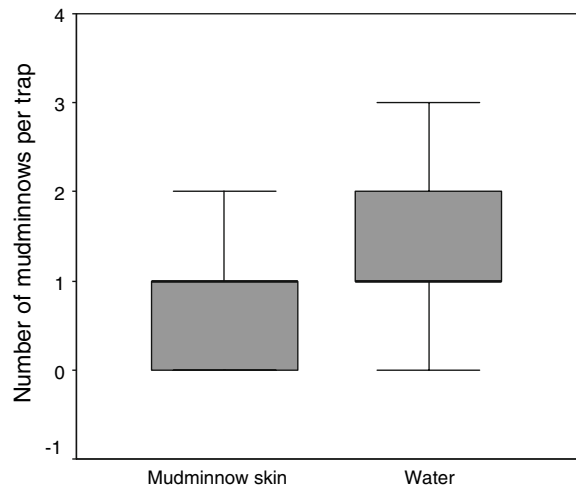


Fig. 1 The median (\pm quartiles and range) number of mudminnows caught in minnow traps chemically labelled with alarm cues in mudminnow skin extract (alarm cue), or water (control). Mann–Whitney U -test and $P = 0.030$

Laboratory experiment

In response to mudminnow skin extract, there was a significant decrease in overall activity (Mann–Whitney U -test, $U = 36$, $P_{(\text{one-tailed})} = 0.001$; Fig. 3) and significant increase in time spent near the bottom (Mann–Whitney U -test, $U = 29.5$ and $P_{(\text{one-tailed})} < 0.001$; Fig. 4) relative to water controls.

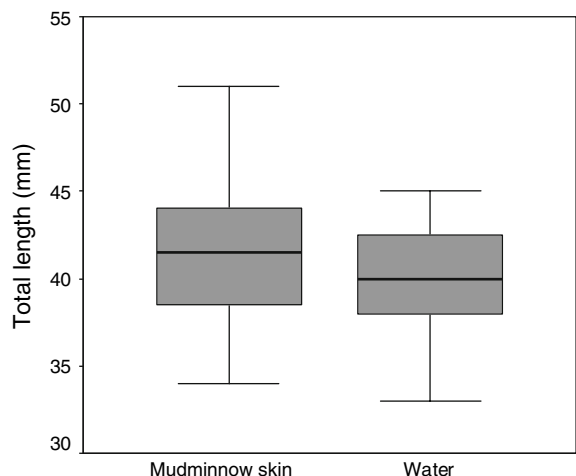
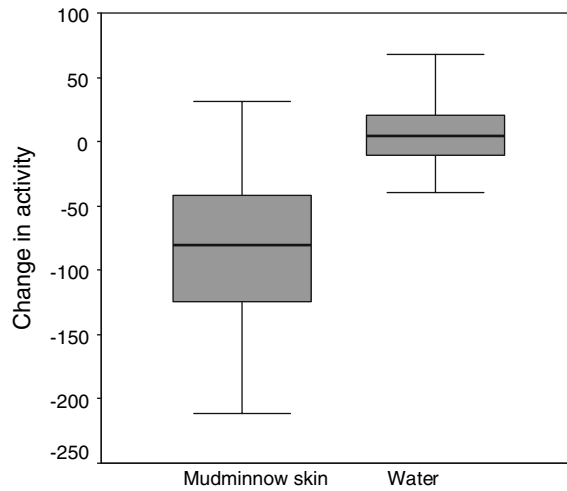


Fig. 2 Median (\pm quartiles and range) total length of mudminnows caught in traps labelled with mudminnow skin extract (alarm cue) or water (control). Mann–Whitney U -test and $P = 0.140$

Fig. 3 Median (\pm quartiles and range) change in activity of mudminnows after the introduction of mudminnow skin extract (alarm cue), or water (control), into the aquarium. Mann–Whitney *U*-test and $P < 0.001$



Thus, field and lab-derived data both indicated an anti-predator response to conspecific alarm cues.

