

The importance of stable schooling: do familiar sticklebacks stick together?

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Preferences for rejoining shoals composed of familiar individuals have recently been documented in a variety of small, shallow-water fish species. Such preferences are assumed to be adaptive, since familiar groups have improved anti-predator defences and more stable dominance hierarchies. However, the design of these studies may have created conditions that elevate preferences for familiar individuals. Furthermore, in natural habitats, where significant opportunities for inter-shoal transfer may exist, it is unclear whether shoals stay together long enough for such preferences to develop. Here we present the results of a laboratory study examining whether prior familiarity influences the subsequent shoal composition of sticklebacks (*Gasterosteus aculeatus*) allowed to re-assort freely in a large arena tank. We show that fish from different familiarity groups associate with familiar conspecifics significantly more than predicted by a model of random assortment, suggesting that even when there is ample opportunity for inter-group transfer, shoal composition can remain stable. We discuss the phenomena that may lead to the formation of familiar groups in natural habitats. In addition, we suggest that familiarity benefits may reduce the relative value of transferring to otherwise more attractive (e.g. larger or more phenotypically matched) groups, and thereby stabilize shoal structure.

Keywords: shoaling; fish; familiarity; group dynamics; shoal composition; three-spined stickleback

1. INTRODUCTION

Recent studies have demonstrated that group-living fishes make facultative choices over group membership decisions. In general, fish appear to exhibit shoal choice decisions to maximize some aspect of their own fitness, and the preferences they show are generally interpreted as having anti-predator or foraging benefits (see reviews by Pitcher & Parrish 1993; Dugatkin & Wilson 1993; Reeb & Saulnier 1997). Specific preferences have been demonstrated for shoals composed of larger numbers of individuals (e.g. Hager & Helfman 1991), shoals that are further away from a predator (e.g. Ashley *et al.* 1993), shoals that contain parasite-free individuals (e.g. Barber *et al.* 1998), and shoals that contain exploitable poor competitors (e.g. Metcalfe & Thomson 1995). In addition, because visual predators often select odd individuals from groups (Landeau & Terborgh 1986), individuals may be expected to prefer to join groups that offer reduced levels of visual 'oddity', and preferences have been shown for shoals composed of size-matched (e.g. Pitcher *et al.* 1985; Ranta & Lindström 1990) and/or conspecific fish (e.g. Wolf 1985).

Recent studies have also suggested that in certain species of fish that naturally form shoals, there is a tendency for individuals to prefer to join groups of familiar conspecifics rather than those composed of individuals of which they have no recent experience (e.g. Van Havre & FitzGerald 1988; Magurran *et al.* 1994; Griffiths & Magurran 1997a,b; Lachlan *et al.* 1998). Unrelated, familiar fish are even preferred as shoal-mates over unfamiliar kin (Griffiths & Magurran 1999). Such

studies examining the role of familiarity in shoaling behaviour have generally used the same kinds of experiments, typically examining the responses of individual fish removed from familiar shoal-mates and given pairwise choices between joining two stimulus shoals, one of which is composed of familiar fish. These experiments have given valuable insight into the existence of familiarity preferences, yet the experimental design is artificial and separation from the shoal is likely to increase the stress level of the isolated fish, potentially enhancing preferences for familiar individuals. In addition, since such preferences may require a significant period to develop (12 days in guppies *Poecilia reticulata*; Griffiths & Magurran 1997b), it is interesting to ask what their significance may be in natural habitats, where inter-shoal distances may be small and separate shoals encounter one another frequently (I. Barber, personal observations). Although the dynamics of shoal membership under such conditions have not been adequately examined, field observations suggest that inter-shoal transfer of individuals under such conditions may be substantial (Helfman 1984; A. Svensson, I. Barber and E. Forsgren, unpublished data). Before the ecological benefits of familiarity can be seriously considered, it is essential that we know whether individual preferences for remaining with familiar group members are replicated in larger groups of unconstrained, freely interacting fish.

Here, we present the results of a study examining the influence of prior familiarity on the group fidelity of fish from separate shoals once they are allowed to interact freely. The aim of our study was to determine whether, when all physical barriers to inter-shoal exchange are removed, familiar members of fish shoals demonstrate preferences for associating with one another.

