PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Phil. Trans. R. Soc. Lond. B 2000 **355**, 1205-1208
doi: 10.1098/rstb.2000.0668

THE ROYAL
SOCIETY

doi 10.1098/rstb.2000.0668

Olfactory assessment of predation risk in the aquatic environment

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The aquatic environment is well suited for the transmission of chemical information. Aquatic animals have evolved highly sensitive receptors for detecting these cues. Here, I review behavioural evidence for the use of chemical cues by aquatic animals for the assessment of predation risk. Chemical cues are released during detection, attack, capture and ingestion of prey. The nature of the cue released depends on the stage of the predation sequence in which cues are released. Predator odours, disturbance pheromones, injury-released chemical cues and dietary cues all convey chemical information to prey. Prey use these cues to minimize their probability of being taken on to the next stage of the sequence. The evolution of specialized epidermal alarm substance cells in fishes in the superorder Ostariophysi represent an amplification of this general phenomenon. These cells carry a significant metabolic cost. The cost is offset by the fitness benefit of the chemical attraction of predators. Attempts of piracy by secondary predators interrupt predation events allowing prey an opportunity for escape. In conclusion, chemical cues are widely used by aquatic prey for risk assessment and this has resulted in the evolution of specialized structures among some taxa.

Keywords: predation risk; chemical cues; olfaction; Schreckstoff; alarm cues

1. INTRODUCTION

Aquatic environments are ideal for the solution and dispersal of chemical cues. Aquatic animals use chemical information from the environment for behavioural decision making relating to foraging, reproduction and the assessment of predation risk. Chemical cues are especially useful in conditions where water is turbid, highly structured, at night, or for species with a poorly developed visual sense.

Predation events escalate along a series of steps beginning with initial detection, leading to attack, capture and finally to prey ingestion (Lima & Dill 1990). At each step in this 'predation sequence' chemical cues are released that can be used by prey to assess and avoid predation risk (table 1). In response to these cues, prey adopt species-specific antipredator behaviour. Typically, these involve any or all of the following: reduction in activity, movement out of the water column, seeking shelter, area avoidance and increased shoal cohesion.

2. DETECTION: PREDATOR ODOURS AND DISTURBANCE PHEROMONES

During the initial stages of a predation event, prey can assess predation risk by the presence of the odour of the predator itself or by disturbance pheromones released by conspecific or heterospecific prey. The ability to respond to the odour of predators has been documented for aquatic prey species among the Protozoa, Arthropoda, fishes and Amphibia (reviewed by Kats & Dill 1998). Disturbance pheromones are released when prey are harassed or threatened (Wisenden *et al.* 1995*a*). The leading hypothesis is that the pheromone is a pulse of urine released by startled prey (Kiesecker *et al.* 1999; K. L. Young and B. D. Wisenden, unpublished data). Both predator odours (also known as kairomones) and disturbance pheromones are released early on, before or at the detection stage of the predation sequence, giving prey the opportunity to avoid completely a predator encounter (Lima & Dill 1990).

3. ATTACK AND CAPTURE: INJURY-RELEASED CHEMICAL CUES

Chemical cues released during the attack and capture stages of the predation sequence are generally injury-released chemical cues. These cues indicate a predator that is actively foraging, thus a more imminent risk of predation, and elicit a more intense antipredator response. Responses to injury-released chemical cues are widespread among aquatic taxa from Protozoa to Amphibia (reviewed by Chivers & Smith 1998), suggesting an ancient origin and universal benefit to this behavioural response (Wisenden 2000). A significant reduction in predation risk in laboratory experiments has been clearly demonstrated for behavioural responses to chemical alarm cues (see Wisenden (2000) and references therein).

4. INGESTION: DIETARY CUES

Prey can smell the diet of their predators, even if they have never before encountered the predator species (reviewed in Chivers & Smith 1998; Wisenden 2000). Injury-released chemical cues from prey pass through the predator's gut and are released from the predator's anus.