Epidermal histology

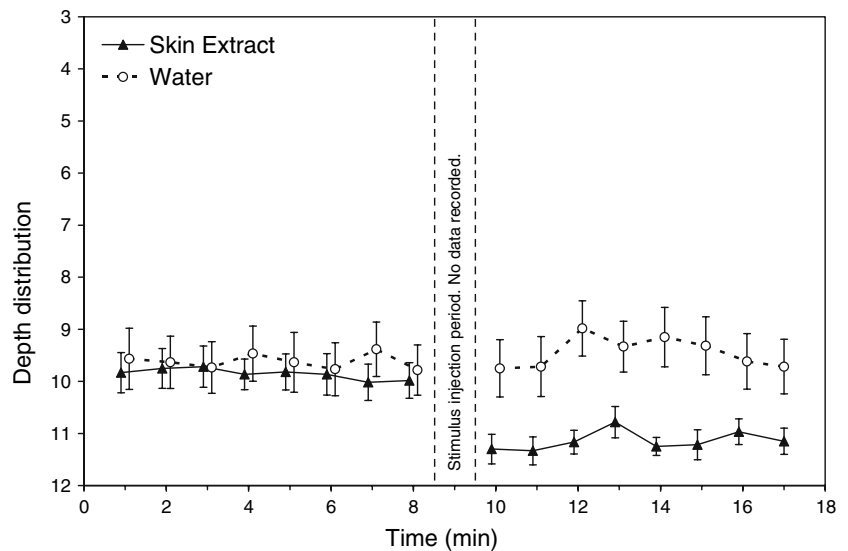
Histological preparations of mudminnow epidermal tissue did not reveal the presence of club cells with staining characteristics similar to club cells in ostariophysan and percid fishes (Fig. 5). The epidermis of mudminnows contains mucus cells and undifferentiated squamous epithelial cells. The skin of juvenile and adult mudminnows appeared to be identical (Fig. 5C, D).

Discussion

This esociform species showed clear evidence of an anti-predator response to conspecific chemical alarm cues in the field (in terms of area avoidance) and in the laboratory (in terms of reduced activity and movement to the bottom). The small size of mudminnows makes them vulnerable to many predators and thus, there is strong selection on mudminnows to detect and respond to chemical public information indicating the presence of predation risk.

A full 36% of the catch in the field experiment occurred in traps scented with alarm cues, suggesting

Fig. 4 Mean (\pm SE) vertical distribution of three mudminnows in test tanks before and after addition of test cues. Minimum distribution score is 3 (*at surface*); maximum score is 12 (*on bottom*). Mann–Whitney *U*-test and $P < 0.001$