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2. MATERIAL AND METHODS

(a) *Fish collection and husbandry*

In September 1998, approximately 500 three-spined sticklebacks *Gasterosteus aculeatus* were caught using a pan net (diameter 90 cm) from a harbour in a channel between the Gullmar and Koljö fjords (58°14' N, 11°33' E) on the West Coast of Sweden. The sample contained fish from many large shoals (each comprising 100s to 1000s of fish) that had gathered in the harbour prior to autumnal offshore migration. Fish were collected from large shoals to minimize the chance that individuals from small, already familiar groups were sampled. Fish were transported together to the laboratory where they were housed in a 600-l tank (1 m × 1 m × 60 cm water depth) prior to standard length measurement of all individuals. The total sample exhibited clear bimodality with respect to body size and only fish from the centre of the upper modal group (total length 48 mm to 56 mm, presumed second-year fish) were used in the experiment; all other fish were returned to the sea. The timing of fish collection ensured that the vast majority of fish were in post-breeding condition. Any males with remnant nuptial coloration, or females visibly carrying eggs, were excluded from the sample, as were any with visible parasitic infections (which are known to affect both shoal membership decisions and schooling ability; Barber *et al.* 1995; Barber & Huntingford 1996; Krause & Godin 1996). The remaining sticklebacks, which were therefore matched for size, neutral sexual appearance and absence of visible parasites, were then split into eight groups of 12 fish, and marked individually with group-specific tags. The tags—made from the coloured plastic outer coating of fine computer wire cut into 0.5 mm sections and pressed onto the left pelvic spine—were non-invasive and effective: none were lost during the study. Fish length did not differ between the familiarity groups (mean length (\pm s.d.) of fish in groups A–H: 52.5 mm (1.2 mm), 51.9 mm (1.2 mm), 52.5 mm (1.0 mm), 52.9 mm (1.0 mm), 51.6 mm (2.0 mm), 52.4 mm (1.8 mm), 52.2 mm (2.4 mm), 52.5 mm (1.3 mm); ANOVA, $F_{7,95} = 0.83$, $p = 0.57$). The fish were maintained in these groups for six weeks, with each group being housed separately in 600-l, 1 m × 1 m holding tanks, filled to a depth of 60 cm with a constant flow of fresh seawater pumped from the fjord. During the familiarization period, and between trials, fish were fed *ad libitum* with chopped blue mussel *Mytilus edulis*.

(b) *Experimental protocol*

From the eight separate familiar groups it was possible to generate 28 different pairs that could be tested together in the experiment. All individuals from two familiar groups were dip-netted from their holding tanks and transferred to separate containers with 1 l of seawater. Both groups of fish were then poured simultaneously into a third container (10 l), to facilitate thorough mixing of the two familiar groups. The 24 fish were then poured quickly into the centre of the experimental arena, a large rectangular tank (3 m (long) × 1 m (wide) × 1 m (deep)) filled to a depth of 80 cm with seawater (figure 1). Sufficient artificial vegetation was positioned in each corner of the tank to provide cover for approximately half of the individuals present (the precise amount of cover required to achieve this was determined empirically during preliminary studies). The trials were recorded by a CCTV camera fitted with a wide-angle lens, mounted on a gantry that also held two 60 W light bulbs which, together with background fluorescent strip lighting, provided constant illumination throughout the tank. Following transfer to the large arena, the group of sticklebacks initially formed a single school, but

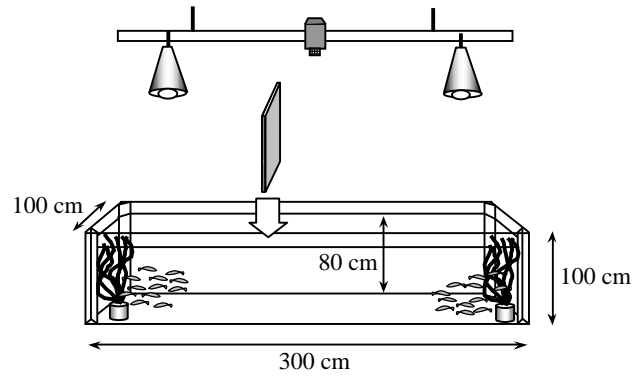


Figure 1. The experimental arena tank (see text for details).

