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Sensory compensation and the detection of predators: the interaction between chemical and visual information

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Recent evidence suggests that environmental conditions may affect whether fishes do or do not respond to the presence of chemical alarm cues in water. We present a simple model which suggests that the combination of risk of predation and information from other sources will determine when fishes should react to these chemical cues. We tested this model with a laboratory experiment which manipulated the risk of predation by altering the animals (hungry or well fed), or their environment (presence or absence of cover). We also altered the availability of visual information by manipulating the water clarity. Consistent with our model, fishes were most likely to react to chemical alarm cues in the absence of visual information and when the perceived risk of predation was high. The manipulation of either parameter was able to extinguish this response.

Keywords: alarm substance; predator detection; sensory compensation

1. INTRODUCTION

Since its discovery, ostariophysan alarm substance (AS) has been assumed to function as an alarm signal designed to alert other shoal members of danger (Von Frisch 1938; Pfeiffer 1962; Smith 1992; Fuiman & Magurran 1994). Individuals which sense AS (also called Schreckstoff) respond with a fright reaction which may involve dashing, freezing, hiding, area avoidance or increased shoaling (Levesley & Magurran 1988; Magurran 1990; Smith 1992; Krause 1993).

Recent papers by Magurran *et al.* (1996) and Irving & Magurran (1997) have demonstrated that, as the environment becomes more natural, the response to AS becomes less significant until the reaction disappears in a completely natural setting. They suggested that the fishes sense AS, but that the magnitude of their response depends upon the context in which it is encountered. Magurran *et al.* (1996) proposed that strong reactions occur in the laboratory because fishes are in an environment which they perceive as very dangerous. They do not react in their natural environment because they perceive it as relatively safe. Smith (1997) argued that these results were atypical as other field experiments have observed reactions to AS (Von Frisch 1938; Mathis & Smith 1992; Chivers *et al.* 1995). Conflict now exists over the importance of AS. The basis of Smith's (1997) argument was that AS is an alarm pheromone and a response should always occur in prey receivers, regardless of whether the reaction is immediate or a long-term behavioural change. Magurran *et al.* (1996) and Henderson *et al.* (1997) proposed AS is a cue which fishes use to detect predators. The response to the cue is dependent on the level of risk.

We propose a simple model which may explain the circumstances under which fishes do and do not react to AS (figure 1). Our model assumes that predators may be visually detected from a long distance when the water is clear (Aksnes & Giske 1993), but that this distance decreases with increasing turbidity. We also assume that the concentration of AS necessary to initiate an alarm response decreases with increasing risk of predation. The basis of the sensory compensation model simply assumes that the concentration of AS necessary to initiate an alarm response will also decrease in response to reduced visual information which is a consequence of increasing turbidity (figure 1). From this model, we predict that reductions in water turbidity or the risk of predation will eliminate the response of fishes to the presence of AS.

2. METHODS

Two experiments were conducted to test the predictions of the model. Experiment 1 manipulated the risk of predation by changing the energetic state of fishes. Hungry fishes are less responsive to predators than satiated fishes (Magnhagen 1988; Gregory 1993). Thus, for this experiment, hungry fishes represented the low-risk environment and satiated fishes represented the high-risk environment. Experiment 2 altered the level of cover. Cover represents a safe area for prey (Savino & Stein 1989) and the trials with cover represented a low-risk environment while no cover was a high-risk environment.

These experiments used wild fathead minnows (*Pimephales promelas*) captured using minnow traps in the autumn of 1997 from the University of Manitoba field station at Delta Marsh, which is located at the southern tip of Lake Manitoba. The environment at Delta Marsh is highly variable in both its turbidity levels (1–15 NTU (nephelometric turbidity units)) (M. V. Abrahams, unpublished data) and the presence of emergent macrophytes. This is typical of the habitat in which fathead minnows are found. The predator for experiment 1 was a 66.5 g yellow perch (*Perca flavescens*). For these experiments, five groups of ten fishes each were held in a 40 l aquarium at 19.5 °C under a 12 h photoperiod for the duration of the experiments. While in the holding tanks, the fishes were fed flake food. The perch was held in a 90 l aquarium and fed one fathead minnow per week.

