

Report for the FSBI Small Research Grant award

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Title of Research:

Preference for familiar fish across different species

1) Publications

- Ward AJW, Axford S & Krause J 2003. Cross-species familiarity in fish. Proceedings of the Royal Society London B (in press).

The grant has also provided the basis for further work on a related topic that has been submitted for publication (Ward, Hart & Krause: “The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks”) to *Animal Behaviour*.

2) Financial statement

The money was spent as outlined in the application on hiring a research assistant and on travelling costs and consumables.

3a) Summary

Preferential association with familiar shoalmates confers a number of potentially important benefits to individuals, including improved anti-predator effects and the amelioration of aggression in competitive interactions. Until now, however, familiarity has been demonstrated purely between conspecifics. This study presents the first evidence of cross-species familiarity. Individual focal fish (chub, *Leuciscus cephalus*) were given a choice of two stimulus shoals of the same size composed of conspecifics or of heterospecifics (minnows, *Phoxinus phoxinus*) in a flow tank. A series of four treatments were carried out investigating the effects of familiarity, induced by a 15-day association between the focal fish and the stimulus fish, on the choices made by the focal fish. Focal fish showed a significant preference for conspecifics over heterospecifics where both stimulus shoals were composed of non-familiar individuals. Focal fish also showed a significant preference for stimulus shoals composed of familiar fish over stimulus shoals composed of non-familiar fish, when both shoals were conspecific as well as when both shoals were heterospecific. Finally, the preference of focal fish for conspecifics disappeared when the alternative, a shoal of heterospecifics, was composed of familiar individuals. This work may have important consequences for species interactions in free-ranging shoals and provides an interesting basis for future fieldwork.

3b) Details of work

Introduction

Shoaling behaviour is widespread amongst fish species, offering individuals a wide range of potential benefits (see Pavlov & Kasumyan, 2000 for a review). It is generally considered that individuals may enhance these benefits by exercising a degree of choice over potential shoalmates (e.g. Krause *et al.*, 2000; Dugatkin & Wilson, 1993). In shoaling species, individuals generally associate preferentially with conspecifics (e.g. Krause *et al.*, 2000; Barber, Downey & Braithwaite, 1998) and in doing so gain a number of advantages, for example single species shoals tend to be more phenotypically homogenous, which may reduce the hunting efficiency of potential predators (Landeau & Terborgh 1986). Furthermore, individuals within single species groups are most likely to have coinciding goal functions, particularly in terms of habitat and foraging preferences (Conradt & Roper 2000).

Individuals may further enhance the benefits accruing from social behaviour if they not only associate with conspecifics, but with familiar conspecifics. Associating with familiars can serve to increase shoal cohesion, which serves to confound predators (Chivers, Brown & Smith 1995). This reduced risk of predation is exemplified by reduced investment in epithelial alarm substance cells by individuals associating with familiars in fathead minnows (*Pimephales promelas*) (Wisenden & Smith, 1998). Furthermore, familiarity among group members stabilises dominance hierarchies in trout (*Salmo trutta*) (Höjesjö *et al.*, 1998) and reduces aggression by mediating competitive interactions in three-spine stickleback, *Gasterosteus aculeatus* (Utne-Palm & Hart, 2000). In addition, familiarity has been shown to promote social learning in the guppy (*Poecilia reticulata*) (Swaney *et al.* 2001).

Familiarity is dependent on the potential for repeated interactions and so develops over time. In the guppy, familiarity develops over a period of 12 days (Griffiths & Magurran 1997). Both olfactory (Brown & Smith, 1994) and visual (Wass & Colgan, 1994) cues are likely to be involved in the discrimination of familiar individuals. A preference for associating with familiar individuals has been reported in a number of fish species both in the natural environment (three-spine stickleback: Ward *et al.*, in press) and in laboratory trials (rainbowfish, *Melanotaenia eachamensis*: Arnold, 2000; salmon, *Salmo salar*: O'Connor, Metcalfe & Taylor, 2000; European minnow, *Phoxinus phoxinus*: Griffiths, 1997).

However until now, the phenomenon of familiarity has been investigated purely in the context of conspecific groups, despite the fact that mixed-species groups are common in a range of taxa (African ungulates: Sinclair, 1985, Fitzgibbon, 1990; shore birds: Metcalfe, 1989; cyprinid fish: Allan & Pitcher, 1986). For example, Hoare *et al.* (2000) reported a majority of shoals in the littoral zone of a freshwater Canadian lake being composed of more than one species. Furthermore, the likelihood of mixed-species shoaling may be increased if the two species are closely related (see Overholtzer & Motta, 2000). In addition, certain periods of fish development are particularly likely to precipitate mixed-species aggregations, for example during the first few months of life when individuals are under high predation pressure, juveniles of different mixed-species tend to aggregate and form shoals in shallow water (Lightfoot & Jones, 1996).