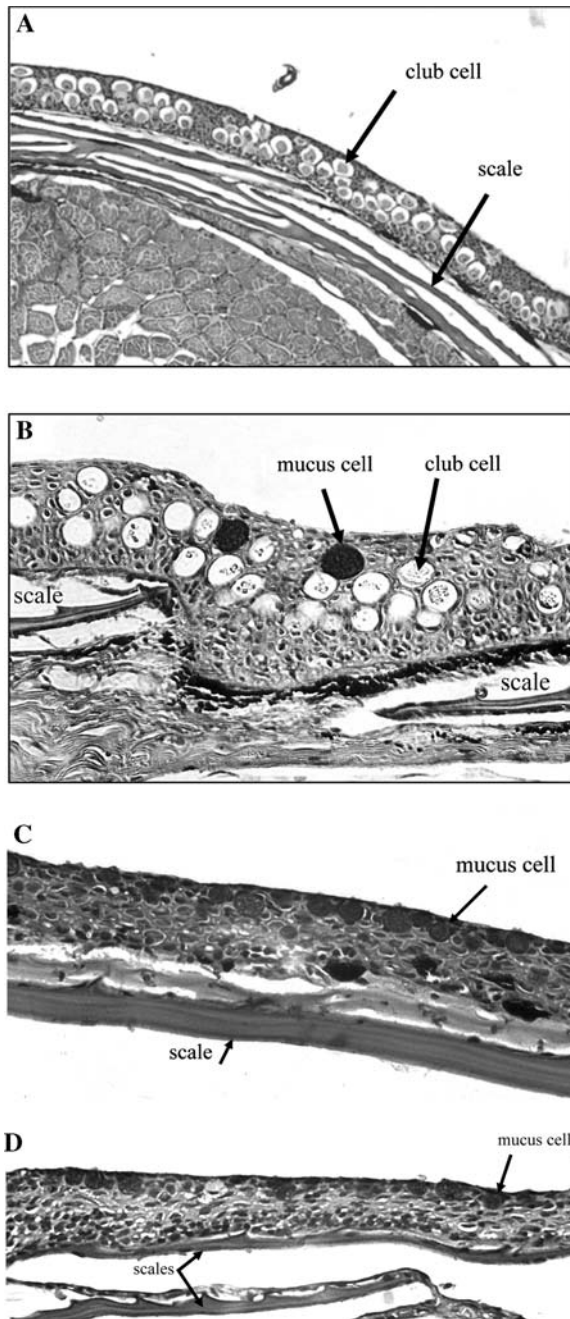


Fig. 5 Histological sections of fish skin reveal abundant club cells in (A) redbelly dace (Ostariophysi, Cyprinidae and *Phoxinus eos* Cope 1862), (B) walleye (Acanthopterygii, Percidae and *Sander vitreum* Oken 1817) but none in the (C) adult mudminnow (Procantopterygii, Umbridae and *Umbra limi* Kirtland 1840) or (D) juvenile mudminnow. Tissues collected from the nape region on the dorsal surface between the head and dorsal fin. Magnification = $\times 400$. All photos by B. D. Wisenden

that area avoidance is not as well developed in mudminnows as it is in ostariophysan species. There may well have been a foraging—anti-predator trade-off that caused fish to enter alarm-scented traps. However, there was no indication that skin extract was interpreted as a food cue by large cannibalistic mudminnows because the median size of fish caught in alarm and water traps was small and did not differ between treatments.

The laboratory experiment was more sensitive to the detection of an anti-predator response to alarm cues than was the field experiment. Most studies on alarm reactions to injury-released chemical cues have been conducted in laboratory aquaria where experimental power to detect an effect is high but ecological realism is potentially compromised by artificial laboratory conditions. Observations of natural populations of free-swimming European minnows, *Phoxinus phoxinus* L., using underwater video cameras sometimes show no (Magurran et al. 1996) or transient (Irving and Magurran 1997) responses to chemical alarm cues. These observations lend support to the hypothesis that alarm reactions are intensified in enclosed spaces such as laboratory aquaria or minnow traps, but less apparent or absent under open field conditions. Although subsequent studies using underwater video observations of unconstrained natural minnow populations have affirmed a field response to chemical alarm cues (Wisenden et al. 2004a; Wisenden and Barbour 2005; Friesen and Chivers 2006), the mudminnow response to alarm cues in the current study suggests that laboratory methods produce more demonstrable alarm reactions than do field methods. In the context of the “confined space” hypothesis, it is important to note that both methods in the current study observed alarm reactions in confined spaces. Three factors may have contributed to the weaker response in the field experiment than the laboratory experiment. First, mudminnows are crepuscular (Martin-Bergmann and Gee 1985) and have limited movement during daylight hours when the field experiment was conducted, and thus, mudminnows may have had limited vulnerability to entrapment. Limited sampling weakens the power of the experiment to detect an effect. Second, the presence of dense macrophytes in the field site may have limited fish movement, and encounter rate, with our minnow traps. Third, the risk-allocation

hypothesis predicts that the degree to which mudminnows live under constant threat of predation in their natural habitat may inure them somewhat to chemical alarm cues and cause mudminnows in the field to allocate more importance to foraging (Lima and Bednekoff 1999). In benign laboratory conditions the sudden appearance of chemical alarm cues may be perceived as a relatively greater change in predation risk causing mudminnows to allocate more importance to risk avoidance (Mirza et al. 2006).

