



A re-evaluation of the effect of shoalmate familiarity on the proliferation of alarm substance cells in ostariophysan fishes

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A re-examination of data from Wisenden & Smith *Journal of Fish Biology* 50, 799–808 suggests that fathead minnows *Pimephales promelas* adjust investment into epidermal alarm substance cells (ASC) facultatively based upon the level of perceived risk. When placed into a container with non-familiar shoalmates (relatively high risk) ASC production increased. This allows these individuals to attract a secondary predator better. When placed into a container with familiar shoalmates (relatively low risk) ASC production decreased. This may reflect increased effectiveness of a group antipredator response by familiar individuals, and/or the cost to inclusive fitness of attracting additional predators to the vicinity of their kin.

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INTRODUCTION

Fishes in the superorder Ostariophysi possess specialized epidermal cells that contain an alarm pheromone (von Frisch, 1941; see Smith, 1992 for review). These alarm substance cells (ASC) present a challenge to evolutionary theory because the alarm substance cannot be released voluntarily (Williams, 1992). Only when the skin is damaged, as occurs during an attack by a predator, is the chemical signal released. Therefore, the presence of alarm substance (*Schreckstoff*) serves as a reliable indicator of predation risk. Nearby conspecifics and heterospecifics respond to the presence of alarm substance with antipredator behaviour (Mathis & Smith, 1992, 1993; Wisenden *et al.*, 1995). What is the benefit to the individual that invests in ASC? A number of hypotheses have been proposed (reviewed in Smith, 1992) and only one, the attraction of secondary predators, has been demonstrated convincingly (Mathis *et al.*, 1995; Chivers *et al.*, 1996). Fathead minnow *Pimephales promelas* Rafinesque skin with ASC is more attractive to predaceous diving beetles (family Dytiscidae [DeGeer]) and northern pike *Esox lucius* L. than minnow skin without ASC (Mathis *et al.*, 1995). Attraction of a second predator increases significantly both handling time of the first predator and the probability that the minnow will escape (Chivers *et al.*, 1996).

When minnows detect injury-released alarm pheromones from members of their own species they adopt antipredator behaviours (von Frisch, 1941; Smith,

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1992). Antipredator behaviour consists of two possible tactics: a group-based response or an individual-based response. Although both tactics include a reduction in movement and a movement toward the bottom, group responses involve increased shoal cohesion while individual responses involve dashing behaviour (erratic swimming) and seeking shelter. When minnows detect alarm pheromones they engage in more group-level antipredator responses when in the company of familiar shoalmates but more individual-level antipredator responses when in the company of non-familiar shoalmates (Chivers *et al.*, 1994).

Minnows prefer to associate with familiar shoalmates (i.e. fish that co-occur in a focal fish's shoal at time of collection) over non-familiar shoalmates (Brown & Smith, 1994). One possible reason is that groups of familiar minnows exhibit a higher degree of co-ordination and co-operation in response to predator risk than non-familiar shoalmates (Chivers *et al.*, 1994). Another possibility for this preference is that familiar individuals are more likely to be kin than non-familiar individuals (Eberhard, 1975; Smith, 1982). If an individual's chemical alarm signal results in a decrease in probability of predation on its kin then genes for ASC investment will be maintained in a breeding population.

The production and maintenance of ASC have a metabolic cost (Wisenden & Smith, 1997). Fathead minnows fed a high food ration exhibited a thicker epidermis and had more ASC and mucus cells than minnows fed a low food ration. Moreover, among minnows fed a high ration, physical condition (residuals from a logarithmic regression of weight on length) was positively correlated with epidermal thickness, and ASC count but not mucus cell count. Thus, fish with abundant resources increase epidermal thickness by investing disproportionately into the production of ASC (Wisenden & Smith, 1997).

Wisenden & Smith (1997) manipulated a second factor: the familiarity of shoalmates but reported no effect of the shoalmate treatment on epidermal quality. Here, we present a further analysis of data from Wisenden & Smith (1997) and report an interaction between food availability and shoalmate familiarity on the degree of proliferation of metabolically-expensive epidermal alarm substance cells. Our new analyses indicate that minnows with abundant resources increase investment into ASC when in the company of non-familiar shoalmates, but decrease their investment into ASC when in the company of familiar shoalmates.

MATERIALS AND METHODS

The materials and methods are described in Wisenden & Smith (1997) and will be restated only briefly here. Young-of-the-year fathead minnows (mean \pm S.E. = 33.5 ± 0.29 mm, $n=40$) were collected from two shoals in Pike Lake, near Saskatoon, Saskatchewan, Canada ($51^{\circ}54'$ N, $106^{\circ}49'$ W). The shoals were separated by *c.* 1 km. One shoal was designated as the focal shoal (familiar), the other as the non-familiar shoal. Individual focal minnows were maintained in 12-l pails together with either two other minnows from their own shoal (familiar shoalmate treatment), two minnows from the second shoal (non-familiar shoalmate treatment) or no other minnows at all (no shoalmate treatment). Forty trials were conducted. Twenty trials received the high food ration of three intact thawed brine shrimp *Artemia franciscana* Kellogg per fish twice per day. The remaining 20 trials received the low food ration of three intact thawed brine

