Association patterns and foraging behaviour in natural and artificial guppy shoals

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Animal groups are often non-random assemblages of individuals that tend to be assorted by factors such as sex, body size, relatedness and familiarity. Laboratory studies using fish have demonstrated that familiarity among shoal members confers a number of benefits to individuals such as increased foraging success. However, it is unclear whether fish in natural shoals obtain these benefits through association with familiars. Here, we investigate whether naturally occurring shoals of guppies (Poecilia reticulata) are more adept at learning a novel foraging task than artificial (where shoal members were selected randomly by the authors) shoals. We used social network analysis to compare the structure of natural and artificial shoals and examine whether shoal organisation predicts patterns of foraging behaviour. We found that fish in natural shoals benefited from increased success in the novel foraging task compared with fish in artificial shoals. Individuals in natural shoals showed a reduced latency to approach the novel feeder, followed more, and formed smaller sub-groups compared to artificial shoals. Our findings show that fish in natural shoals do gain foraging benefits, and that this may be facilitated by a reduced perception of risk among familiarised individuals, and/or enhanced social learning mediated by following other individuals and small group sizes. Although the structure of shoals was stable over time, we found no direct relationship between shoal social structure and patterns of foraging behaviour.

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Wild animal groups commonly display non-random patterns of social structure. Within a species, individuals are known to associate on the basis of kinship (Ward & Hart 2003, Silk et al. 2006), body size (Ward & Krause 2001), parasite load (Krause & Godin 1994), disease status (Behringer et al. 2006) and colour (McRobert & Bradner 1998), resulting in groups that are assorted by phenotypic characteristics. These association patterns are thought to confer anti-predator benefits, such as a reduction in risk through predator confusion; and foraging benefits, such as reduced competition for resources (Krause & Ruxton 2002). A further level of social organisation can arise when individuals preferentially associate with, or avoid, conspecifics based on previous interactions (Griffiths 2003).

Preferences for associating with familiars have been reported in a number of animal groups (e.g. mammals: Porter et al. 2001, birds: Senar et al. 1990, reptiles: Bull et al. 2000, insects: Clarke et al. 1995), but have been particularly well studied in shoaling fishes. These studies have revealed that associations based on familiarity occur both in the laboratory (e.g. Barber & Ruxton 2000, Barber & Wright 2001) and the field (Griffiths & Magurran 1997a, reviewed in Griffiths 2003). A number of benefits to associating with familiars have been demonstrated, including enhanced predator escape responses (Chivers et al. 1995), reduced levels of aggression (fishes: Utne-Palm & Hart 2000; birds: Temeles 1994), increased foraging success (reviewed in Krause & Ruxton 2002, Griffiths 2003, Ward & Hart 2003) and an elevated performance in learning tasks (Swaney et al. 2001, Galef & Giraldeau 2001).

Previous work has demonstrated that animal groups contain pairs or small groups of individuals that are linked by stable interactions (Croft et al. 2004, 2006,
However, the majority of work investigating the benefits of familiarity has focused on groups of fish that are artificially familiarised in the laboratory (by holding fish together in small groups for a period of two weeks or more), and it is unclear whether wild (i.e. naturally assorted) shoals would gain the same benefits. Wild shoals of fathead minnows (*Pimephales promelas*) show more cohesive anti-predator behaviour than unfamiliar, laboratory-assembled shoals (Chivers et al. 1995), but other putative benefits of familiarity have not yet been studied in natural groups. Here, we investigate whether natural shoals of guppies (*Poecilia reticulata*) benefit from enhanced foraging success, when compared to artificial (laboratory-assembled) shoals. We used female guppies, as they display greater within-shoal fidelity than males (Griffiths and Magurran 1998), are more likely than males to exploit novel foods (Laland & Reader 1999a), and learn new foraging tasks more rapidly than males (Laland and Reader 1999b). We present the shoals with a novel foraging task, consisting of a novel foodstuff hidden within a feeder, and investigate the success of shoal members in completing the task.

