Fish pheromones and evolutionary enigmas: a reply to Smith

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Smith (1997) comments, in his response to our paper, that few field tests of Schreckstoff have been published 'because the question was assumed to have been settled'. When we embarked on our investigation of the function and evolution of Schreckstoff, we too subscribed to the assumption that skin extract would readily elicit the classic fright response in natural communities of fish. Our initial motivation was not to test the efficacy of Schreckstoff *per se*, but rather to learn more about the behaviour of wild fish under threat of predation. Preliminary work with European minnows, *Phoxinus phoxinus*, failed to uncover a marked fright response and prompted us to design the experiment reported in Magurran *et al.* (1996). This led us in turn to consider why the fright response might be context-dependent, and also to question the received wisdom that club cells evolved to signal alarm. On the basis of our results we concluded that Schreckstoff should no longer be termed an alarm pheromone.

In his reply, Smith disputes this conclusion. The fact that Schreckstoff induces a fright response in some contexts but not in others does not, in his view, disqualify it as a pheromone. Neither is there, he argues, any need to prove that Schreckstoff evolved primarily as an alarm signal.

In some ways the issue is a semantic one because biologists disagree on the definition of a pheromone. We choose to follow Williams (1992) because, like him, we feel that the term pheromone is only useful if it is restricted to chemical substances that are adaptive for the sender and that are produced, released and received by structures that have evolved for the purpose of communication. Williams (1992) makes the point that many female sex 'pheromones' do not meet these criteria, as they are incidental products of reproductive physiology rather than signals designed to convey information to potential mates.

The crucial question must be whether Schreckstoff evolved to signal alarm. In contrast to other alarm signals, Schreckstoff is not produced voluntarily. This substance is only released when the skin of a fish is damaged and the club cells are ruptured. The only plausible evolutionary explanation for an alarm signalling system that relies on damage to the sender would be kin selection. As we pointed out previously,

there is, to date, no evidence to support the hypothesis that European minnow schools are kin groups (Naish *et al.* 1993). Indeed, the reproductive behaviour of this species makes such a possibility unlikely (Frost 1943; Maitland & Campbell 1992). Smith also argues that the ability of fish to recognize and preferentially associate with familiar individuals could favour the evolution of Schreckstoff. But this would require levels of reciprocal altruism not yet recorded for the lower vertebrates. If the primary function of Schreckstoff is to signal alarm to kin or to familiar individuals, then why has a secretory system not evolved? Such a system would allow the sender to more effectively target the message at kin or comrades, as is seen in the Belding's ground squirrel, *Spermophilus beldingi*, where females increase their frequency of alarm calls when there are close relatives nearby (Sherman 1977). An alarm system that did not rely on skin damage would also be advantageous for the sender in other ways. Minnows are adept at predicting attacks from pike (Magurran & Pitcher 1987; Pitcher & Parrish 1993) and it would make good adaptive sense for a wary individual to signal in anticipation of the strike, rather than waiting until it is already in the predator's jaws. As Endler (1986) notes, antipredator defences are more efficient if they interrupt the predation sequence at an early stage. Once again we concur with Williams ((1992), p. 115) who writes that 'the traditional view that alarm substance is produced for a communicative function is dubious'.

The context-dependent nature of the response to Schreckstoff has important implications. In our work on European minnows, the intensity of the fright reaction diminished as the conditions in which the fish were kept became more natural. A strong response in laboratory conditions gave way to a weak one in a fluvium (Irving & Magurran 1996) and was lost entirely in the wild (Magurran *et al.* 1996). We argue that the degree of naturalness and confinement can influence the response. Smith disagrees but offers no equivalent study that shows a response under natural conditions. More research is needed to resolve this debate. This research should be designed to observe fish in as natural and unconstrained a state as possible. It should cover a variety of the species which have been the focus of intensive Schreckstoff work in the past. We strongly advocate the use of underwater video in future investigations, as this provides a detailed and con-