Given the advantages conferred by preferential association with familiars in a conspecific context, we examined the possibility that this phenomenon may occur across species using juveniles of two species of cyprinid fish. We investigated (a) species preference (b) preference for familiar fish in conspecifics (c) preference for familiar fish in heterospecifics, and (d) trade-offs between species preference and familiarity.

Methods

Fish and Holding Conditions

We captured approx. 150 juveniles of each of two species, chub (*Leuciscus cephalus*) and the European minnow (*Phoxinus phoxinus*), using hand nets in the river Wharfe at Arthington in West Yorkshire, U.K. (grid reference SE2630 4550) during October 2001. We opted to use these species as models because they comprise approximately 70% of the year 0+ fish population (UK Environment Agency, 2001)(see Fig. 1) at their site of capture and are morphologically similar (Ward, Axford & Krause, 2002). The mean \pm standard deviation body length was measured for each species (minnows: 29.2 \pm

mm; chub 30.1 ± 3 mm). Only fish measuring 30 ± 3 mm were used in the study in order to avoid the potentially confounding effect of assortment by body length (Ward & Krause 2001). Juveniles of the two species were observed to occur in mixed-species aggregations at the study site in slow flowing (0.02 m/s to 0.1 m/s), shallow (0.05 - 0.4 m) water.

A total of 12 fish, made up of 6 chub and 6 minnows, were allocated to each of fifteen 20 l aquaria in a temperature-controlled room at 12°C on a 12:12h light:dark cycle. They were fed live and frozen bloodworm and commercially available Aquarian flaked food *ad libitum*. Each group of fish was maintained for 15 days in the holding tank. After the laboratory work was finished the fish were released at the site of capture.

Shoal choice Experiments

Binary choice tests were carried out using a flow channel to simulate the natural lotic conditions under which both species exist at the river Wharfe. The flow channel (see Fig. 2) measured 3.5 m × 0.5 m with a water depth of 0.1 m. A pump circulated the water giving a flow speed of 0.05 ms⁻¹. A series of mesh baffles were used within the channel to minimise turbulence, this had the additional effect of restricting the experimental arena to an area of 1.5 m × 0.5 m. Two separate compartments measuring 0.16 m × 0.08 m were constructed using netting material (mesh size 2 mm) within this arena to contain the stimulus shoals. The use of mesh allows for olfactory, as well as visual, stimulation of a focal fish. The stimulus shoal compartments were sited 0.5 m downstream from the upper baffles, one at either side of the flow channel, separated from each other by a distance of 0.32 m. We drew lines on the base of the flow channel demarcating a preference zone of 60 mm around each stimulus shoal compartment. This distance represents two body lengths of a 30 mm focal fish which falls within the range of inter-individual distances observed in free-ranging fish shoals (Pitcher & Parrish 1993).

For each replicate, a shoal of 5 stimulus fish was added to each of the netting compartments. The stimulus fish were then given 5 minutes to acclimatise to the conditions before a single focal fish was added. Each focal fish was introduced to the flow channel in a mesh cylinder situated centrally in the flow channel at a distance of 0.3 m downstream of the stimulus shoals. The focal fish was also given 5 minutes to acclimatise before being liberated by the removal of the mesh cylinder. The time spent by the focal fish within 2 body lengths of a stimulus shoal was recorded for each stimulus shoal for a total of 5 minutes. Each focal fish and each stimulus shoal were used only once per treatment to prevent pseudoreplication and trial order was randomised.

A total of four treatments were carried out, involving binary choices between stimulus shoals of: (a) Non-familiar conspecifics v. non-familiar heterospecifics; (b) Familiar conspecifics v. non-familiar conspecifics; (c) Familiar heterospecifics v. non-familiar heterospecifics; (d) Familiar heterospecifics v. non-familiar conspecifics.

