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Is there a fish alarm cue? Affirming evidence from a wild study

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Chemical alarm cues released from injured tissue are not released under any other context and therefore reliably inform nearby prey of the presence of a predator. Laboratory and field studies have demonstrated that most aquatic taxa show antipredator responses to chemical alarm cues. Ostariophysan fish (e.g. minnows) possess specialized skin cells that contain an alarm chemical. Magurran et al. (1996, *Proceedings of the Royal Society of London, Series B,* **263,** 1551–1556) were the first to use underwater video to carefully document the behavioural response of free-ranging wild populations of minnows to minnow alarm cues. They found no evidence of an antipredator response, and challenged the assumption that the contents of these cells indicate risk in the field. They proposed that alarm responses are context dependent in that they are an artefact of enclosed environments such as laboratory aquaria and field traps. Here, we repeat their experiment on free-swimming field populations of littoral fish and report a significant decrease in the number of fish in areas where chemical alarm cues of blacknose shiners, *Notropis heterolepis* (Ostariophysi: Cyprinidae) were released. The effect of these chemical cues was equal in magnitude to the effect of the presentation of a model predator. The response to the approach of a model predator (visual cue) was intensified by pre-exposure to chemical alarm cues. We corroborated this interaction between chemical and visual indicators of predation risk in a laboratory study using glowlight tetras, *Hemigrammus erythrozonus* (Ostariophysi: Characidae). Response to the visual stimulus of a predator was significantly intensified by previous exposure to conspecific chemical alarm cues. We conclude that ostariophysan skin indeed contains an alarm cue that (1) informs nearby prey of imminent predation risk, (2) induces some form of antipredator behaviour in most contexts, and (3) affects subsequent behavioural responses to stimuli in other sensory modalities.

The selection gradient to detect and respond adaptively to predation risk is steep and unforgiving. Organisms whose many generations of ancestors have survived to reproduce and pass on their behavioural strategies can be expected to be acutely tuned to subtle indicators of predation risk. Aquatic invertebrates and vertebrates are well endowed with sensitive olfactory receptors for biologically important molecules, including those associated with the detection of predation risk (Dodson et al. 1994; Chivers & Smith 1998; Kats & Dill 1998). Predatory attack causes injury and the passive release of chemical compounds from internal body tissues of prey. The release of these cues is confined to the context of predation. These chemicals therefore serve as alarm cues and induce antipredator behaviour in nearby prey. As testimony to the pervasive impact of predation on natural selection, and the role of chemical cues in risk assessment, a diverse array of aquatic taxa ranging from ciliates to amphibians have antipredator responses to injury- or damage-released cues (reviewed in: Chivers & Smith 1998; Wisenden 2003).

Given the ubiquitous nature of injury-released alarm cues among aquatic taxa, it is not surprising that minnows use them too. The ostariophysan alarm 'signalling' system (Smith 1992; Wisenden & Stacey, in press) is of interest to evolutionary biologists because these cells exact a significant metabolic cost for their production and maintenance (Wisenden & Smith 1997). However, the mechanism by which the investor benefits from its investment is not immediately obvious (Williams 1992). A number of hypotheses have been advanced for mechanisms for selfish benefits from alarm substance cells (Smith 1992), one of which has received experimental support (Mathis et al. 1995; Chivers et al. 1996).

The alarm cue function of ostariophysan skin extract is well established in the literature (Chivers & Smith 1998).

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Laboratory (e.g. Lawrence & Smith 1989), seminatural fluvarium (Irving & Magurran 1997) and field (e.g. von Frisch 1938; Mathis & Smith 1992; Wisenden et al. 1995) studies demonstrate clear behavioural responses to these cues. Magurran et al. (1996) used underwater video cameras to observe the behavioural responses of free-swimming natural populations of minnows, *Phoxinus phoxinus*, and surprisingly, found no evidence of an antipredator response to chemical alarm cues. Based on these results, Magurran et al. (1996) proposed that the alarm response of littoral fish may be an artefact of the confining context of laboratory aquaria and field traps. In another study, Irving & Magurran (1997) tested alarm responses of minnows in a seminatural fluvarium and found that minnows that received skin extract showed a significant increase in shoal cohesion and a significant decrease in foraging, but showed no skittering (dashing) or cessation of activity typical of alarm behaviour in captivity. These results were interpreted as a weak response (relative to laboratory tests). Henderson et al. (1997) later summarized context-dependent alarm behaviour in fish as follows: strong responses in the laboratory, weak responses in seminatural conditions (fluvarium) and no response in the wild.

Magurran et al.'s (1996) results stirred some controversy, and the ensuing exchange (Henderson et al. 1997; Smith 1997) produced confusion over the nature of the principal question. There are, in fact, two questions at the heart of the matter. One question pertains to the evolutionary origin of alarm substance cells and the function, or multiple functions, that they have at present and may have had in the evolutionary past. The other question pertains to whether or not alarm substance cells of present-day ostariophysans induce overt antipredator behaviour in wild populations of fish. Although the evolutionary origin of alarm substance cells is unresolved, their function in alerting littoral fish to the presence of predation risk cannot be in doubt, Magurran et al. (1996) notwithstanding. The sheer volume and diversity of observational data from laboratory and field trap studies on chemically induced alarm behaviour among aquatic animals indicate adaptive use of public information to reduce predation risk. Henderson et al. (1997) concluded with a call for more field experiments to explore this phenomenon further, especially those that make use of underwater video. In this paper, we set out to do exactly that, by repeating the experiment by Magurran et al. (1996) using an underwater video camera to record the response of wild populations of free-swimming fish to minnow skin extract.