The experiments were conducted in a square (76 cm × 76 cm × 30.5 cm) aquarium (figure 2) mounted on a light table (a frame with sandblasted glass diffusing light from 12 × 30 W

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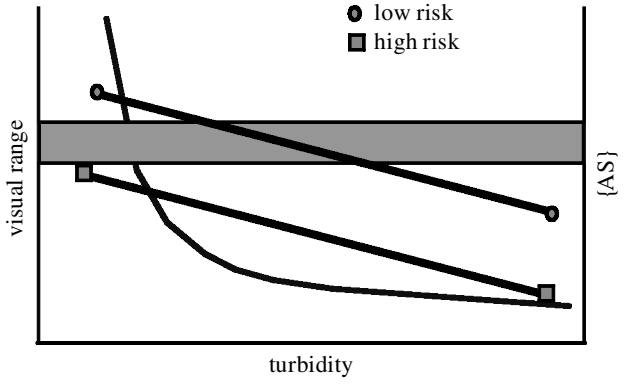

Figure 1. The sensory compensation model. The grey bar indicates the concentration of AS released. As indicated by the two parallel lines, the concentration of AS necessary to generate an anti-predator response decreases as the level of predation risk increases. This threshold concentration also decreases in response to diminishing visual information due to increased turbidity. When faced with a high risk of predation, the minnows should respond to a given concentration of AS in both clear and turbid water. At low risk, the minnows should only respond to AS in turbid water.

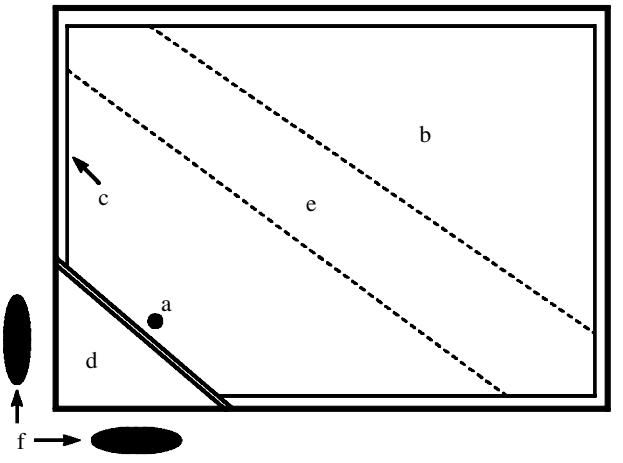

Figure 2. The overhead view of the apparatus which includes (a) the stimulus introduction point, (b) the test area, (c) the Plexiglas insert, (d) the predator area, (e) the standardization zone and (f) the halogen lights for illuminating the predator.

fluorescent bulbs set in six, 90 cm strip fixtures placed 25 cm below the tank). The aquarium walls were lined with cardboard to minimize disturbance to the fishes. Plexiglas walls were placed 3 cm inside the aquarium to keep the minnows away from the shadows generated by the wood supports for the aquarium. The apparatus was filled to a depth of 6.5 cm, which required the fishes to detect and respond to stimuli in the horizontal axis while we monitored their behaviour in the vertical axis with a Hi8 video camera mounted 1.75 m above the apparatus. This allowed us to observe their responses to AS, even when the water was turbid. A triangular area for the live predator was built into the corner of the tank using a two-way mirror, making the predator visible only when it was illuminated. The bottom of this area was covered with black Plexiglas to block light from the light table. Two parallel lines were drawn on the bottom of the apparatus 20 and 45 cm away from the predator partition. Once all of the fishes were between these lines, the exposure period began. We did this to provide some limitation on the diffusion gradient (and, hence, the concentration) of AS presented to these fishes, as well as standardizing the distance between the fishes and the live predator used in these experiments.