Mudminnow skin does not contain club cells analogous to the cells that have been implicated in the alarm cue system of ostariophysans (Pfeiffer 1977), percids (Smith 1979, 1982; Wisenden et al. 2004b), poeciliids (Garcia et al. 1992) and eleotrids (Kristensen and Closs 2004). Club cells have been a distraction from the study of chemical alarm cues because it has been long assumed that club cells are causally linked to the production of an alarm substance, or *Schreckstoff*, that signals alarm to nearby conspecifics. However, club cells are likely maintained by non-alarm functions (Smith 1986) such as wound healing following parasite attack, exposure to ultraviolet radiation or other damage to epidermal tissue (Wisenden and Stacey 2005; but see Iger and Abraham 1990). For species with club cells, there is selection on receivers to elaborate receptors to detect chemicals released from damaged epithelial tissue because this is useful public information about predation risk (Wisenden and Stacey 2005; Wisenden and Chivers 2006). However, there is no selection on senders to create a special class of cell to produce compounds with ideal properties for transport through water or that maximally stimulate olfactory receptors because individuals that produce the cells (senders) do not benefit from the behavioural response of the receivers. So-called “alarm substance cells” of the ostariophysans, percids, et al., have assumed an alarm function secondarily. Their primary function is for healing damage caused by parasite attack and exposure to ultraviolet radiation (Wisenden, Alemadi, James, Corwin, Savaloja and Goater unpublished data). Data from the current study demonstrate that Umbrids can be added to the growing list of fish groups without epidermal club cells (see Chivers and Smith 1998 for review) that nevertheless exhibit alarm reactions to conspecific alarm cues. Most aquatic animals use conspecific

alarm cues to guide anti-predator responses (e.g. Chivers and Smith 1998; Wisenden 2003). Because alarm reactions without specialised cells are the general norm among aquatic non-fish taxa, it follows logically that epidermal club cells did not have an origin related to alarm function and are not maintained by their role as a contributor to alarm cue today.

Acknowledgements Funding was provided by faculty research grants to BDW from the MSUM College of Social and Natural Sciences. The authors thank the Steven Zissou Foundation.

References

- Bry C, Basset E, Rognon X, Bonamy F (1992) Analysis of sibling cannibalism among pike, *Esox lucius*, juveniles reared under semi-natural conditions. *Environ Biol Fish* 35:75–84
- Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Écoscience* 5:338–352
- Danchin É, Giraldeau L-A, Valone TJ, Wagner RH (2004) Public information: from nosy neighbors to cultural evolution. *Science* 305:487–491
- Friesen RG, Chivers DP (2006) Underwater video reveals strong avoidance of alarm cues by prey fishes. *Ethology* 112:339–345
- Garcia C, Rolan-Alvarez E, Sanchez L (1992) Alarm reaction and alert state in *Gambusia affinis* (Pisces, Poeciliidae) in response to chemical stimuli from injured conspecifics. *J Ethol* 10:41–46
- Iger Y, Abraham M (1990) The process of skin healing in experimentally wounded carp. *J Fish Biol* 36:421–437
- Irving PW, Magurran AE (1997) Context-dependent fright reactions in captive minnows: the importance of naturalness in laboratory experiments. *Anim Behav* 53:1193–1201
- Kristensen EA, Closs GP (2004) Anti-predator response of naïve and experienced common bully to chemical alarm cues. *J Fish Biol* 64:643–652
- Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am Nat* 153:649–659
- Magurran AE, Irving PW, Henderson PA (1996) Is there a fish alarm pheromone? A wild study and critique. *Proc R Soc Lond Ser B* 263:1551–1556
- Martin-Bergmann KA, Gee JH (1985) The central mudminnow, *Umbra limi* (Kirtland), a habitat specialist and resource generalist. *Can J Zool* 63:1753–1764
- 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, Mathis A, Chivers DP (2006) Does temporal variation in predation risk influence the intensity of