Table 1. *Relationship between escalating stages of the predation sequence and the role of chemical cues in mediating predator–prey interactions in aquatic environments*

		stage of predation sequence			
chemical cue	source	detect	attack	capture	ingest
disturbance pheromones	prey	yes	yes	?	?
injury-released chemical cues	prey	no	no	yes	yes
dietary cues	prey from within predator	yes	yes	?	?
predator odour	predator	yes	yes	yes	yes
learned recognition of risk	prey or predator	yes: by disturbance pheromone?	yes	yes	yes

These cues are recognizable by prey and indicate that the releaser of these cues is a predator of conspecifics (or ecologically similar heterospecifics). For example, larval damselflies from a pond where northern pike are absent do not respond with antipredator behaviour to pike odour. However, if a pike is fed damselflies its odour elicits antipredator behaviour (Wisenden 2000).

5. LEARNED RECOGNITION OF PREDATOR ODOUR

When prey simultaneously encounter injury-released chemical cues from their own species (which are known to indicate danger) along with a novel chemical cue (until then, neutral), they associate the novel cue with risk. Thereafter, the novel cue comes to be interpreted as an indicator of danger (Chivers & Smith 1998; Wisenden 2000). Injury-released chemical cues that allow the formation of learned associations can be cues released at the time of attack or from dietary cues released at the post-ingestion stage of the predation sequence. For example, pike-naive damselfly larvae learn to recognize pike odour as dangerous when pike odour is paired with the odour of crushed damselflies, or when exposed to the odour of pike fed a diet of damselflies (Wisenden 2000).

The remarkable aspect of this recognition learning is that a single trial is sufficient. This makes ecological sense in the context of a predation event. Prey that require second or third opportunities to learn an association may not survive to make use of the knowledge.

Predator species vary both spatially and temporally across a prey species' range. A flexible learning paradigm gives prey the ability to associate novel stimuli with risk and allows prey to adapt quickly to the local predation climate. Thus, after a single close encounter and learned recognition of a novel cue, prey can use predator odour at the detection stage of the predation sequence.

Fathead minnows *Pimephales promelas*, whose chemical ecology has received much attention, have a remarkable ability to form associations between risk and novel stimuli. In the laboratory context minnows can easily be tricked into forming associations with non-biological or irrelevant stimuli such as a goldfish, a cichlid, a random water sample, a red light or morpholine (see Chivers & Smith 1998; Wisenden (2000) and references therein). Minnows therefore run the risk of forming maladaptive associations with irrelevant stimuli. The question then becomes: How do minnows decide which stimuli to fear among the many present in the environment when they detect chemical alarm cues? Clearly, some kind of internal hierarchy of salience is associated with novel stimuli making some more easily associated with risk than others. Recent data indicate that motion facilitates the association of risk to novel visual objects (B. D. Wisenden and K. R. Holman, unpublished data).

6. FIELD STUDIES

The original observation of chemical alarm cues was observed in the field (Von Frisch 1938) but most of the studies since then have been conducted in the laboratory (Smith 1992; Chivers & Smith 1998; Kats & Dill 1998). In the last few years there has been a welcome shift back to field observations through careful manipulation experiments that combine the experimental rigour of the laboratory with the ecological realism of data collected from wild animals in their natural habitat. Initial field experiments verified the repellent nature of skin extract. Cross-species reactions, in which ecologically similar species recognize each other's skin extract as an indicator of danger, are known from field experiments (reviewed in Wisenden 2000). Separate field experiments showed that fewer minnows, or fewer brook sticklebacks *Culaea inconstans* use chemically labelled 'risky' areas after the release of minnow alarm cues (Wisenden *et al.* 1995*b*) (figure 1). After cue removal, suppression of activity lasts for 2–4 h, after which the number of fishes in a risky area is the same as in areas where no alarm cue was released. However, individual 'resident' fishes that experience the cue directly remain away from areas for 6–8 h. Experienced fishes remain away even after other individuals resume activity in the risky area, presumably because of the possibility that areas remain risky long after the alarm cue has dissipated (Wisenden *et al.* 1995*b*).

