

Cross-species familiarity in shoaling fishes

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Preferential association with familiar shoal mates confers a number of potentially important benefits to individuals, including improved anti-predator effects and the reduction of aggression in competitive interactions. Until now, however, familiarity has been demonstrated purely between conspecifics. Here, we present evidence that familiarity preferences can override natural preferences for conspecifics. Individual focal fishes (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 was carried out to investigate the effects of familiarity, induced by a 15 day association between the focal fish and the stimulus fishes, on the choices made by the focal fish. Focal fishes showed a significant preference for conspecifics over heterospecifics when both stimulus shoals were composed of non-familiar individuals. Focal fishes also showed a significant preference for stimulus shoals composed of familiar fishes over stimulus shoals composed of non-familiar fishes when both shoals were conspecific and when both shoals were heterospecific. Finally, the preference of focal fishes for conspecifics disappeared when the alternative, a shoal of heterospecifics, was composed of familiar individuals. The importance of this work is discussed in the context of species interactions in free-ranging shoals.

Keywords: school; schooling; group composition; individual recognition

1. INTRODUCTION

The ability to recognize familiar individuals exists in social species representing a range of taxa (mammals, Porter *et al.* (2001); birds, Cristol (1995) and Wiley *et al.* (1999); reptiles, Bull *et al.* (2000); fishes, Barber & Ruxton (2000); insects, Clark *et al.* (1995)). The preference for such individuals is explicable in terms of the benefits available to individuals associating with familiars. Associating with familiars can increase shoal cohesion, which serves to confound predators (Chivers *et al.* 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 stabilizes dominance hierarchies in brown trout (*Salmo trutta*; Höjesjö *et al.* 1998) and reduces aggression by mediating competitive interactions in the three-spined 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 (Waas & 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-spined stickleback, Ward *et al.* (2002b)) and in laboratory trials (bluegill sunfish, *Lepomis macrochirus*, Dugatkin & Wilson (1992); Atlantic salmon, *Salmo salar*,

O'Connor *et al.* (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) and Fitzgibbon (1990); shore birds, Metcalfe (1989); cyprinid fishes, Allan & Pitcher (1986)). For example, Hoare *et al.* (2000) reported that most shoals in the littoral zone of a freshwater Canadian lake were 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 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 the following:

- (i) species preference;
- (ii) preference for familiar fishes in conspecifics;
- (iii) preference for familiar fishes in heterospecifics; and
- (iv) trade-offs between species preference and familiarity.

2. MATERIAL AND METHODS

(a) Fishes and holding conditions

We captured *ca.* 150 juveniles of each of two species, chub (*Leuciscus cephalus*) and the European minnow (*P. phoxinus*),

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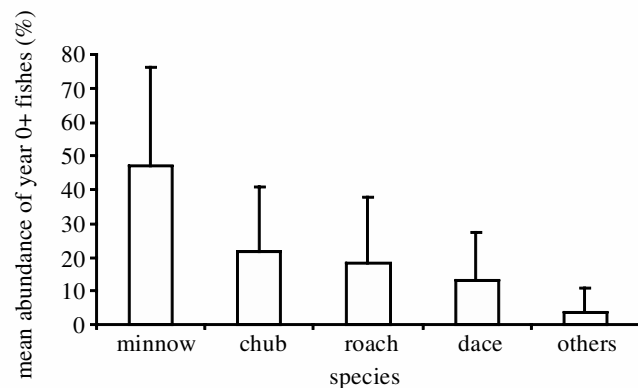


Figure 1. Mean (\pm s.d.) percentage of the total catch of year 0+ fishes of different species caught annually at the study site between 1981 and 2000. Data source: UK Environment Agency (2001).

using hand nets in the river Wharfe at Arthington in West Yorkshire, UK (grid reference SE2630 4550), during October 2001. We opted to use these species as models because they comprise *ca.* 70% of the year 0+ fish population (UK Environment Agency 2001) (see figure 1) at their site of capture. In addition, these species of cyprinids have been used in previous mixed-species studies (Allan & Pitcher 1986) and are morphologically similar (Ward *et al.* 2002a). The mean \pm standard deviation body length was measured for each species (minnows 29.2 ± 1.4 mm; chub 30.1 ± 1.8 mm). Only fishes measuring 30 ± 3 mm were used in the study 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 – 0.1 m s⁻¹) shallow (0.05 – 0.4 m) water.

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

(b) Shoal choice experiments

Binary choice tests were carried out using a flow channel to simulate the natural lotic conditions under which both species exist in the river Wharfe. The flow channel (see figure 2) measured 3.5 m \times 0.5 m with a water depth of 0.1 m. A pump circulated the water giving a flow speed of 0.05 m s⁻¹. A series of mesh baffles were used within the channel to minimize turbulence; this had the additional effect of restricting the experimental arena to an area of 1.5 m \times 0.5 m. Two separate compartments measuring 0.16 m \times 0.08 m were constructed using netting material (mesh size of 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 of 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 preference zones of 60 mm around each stimulus-shoal compartment. This distance represents two body lengths of a 30 mm focal fish and falls within the range of inter-individual distances observed in free-ranging fish shoals (Pitcher & Parrish 1993).