Previous work with fishes has shown that foraging success and performance in a novel learning task are influenced by social structure, specifically group size. For example, individuals in larger groups are generally more successful at locating food patches e.g. Pitcher et al. 1982), and learning to escape from a moving net (Brown & Warburton 1999) than individuals in smaller groups, but individuals in smaller groups can locate food more quickly when the food is hidden (the conformity effect; Day et al. 2001). The influence of familiarity on learning has been well studied: In fish, individuals learn more successfully from familiar demonstrators than from unfamiliar conspecifics (where familiar groups are created by holding individuals together in the
laboratory over a period of time; Swaney et al. 2001, Ward & Hart 2005), and in birds, young learn how to handle a new food source more effectively from familiar adults than from unfamiliar ones (Cadieu & Cadieu 2004).

One method which is becoming increasingly useful for understanding associations and structure in animal populations is social network analysis (e.g. Croft et al. 2004, Lusseau & Newmann 2004, Wolf 2005). Previous work on guppies using this approach has demonstrated that individuals have preferred associations (i.e. associations are non-random), even within small shoals of fish (Croft et al. 2004, 2006). We use a social networks approach to investigate the influence of social structure on the performance of guppies in a novel foraging task. To our knowledge, this is the first time these techniques have been used in this way. Firstly, we investigate differences in social structure between natural and newly-created artificial shoals, and success in the foraging task. Secondly, we investigate whether social association patterns are linked to patterns of foraging and information transfer within groups. We predict that the social structure of natural shoals will reflect the non-random associations occurring within them (Croft et al. 2006), and thus will differ from association patterns in artificial shoals, where individuals will be unfamiliar, and preferred associations will not have been established. We also predict that individuals in natural shoals will forage more successfully (Swaney et al 2001, Ward & Hart 2005), and that within shoals, individuals will be more likely to foraging with and obtain social information from those shoal members with whom they have close associations.

METHODS
Study Site and Holding Conditions

Fish were captured from the Arima River in the Northern Mountain Range of Trinidad (within 500m of Verdant Vale Village, 10°41´N, 61°17´W) during May 2005, between 09.00 – 16.00 hours, using a 2m beach seine. In our investigation we used natural and artificial shoals of fish, each containing 8 adult females. Shoals of 8 were chosen as being representative of natural shoal sizes (2-20 individuals; Croft et al. 2003a). Natural shoals (N=10) consisted of groups captured together in the wild, and artificial shoals (N=10) were groups assembled in the laboratory from over 300 fish (see below).

Natural shoals consisting of at least 10 individuals were captured from the river in their entirety, any males were released back into the river and 8 females of a similar body size (mean ± SD size of females in natural shoals = 27.99 ± 2.97mm) were selected from each shoal. The remaining fish were returned to the river. A shoal of guppies was defined as individuals that occurred within 4 body lengths of each other (Pitcher & Parrish 1993). The 8 fish were kept together, isolated from other shoals, and returned to the laboratory, where each shoal was placed in a large artificial pool (diameter = 120cm, water depth = 5cm, water temperature = 26ºC), and allowed to acclimatise for 24 hours.

The 10 artificial shoals were created by collecting over 300 females using 36 seine hauls from a 500m stretch of river. The fish were returned to the laboratory and placed in an artificial pool (as above) for 24 hours to allow complete mixing. From this population 80 individuals of similar body size (mean ± SD size of females in
artificial shoals = 28.09 ± 4.07mm) were selected and assigned haphazardly to 10 shoals. Each shoal was then placed in a visually isolated pool for 24 hours, as above. Body size did not differ significantly between fish in natural and artificial shoals (independent samples t-test: \( t_{1,158} = -0.178, p = 0.859 \)), and there was no significant different in within-shoal standard deviation between natural and artificial shoals (\( t_{1,18} = -1.437, p = 0.168 \)). Unused fish were placed in two large outdoor pools, and all test shoals were maintained on a diet of commercially available flake food, given twice per day.