Results

Shoal Choice

Focal fish showed a significant preference for a stimulus shoal composed of non-familiar conspecifics over one composed of non-familiar heterospecifics (Wilcoxon Signed Ranks: $Z = 2.15$, $N = 15$, $P = 0.032$; Fig. 3a). Focal fish also showed a significant preference for stimulus shoals composed of familiar fish over stimulus shoals composed of non-familiar fish, when both shoals were conspecific (Wilcoxon Signed Ranks: $Z = 2.07$, $N = 15$, $P = 0.039$; Fig. 3b) and when both shoals were heterospecific (Wilcoxon Signed Ranks: $Z = 2.56$, $N = 15$, $P = 0.011$; Fig 3c) to the focal fish. Focal fish showed no preference for a stimulus shoal composed of non-familiar conspecifics over a stimulus shoal composed of familiar heterospecifics (Wilcoxon Signed Ranks: $Z = 1.25$, $N = 15$, $P = 0.21$; Fig 3d).

In the following we re-analysed the above data to facilitate direct comparisons of shoaling preferences. To test the responses of focal fish to heterospecifics in different contexts, we compared time shoaling with familiar heterospecifics against non-familiar heterospecifics between different treatments where in both cases the alternative stimulus shoal was composed of non-familiar conspecifics (see Figs. 3a & 3d). Focal fish spent significantly greater amounts of time in proximity to stimulus shoals composed of familiar heterospecifics compared to stimulus shoals composed of non-familiar heterospecifics when

the alternatives were stimulus shoals composed of non-familiar conspecifics (Mann-Whitney U-test: $Z_{15,15} = 2.8$, $P = 0.005$).

To test possible trade-offs between species preferences and familiarity in a heterospecific context we compared time shoaling with non-familiar conspecifics and familiar heterospecifics between different treatments where in both cases the alternative shoal was composed of non-familiar heterospecifics (see Figs. 3a & 3c). There was no difference between the amounts of time spent by a focal fish in proximity to stimulus shoals composed of familiar heterospecifics compared to stimulus shoals composed of non-familiar conspecifics when the alternatives were stimulus shoals composed of non-familiar heterospecifics (Mann-Whitney U-test: $Z_{15,15} = 0.8$, $P = 0.5$).

Field Data

Analysis of population data provided by the UK Environment Agency (2001) shows that the percentage representation of minnows in the total sample is inversely proportional to the percentage representation of chub over a 20 year period (Spearman Rank: $r_s = -0.59$, $N = 20$, $P = 0.006$; Fig. 4).

Discussion

Juvenile chub assorted preferentially with familiar fish, regardless of whether those familiars were conspecifics or heterospecifics. The amount of time spent with heterospecifics was significantly greater when the heterospecific stimulus shoal was familiar to the focal fish. The preference of focal fish for conspecifics over heterospecifics when both stimulus shoals were non-familiar disappeared when the heterospecific shoal was composed of individuals familiar to the focal fish.

Focal fish were able to recognise familiar individuals, whether these were conspecifics or heterospecifics. The ability to recognise familiar individuals is present in social species from a range of taxa (mammals: Porter *et al.*, 2001; birds: Wiley *et al.*, 1999, Cristol, 1995; reptiles: Bull *et al.*, 2000; insects: Clark, Beshear & Moore, 1995). The preference for such individuals is explicable in terms of the benefits available to individuals associating with familiars. Barber & Wright (2001) described apparent trade-offs made by European minnows, wherein fish opted to associate with a shoal of familiars even though an alternative shoal of non-familiar fish contained almost twice as many individuals, suggesting significant benefits. The high predation regime experienced by juvenile cyprinids in their natural environment potentially ascribes high potential benefits to associating with familiars given that shoals composed of familiars display more effective anti-predator strategies (Chivers, Brown & Smith 1995). The overlapping habitat utilisation of Year 0+ chub and minnows (*pers. obs.*) provides the opportunity for such preferences to develop. However, as the two species grow, their habitat preferences diverge and mixed-species shoals become less common in year 1 and subsequent year-group fish. This may suggest the use of older fish as experimental subjects to investigate the effects of ontogeny on the preference for familiars.

Theory predicts that individuals should demonstrate a preference for conspecifics (see Krause & Ruxton 2002). Indeed, a number of studies have reported such a preference (Allan & Pitcher, (1986): minnow, *P. phoxinus*; Brown *et al.* (1993): rainbow trout, *Oncorhynchus mykiss*; Krause & Godin (1994): banded killifish, *Fundulus diaphanus*; Barber, Downey & Braithwaite (1998): threespine stickleback, *G. aculeatus*). Interestingly, fish in this study showed no preference for a group of conspecifics over one composed of heterospecifics when the latter were familiar. This again hints at the broad advantages that may potentially be realised by assorting with familiars. Field population data at the site (Environment Agency, 2001) show cyclical population fluctuations of both juvenile minnows and chub across a period of 20 years. Furthermore Year 0+ populations of the two species are inversely correlated. In years where conspecifics are rare but other cyprinid species are relatively abundant, the ability to recognise and subsequently assort with familiar heterospecifics may well be an adaptive strategy.