separated into two groups around the two artificial structures in the arena within several minutes (mean time taken to separate (\pm s.d.) = 680 s (344 s)). Such behaviour is typical of sticklebacks faced with such conditions (e.g. Ranta & Lindström 1990). By watching the live image on a video monitor the observer was able to track the movements of the fish around the experimental arena. The specific criterion used to determine when the group had split into two separate shoals was that at least eight of the fish from the group of 24 were separated from the remainder by at least 2 m (effectively when the two separate shoals occupied opposite ends of the tank). When this had occurred, a divider was lowered quickly into the tank and all of the fish in the group around one of the refuges (one of the two 'associating groups', chosen at random) were then caught and removed.

The fish in the associating group removed from the experimental arena were then examined and identified using the coloured bands on their pelvic fins. The number of associating fish from each familiar group was recorded. The remaining fish were then removed from the experimental arena and separated into their original familiarity groups. All fish were then returned to their 'home' tanks. No group was used more than once on any day. After all trials had been completed, tags were removed and fish were returned to the fjord.

(c) *Calculation of the assortment index*

We developed an assortment index (I_a) to express the absolute difference in the proportions of fish from the two familiar groups that were found at one side of the tank, calculated using the equation below

$$I_a = \left| \left(\frac{n_x}{12} \right) - \left(\frac{n_y}{12} \right) \right|, \quad (1)$$

where n_x and n_y are the numbers of fish from the two original familiar groups (x and y) in the newly formed associating group at one side of the tank. Note that it does not matter which side of the tank is chosen: the indices work out to be the same, since

$$\left| \left(\frac{n_x}{12} \right) - \left(\frac{n_y}{12} \right) \right| = \left| \left(\frac{12 - n_x}{12} \right) - \left(\frac{12 - n_y}{12} \right) \right|. \quad (2)$$

(d) *Predictions from a null model*

Simulation was used to generate the frequency distribution of assortment index values that would be expected if the 24 fish always assorted randomly with respect to familiarity status, i.e. if they split into two groups on a completely individual basis. The number of fish from the two groups expected at one side of the tank under such random assortment in 1000 simulated trials was calculated in the following way. Each of the two familiar

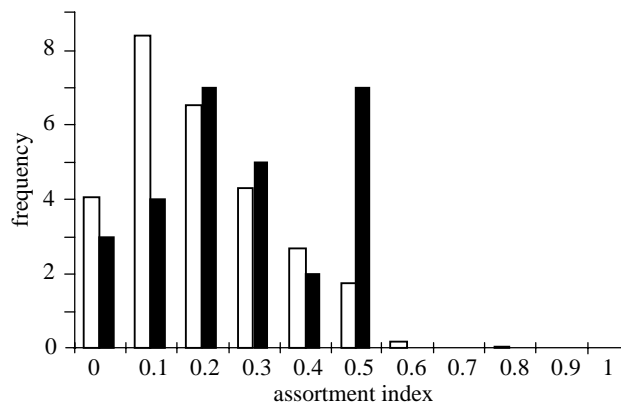


Figure 2. The frequency distribution of assortment indices estimated from simulations based on a model that assumes no effect of familiarity (open bars), and the frequency distribution of assortment indices from experimental trials (black bars). The distributions differ significantly (see text for statistical details).

groups of fish in the simulated trial was represented by 12 randomly generated numbers: 1 (representing those present at one side of the tank) or 0 (representing those absent, i.e. in the other associating group). Ones and zeros each occurred with a probability of 0.5, so that each fish in the simulation had an equal probability of being found at one end of the tank than the other. By summing the total number of ones for each group, data equivalent to that obtained from the experimental set-up was generated. Calculation of the assortment index was then carried out for the 1000 simulated trials in precisely the same way as for the experimentally derived data. The expected frequency distribution of association index values obtained in this way could then be compared with that of the experimentally determined data using a χ^2 -test (Sokal & Rohlf 1995).