7. EVOLUTION OF ALARM SUBSTANCE CELLS IN OSTARIOPHYSAN FISHES

The Ostariophysi represent about 64% of all freshwater fish species and include all the minnows, characins, catfish, suckers and sundry other groups. One of the

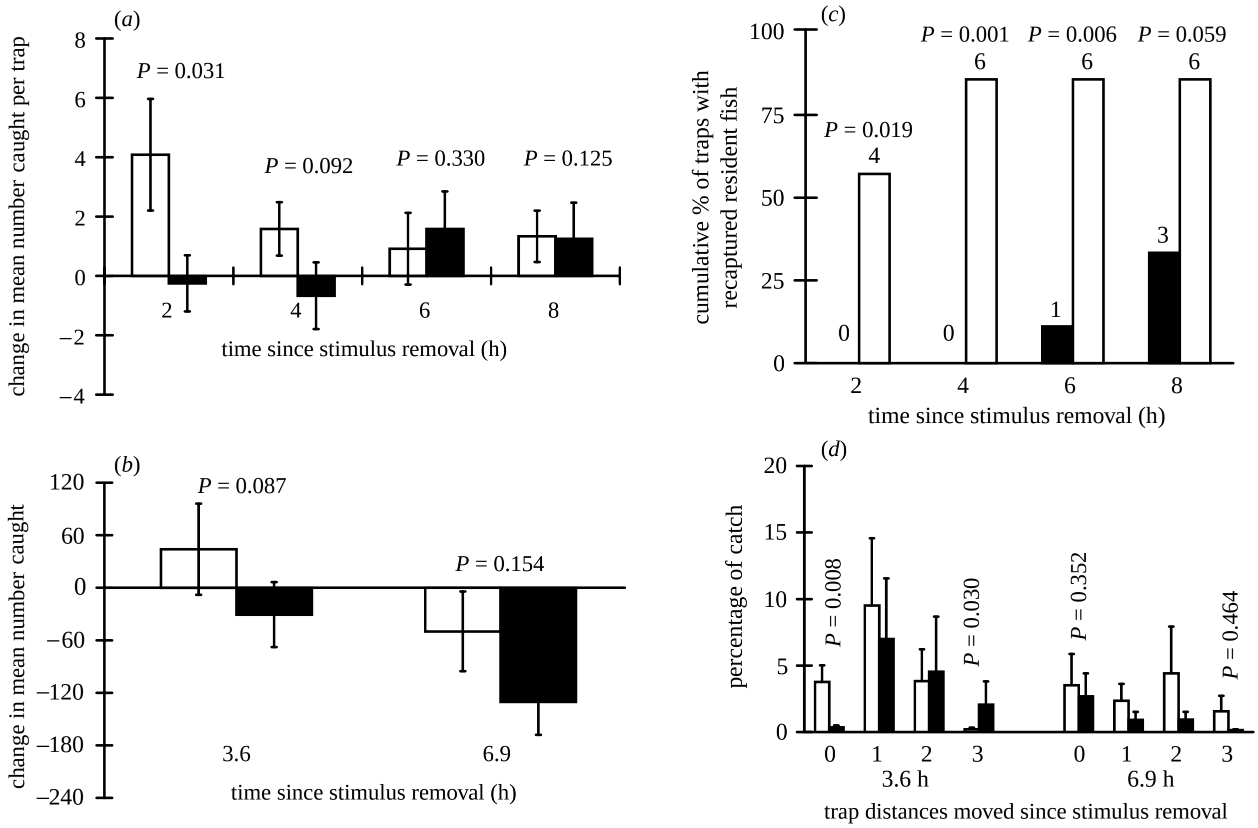

Figure 1. Areas were chemically labelled as risky by the addition of a sponge soaked in skin extract of fathead minnows. After sponge removal, the number of fishes captured in unscented minnow traps in risky areas showed significantly reduced fish activity (compared with control areas labelled with water-soaked sponges) for the next 2–4 h by (*a*) brook sticklebacks in a small stream and (*b*) minnows in a pond. However, individual 'resident' fishes present at the time of pheromone release did not return until 6–8 h after removal of the sponges. This was true for both (*c*) sticklebacks and (*d*) minnows. For sticklebacks (*c*), return of resident fishes was determined by percentage of traps recapturing at least one resident fish. For minnows (*d*), resident fishes from control areas were more likely to move zero trap distances (stay in same place) and less likely to disperse three trap locations than resident fishes from alarm locations. These data suggest that fishes using risky areas between 4 and 8 h after sponge removal were either risk-naive or had cost-to-benefit ratios that caused them to be insensitive to risk (after Wisenden *et al.* 1995*b*). Open bars, trap location marked with water sponges; solid bars, trap locations marked by sponges with minnow alarm cue.

defining characters of this group is the presence of specialized epidermal club cells that contain an alarm pheromone. These cells are called alarm substance cells (ASCs). ASCs lack a duct to the exterior; thus the pheromone can be released only when the cells are ruptured by a predator grasping its prey. These cells pose a problem for evolutionary biologists because it is not immediately clear how individuals accrue a fitness benefit from investment in ASCs. One mechanism for a fitness benefit is the chemical attraction of predators (Smith 1992). Minnow skin with ASCs attracts predators more than minnow skin without ASCs (Mathis *et al.* 1995). When a second predator is attracted to a predation event in progress the subsequent bullying and threatening between predators provide the minnow prey an opportunity for escape (Chivers *et al.* 1996).