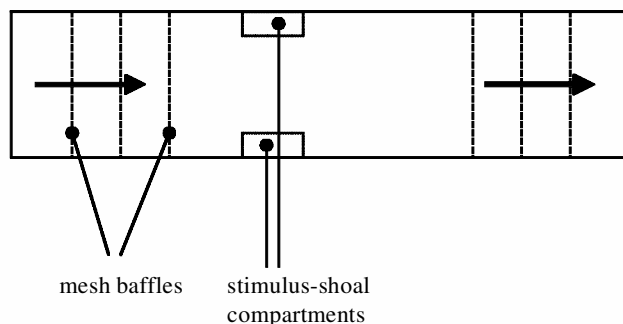


Figure 2. Aerial view of flow-tank apparatus. The arrows show the direction of the water flow.

For each replicate, a shoal of five stimulus fishes was added to each of the netting compartments. The stimulus fishes were then given 5 min to acclimatize 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 min to acclimatize before being liberated by the removal of the mesh cylinder. The time spent by the focal fish within two body lengths of either stimulus shoal was recorded for each stimulus shoal for a total of 5 min. Each focal fish and each stimulus shoal were used only once per treatment to prevent pseudoreplication, and trial order was randomized.

A total of four treatments were carried out, involving binary choices between stimulus shoals of:

- (i) non-familiar conspecifics versus non-familiar heterospecifics;
- (ii) familiar conspecifics versus non-familiar conspecifics;
- (iii) familiar heterospecifics versus non-familiar heterospecifics; and
- (iv) familiar heterospecifics versus non-familiar conspecifics.

3. RESULTS

(a) Shoal choice

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

In the following, we re-analysed these data to facilitate direct comparisons of shoaling preferences. To test the responses of focal fishes to heterospecifics in different contexts, we compared time shoaling with familiar heterospecifics against non-familiar heterospecifics in different treatments where, in both cases, the alternative stimulus shoal was composed of non-familiar conspecifics (see figure 3a,d). Focal fishes spent significantly more time in