- anti-predator responses? A test of the risk allocation hypothesis. *Ethology* 112:44–51
- Nelson JS (1994) *Fishes of the world*, 4th edn. Wiley, New York
- Pfeiffer W (1960) Über die Schreckreaktion bei Fischen und die Herkunft des Schreckstoffes. *Z vergl Physiol* 43: 578–614
- Pfeiffer W (1977) The distribution of fright reaction and alarm substance cells in fishes. *Copeia* 1977:653–665
- Schutz F (1956) Vergleichende Untersuchungen über die Schreckreaktion bei Fischen und deren Verbreitung. *Zeitschr vergl Physiol Psychol* 38:84–135
- Smith RFJ (1979) Alarm reaction of Iowa and Johnny darters (*Etheostoma*, Percidae, Pisces) to chemicals from injured conspecifics. *Can J Zool* 57: 1278–1282
- Smith RJF (1982) Reaction of *Percina nigrofasciata*, *Ammocrypta beani*, and *Etheostoma swaini* (Percidae, Pisces) to conspecific and intergeneric skin extracts. *Can J Zool* 60:1067–1072
- Smith RJF (1986) The evolution of chemical alarm signals in fishes. In: Duvall D, Schwarze D, Silverstein RM (eds) *Chemical signals in vertebrates vol 4*. Wiley, New York, pp 99–115
- Smith RJF (1992) Alarm signals in fishes. *Rev Fish Biol Fish* 2:33–63
- Tonn WM (1985) Density compensation in *Umbra-Perca* fish assemblages of northern Wisconsin lakes. *Ecology* 66:415–429
- Tonn WM, Paszkowski CA (1987) Habitat use of the central mudminnow (*Umbra limi*) and yellow perch (*Perca flavescens*) in *Umbra-Perca* assemblages: the roles of competition, predation, and the abiotic environment. *Can J Zool* 65:862–870
- von Frisch K (1938) Zur Psychologie des Fisch-Schwarmes. *Naturwissenschaften* 26:601–606
- von Frisch K (1941) Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Zeitschr vergl Physiol* 29:26–145
- Wisenden BD (2003) Chemically-mediated strategies to counter predation. In: Collin SP, Marshall NJ (eds) *Sensory processing in aquatic environments*. Springer, New York
- Wisenden BD, Barbour KA (2005) Antipredator responses to skin extract of redbelly dace by free-ranging populations of redbelly dace and fathead minnows. *Environ Biol Fish* 72:227–233
- Wisenden BD, Chivers DP (2006) The role of public chemical information in antipredator behaviour. In: Ladich F, Collins SP, Moller P, Kapoor BG (eds) *Communication in fishes*. Science Publisher, Moscow
- Wisenden BD, Smith RJF (1997) The effect of physical condition and shoal-mate familiarity on proliferation of alarm substance cells in the epidermis of fathead minnows. *J Fish Biol* 50:799–808
- Wisenden BD, Stacey NE (2005) Fish semiochemicals and the network concept. In: McGregor PK (ed) *Animal communication networks*. Cambridge University Press, Cambridge
- Wisenden BD, Vollbrecht KA, Brown JL (2004a) Is there a fish alarm cue? Affirming evidence from a wild study. *Anim Behav* 67:59–67
- Wisenden BD, Klitzke J, Nelson R, Friedl D, Jacobson P (2004b) Fisheries enhancement potential and logistical constraints of using chemical alarm cues to condition hatchery-reared walleye to avoid pike odour. *Can J Fish Aquat Sci* 62:2144–2150