We also set out to determine the natural responses of fish to a visual presentation of threat from a model predator, in the context of a wild population. Even though model predators induce antipredator behaviour in laboratory aquaria, minnows in the wild may ignore the presence of a predator until an attack occurs. Missing from this discussion of context-dependent alarm behaviour is the unstated assumption that fish should flee a chemical alarm stimulus. Minnows naturally shoal because they are under constant threat and a shoal remains the safest place to be when predation risk is detected. An overt behavioural response to chemical or visual indicators of predation risk may be maladaptive in some contexts. Moreover, response to the visual presence of a predator may be influenced by previous release of chemical alarm cues (Magurran & Pitcher 1987; Levesley & Magurran 1988; Brown & Godin 1999a; Wisenden et al. 2003). Therefore, we designed our experiment to test the independent effects of chemical and visual sources of information about predation risk, and their interaction.

Having accomplished our first two objectives, we took the additional step of repeating our field experiment on the interaction between chemical and visual cues in the laboratory setting. By combining two related data sets within the same paper, we hoped to combine the ecological realism of the field setting with the experimental power of the laboratory setting.

METHODS

Field Experiment

Field data were collected at three sites in Minnesota: Lake Ozawindib (47°14′ N, 95°16′ W, 61-ha area), Long Lake (47°17′ N, 95°18′ W, 67-ha area) and Big Cormorant Lake (46°46′ N, 96°03′ W, 1.384-kha area). Each lake is host to a diverse ichthyofauna, including large shoals of cyprinids, primarily blacknose shiners, *Notropis heterolepis* (Ostariophysi: Cyprinidae) and piscivores (northern pike, *Esox lucius*; muskellunge, *Esox musquinongy*; largemouth bass, *Micropterus salmoides*; rock bass, *Ambloplites rupestris*; pumpkinseed sunfish, *Lepomis gibbosus*; walleye, *Stizostedion vitreum*; yellow perch, *Perca flavescens*) and, in Long Lake, stocked rainbow trout, *Oncorhynchus mykiss*. The relative abundance of piscivores varies among lakes, regions within each lake, and over annual, seasonal and daily time scales. However, it is safe to assume that small fish in all the study lakes face intense predation pressure. Loons, *Gavia immer*, kingfishers, *Ceryle alcyon*, herons, *Ardea herodias*, and larval dragonflies (Insecta: Odonata) also prey on small fish of the littoral zone.

The behaviour of littoral fish was viewed using an Atlantis 'mini' underwater video camera (model number AUW-401) submerged at a depth of about 50 cm, about 2 m from the shore (Fig. 1). Camera placement was in an open area away from structures that might obstruct the view of the camera. The area viewed by the camera was about 2×2 m. The weighted camera rested on the substrate. A 15-m waterproof cable led from the camera to shore, where it was out of view of the fish, attached to a monitor and an analogue video-cassette recorder (Sony TRV65 camcorder). All trials were recorded on Hi8 video cassettes and scored later. Standard aquarium airline tubing (internal diameter = 4 mm, total internal volume of the airline tubing was 60 ml) was attached at one end to a small stick. The stick was used as a stake to position the end of the tube about 15 cm above the substrate and 30 cm in front of, and in full view of, the camera. The tubing led to shore where test chemical cues could be injected surreptitiously (Fig. 1).

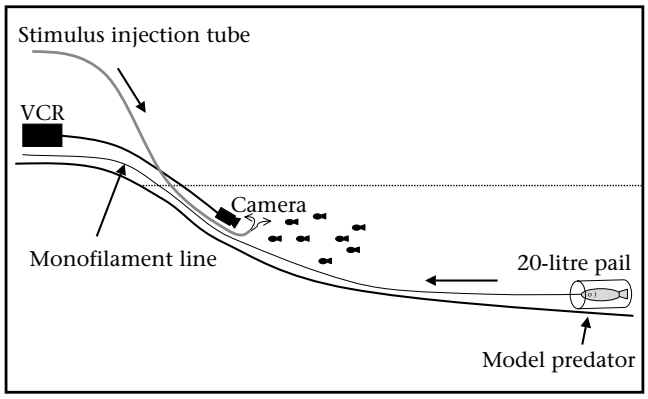

Figure 1. Arrangement of the underwater video camera, tube for injection of chemical stimuli and location of the model predator during the field experiment.

A wooden model predator (total length = 43 cm) that resembled a rainbow trout was weighted along the ventral edge to keep the model oriented upright and close to the substrate. The model was attached to the inside bottom of a 20-litre pail submerged on its side about 10 m away in deep water to shield the predator's presence until needed. At the appropriate time the model was pulled along a taut monofilament line that ran at an oblique angle from the pail to shore, passing through the area in view of the camera where the injection hose was also located (Fig. 1).