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tinuous record of the behavioural responses of wild fish in the absence of human intrusion. The method of extracting, storing and presenting Schreckstoff, and the season in which the fish were tested, was the same in all our experiments, so it is unlikely that differences in protocol could account for the results. Nonetheless, we can find common ground in the observation that fish do not invariably show an overt response to Schreckstoff. Brown & Smith (1996) discovered that the fright reaction in fathead minnows, *Pimephales promelas*, is suppressed by hunger and Smith makes the point that fasting minnows that show no overt reaction to Schreckstoff may exhibit 'covert responses such as learned predator recognition'. However, this finding also raises problems for the hypothesis that Schreckstoff evolved as a signal. The sender cannot rely on behavioural changes in other nearby prey to disrupt the attack. Instead, its only hope is that predators will congregate and thereby facilitate its escape (Mathis *et al.* 1995; Chivers *et al.* 1996).

In our original paper we argued that more Schreckstoff was released during our experiment than would typically be the case in a natural predation event in the River Frome (where we conducted the work), and therefore that the absence of a response in the fish we tested could not be attributed to a shortage of alarm substance. Smith disputes this comment and refers to the long handling times displayed when small pike consume minnows in his laboratory (a mean of 104 s for pike of 9.2 cm mean fork length when eating minnows 4–5 cm long). However, thanks to work by Mann (1982) we have data on the feeding behaviour of wild Frome pike which show that these laboratory observations are not representative of the situation in the wild. Minnows comprise almost 50% of the diet of one-year-old pike in the section of the river that includes our test site. (Younger pike specialize on invertebrates.) Over 80% of pike in this age group measure > 200 mm and the mean individual weight of minnows consumed is 1.3 g. In a laboratory study, Hart & Connellan (1984) found that the median time taken by pike of this size to manipulate (i.e. handle) minnows in the 1–1.9 g size range was 3.1 s. We can thus confirm that the probability of prolonged skin damage during natural predation is low. Mann also estimated that Frome pike consume, on average, > 4300 minnows ha⁻¹ yr⁻¹. Given the severity of the predation risk we might expect Frome minnows to respond strongly to Schreckstoff, but they don't. Smith cites habituation as one of the conditions that can inhibit Schreckstoff responses, yet again it seems counter-intuitive that, if it genuinely is an alarm pheromone, fish should ignore vital information in circumstances where it should be most important. Incidentally, the Frome predation data also help refute the proposal that 'there could even be selection for alarm signals that elicit a broad taxonomic response because senders, and possibly their kin, could benefit if predators are generally unsuccessful in the area' (Smith 1997).

Smith is correct in observing that there is still much to be learnt about the proposed antipathogenic function of club cells. The fact that such work is in its infancy does not, however, mean that it should be

dismissed. In particular, the immunocompetence handicap hypothesis (Folstad & Karter 1992) offers some promising avenues of research. This hypothesis states that the androgens needed for the expression of secondary sexual characteristics also suppress the immune system (Sheldon 1996). The idea is essentially an extension of the good genes model proposed by Hamilton & Zuk (1982) because it means that females have reliable information on the genetic quality of their mates; males with bright coloration are able to stay healthy despite an impaired immune system. Like other ostariophysans (Nelson 1994), male European minnows become ornamented during the breeding season. They also lose their club cells at this time (Irving 1996). The conventional explanation for the seasonal loss of club cells is that skin damage might occur during abrasive spawning and any release of Schreckstoff could disrupt mating (Smith 1992). However, European minnows are not abrasive spawners and we do know that in this species (as in many others) club cells are under androgen control (Irving 1996). If club cells do prove to have an antipathogenic function, then we have an alternative explanation for the loss of club cells during the breeding season, and for the evolution of Schreckstoff itself.

We hope that our experiment and this exchange of views will stimulate others to think about this fascinating evolutionary problem. The question of when, and why, wild fish react to skin extract is clearly not settled. More field experiments, especially those that make use of underwater video, are urgently needed.

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