Associations with heterospecifics may provide a number of general benefits. Where species share the same predator, individuals within mixed-species groups can all potentially benefit from being able to reduce vigilance (Metcalf, 1989). This might be especially beneficial where the species involved exploit different resource niches, as in mixed-species flocks of tits (Sasvari, 1992). Fitzgibbon

(1990) studied mixed-species grouping in Grant's and Thomson's gazelles, concluding that both species benefit from the anti-predator benefits of being in larger groups, rather than assorting into monospecific, smaller herds. Guppies show an active preference for swordtails (*Xiphophorus helleri*) when juveniles of the former species are raised with the latter, potentially suggesting imprinting as a mechanism (Warburton & Lees, 1996). FitzGerald & Morrissette (1992) reported the absence of any preference for conspecifics in the threespine stickleback, *G. aculeatus* where the alternative was the closely related blackspotted stickleback, *G. wheatlandi*. In this study, however, focal fish clearly associated preferentially with conspecifics over heterospecifics when the choice was between two non-familiar stimulus shoals. This preference for conspecifics only disappeared when the alternative shoal was composed of familiar heterospecifics, indicating that the fish made a trade-off between species preference and a preference for familiar fish.

Cross-species familiarity may be more likely between closely related species for a number of ecological reasons. Useful further work could consider and investigate the possibility of familiarity developing between sympatric but unrelated species, for example minnows and three-spine stickleback.

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Figure 1: Mean (\pm st. dev.) percentage of total catch of Year 0+ fish of different species caught annually at study site from 1981-2000. (Data source: Environment Agency).

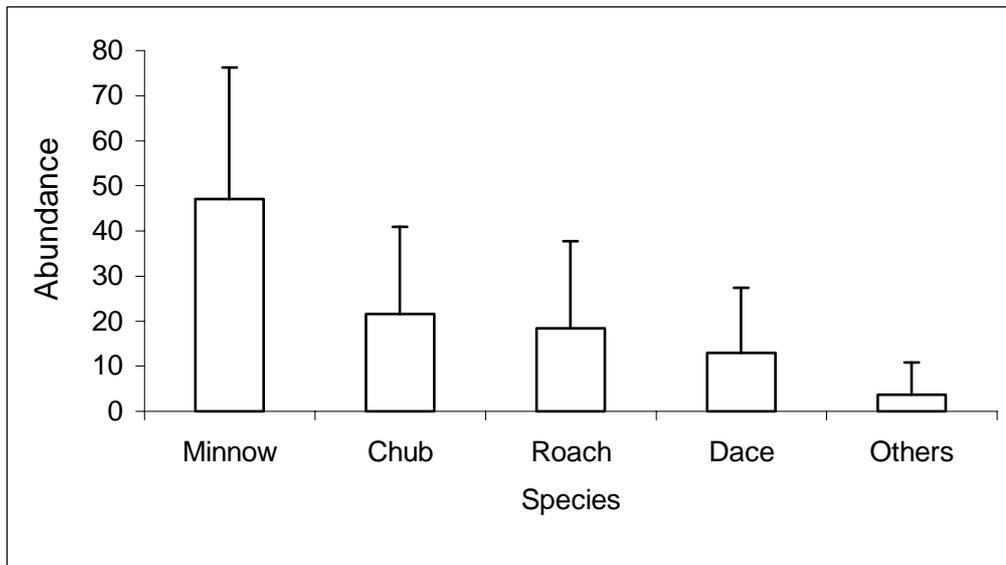


Figure 2: Aerial elevation of flow tank apparatus. Arrows represent direction of water flow.