We captured some blacknose shiners with a net outside the study area before the trial to serve as stimulus donors. To prepare chemical alarm cue stimulus, shiners were humanely killed by cervical dislocation with a razor blade. The skin on each flank was cut lightly 10 times and the fish was rinsed with 60 ml of lake water. The rinse water, or skin extract, constituted the chemical alarm cue stimulus. A single donor fish was used per trial. As a control, blank lake water was used. To prevent potential contamination, separate syringes were used for chemical alarm and water control test stimuli, and the injection tubing was replaced after each trial.

Experimental protocol

The camera, injection tube and model predator were set in place 15 min before any data were collected. The tubing was rinsed with lake water by drawing 60 ml through the tube three times and discarding the water on shore. A final 60 ml of lake water was retained and used to flush the test cue out of the injection tube during the trial. Video recording ran continuously throughout the trial. We recorded prechemical stimulus behaviour for 5 min. During the sixth minute, we injected 60 ml of either water control stimulus or skin extract followed by 60 ml of lake water to flush the cue from the tube. At the start of the seventh minute, we recorded postchemical stimulus behaviour for 2 min. At the start of the ninth minute, we pulled the model predator towards the shoreline in view of the camera (time ~10 s). When the model predator was at the shoreline, we recorded postpredator behaviour for 3 min. We conducted 11 trials using water stimulus and 11 trials using skin extract stimulus (three of each at Ozawindib Lake, and four of each at Long and Big Cormorant Lakes).

Data analysis

All trials were videotaped, and behavioural data were scored from video playback. We counted the number of fish in view of the camera at 10-s intervals. These scores were summed and averaged to produce a score of area use expressed as the number of fish per minute in the field of view of the camera. We could not always confidently identify fish species from the video image. It was apparent that more than one species of small littoral fish passed the camera during the trials. However, if these fish occur in the same habitat and are presumably vulnerable to similar predators, then chemical alarm cues from one species indicate risk to them all. Cross-species reactions within a prey guild are well known (see Chivers & Smith 1998 for review). We used Wilcoxon matched-pairs signed-ranks tests (Siegel & Castellan 1988) to assess the magnitude of the change in area use after presentation of chemical cues, and later, after the visual presentation of the model predator. We used Mann–Whitney tests to compare the effect of cue (water or alarm) on the magnitude of the change in area use after chemical stimulus, and again after the presentation of the model predator. We followed this analysis with analysis of covariance (ANCOVA) on the effect of chemical stimulus (by itself, or as a primer of the response to the model predator) on area use, with prechemical stimulus area use as a covariate. All statistical tests are based upon two-tailed probability distributions.

Laboratory Experiment

We purchased glowlight tetras, *Hemigrammas erythrozonas* (Ostariophysi: Characidae) from a commercial supplier. Before testing, glowlight tetras were held in 75-litre aquaria at 26 °C on a 12:12 h light:dark cycle and fed commercial flake food. Test aquaria were 37 litres in volume and contained a 100-W heater, an air-powered sponge filter and a thin layer of naturally coloured sand. A 5×10 grid (cells = 5×5 cm) was drawn on the front (long) pane of each test aquarium for use in scoring fish position and movement (see below). A second airline tube was wedged into the lift tube of the sponge filter through which test stimuli could be injected. The rising air and water currents from the sponge filter quickly dispersed test stimuli throughout the aquarium and masked any turbulence associated with stimulus injection. Chemical stimuli were prepared before each trial. An individual tetra was humanely killed by cervical dislocation and each flank was scored 10 times with a razor blade. We dribbled 10 ml of dechlorinated tap water over the fish and collected the water in a glass beaker. This skin extract was used as the chemical alarm stimulus. Control trials used 10 ml of blank dechlorinated tap water.

Experimental protocol

Two glowlight tetras were added to each test aquarium. Adjacent to each test aquarium was a second 37-litre aquarium called the visual presentation tank. It was

similarly equipped with a sponge filter, a 100-W heater and a thin layer of gravel. The test and visual presentation aquaria were placed with their small sides together, but separated by an opaque, removable barrier. We recorded three behaviours for 5 min (prechemical stimulus behaviour). Activity was determined by counting the total number of grid lines crossed by the pair of tetras. Vertical distribution, taken at 15-s intervals, was recorded as the horizontal zones formed by the grid that was occupied by each fish. The five zones were assigned values from 1 (bottom) to 5 (uppermost). These values were summed for the two fish in each trial over each minute. Vertical distribution scores ranged in value from 200 (near the surface for all samples) to 40 (near the tank bottom for all samples). Distance to the presentation aquarium, taken at 15-s intervals, recorded which of the 10 vertical grid zones was occupied by each tetra. Proximity to the presentation aquarium was scored as risk (i.e. the zone nearest the predator was assigned a value of 10, the zone farthest from the predator was assigned a value of 1). Scores for both fish in each trial were summed for each minute; thus, horizontal distribution (risk) scores ranged from 400 (next to the predator tank for all samples) to 40 (at the end opposite to the predator presentation tank for all samples). After prechemical stimulus behaviour was recorded, we added 10 ml of test stimulus, flushed through the injection line with 60 ml of tank water retained from before the trial began. Chemical stimulus was slowly infused over 1 min, after which we immediately recorded activity, vertical distribution and horizontal distribution (postchemical stimulus behaviour) for 5 min. At the 11th min, we removed the barrier separating the test aquarium from the presentation aquarium. We waited 1 min to allow recovery from the disturbance of barrier removal, then recorded 5 min of postbarrier removal behaviour. The presentation aquarium was either a tank that contained an adult female convict cichlid, *Archocentrus nigrofasciatus* (average total length = 9.5 cm, range 7.6–11 cm) that could be perceived as a potential predator or an identical tank with no fish. We conducted 59 trials: 14 for the combination of water cue and predator in the presentation tank and 15 for each of the other three combinations of chemical and visual cues.