Female guppies do not have any natural markings that can be used for identification purposes and thus individuals from all shoals were anaesthetised using tricane methanesulfonate (MS222), and given individual identity marks using a visible implant elastomer injected in the dorsal epidermis (a standard procedure for marking fish: see Croft et al. 2003b). All fish recovered quickly from the anaesthetic, normal swimming behaviour was quickly resumed, and no mortality was observed as a result of the marking process. Previous work has demonstrated that the procedure does not affect shoaling decisions in guppies (Croft et al. 2004). After marking, shoals were allowed to acclimatise for 12 – 16 hours before experimental procedures began. The experimental protocol for each shoal consisted of 1) quantifying the social structure and shoaling associations (see below), 2) introducing a novel foraging task to the pool and observing the success of fish in completing the task, and 3) re-quantifying the social structure. For each shoal, the three elements of the experimental protocol were carried out sequentially, separated by periods of 10 minutes.

Quantifying Social Structure
We quantified patterns of association by visually observing and recording the membership of sub-groups within the shoal once per minute over a 30 minute period (Croft et al. 2004; Thomas et al. in press). Previous work has shown this to be a sufficient time period to quantify the non-random social network structure of guppy shoals (see Croft et al. 2004 online supplementary material). We defined an association between two fish as occurring when the fish were positioned within four body lengths of each other, a distance that falls within the range of inter-individual distances most commonly observed in shoaling fishes in nature (Pitcher & Parrish 1993). All fish in a sub-group were within 4 body lengths of at least one other member of the group (thus, if fish A and C were 6 body lengths apart, but fish B was positioned between them, then all were considered members of a single sub-group).

One observer sat motionless, close to the edge of the pool, and a second person was positioned further back from the pool to record the observations relayed to them by the observer.

**Novel Foraging Task**

Ten minutes after quantifying association patterns, we introduced a novel foraging task to the experimental arena. The task consisted of a white, opaque, plastic cylinder (the ‘feeder’, height = 85mm, diameter at base = 75mm, diameter at top = 68mm) with a 20x20mm entrance hole located in the lower wall. The feeder was placed with its centre 30cm from the edge of the pool closest to the observer, with the entrance hole facing the observer. At the beginning of the trial, a pinch of freeze-dried bloodworm (*Chironomus* spp.) was placed in the feeder, where it floated on the surface of the water, but was constrained within the feeder. Any odour cues from the bloodworm are likely to be well contained within the feeder (Laland, K.N.,
unpublished data). Bloodworm represents a high-protein food source, readily consumed by the fish, and similar food items are likely to make up part of their natural diet (Magurran 2005). As the bloodworm floated on the water surface, fish feeding at the surface inside the feeder were not visible to fish outside the feeder.

Following the introduction of the feeder, we recorded the time taken and the identity of the first fish to approach the feeder within four body lengths. After this initial approach observations were made over a 30 minute period. The delay before commencing the observation period ensured that the fish were settled following the introduction of the feeder into the pool, and that they had identified the presence of this novel object. Each time a fish entered the feeder, or fed on the bloodworm at the surface, we recorded the time since the start of the observation period and the identity of the fish.

After 30 minutes, we removed the feeder and any remaining bloodworm from the pool using a fine-mesh dip net, and after a 10 minute settling period, we re-quantified social structure and association patterns using the same procedure as above. This allowed us to check whether social structure was stable over the time period of the experiment. Previous work has demonstrated that in the wild guppies can move between shoals very rapidly, changing shoals up to once per minute (Croft et al. 2003a), and that associations quantified using this method are based on active preferences (Croft et al. 2004). The 30-minute time interval between the two measures of (pre- and post-foraging task) social stability is therefore sufficient to allow ample opportunity for individuals to move among groups. At the end of the experiment, fish
were removed from the experimental arena and placed in large artificial outdoor pools, isolated from the river system.

