

Antipredator responses to skin extract of redbelly dace, *Phoxinus eos*, by free-ranging populations of redbelly dace and fathead minnows, *Pimephales promelas*

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Synopsis

Fishes in the superorder Ostariophysi possess specialized epidermal cells that contain a chemical alarm cue. The alarm cue is released when the skin is damaged during a predatory attack. Therefore, the cue serves as a reliable indicator of predation risk to nearby conspecifics and ecologically similar heterospecifics with which it shares predators. Antipredator behavior in response to these alarm cues has been demonstrated in numerous studies in confined spaces (laboratory aquaria, field traps, fluvarium). When tested on a natural field population however, behavioral response has been inconsistent. Here, we expose free-ranging redbelly dace and fathead minnows to skin extract of redbelly dace and record their behavioral response with an underwater video camera. We observed avoidance of areas in which skin extract was introduced, but no avoidance of areas in which water (control) was introduced. These data confirm the ecological function of skin extract in mediating predator–prey interactions in aquatic habitats, and argue against the hypothesis that alarm reactions are an artifact of confined spaces.

Introduction

By any measure, the fishes of the superorder Ostariophysi are an evolutionary success story. They represent 64% of all freshwater fish species and include the speciose orders of the minnows (Cypriniformes), catfishes (Siluriformes), and the tetras (Characiformes) (Nelson 1994). Two anatomical features distinguish this group from other fishes. First, most subgroups (including those listed above) are also in the Otophysi (vertebral extensions connect the air bladder to the inner ear) and are able to detect a wide range of sound stimuli. Second, all subgroups except the weakly electric Gymnotiformes possess specialized epidermal cells that contain an ‘alarm substance’ or *Schreckstoff* (Pfeiffer 1977, Smith 1992).

Chemical alarm cues are released only when the epidermis is damaged. Its release is a chemical form of public information that reliably informs nearby prey about the presence of predation risk (Wisenden & Stacey 2004).

Ostariophysan alarm substance cells have attracted the attention of evolutionary ecologists because the cost of their production (Wisenden & Smith 1997) is offset by novel fitness benefits. The chemical(s) in these cells not only warn nearby conspecifics of predation risk, but also signal to nearby predators the opportunity to pirate a prey item (Mathis et al. 1995). When a captured minnow is contested among multiple predators, the minnow can often escape, thereby promoting genes that produce and maintain alarm substance cells (Chivers et al. 1996).

Most studies of this system have focussed on the ecological role of alarm cues in skin extract in mediating antipredator responses of Ostariophyan fishes (see Chivers & Smith 1998 for review). Dozens of studies on various species by many laboratories report antipredator responses to skin extract. In 1996, Magurran et al. published a study of European minnows, *Phoxinus phoxinus*, in which no behavioral response to skin extract could be detected. What distinguished Magurran et al. (1996) from studies that came before, was that they quantified the behavioral response of a wild, free-ranging minnow population using underwater video. In their study, minnows in open water in nature did not respond to skin extract with any noticeable antipredator behavior. Although there are conditions in laboratory aquaria that suppress alarm reactions to skin extract (Smith 1977), Magurran et al. (1996) nevertheless threw into question the validity of studies based on the alarm behavior of confined animals. Magurran et al. (1996) postulated that alarm responses reported in earlier studies may have been an artifact of the confined nature of laboratory aquaria or of minnow traps scented with alarm cues.

Wisenden et al. (2004) repeated the experiment of Magurran et al. (1996) using under water video to record behavioral responses of free-swimming blacknose shiners, *Notropis volucellus*, to conspecific chemical alarm cues and the visual approach of a model predator. Wisenden et al. (2004) observed a statistically significant avoidance response to conspecific alarm cues and an intensified response to an approaching model predator if shiners were first exposed to alarm cues. However, response intensity was variable because the data were collected in large water bodies with well-developed predator communities and consequently a relatively low density of shiners.

The two studies to date on free-swimming field populations of minnows report contradictory conclusions. Additional observations are needed to verify the ecological importance of chemical alarm cues in assessment of predation risk. Here, we test the behavioral response of two cyprinid species in Budd Lake; a small boreal lake in which large piscivorous fish species are absent. Fish density in this lake is high, affording a more sensitive test of a behavioral response to chemical

indicators of predators risk than was the case in Wisenden et al. (2004).