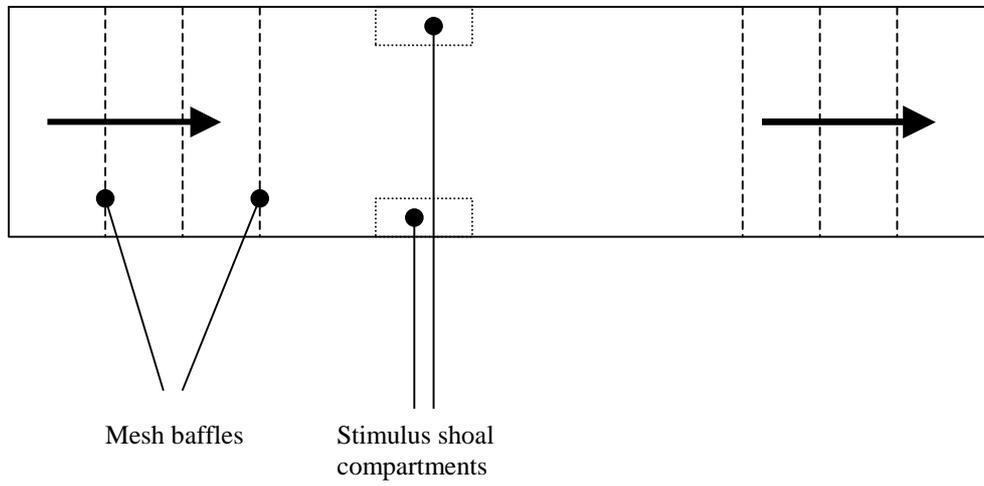
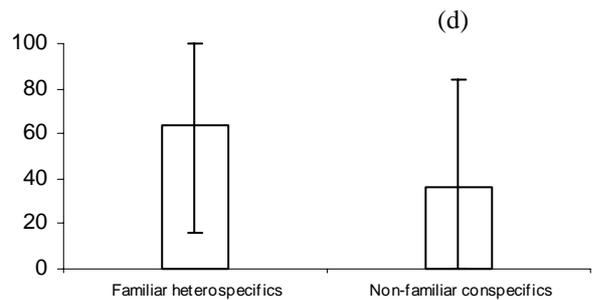
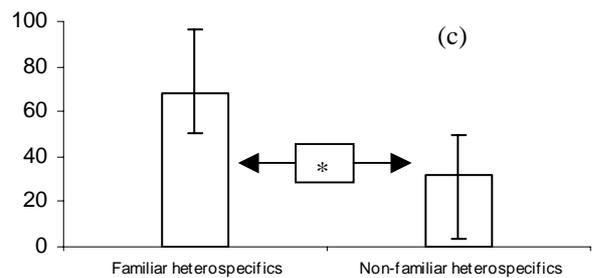
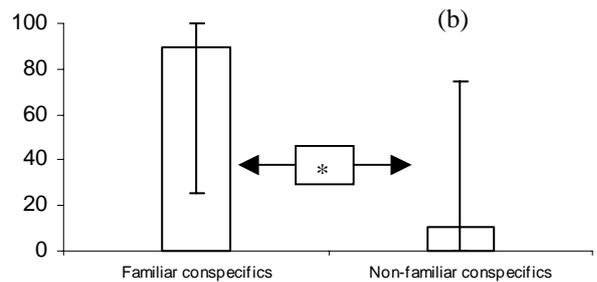
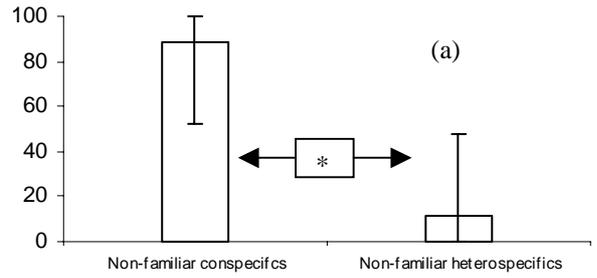


Figure 3: The median time \pm quartiles spent by focal fish shoaling with each stimulus shoal is shown as a percentage of overall time spent shoaling. Median (\pm quartiles) time spent by the focal fish in proximity to 1 (a) non-familiar conspecifics against non-familiar heterospecifics; (b) familiar conspecifics against non-familiar conspecifics; (c) familiar heterospecifics against non-familiar heterospecifics; (d) familiar heterospecifics against non-familiar conspecifics. Significant differences in the percentage time spent shoaling by focal fish between the two stimulus shoals are shown: $P < 0.05$; $** P < 0.01$ Test results generated with Wilcoxon signed ranks test, comparing (% time spent with shoal a) – (% time spent with shoal b) against zero. $n = 15$ for each treatment.



Median % time spent with stimulus shoal

Figure 4: Percentage of total sample of Year 0+ fish made up by minnows and chub in an annual sample from 1981-2000. (Data source: Environment Agency).