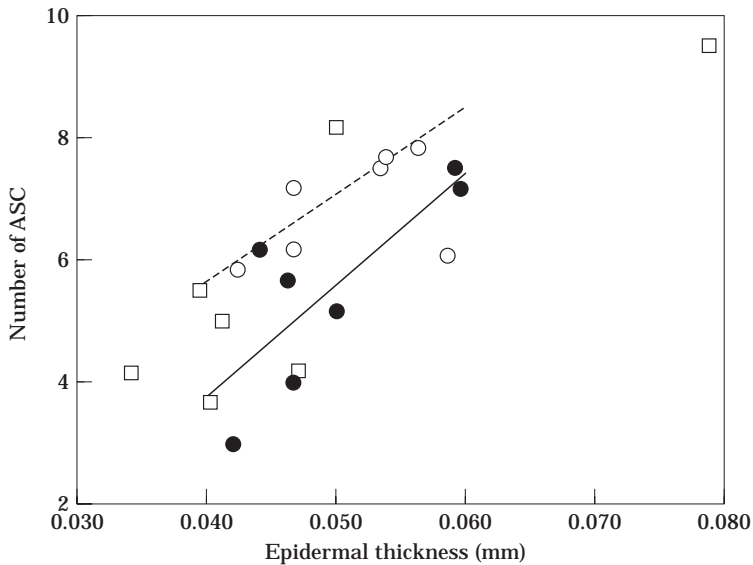


FIG. 1. The number of alarm substance cells (ASC) in minnow epidermis for fish on the high ration diet. ○ and ---, fish kept with non-familiar shoalmates; ● and —, fish kept with familiar shoalmates; □, fish kept with no shoalmates.

shrimp per fish once every second day. Sample sizes for the shoalmate treatments were assigned to each ration treatment as follows. For the high ration: familiar shoalmates, $n=7$; non-familiar shoalmates, $n=6$; no shoalmates $n=7$; and for the low ration: familiar shoalmates, $n=6$; non-familiar shoalmates, $n=7$; no shoalmates $n=7$. After 16 days, focal fish were killed by an overdose of methane tricaine sulphonate (MS-222), weighed, measured and preserved in 10% formalin. A section of epaxial epidermis and musculature was then excised, sectioned ($7\ \mu\text{m}$ thick) and stained using periodic acid-Schiff's reagent and counterstained with Lillie's haematoxylin (PAS-H). ASC are PAS-negative with central nuclei while mucus cells are PAS-positive with basal nuclei. Six sections were taken from each fish and scored in a central interval of skin of 0.127 mm wide. A single average score was calculated for each fish. We recorded (blind) the number of ASC, the number of mucus cells and the epidermal thickness from the basement membrane to the surface of the epidermis.

Among-shoalmate treatment groups were compared by performing analyses of covariance (ANCOVA), using ASC or mucus cell count as response variables, shoalmate treatment as a class variable, and epidermal thickness as a covariate. Separate ANCOVAs were performed for each level of the ration treatment.

RESULTS

There was a significant interaction between the shoalmate treatment and ration, but not the one predicted in Wisenden & Smith (1997). Minnows on the high food ration showed a significant effect of the shoalmate treatment on the number of ASC (ANCOVA $F_{2,16}=3.61$, $P=0.05$; Fig. 1) but not on the number of mucus cells (ANCOVA $F_{2,16}=1.21$, $P=0.33$; Fig. 2). Although there was an overall effect of epidermal thickness on number of mucus cells ($r=0.45$, $F_{1,18}=4.60$, $P=0.046$), there was no significant correlation between epidermal thickness and number of mucus cells for either familiar or non-familiar shoalmate groups (familiar: $r=0.40$, $F_{1,5}=0.95$, $P=0.374$; non-familiar: $r=0.41$,

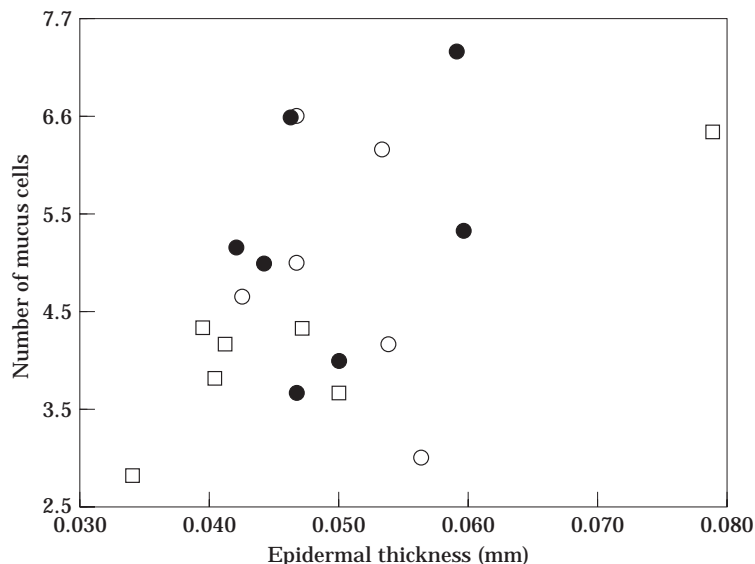


FIG. 2. The number of mucus cells in minnow epidermis for fish on the high ration diet. ○ Fish kept with non-familiar shoalmates; ● fish kept with familiar shoalmates; □ fish kept with no shoalmates.