Materials and methods

Study site

The site for this study was Budd Lake (ca. 5 ha), located within the boundaries of Itasca State Park, MN (47°11' N, 95°9' E) and near the University of Minnesota's Itasca Biological Field Station. Budd Lake contains only two fish species; fathead minnows, *Pimephales promelas*, and redbelly dace, *Phoxinus eos*. Budd Lake is also home to a large population of central newts, *Notophthalmus viridescens louisianensis*, and a family of beavers, *Castor canadensis*.

The most likely predators of cyprinids in Budd Lake are birds such as common loons, *Gavia immer*, great blue herons, *Ardea herodias*, and belted kingfishers, *Ceryle alcyon*, all of which have been observed foraging at Budd Lake. In addition, newts and invertebrate predators such as odonates, belostomatids and dytiscids likely prey on small size classes of these fishes.

Water clarity in Budd Lake is excellent. In a separate study, we easily filmed the spawning behavior of fathead minnows at depths of up to 1 m from above the water surface, using a conventional camcorder equipped with a polarizing filter (Wisenden & Alemadi, unpublished data). Aquatic vegetation is sparse in Budd Lake, limited to isolated stands of lily pads (*Nuphar* sp. and *Brasenia schreberi*). Small (2 cm diam.) water-logged sticks are strewn along the littoral zone by beaver activity, forming tangled heaps at their points of entry and exit at the shore.

Chemical stimuli

Each day, we collected dace from Budd Lake with a seine net outside of the study area and saved them temporarily in a 20-l pail until needed, and returned unused fish to the lake after a few hours. For each trial using alarm cues, we killed one adult dace by severing the spinal cord behind the head with a razor blade, and made twelve superficial incisions across each side with the razor blade. The dace was then placed in a 973-ml mason jar that

contained 60 ml of lake water, and swirled gently for 10 s to release alarm cues from the damaged epidermal tissue. The resulting alarm cue stimulus was collected into a 60 cc syringe and used within 15 min of preparation. In control trials, 60 ml of lake water was retained in separate 60 cc syringe.

Experimental protocol

We collected data from thirty different locations in Budd Lake in June 2003; each location was separated by at least 5 m. We selected locations of the lake where shoals of cyprinids were clearly visible. An underwater camera was affixed to a small branch and staked about 15 cm above the substrate. Mean (\pm SE, $n = 30$ for all) depth (water surface to substrate) at cue release was 0.65 ± 0.04 m, distance to shore was 1.71 ± 0.17 m and distance to a water depth of 1 m was 6.65 ± 0.15 m. A cable from the underwater camera led to an analogue Hi8 camcorder video cassette recorder. A 3-m length of conventional aquarium airline tubing was attached with a rubber band to a second small stick and staked about 50 cm away from the camera and with its end about 15 cm above the substrate. Fresh injection tubes were used for each trial.

We installed the underwater camera and stimulus-injection tube and waited at least 10 min for fish to acclimate to the disturbance. During this time, a separate 60 cc 'flush' syringe was used to extract lake water through the injection tube to rinse the tube of any residues left there by the manufacturer. The injection tube was rinsed three times in this fashion, discarding the water on shore each time. Lake water on the fourth rinse was retained in the flush syringe.

For 10 min we recorded fish activity in view of the camera, a view that included the end of the injection tube (pre-stimulus area use). Then, for about 1 min, either 60 ml of alarm cues or 60 ml of water (control) stimulus was injected into the injection tube, followed by 60 ml of the retained lake water to flush the test stimulus from the injection tube. Fish area use was recorded for another 10 min (post-stimulus area use). We tested 30 locations, injecting 15 locations with chemical alarm cues, and injecting the other 15 locations with water control stimulus. To describe any spatial containment experienced by these fish, we

measured the distance from the stimulus injection tube to shore, and the distance from the injection tube to the point at which water depth reached 1 m.

The number of fish in view of the camera was scored later from videotape playbacks on a TV monitor at the Itasca Biological Field Station. Area use was scored by recording the number of fish present on screen at 10-s intervals. In fish counts, we did not distinguish between fathead minnows and redbelly dace because we could not always confidently identify fish species from the video. Moreover, both species respond to ostariophysan alarm cue because of shared phylogeny and shared vulnerability to the same predators (Wisenden et al. 2003).