Data Analysis

*Group Sizes and Social Stability*

From our observations of association patterns prior to the foraging task (see ‘Quantifying social structure’, above) we calculated the number and size of sub-groups occurring at each time interval (i.e. every minute for 30 minutes, yielding 30 observations of group size for each shoal). From these data, the mean group size was calculated for every time interval and we calculated the median value for each shoal independently. We compared the median group sizes of natural and artificial shoals using a Mann-Whitney U test. For each shoal, we also calculated the mean percentage of individuals that were present in groups of sizes 1 to 8, over the 30 observations, to give frequency distributions of group sizes for natural and artificial shoals. To test whether shoal associations are stable over time (i.e. before and after the foraging task), we created association matrices for each shoal, describing association patterns before and after the foraging trial. We compared the two association matrices (before and after the foraging task) for each shoal using Mantel Tests for matrix correlations. Where appropriate, p-values were combined using Fisher’s Omnibus Test to examine patterns across shoals. Throughout, ‘shoal’ refers to all 8 individuals in a pool, and ‘group’ is used when the shoal divides into sub-units.
For each shoal, we calculated the proportion of times that each individual fish was observed with each other fish (i.e. the proportion of times all possible pairs were associated) during the observation period (association strength, AS). The mean of these values gives an overall AS for each shoal, which is an additional measure of shoal cohesion. To investigate the variation in association scores, we calculated the coefficient of variation of the association strengths for each shoal. In each shoal, all fish were observed together one or more times over the 30min observation period, giving fully interconnected social networks. As such, measures of social network structure based on the presence or absence of interactions between individuals (such as mean degree, path length and clustering coefficients; e.g. Newman 2003; Croft et al. 2004) are not informative and therefore have not been calculated.

Patterns of Foraging

If individuals learn socially from one another, we predict that they are likely to follow one another into the feeder. To investigate whether individuals solved the task by following another individual, or entering the feeder alone, we identified events where an individual first entered the feeder shortly after another individual. We used three definitions of following: 5, 10 and 20 seconds, and all following events were included. Thus, an individual that entered the feeder 4 seconds behind another would be included in all three analyses, while an individual entering 14 seconds behind another would only be included in the 20 second analysis. A definition of following within 5 seconds represents a situation where individuals enter closely behind another individual (within about 4 body lengths), and therefore could be considered members of the same shoal (Pitcher and Parrish 1993). A definition of following within 20 seconds, on the other hand, allows for the possibility that individuals can observe
another entering the feeder from some distance away, then approach and enter the
feeder themselves. In the most successful shoal, the total number of feeder entries
over the 30 minutes was 59. This means that on average, one fish fed every 30.5
seconds, a greater time interval than our longest following definition of 20 seconds.
Within a shoal type (natural or artificial) we corrected p-values using Benjamini and
Hochberg’s (1995) method for false discovery rate (FDR) control. Adjusted p-values
are presented.

If individuals do follow one another into the feeder, rather than foraging
independently, we would predict that entries to the feeder to be more closely clustered
in time than would be expected by chance (i.e. the null hypothesis would be that
foraging events are independent of one another). To investigate whether this is the
case, we performed a randomisation test. We used only foraging events occurring
within the interquartile range of foraging times (i.e. the ‘middle half’ of the each trial)
to control for differences in response towards the feeder after it was placed in the
pool, and any effect of satiation or food depletion on foraging towards the end of the
trial. For each shoal, we calculated the total number of observed feeder entries and the
number of occasions on which the difference between one entry time and the
preceding one (the entry lag) was less than 5, 10 or 20 seconds (possible following
events). We then generated a random set of feeder entry times (within the time
available in the interquartile range), containing the observed number of feeder entries,
and calculated the number of times the entry lags were less than 5, 10 or 20 seconds.
This was repeated 999 times. We calculated the total number of randomisations where
the predicted number of following events was greater than or equal to the number
observed in the shoal, to give a conservative probability that entries were more
clustered in time in the observed shoal than expected by chance (one-tailed test). This
was repeated for all 10 natural shoals, and the 6 artificial shoals where entry events
were observed.