Data analysis

We used Wilcoxon matched-pairs signed-ranks tests (Siegel & Castellan 1988) to determine whether response behaviours for individual treatments and time combinations differed significantly from zero. We used Kruskal–Wallis one-way analyses of variance (ANOVAs) to compare the magnitude of the behavioural change between treatments within each time interval. All tests were based upon two-tailed probability distributions.

RESULTS

Field Experiment

There were significantly fewer fish in the view of the camera after the release of skin extract than after the release of water (Figs 2, 3). The change in area use did not deviate significantly from zero when alarm cue was presented (two-tailed Wilcoxon matched-pairs signed-ranks test: $T = -45$, $N = 9$, $P = 0.084$) or when water

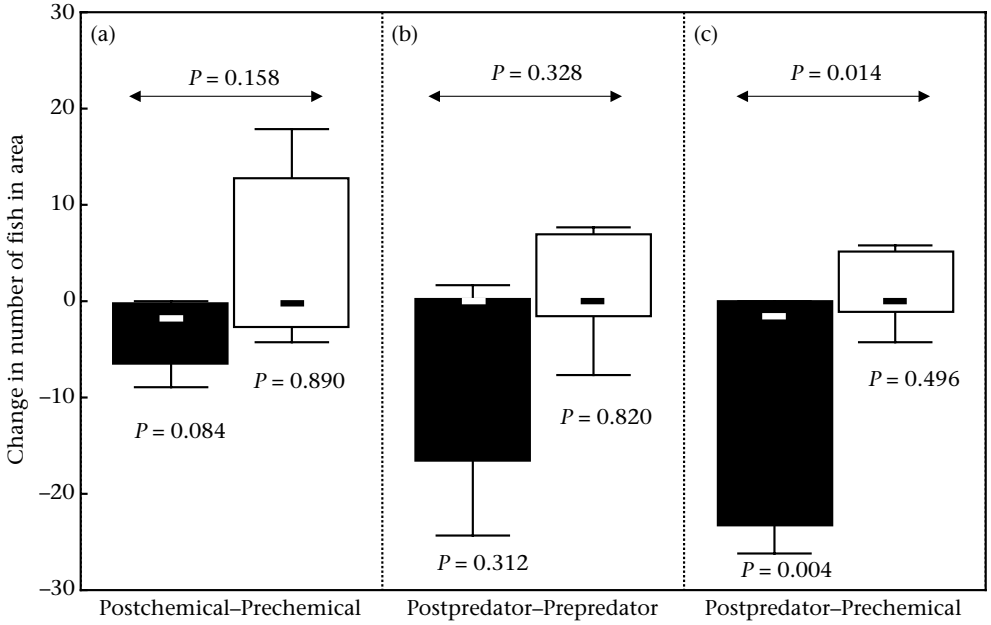

Figure 2. Change in the number of fish in natural habitats visible to the underwater camera. Horizontal lines are medians, boxes are upper and lower quartiles, vertical lines represent maximum and minimum values. Change in area use before and after introduction of (a) chemical alarm cues or water and (b) a model predator in areas scented with chemical alarm cues or water. (c) Overall change in area use by fish before the introduction of chemical cues and after chemical and visual stimuli were presented. ■: Alarm cue trials; □: water trials. Probability values above bars are from Mann–Whitney tests comparing the magnitude of the change between chemical cue treatments. Probability values below bars are from Wilcoxon matched-pairs signed-ranks tests comparing each bar's deviation from zero.