Data analysis

We quantified the number of fish in each 10-min observation period as the sum of the 60 fish counts from the 10-s point samples. We conducted an analysis of covariance (ANCOVA) using the number of fish in view in the post-stimulus period as the response variable, the number of fish in the area during the pre-stimulus period as a covariate, and cue type (alarm cues or water) as a categorical predictor. Basin morphology might contribute to response intensity because it may represent a form of spatial confinement. We tested the effects of: (1) water depth at cue release, (2) distance to shore, and (3) distance to where water depth reached 1 m by adding these variables as additional covariates to the model. We had only enough degrees of freedom to test for main effects and all two-way interactions. We began with this model and repeatedly re-ran the ANCOVA sequentially dropping nonsignificant terms, with each iteration deleting the interaction term with the lowest significance (highest p -value). All interactions terms but one eventually fell out of the model ($p > 0.05$).

Results

Cue type significantly affected the number of fish in view of the camera after cue injection. There was a significant interaction between cue type and pre-stimulus fish number (ANCOVA: Cue type $F_{1,23} = 0.30$, $p = 0.592$, Prefish No $F_{1,23} = 12.55$, $p = 0.002$, Cue type * Prefish No $F_{1,23} = 11.32$,

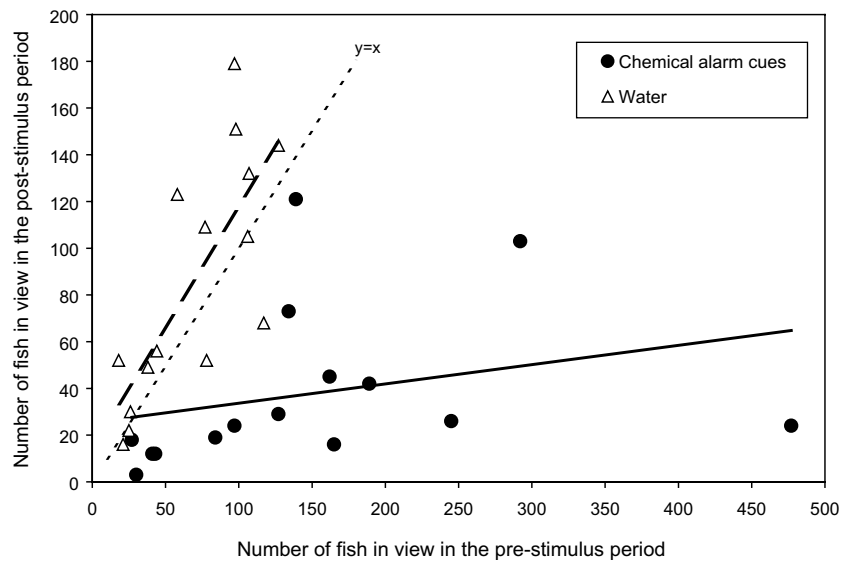


Figure 1. The number of minnows (redbelly dace + fathead minnows) in view of the camera per 10 min before and after the addition of chemical alarm cues from the skin of redbelly dace (solid circles, solid line) or the addition of lake water (open triangles, dashed line). The faint dotted line represents the line of equality (slope = 1).

$p = 0.003$; Figure 1). This can be interpreted to mean that the number of fish present after the release of test cues depended on the type of cue released. The number of fish present after stimulus introduction was correlated with the number of fish present before stimulus introduction in control trials (least squares regression: $\text{Postfish} = 1.02 * \text{Prefish} + 15.13$, $R^2 = 0.57$, $F_{1,14} = 1.14$, $p = 0.001$) but not for alarm cue trials ($\text{Postfish} = 0.08 * \text{Prefish} + 25.40$, $R^2 = 0.08$, $F_{1,14} = 17.53$, $p = 0.305$). All trials that received chemical alarm cues had fewer fish in view after stimulus introduction than during the pre-stimulus fish count, whereas only 5 of 15 control trials demonstrated reduced fish counts after stimulus injection (Figure 1; Fisher exact test $P < 0.001$). ANOVA factors of Depth at Cue Release ($F_{1,23} = 0.97$, $p = 0.335$; Figure 2), Distance to Shore ($F_{1,23} = 0.32$, $p = 0.577$; Figure 3), and Distance to a Depth of 1 m ($F_{1,23} = 2.04$, $p = 0.167$; Figure 4) did not contribute significantly to variation in the number of fish in view in the post-stimulus period.