During the trials, water was pumped through the tank at a rate of 4 ml s^{-1} with a Manostat Varistaltic pump. During the acclimation period, air stones were used to keep the water aerated. Three millilitres of AS (0.450 cm^2 of minnow skin per millilitre) was added directly to the tubing with a 3 cm^3 syringe and was introduced into the tank at point a (figure 2). AS was prepared following the methods of Magurran *et al.* (1996) and Irving & Magurran (1997) at a concentration similar to other studies (Mathis & Smith 1992; Brown & Smith 1996; Irving & Magurran 1997). A control stimulus was prepared as above but using an equivalent mass of muscle from the caudal peduncle. The final solutions of AS and the muscle control were divided into 1 ml aliquots and stored in a freezer at $-74\,^{\circ}C$.

(a) *Experiment 1: manipulation of hunger and its effect on the use of danger stimuli*

Five groups of ten fathead minnows were randomly exposed to 16 different environmental conditions involving water clarity (clear or turbid), hunger level (hungry or satiated), visual stimuli (predator or no predator) and chemical stimuli (AS or muscle control). The water clarity treatment was randomly determined for each week while the other three treatments were randomized daily for each group of minnows. There was a minimum of 48 h between subsequent exposures to AS for each group and each group was only exposed to AS eight times over three months.

The tests involving clear water had a turbidity level of < 1 NTU. Turbid water was created by suspending 7 g of bentonite clay in 1200 ml of water to generate a turbidity of 21 NTU (0.24 s.e.).

The trials were conducted by placing a group of minnows in the test apparatus and allowing them to acclimatize for 90 min. After 75 min, the air stones were removed, the pump and the light table was turned on, the flake food was added for the satiated fishes and the predator was added (if necessary). Fifteen minutes after the lights were turned on, we recorded a 15 min pre-exposure period. Filming was stopped until all of the fishes were within the standardization zone (figure 2). To present the predator visually, the halogen floodlights were turned on for 2 min. The chemical stimulus was presented by injecting it into the tygon tubing with a 3 cm^3 syringe. We recorded the behaviour of the fishes for 15 min following the introduction of the stimulus. We concluded that the fishes detected the stimulus if there was a significant increase in the number of dashes (a rapid movement in a random direction) between the pre- and post-exposure periods.

After each trial, the apparatus was drained and fresh water pumped through the tygon tubing to flush out any chemical residue. The apparatus was then filled with dechlorinated water for the following trial. We conducted a maximum of three trials per day (between 09.00 and 19.00). Using regression analysis, we tested for habituation by plotting the number of responses per trial against order for those trials where a response to AS was predicted.

(b) *Experiment 2: effect of cover on the relative use of stimuli*

Cover was created by cutting a 68 cm × 68 cm sheet of clear Plexiglass and drilling 160 staggered holes 5 cm apart. A 7 cm strand of polypropylene rope was put in each hole. This provided fish with cover while allowing observations from above.

Table 1. *The response to AS observed in experiment 1*

(The response to AS measured by the mean number of dashes ± 1 s.e. (post-exposure − pre-exposure) observed for five groups of minnows. The arrows and cells containing values in bold indicate the environmental combinations where the fishes switch from a negative to a positive response to AS. In this experiment, risk was manipulated with hunger levels: satiated, high risk, and hungry, low risk. C+, alarm substance, and C−, muscle control. V+, predator present, and V−, predator absent.)

	turbidity			
	low		high	
risk	C+	C−	C+	C−
high				
V+	41.2 ± 3.93	30.2 ± 1.02	33.0 ± 3.35	0.2 ± 1.28
V−	**40.0 ± 6.80**	4.6 ± 2.20	35.0 ± 2.77	1.2 ± 1.02
low				
V+	44.8 ± 2.75	35.6 ± 1.63	30.0 ± 9.66	4.2 ± 2.13
V−	**2.0 ± 0.45**	4.4 ± 2.16	**39.8 ± 2.08**	1.2 ± 0.80

The procedure was the same as for experiment 1. Five groups of ten fathead minnows were randomly exposed to eight different environmental conditions involving water clarity (clear (< 1 NTU) or turbid (22 NTU ± 0.39 s.e.)), cover (present or absent) and chemical stimuli (AS or control).