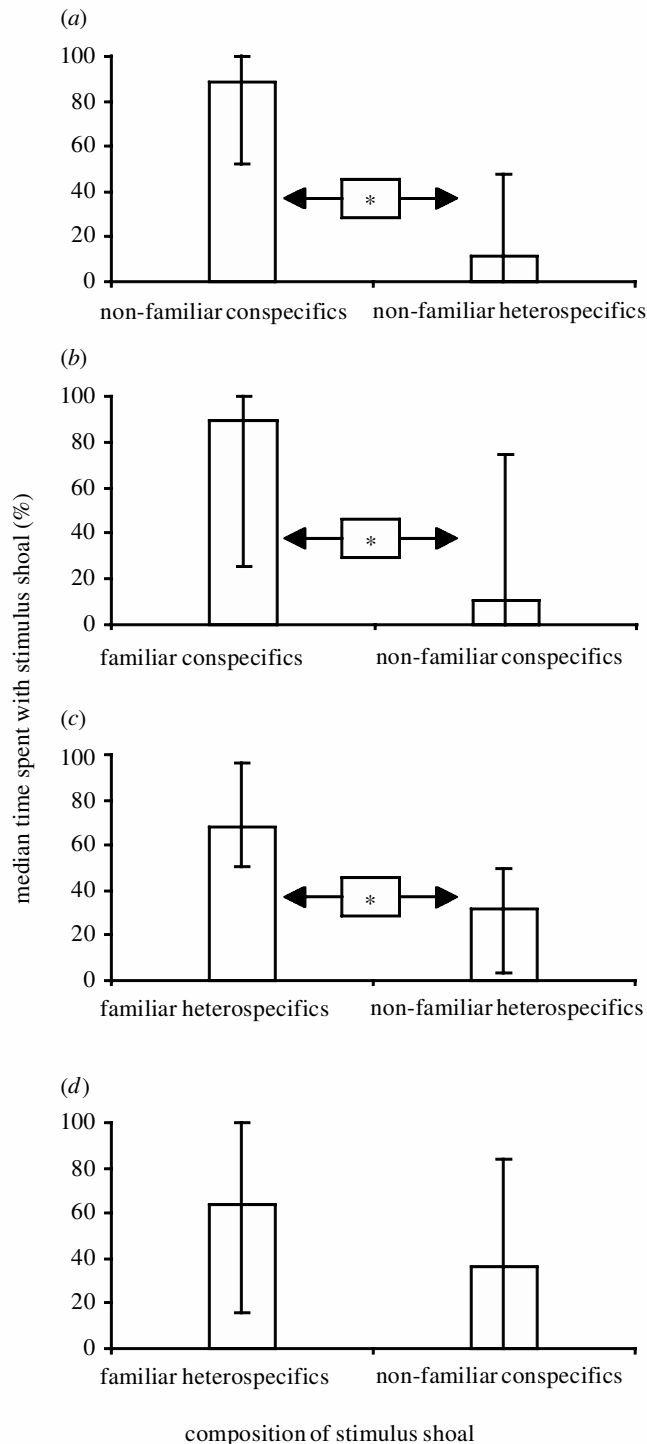


Figure 3. The median time \pm quartiles spent by focal fishes shoaling with each stimulus shoal as a percentage of the overall time spent shoaling. Median (\pm quartiles) time spent by the focal fishes in proximity to (a) non-familiar conspecifics against non-familiar heterospecifics; (b) familiar conspecifics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

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

(b) 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's rank: $r_s = -0.59$, $N = 20$, $p = 0.006$; figure 4).

4. DISCUSSION

Juvenile chub assorted preferentially with familiar fishes, 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 fishes 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 fishes were able to recognize and show preferences for familiar individuals, whether these were conspecifics or heterospecifics. Barber & Wright (2001) described apparent trade-offs made by European minnows, wherein fishes opted to associate with a shoal of familiars even though an alternative shoal of non-familiar fishes contained almost twice as many individuals, suggesting significant benefits. The high predation regime experienced by juvenile cyprinids in their natural environment ascribes high potential benefits to associating with familiars, given that shoals composed of familiars display more effective anti-predator strategies (Chivers *et al.* 1995). The overlapping habitat utilization of year 0+ chub and minnows (A. J. W. Ward, personal observations) 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 fishes. This may suggest the use of older fishes 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*;

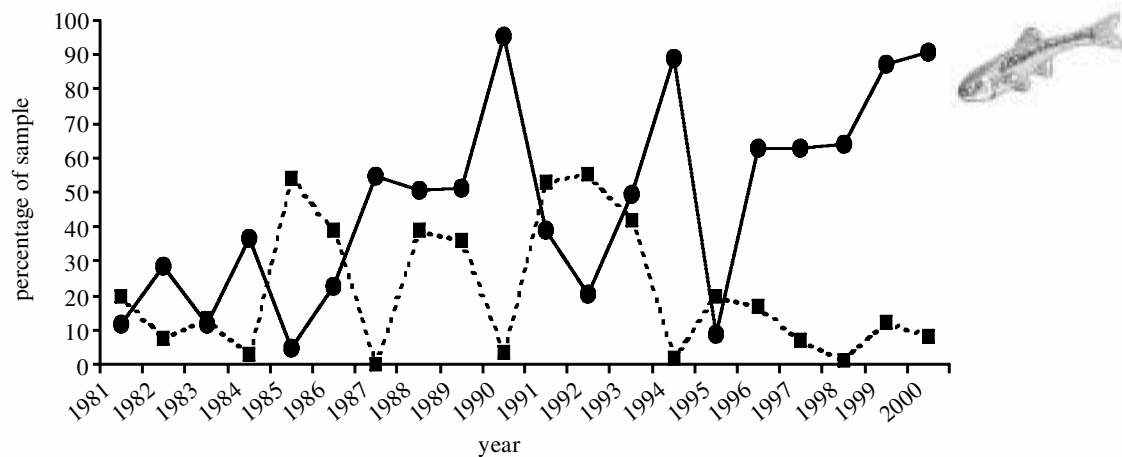


Figure 4. The percentage of the total sample of the year 0+ fishes made up by minnows (circles) and chub (squares) in an annual sample from 1981 to 2000. Data source: UK Environment Agency (2001).

Krause & Godin (1994), banded killifish, *Fundulus diaphanus*; Barber *et al.* (1998), three-spined stickleback, *G. aculeatus*). Interestingly, fishes 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 realized by assorting with familiars. Field population data at the site (UK Environment Agency 2001) show cyclical population fluctuations of both juvenile minnows and chub over 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 recognize 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 profit 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 & Morrisette (1992) reported the absence of any preference for conspecifics in the three-spined stickleback, *G. aculeatus*, when the alternative was the closely related black-spotted stickleback, *G. wheatlandi*. In this study, however, focal fishes clearly associated preferentially with conspecifics over heterospecifics when the choice was between two non-familiar stimulus shoals, thus showing that focal fishes could discriminate between the two species. This preference for conspecifics disappeared only when the alternative shoal was composed of familiar heterospecifics, indicating that the fishes made a trade-off between species preference and a preference for familiar fishes.

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-spined sticklebacks.

The authors thank G. Ruxton for stimulating discussions, and two anonymous referees for their comments on this paper.

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