Discussion

Free-ranging populations of redbelly dace and fathead minnows in Budd Lake clearly avoided

areas chemically labelled with chemical alarm cues from the skin of redbelly dace. These observations concur with the original *shreckreaktion* observations of von Frisch (1939) and subsequent field studies on unconfined littoral fishes (Newsome 1975, Wisenden et al. 2004). Wisenden et al. (2004), using the same underwater video as used here, observed that the effect of chemical alarm cues on blacknose shiners was equal to that of the visual approach of a model predator.

The three studies to date using underwater video (Magurran et al. 1996, Wisenden et al. 2004, current study) were possible because they were conducted in relatively clear water with high visibility. Although Hartman & Abrahams (2000) found that turbidity is a factor that increases the intensity of alarm reaction to alarm cues, the lack of turbidity did not play a role in the avoidance behaviour recorded in our studies. The suggestion by Magurran et al. (1996), that injury-released chemicals may not function as alarm cues in nature, are not consistent with our findings. The absence of a detectable avoidance response to alarm cues by Magurran et al. (1996) was probably due to low sample size ($n = 8$) and low population density. The current study provides an important affirmation of Wisenden et al.

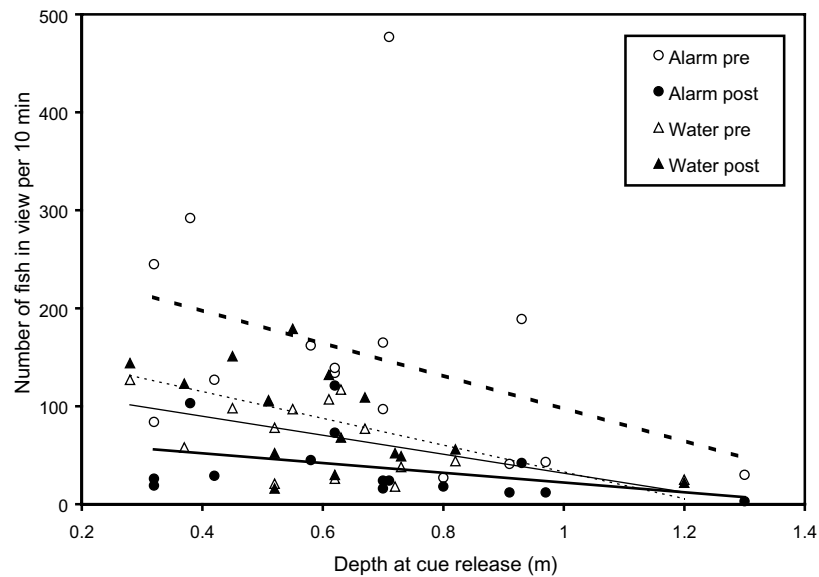


Figure 2. The number of fish in view per 10 min before and after the addition of test stimuli (alarm cues from redbelly dace, or water) as a function of the depth at which the cues were released. Open symbols and dashed lines, pre-stimulus fish counts; solid symbols and solid lines, post-stimulus fish counts; circles and bold lines, alarm cue trials; triangles and light lines, water trials.