The videotapes for these experiments were analysed in order to determine whether there were significant differences in the distance travelled between clear and turbid water. At 5 and 10 min, one fish was randomly chosen and its movement traced for 1 min onto acetate with a felt pen. The distance moved and the number of direction changes (< 90°) were determined by tracing the line with Jandel Sigma Scan v. 3 (1990).

3. RESULTS

(a) *Experiment 1*

When the fishes reacted to the predator and AS, the dashes were very rapid (i.e. Mauthner-mediated, S-start fast response) and 86% of the dashes occurred within the first 2 min. Four of the five groups exhibited no evidence of habituation ($F_{4,4} = 0.86$ and $p = 0.56$), but one group did exhibit a slight reduction ($F_{1,6} = 9.93$ and $p = 0.02$). This was due to a large number of responses in the first trial. The removal of this trial eliminated any evidence of habituation in all subsequent trials ($F_{1,5} = 4.03$ and $p = 0.10$).

The unique predictions of the sensory compensation model were that the minnows would not respond to AS at low risk levels in low turbidity but would in high turbidity. When presented with only AS in clear water, satiated fishes displayed obvious responses while the low-risk, hungry fishes did not show a significant response (paired *t*-test, $t_4 = 5.815$ and $p = 0.002$; table 1). Increased turbidity caused hungry fishes to react to AS with significantly more dashes than they did in clear water (paired *t*-test, $t_4 = 17.182$ and $p < 0.001$). The number of dashes observed with hungry fishes in turbid water was not significantly different from the number observed when satiated minnows responded to AS in turbid water (paired *t*-test, $t_4 = 1.672$ and $p = 0.170$). No significant responses were observed after the addition of only the muscle control (table 1).

Table 2. *The response to AS observed in experiment 2*

(The response to AS measured by the mean number of dashes ± 1 s.e. (post-exposure − pre-exposure) observed for five groups of minnows. The arrows and cells containing values in bold indicate the environmental combinations where the fishes switch from a negative to a positive response to AS. In this experiment, risk was manipulated with levels of cover: no cover, high risk, and cover, low risk. C+, alarm substance, and C−, muscle control.)

	turbidity			
	low		high	
risk	C+	C−	C+	C−
high	**27.80 ± 4.40**	1.20 ± 0.66	30.2 ± 1.43	−0.6 ± 0.40
low	**6.40 ± 2.56**	0.20 ± 0.86	**24.6 ± 2.44**	0.8 ± 0.97

The minnows always displayed strong responses to the presence of the visual predator stimulus in clear water (table 1). When the water was turbid, the minnows displayed significantly fewer dashes in response to the visual predator stimulus than were observed in clear water (paired *t*-test, $t_4 = 19.117$ and $p < 0.001$).

(b) *Experiment 2*

None of the groups used in this experiment exhibited any evidence of habituation ($F_{1,1} = 2.73$ and $p = 0.35$). The behaviour of the minnows was significantly affected by changing the turbidity of the water. In turbid water the fishes swam over a larger area than when in clear water. The fishes in turbid water moved significantly further and faster (936 ± 39.01 cm s.e.) during the 1 min observations than the fishes in clear water (466 ± 25.63 cm s.e.) ($t_{39} = 9.695$ and $p < 0.001$). The fishes in clear water swam in irregular patterns, regularly changing direction (< 90°), while the minnows in the turbid water trials swam in circular patterns with few changes in direction. The fishes in clear water changed direction significantly more than the fishes in turbid water ($t_{39} = 9.238$ and $p < 0.01$).

The fishes reacted rapidly to the addition of a stimulus, with 83% of the total number of dashes occurring within the first 2 min. To support the model, fishes should not respond to AS in clear water with cover but when the water is turbid they should respond. The minnows in clear water with cover showed no significant differences between the number of dashes in response to AS or the muscle control (paired *t*-test, $t_4 = 1.928$ and $p = 0.126$; table 2). Fishes which did not react to AS in cover when the water was clear demonstrated a significant increase in the number of dashes when the water became turbid (paired *t*-test, $t_4 = 5.202$ and $p = 0.004$).