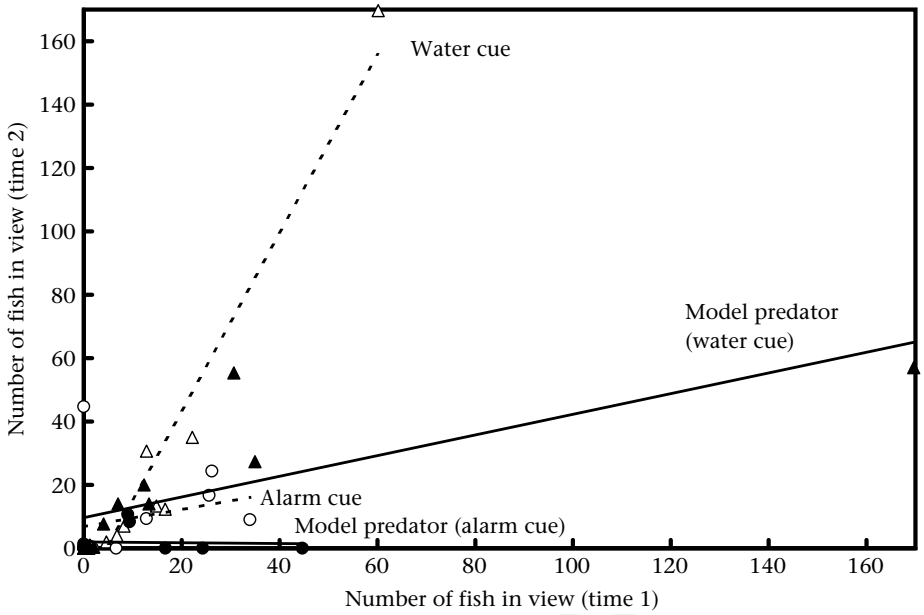

Figure 3. The number of fish in videotaped areas in natural habitats after a manipulation (time 2) versus before the manipulation (time 1). Dashed lines denote the effects of chemical cues alone and solid lines denote the change in area use after the approach of a model predator. △: Water cue alone; ▲: water cue trials before and after the approach of the model predator; ○: effect of chemical alarm cues; ●: chemical alarm cue trials before and after the approach of the model predator. Statistical analyses were performed on ln +1 transformed data.

was presented ($T = 24$, $N = 9$, $P = 0.890$; Fig. 2). Therefore, by this analysis, magnitude of the change in area use was not significantly affected by cue alone (Mann–Whitney test: $Z = 1.41$, $N_1 = N_2 = 11$, $P = 0.158$). The magnitude of the additional change in area use in response to the model predator did not differ from zero for fish prewarned with chemical alarm cues ($T = -26$, $N = 8$, $P = 0.312$) or for fish prewarned with water ($T = 24.5$, $N = 9$, $P = 0.820$; Fig. 2). Not surprisingly, the magnitude of the effect of predator did not differ between the cue treatments ($Z = 0.985$, $N_1 = N_2 = 11$, $P = 0.328$). The combined effect of chemical and visual cues (i.e. from the prechemical stimulus period to the postpredator period) reduced the number of fish using the area for alarm cue trials ($T = -45$, $N = 9$, $P < 0.004$) but not for water trials ($T = 30$, $N = 9$, $P = 0.496$). The magnitude of change in area use differed significantly between the cue treatments for the effect of cue and predator combined ($Z = 2.46$, $N_1 = N_2 = 11$, $P < 0.014$; Fig. 2).

Comparing post- and prechemical stimulus area use, the slope of the alarm cue trials differed significantly (interaction term) from the slope of the water trials (ANCOVA: cue: $F_{1,18} = 6.17$, $P = 0.023$; prechemical stimulus area use: $F_{1,18} = 50.18$, $P < 0.001$; cue∗prestimulus area use: $F_{1,18} = 33.75$, $P < 0.001$; Fig. 3). The change in the number of fish in view of the camera in response to chemical alarm cues was not different from the response to the visual presentation of the model predator in trials where water was first presented (ANCOVA: cue: $F_{1,18} = 1.47$, $P = 0.706$; prechemical stimulus (min 1–5 for chemical alarm cue trials, min 7–9 for water cue trials): $F_{1,18} = 2.654$, $P = 0.12$; cue∗prestimulus area use: $F_{1,18} = 0.019$, $P = 0.89$; Fig. 2). Therefore, skin extract of cyprinids functioned as an alarm cue. The ANCOVA approach did not detect an effect of chemical cue on additional response to the visual cue (cue: $F_{1,18} = 2.12$, $P = 0.16$; premodel behaviour: $F_{1,18} = 1.72$, $P = 0.21$; cue∗premodel behaviour: $F_{1,18} = 1.97$, $P = 0.18$; Fig. 3).

Laboratory Experiment

Chemical alarm cues caused tetras confined in laboratory aquaria to significantly reduce activity (Table 1, Fig. 4) and move towards the top of the tank (Table 1, Fig. 5) but had no effect on distance to the barrier shielding the predator presentation tank (Table 1, Fig. 6). When the barrier was removed to reveal the presentation tank, the activity of tetras previously given alarm cues significantly increased (Table 1, Fig. 4). Tetras that had received chemical alarm cues moved from the surface towards the midcolumn (Table 1, Fig. 5) and they significantly increased distance from the presentation tank (i.e. reduction in risk) when it contained a predator (Table 1, Fig. 6). The behavioural response to the visual presentation of a cichlid in the presentation tank was significantly affected by chemical stimulus.