$F_{1,4}=0.83$, $P=0.415$). There was no significant difference between the mean number of mucus cells in the three shoalmate treatments (ANOVA $F_{2,17}=1.37$, $P=0.280$). Those given the low food ration did not show any effect of the shoalmate treatment on ASC (ANCOVA $F_{2,16}=1.00$, $P=0.390$) or number of mucus cells (ANCOVA $F_{2,16}=0.39$, $P=0.684$). In the high ration fish, the number of ASC per unit area was significantly greater for those in the company of non-familiar shoalmates than those with familiar shoalmates (Bonferroni-adjusted *post hoc* pair-wise comparisons of least-square means: familiar *v.* non-familiar, $P=0.05$; familiar *v.* none, $P=0.98$; non-familiar *v.* none, $P=0.34$). Focal fish without shoalmates were intermediate between the two other shoalmate groups. There were no significant interaction terms in the ANCOVAs ($P>0.05$).

Weight gain over the 16-day test period did not differ among the shoalmate groups (ANOVA high food: $F_{2,17}=0.51$, $P=0.611$, low food: $F_{2,17}=0.46$, $P=0.640$), although there was a trend among high ration fish toward greater weight gain when in the company of familiar shoalmates, and lower weight gain when in the company of non-familiar shoalmates (familiar *v.* non-familiar: $t=1.44$, d.f. = 11, $P=0.087$; Table I). Similarly, physical condition did not differ among shoalmate groups (ANOVA high food: $F_{2,17}=0.09$, $P=0.914$, low food: $F_{2,17}=1.61$, $P=0.228$; Table I).

DISCUSSION

It is proposed that the best explanation of these data is a combination of two known phenomena. The first is the increased efficacy of group antipredator behaviour among familiar *v.* non-familiar shoalmates (Chivers *et al.*, 1994). The second is the function served by distress signals (acoustic and chemical) in

TABLE I. Mean (S.E.) change in weight of fathead minnows over 16 days and final physical condition, when fed one of two rations and kept with either familiar, non-familiar or no shoalmates (SM)

Ration	Weight change (g)		Condition residuals	
	High	Low	High	Low
Familiar SM	0.153 (0.0181)	- 0.007 (0.0084)	0.00631 (0.02430)	- 0.04164 (0.02921)
Non-familiar SM	0.118 (0.0159)	0.007 (0.0036)	0.00416 (0.02469)	0.00553 (0.03029)
No SM	0.141 (0.0329)	0.010 (0.0111)	- 0.00987 (0.03673)	0.03016 (0.02455)

attracting secondary predators as a selfish strategy for escaping from the clutches of a predator (Högstedt, 1983; Schuett & Gillingham, 1990; Mathis *et al.*, 1995; Chivers *et al.*, 1996; Wisenden, 1998). When in the company of familiar shoalmates, a focal individual can rely more upon the efficacy of a group antipredator response, thus it need not invest as much into the maintenance and production of ASC. When in the company of non-familiar shoalmates that do not execute an effective, well-coordinated group response to predation risk (Chivers *et al.*, 1994), there is an increased importance placed upon the ability to attract secondary predators. Thus, the presence of non-familiar shoalmates stimulates somatic investment into ASC proliferation.

There are two predictions that can be made from the kin selection hypothesis. If kin are among the shoalmates that benefit from an injury-released alarm signal, then the inclusive fitness of the signaller accrues benefit (Smith, 1992). From this interpretation of the kin selection hypothesis, Wisenden & Smith (1997) predicted that if ASC were maintained by kin selection, and familiarity served as a surrogate for estimating kinship (Eberhard, 1975; Smith, 1982), then minnows fed the low ration would continue to invest into ASC only when in the company of familiar shoalmates, and minnows fed the high ration would invest maximally into ASC under all social contexts because of abundant resources. These predictions were not supported by the data. Minnows held with familiar shoalmates should have increased resource allocation to ASC, not decreased it.

Minnow alarm pheromone attracts predators (Mathis *et al.*, 1995; Chivers *et al.*, 1996). To the extent that familiar shoalmates may also be kin, there may be a cost to inclusive fitness of attracting secondary predators that may then increase the risk of predation to shoalmates. Thus, an alternative prediction of the kin selection hypothesis is that the presence of familiar shoalmates should suppress proliferation of ASC. This second prediction is supported by the data in this study and may explain the intermediate ASC values for fish in the no-shoalmate treatment of the high ration group. As in Wisenden & Smith (1997), the effect was observed only in the high food group. This confirms that ASC are metabolically expensive to maintain and further suggests that investment into ASC proliferation is a conditional strategy afforded only by those with access to abundant resources.

These data are highly suggestive of both an intriguing proximate mechanism for determining allocation of somatic resources and a new research avenue to resolve this perplexing question in evolutionary ecology.

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