4. DISCUSSION

Our experiments were unique in being able to predict and generate the environmental conditions where fishes

will and will not respond to AS. In clear water, decreasing the level of risk resulted in a significant reduction in the number of dashes performed in response to the introduction of AS. Similar results were observed by Brown & Smith (1996), Magurran *et al*. (1996) and Irving & Magurran (1997). However, unlike previous experiments, we were then able to manipulate the environment by decreasing the amount of visual information and increasing the sensitivity of these fishes to the same AS cues. These data are consistent with our model predictions.

Data consistent with the sensory compensation model have been observed elsewhere. Diving beetles (*Acilius sulcatus*) in a fluvarium did not respond to the scent of a hungry perch predator in the absence of a visual stimulus during the daytime but, with reduced light, the beetles reacted strongly to the predator scent (Åbjörnsson *et al*. 1997). Diving beetles have well-developed eyes which suggests they may rely mainly on visual information (Åbjörnsson *et al*. 1997). When input to this sense is reduced, the beetles demonstrated an increased sensitivity to chemical cues.

Our sensory compensation model provides a resolution to the controversy surrounding the use of AS. Smith (1997) argued that AS is an alarm pheromone designed to convey information about the presence of a predator. Smith (1997) cited previous field observations and field experiments involving the use of traps as his evidence (Von Frisch 1938; Smith 1976; Mathis & Smith 1992; Chivers *et al*. 1995). Many previous laboratory experiments have demonstrated that fishes respond to AS without a predator present (Pfeiffer 1962; Brown & Smith 1996; Irving & Magurran 1997). As proposed by Magurran *et al*. (1996), our experiments demonstrated it was due to the relative risk of predation. This level of risk may have been a significant factor influencing the responses observed in the field experiments cited by Smith (1976) (Von Frisch 1938). In both of these examples, human observers made direct observations during the experiments. The minnows in the experiments conducted by Von Frisch (1938) responded to the release of AS from an injured shoal member. This reaction may have been observed due to the presence of AS and the presence of the observer which may have represented a large predatory stimulus. The increased risk of predation associated with the trial would result in the minnows being more sensitive to the release of AS. The experiments by Smith (1976) demonstrated similar reactions which, again, could be due to the presence of the observer combined with the AS.

The trap experiments cited by Smith (1997) did not discuss the immediate responses of the minnows (Mathis & Smith 1992; Chivers *et al*. 1995). There were significantly more minnows caught in the control traps than in the traps marked with AS. This could have been a result of the confined nature of the traps. A trap may represent a safe area for the minnows to hide in, but they were not willing to enter that confined area when scented with AS. This situation would represent a greater risk of predation which would result in fewer trapped minnows.

Our model elaborates upon the hypothesis proposed by Magurran *et al*. (1996) by introducing the concept of sensory compensation. Magurran *et al*. (1996) proposed that the level of risk in which AS is encountered may mediate the response. They suggested that AS is a cue and the minnows should only respond when the level of risk is increased. In a natural setting minnows are less likely to respond to AS because there is a low level of risk associated with the familiarity of their habitat (Magurran *et al*. 1996). When the level of risk is increased, the minnows are more likely to respond to AS. Our model predicted and the results confirmed that fishes do respond to AS in low-risk situations but only when the availability of visual information becomes significantly reduced. We determined that their sensitivity to AS is not only affected by the level of risk but also by the availability of information to the senses. When the primary source of information is reduced, minnows are more willing to use alternate cues. The variability of the minnows' environment forces minnows to use a variety of inputs. The ability to use AS gives the prey an advantage when attempting to detect a predator in turbid conditions.

Sensory compensation may also be an important evolutionary force. Huber & Rylander (1992) and Van Staaden *et al*. (1995) found that fish species inhabiting turbid water had better-developed olfactory apparatus when compared with species from clear water. The increased development of these structures suggests a greater reliance on the use of chemicals in obtaining information from a more turbid environment (Van Staaden *et al*. 1995) and potentially a greater ability for using AS to detect predators.

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