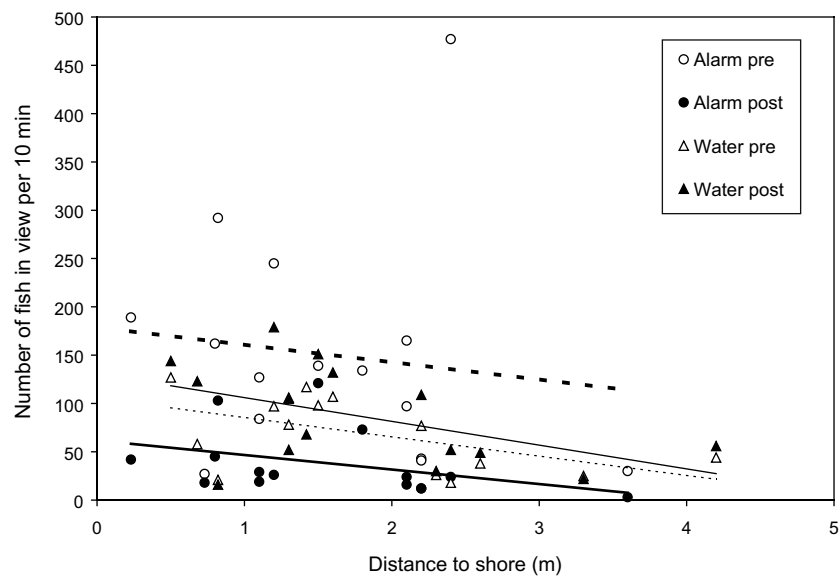


Figure 3. The number of fish in view per 10 min before and after the addition of test stimuli (alarm cues from redbelly dace, or water) as a function of the distance from shore at which the cues were released. Open symbols and dashed lines, pre-stimulus fish counts; solid symbols and solid lines, post-stimulus fish counts; circles and bold lines, alarm cue trials; triangles and light lines, water trials.

(2004) and the role of chemical alarm cues in mediating predator-prey interaction in aquatic habitats.

We recorded depth at cue release (surface to substrate), distance to shore and distance to a depth of 1 m because spatial confinement may

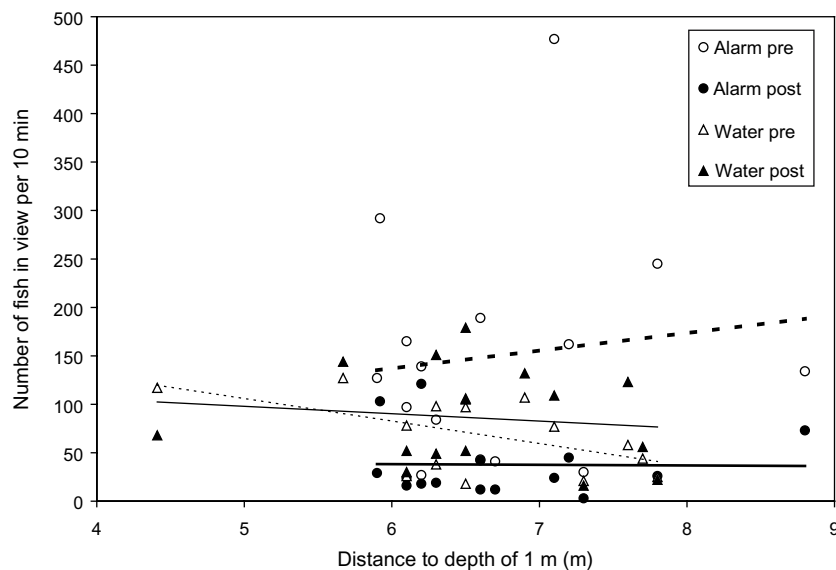


Figure 4. The number of fish in view per 10 min before and after the addition of test stimuli (alarm cues from redbelly dace, or water) as a function of the distance to a depth of 1 m from the point at which the cues were released. Open symbols and dashed lines, pre-stimulus fish counts; solid symbols and solid lines, post-stimulus fish counts; circles and bold lines, alarm cue trials; triangles and light lines, water trials.

contribute to intensity of alarm response (Magurran et al. 1996, Irving & Magurran 1997, Henderson et al. 1997). We were unable to detect any significant contribution of these parameters to response intensity in this study. However, we did not set out to explicitly test these parameters and the limited range of depths and shore slopes used in our study may not have permitted a powerful test of their influence on alarm reaction intensity.

The high population density of cyprinids in Budd Lake created conditions for a sensitive test of the effect of chemical alarm cues, but the findings here are not necessarily only a function of high population density. We argue that the same ecological function of chemical alarm information occurs among all minnow populations. It is merely more difficult to measure and detect avoidance behaviour when populations are dispersed. Dispersed minnow populations travel in discrete shoals. In these situations, underwater video records the occasional presence and frequent absence of shoals, leading to high variance and low statistical power (Wisenden et al. 2004).

In conclusion, natural populations of unrestrained minnows respond to chemical alarm cues

with antipredator behavior. When predation occurs, chemicals released from damaged tissue are released into the water. This is public information about predation risk. There is tremendous selective pressure for conspecifics and ecologically similar heterospecifics to be able to detect and respond to these cues. Not surprisingly, most aquatic taxa tested to date demonstrate antipredator responses to injury-released chemical cues from conspecifics (Chivers & Smith 1998, Wisenden 2003). Minnows and other members of the fish superorder ostariophysi are no exception.

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