The overall effect of the chemical and visual cues combined (i.e. comparing the change in behaviour from the prechemical stimulus period to the postbarrier removal period) revealed conflicting responses to the two sensory modalities. Activity was reduced in response to the chemical alarm cue alone but increased when a cichlid was presented (Table 1), resulting in no net change (Table 1, Fig. 4). Similarly, alarm cues caused tetras to move towards the surface but the visual presence of a predator caused them to move towards the bottom. The net effect of both alarm and predator cues was no overall change (Table 1, Fig. 5). Only tetras that received chemical alarm

Table 1. Change in activity, vertical distribution and distance to predator (risk) between (1) pre- and postchemical stimulus (effect of chemical cues), (2) postchemical stimulus and postbarrier removal (effect of visual cues) and (3) prechemical stimulus and postbarrier removal (effect of chemical and visual cues combined)

	Effect of											
Treatment	Chemical cues				Visual cues				Chemical and visual cues			
Chemical/visual	*T*	*N*	*P*	H_3*	*T*	*N*	*P*	H_3*	*T*	*N*	*P*	H_3*
Activity												
Water/E	−82.5	15	0.230	a	−66	15	0.762	a	+71	15	0.562	a
Water/P	+62	14	0.584	a	+54	14	0.952	a	+60.5	14	0.670	a
Alarm/E	−110	15	0.003	b	−74	15	0.454	a	−116	15	0.001	b
Alarm/P	−120	15	0.001	b	+104	15	0.010	a	−72	15	0.524	a
ANOVA†	$H_3 = 28.84$, $P<0.001$				$H_3 = 7.01$, $0.1 > P > 0.05$				$H_3 = 16.10$, $P<0.001$			
Vertical distribution												
Water/E	−57	14	0.808	a	+84	14	0.494	a	+81.5	15	0.229	ab
Water/P	−82	14	0.068	a	−80.5	14	0.085	ab	−93.5	14	0.008	b
Alarm/E	+96	14	0.004	b	−27	8	0.250	a	+94.5	14	0.006	a
Alarm/P	+105	14	0.001	b	−103	14	0.001	b	−77	13	0.027	b
ANOVA†	$H_3 = 14.16$, $P<0.010$				$H_3 = 22.10$, $P<0.001$				$H_3 = 18.59$, $P<0.001$			
Risk												
Water/E	+67	15	0.720	a	−67	15	0.720	a	+66	15	0.762	a
Water/P	+72	14	0.241	a	−71	14	0.268	a	−60	14	0.670	a
Alarm/E	+69	15	0.639	a	−89	15	0.107	a	−87	15	0.135	a
Alarm/P	−89	15	0.054	a	−117	15	0.001	a	−120	15	0.001	b
ANOVA†	$H_3 = 4.21$, $0.3 > P > 0.2$				$H_3 = 7.49$, $0.1 > P > 0.05$				$H_3 = 18.87$, $P<0.001$			

E: empty presentation tank; P: predator in presentation tank. Wilcoxon matched-pairs signed-ranks test for deviation from zero, sample size usable for test and two-tailed probability of random chance.
*Post hoc alpha-adjusted pairwise comparisons following a Kruskal–Wallis one-way ANOVA (shared letters indicate no difference at $P<0.05$).
†Overall Kruskal–Wallis ANOVA for comparisons between treatments.

cues and that were subsequently shown a cichlid showed a significant aversive reaction to the presentation tank (Table 1, Fig. 6). The laboratory data, like those of the field experiment, indicate that chemical alarm cues primed fish for a behavioural response to a visual stimulus.

DISCUSSION

There are two principal findings in this study. First, chemical cues in ostariophysan skin alert wild, free-ranging populations of littoral fish to the presence of

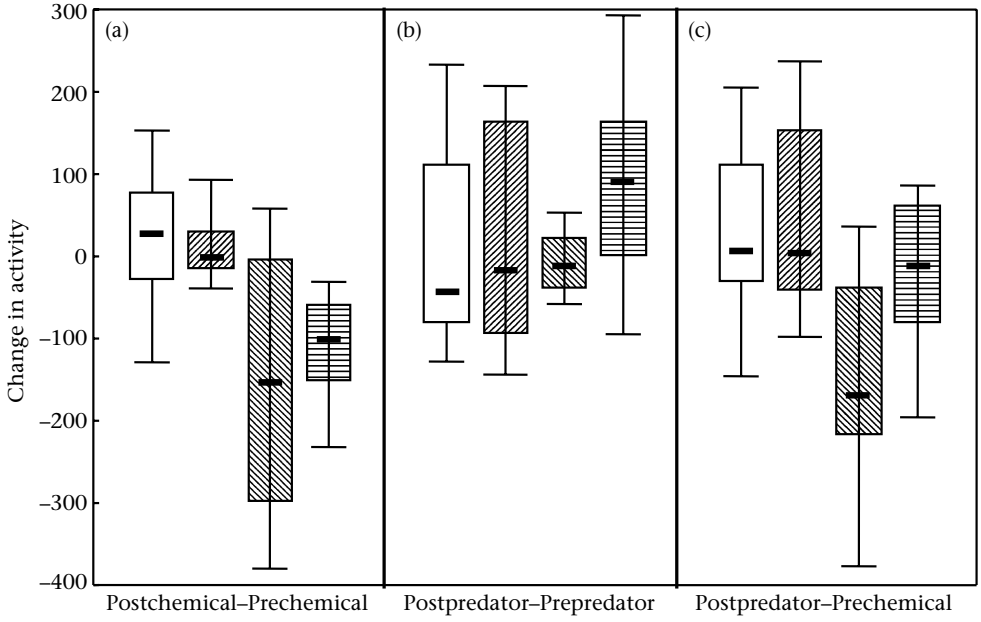

Figure 4. Change in activity of two glowlight tetras after (a) the introduction of a chemical cue and (b) the removal of a barrier that revealed a second aquarium that contained a cichlid fish or nothing. (c) Overall effect of chemical and visual indicators of predation risk. Figure format is as described for Fig. 2. □: Water cue + no predator; ▨: water cue + predator; ▧: alarm cue + no predator; ▤: alarm cue + predator.