3. RESULTS

The distribution of association index values from the outcome of experimental trials differed significantly from the distribution of association index values calculated from probabilities generated by the simulation model (that assumes no effect of familiarity) (χ^2 -test, $\chi^2 = 49.04$, d.f. = 10, $p < 0.05$; figure 2). To test the directionality and statistical significance of the difference between the observed and expected frequency distributions, 100 sets of 28 assortment indices were selected at random from the population of 1000 simulated 'trials', and the mean assortment index calculated for each set. The frequency distribution of the mean assortment indices generated from these 100 sets of 28 simulated trials is shown in figure 3, and is compared in that figure with the mean level of assortment observed from the experimental trials. Ninety-nine out of the 100 simulated 'experiments' had mean assortment indices lower than the observed mean value. We therefore conclude that the observed mean level of assortment was significantly higher than the values predicted from the random assortment model (randomization test, estimated $p = 0.01$).

4. DISCUSSION

In this study we have demonstrated that sticklebacks maintained together in shoals over a period of six weeks

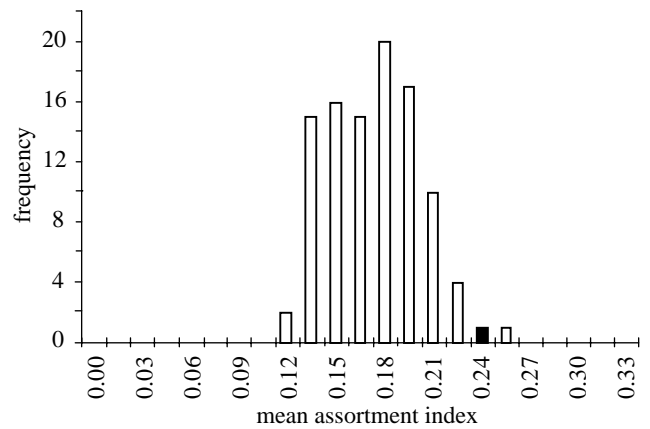


Figure 3. Frequency distribution of mean association indices from 100 sets of 28 simulated trials (= 100 simulated 'experiments') drawn at random from a population of 1000 simulated trials (open bars) and the position of the observed mean association index from the 28 trials carried out (black bars). Ninety-nine out of the 100 expected mean values fall below the observed mean value, resulting in an estimated probability value of 0.01 (see text for statistical details).

subsequently associated preferentially with each other, following the removal of any physical barriers to their separation from other shoals. Although there was no evidence of perfect assortment amongst the 28 trials carried out (in other words in none of trials did assortment groupings completely match familiarity groupings), the assortment indices obtained from the experimental trials were significantly higher than those predicted by a model of random assortment. Clearly a significant proportion of fish from each group were associating preferentially with familiar individuals.

Experimental tests examining the fidelity of freely associating groups of fish are scarce and have provided equivocal data. Mark-recapture studies suggest that large skipjack tuna *Katsuwonus pelamis* schools have a rapid turnover rate (up to 63% per day) whereas smaller subunits may be substantially more stable (Hilborn 1991). Griffiths (1997) took minnows *Phoxinus phoxinus* from two distant sites in the River Frome, England, and found that, when groups from the two sites were subsequently mixed in a flume tank, 75% of individuals in small foraging shoals (four to five fish) were from the same location. However, since these fish were from shoals collected at the separate locations, it is not clear that observed preferences for shoal-mates in the experiment were based solely on familiarity. For example, local population- or subpopulation-level variation in a morphological or conditional trait (such as body coloration or nutritional condition) could lead to the same results, if individuals adopted phenotype-matching behaviour (Brown *et al.* 1993) during shoaling. In our study, familiarity groups were generated at random from fish sampled from one location, and maintained together over a period of six weeks in standardized holding tanks, in complete sensory isolation from other groups. Because of this, we are confident that familiarity developed during the holding period, rather than any population-level variation, was responsible for the shoaling preferences we report.