The metabolic cost of ASCs has been verified by a simple experiment. Epidermal thickness is proportional to physical condition in minnows (see Wisenden (2000) for references). Minnows fed a maintenance ration have a thinner epidermal layer, fewer mucus cells and fewer ASCs than minnows on a high ration. Social context influences investment into ASCs. Familiar shoal-mates are designated as those travelling in the same shoal at the time of collection. Minnows held with familiar shoal-mates make fewer ASCs than minnows held with non-familiar shoal-mates. One explanation for this finding is that familiar shoal-mates execute effective group-level antipredator responses whereas assemblages of non-familiar minnows do not (see Wisenden (2000) for references). When in the context of non-familiar shoal-mates, minnows increase their reliance on their own ability to attract secondary predators.

8. FUTURE RESEARCH PRIORITIES

There has been much recent progress in unravelling the ecological and evolutionary underpinnings of the ostariophysan Schreckstoff–Schreckreaktion system. The evolutionary origin and any additional functions of ASCs remain interesting and untested questions.

Chemical ecology of predator–prey interactions has thus far concentrated on behavioural and ecological questions. The time is nigh to direct research efforts towards a greater understanding of the nature of the chemical messengers themselves and the olfactory receptors that detect them. Knowledge of the metabolic pathways of production, cell contents and additional functions of ostariophysan ASCs is still in its infancy. In addition, analogous epidermal club cells are present in North American darters (Percidae) and at least some poecilliids. Comparison among these evolutionary disparate fish taxa may reveal common selection agents for the origin of these cells (antipathogen, ultraviolet protection, etc.).

I thank Justin Marshall and Shaun Collin for organizing the Heron Island symposium and inviting me to present these recent findings. Due to limits on the number of references permitted in this paper, due credit could not always be made to the scientists who contributed to my thinking and collaborated with me on recent advances in the field of chemical ecology of predator–prey interactions. They are Douglas P. Chivers, R. Jan F. Smith, Grant E. Brown and Alicia Mathis. Complete reference lists appear in recent reviews cited here. Research funding was provided by the Natural Sciences and Engineering Research Council of Canada, the University of Saskatchewan, the University of Kentucky and Moorhead State University.

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