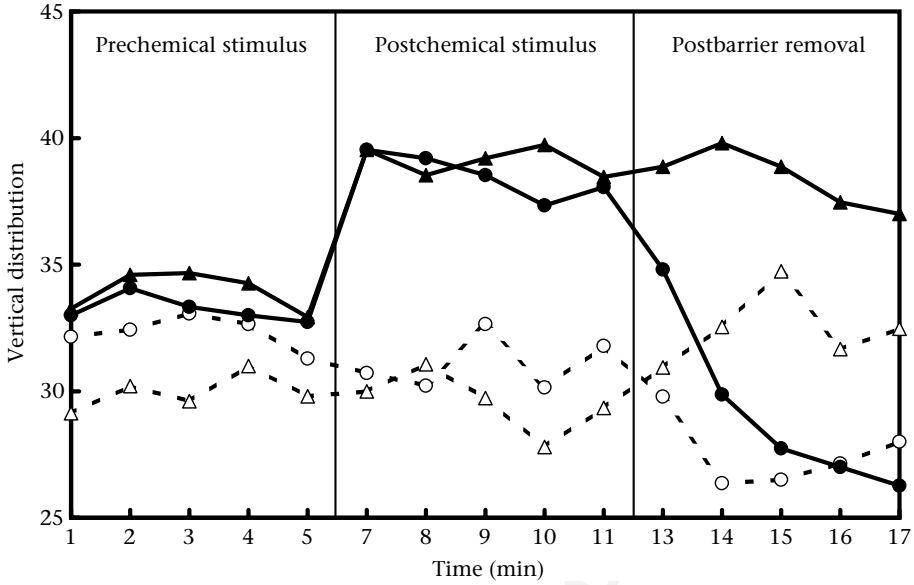

Figure 5. Mean vertical distribution for pairs of glowlight tetras (a) before the introduction of a chemical cue and (b) after the introduction of a chemical alarm cue (solid symbols and lines) or water (open symbols, dashed lines), and (c) after the removal of a barrier that revealed a second aquarium that contained nothing (triangles) or an adult convict cichlid (circles). Larger scores of vertical distribution indicate greater distance from the tank bottom.

predation risk. In the context of wild field populations, the effect of chemical alarm cue alone was the same as the effect of a model predator alone (Fig. 3). The hypothesis that alarm reactions are an artefact of enclosed spaces is therefore refuted by these data. The alarm response was indeed context dependent, in that the response intensity in the field was less than that typically seen in laboratory aquaria. However, there can be no doubt that chemical alarm cues function to alert wild prey fish to the presence of predation risk (see Brown & Godin 1999b for a non-ostariophysan example). The change in area use observed in this study (Fig. 3) also appeared to be present in nascent form in the data published by Magurran et al. (1996, Table 1, page 1554). A plot of the number of fish in view before versus after release of alarm cues produced a slope of 0.613, whereas the change in area use before versus after release of muscle tissue (control) produced a slope of 1.32. In other words, for any given level of area use before cue

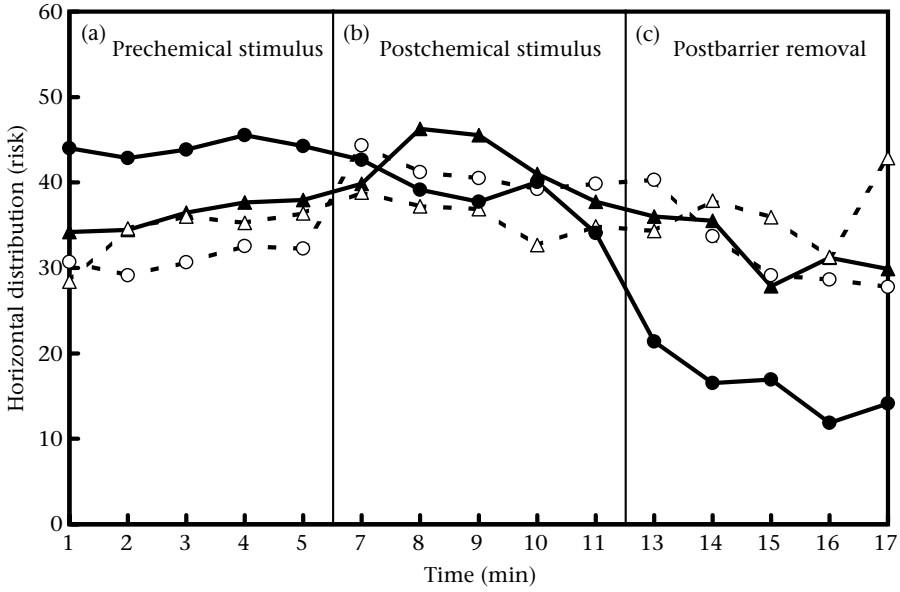

Figure 6. Mean horizontal distribution for pairs of glowlight tetras (a) before the introduction of a chemical cue and (b) after the introduction of a chemical alarm cue (solid symbols and lines) or water (open symbols, dashed lines), and (c) after the removal of a barrier that revealed a second aquarium that contained nothing (triangles) or an adult convict cichlid (circles). Larger scores of horizontal distribution indicate closer proximity to the presentation tank, and thus, greater predation risk for trials in which the presentation tank contained a cichlid.