Preferences for familiar conspecifics are argued to be adaptive, since it is likely that there are benefits to be gained from grouping with familiars, including the possible maximization of anti-predator responses. These benefits are thought to exist because knowledge of the particular behaviour and skills of recognizable individuals, gained and reinforced through direct experience, may be used to maximize the group's response to stimuli. Experimental studies have demonstrated such putative benefits. Groups of familiar fathead minnows *Pimephales promelas* form more cohesive shoals than non-familiar fish and appear to have better group anti-predator responses (Chivers *et al.* 1995). Groups of familiar salmonids are known to be less aggressive (Johnsson 1997), and familiar groups also exhibit stable dominance ranks, reduced nearest-neighbour distance and higher food intake for all fish than in unfamiliar groups (Höjesjö *et al.* 1998).

The ability to recognize individuals, or at least base grouping decisions on preferences for familiar individuals, appears to be restricted to vertebrate taxa. Cuttlefish *Sepia officinalis*, for example, are unable to recognize individuals under experimental conditions (Boal 1996) and social squids *Sepioteuthis lessoniana* do not shoal with familiar individuals when allowed to freely associate (Boal & Gonzalez 1998). The development of recognition mechanisms in fishes is not well understood, but it seems that both visual and chemical cues are important in distinguishing between familiar and non-familiar individuals (e.g. Waas & Colgan 1994; Brown & Smith 1994). In guppies, the tendency of females to school with familiar fish declines as the group size in which they live naturally increases (Griffiths & Magurran 1997b), suggesting a limit to the number of individuals with which they may familiarize.

Inter-shoal distances are often very small in three-spined sticklebacks and other small, shoaling inshore fishes inhabiting shallow-water ecosystems, and separate shoals encounter one another frequently (I. Barber, personal observations). The opportunity for movement of individuals between shoals in such situations is likely to be considerable (A. Svensson, I. Barber and E. Forsgren, unpublished data), yet little is known about shoal fidelity or shoal dynamics in small fishes such as those examined in these studies. Given that fish show measurable state- and condition-dependent preferences for so many shoal attributes, it seems likely that for any individual shoal member there will frequently be a more 'attractive' (e.g. larger, more phenotypically matched) shoal within easy reach. In this scenario, which is likely to be true for the vast majority of small shoaling prey fishes that occupy shallow inshore areas, extensive, continuous mixing between shoals may be predicted. This would prevent long-term fidelity and reduce the ecological importance of any benefits resulting from the development of familiarity. The main factors limiting transfer between shoals, and hence the major forces stabilizing shoal composition, are generally thought to be the increased predation risks involved with breaking away from a group, existing as an individual for a short time whilst 'in transit', and joining a new group (Pitcher & Parrish 1993). There may also be additional costs to being a new shoal member, such as reduced performance during anti-predator group manoeuvres or, since fish can recognize

familiar shoal-mates (and hence non-familiar ones), possible initial exclusion from food or other resources. Whilst these transfer and joining costs are almost certainly important, the benefits that may be accrued by remaining with familiar shoal-mates may also help offset any advantages gained by leaving a familiar group in order to join an unfamiliar but otherwise apparently more 'attractive' shoal. The existence of such familiarity benefits would then stabilize shoal structure, and limit inter-shoal fish transfer until the gross fitness benefit of doing so was large enough to offset them. Unlike the proposed transport costs of shoal switching, familiarity benefits would be independent of inter-shoal distance and would be important stabilizers of shoal composition even when shoals were in very close proximity.

Although we have demonstrated that freely interacting fish show significant preferences for remaining together, it is still unclear what causes a group, once formed, to remain together long enough for familiarity to develop. One possibility is that simple transfer costs may initially limit transfer between shoals of juvenile fish. As the fish grow these transfer costs may become less important and familiarity benefits take over as the main stabilizer of shoal structure. Titration-style experiments would be valuable in developing our understanding of how important familiarity benefits are in relation to other factors known to be important in shoaling decisions, such as shoal size and phenotype matching. In addition, the improvement (in terms of miniaturization and affordability) of implanted microchip-tags—which can be detected by in-water sensors and provide constantly updated data on shoal composition—will provide an enormous potential for the development of research into long-term shoal structure and dynamics.

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