release in Magurran et al.'s (1996) study, less than half as many fish were in the same area after the release of alarm cue compared with the number before and after the release of the muscle tissue control (ANCOVA interaction term of ln +1 transformed data: $F_{1,12} = 0.83$, $P = 0.38$). One possible explanation for the absence of a detectable response reported by Magurran et al. (1996) may be that they summed videotaped behavioural responses over 30-min observation periods, which may have included about 2 min of response and 28 min of normal shoaling behaviour. In the context of a wild population, the omnipresent threat of predation may select for a brief response and quick recovery once the immediate danger has passed, with perhaps extended periods of heightened vigilance (Bryant 1987; Wisenden et al. 1995).

Fathead minnows, *Pimephales promelas*, and brook stickleback, *Culaea inconstans*, do not enter unscented traps for at least 2 h after trap sites are chemically labelled with minnow alarm cue (Wisenden et al. 1995). The time frame of 30 min used by Magurran et al. (1996) should have detected avoidance if unstructured areas (i.e. no traps) were also avoided for 2 h. We therefore concur with Henderson et al. (1997) that enclosure probably explains the greater intensity of alarm reaction in laboratory aquaria and the greater duration of area avoidance in trap studies than in open water. We predict that relative to alarm reactions in open water, field populations of fish should show more intense alarm responses in very shallow water, small pools, or other naturally occurring confined spaces.

Alarm behaviour is more intense in laboratory aquaria with turbid water than in clear water (Hartman & Abrahams 2000). Although we do not have measures of water clarity for our study sites, the water was sufficiently transparent for the camera to record fish images. We can only assume that this was also the case for similar studies using underwater video (Magurran et al. 1996; Irving & Magurran 1997). Hunger may also reduce or eliminate an overt behavioural response to chemical alarm cues (Smith 1982a, 1997; Brown & Smith 1996; Chivers et al. 2000). In the absence of an overt behavioural response, chemical alarm cues can nevertheless affect future behaviour by facilitating learned association of risk with correlates of predation events (Brown & Smith 1996; Brown et al. 2001a).

The second principal conclusion of this study is that the effects of chemical alarm cues extend beyond the inducement of immediate, overt behavioural antipredator responses. Our behavioural observations from the field and the laboratory indicate that the behavioural response to visual indicators of predation risk is enhanced by preexposure to chemical alarm cues. In the field, the interaction between chemical and visual cues was manifest as an intensification of the avoidance response to the model predator. In the laboratory, tetras preconditioned with chemical alarm cues were the only ones to show a significant avoidance response to the cichlid. In a similar laboratory experiment, Brown & Godin (1999a) showed that glowlight tetras approach convict cichlids more cautiously when the cichlids have fed on ostariophysan fish than when they have fed on a nonostariophysan fish. Chemical alarm cues heighten anticipation of a predatory attack (Magurran & Pitcher 1987; Levesley & Magurran 1988). Rapid response to the visual presence of a predator translates into reduced probability of predation (Lima & Dill 1990). This has been demonstrated in various aquatic taxa in laboratory studies (e.g. Hews 1988; Mathis & Smith 1993; Wisenden et al. 1999; Gazdewich & Chivers 2002).

Sunfish appeared after injection of alarm cues in two trials in Ozawindib Lake, and a small largemouth bass appeared during one of the alarm cue trials in Big Cormorant Lake. Although not statistically significant (Fisher's exact test: $P = 0.107$), this rate of attraction of potential predators may be biologically significant in maintaining ostariophysan alarm substance cells over evolutionary time (Mathis et al. 1995). Senders of chemical alarm cues benefit by attracting predators because predator–predator interactions create escape opportunities for the sender (Chivers et al. 1996). This provides a mechanism for a fitness benefit to offset the cost of making alarm substance cells (Wisenden & Smith 1997). Predator attraction to ostariophysan skin extract has been noted in other field studies (Brown et al. 2001b; Wisenden & Thiel 2001).

That chemical alarm cues are released by the skin or from specialized epidermal cells (in the case of the Ostariophysi) does not automatically lead to the conclusion that alarm signalling is the original or sole selection pressure that maintains their existence. Alarm substance cells in these fish may provide fitness benefits through defence against parasites (Smith 1982b), bacterial infection (Al-Hassen et al. 1985), protection against ultraviolet radiation (Blazer et al. 1997), or if the absence of epidermal alarm cells is detectable by females, perhaps as a courtship signal of handicapped immunological competence (Irving 1996). The full range of functions of these cells will be unravelled in the fullness of time, and it may well be the case that these cells serve multiple functions. In the meantime, there can be no dispute that alarm substance cells release chemical alarm cues that alert prey fish to the presence of predation risk, mediate an immediate antipredator response and alter behavioural responses to subsequent stimuli.

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