We used a further randomisation technique to investigate whether individuals
that first entered the feeder by following a leader (‘lead-follow pairs’) were
significantly more highly associated with that individual than would be expected if
they learnt the task by following another individual at random. To control for
differences between shoals in overall levels of association, we calculated the total of
the association strengths of all lead-follow pairs for each shoal. We defined lead-
follow pairs as two fish that entered the feeder within 20 seconds of each other,
assuming that individuals followed the immediately preceding fish (i.e. if three
individuals enter closely in time, in the order A, B and then C, we assume that B
follows A, and C follows B). The 20 second rule only was used due to low numbers
of following events for other rules in some shoals. For each shoal, we then randomly
selected the same number of pairs as were observed in lead-follow events, and
summed their association strengths. This randomisation was repeated 999 times for
each shoal. We then calculated how many of the randomly generated pairs had a
higher sum of association indices than the observed pairs, generating a probability (p-
value) that the observed pairs were significantly more associated than random pairs
(one-tailed test).

RESULTS
Grouping behaviour differed between natural and artificial shoals. The median group size was significantly larger in artificial shoals than in natural shoals (Mann-Whitney U: \( z = -3.659, N_{\text{natural}} = N_{\text{artificial}} = 10, P < 0.001 \)), and small groups were commonly observed in shoals of naturally co-occurring fish, while larger groups were most commonly found in artificial shoals (figure 1).

Natural shoals had a significantly smaller mean shoal association strength (AS) than artificial shoals (Mann Whitney U: \( z = -3.628, N_{\text{natural}} = N_{\text{artificial}} = 10, P < 0.001 \); figure 2a). Associations in natural shoals were also more variable than in artificial shoals: the coefficient of variation in AS was higher in natural than in artificial shoals (Mann Whitney U: \( z = -3.175, N_{\text{natural}} = N_{\text{artificial}} = 10, P = 0.001 \); figure 2b). Both natural and artificial shoals thus showed variation in AS between pairs of individuals within a shoal, giving us the opportunity to investigate patterns of learning in relation to patterns of social structure.

After the feeder had been placed in the pool, fish from natural shoals approached it significantly more rapidly than fish from artificial shoals (Mann Whitney U: \( z = -2.117, N_{\text{natural}} = N_{\text{artificial}} = 10, P = 0.035 \); figure 3a), and more fish from natural than artificial shoals entered and fed from the feeder (entering the feeder: Mann Whitney U: \( z = -2.701, N_{\text{natural}} = N_{\text{artificial}} = 10, P = 0.007 \); not shown, feeding: Mann Whitney U: \( z = -3.752, N_{\text{natural}} = N_{\text{artificial}} = 10, P < 0.001 \); figure 3b). In all 10 of the natural shoals at least three fish fed, successfully completing the task. In one shoal, all
fish fed. In comparison, we observed feeding in only 5 of the artificial shoals, where a maximum of three individuals fed.

Can Patterns of Association Predict Patterns of Foraging?

Patterns of association in the pre-foraging trial (first) social network were significant predictors of association patterns in the post-foraging trial (second) social network (network correlations analysed using Mantel Test for matrix correlations, \( P \) values combined using Fishers Omnibus test: wild shoals, \( F_{20} = 63.45, P<0.001 \), Table 1a; random shoals, \( F_{20}=35.64, P=0.02 \). This suggests that associations are stable over the time of the experiment, and all further analysis is based on pre-foraging trial associations only.

Do individuals follow others?

We found strong evidence that entries to the feeder were more closely clustered in time than would be expected by chance in both natural and artificial shoals, regardless of the definition of following used (Natural shoals: 5 seconds: \( F_{20}=83.989, p<0.001 \). 10 seconds: \( F_{20} = 84.011, p<0.001 \). 20 seconds: \( F_{20}=54.200, p<0.001 \). Artificial shoals: 5 seconds: \( F_{12}=27.522, p=0.006 \). 10 seconds: \( F_{12}=44.190, p<0.001 \). 20 seconds: \( F_{12}=46.207, p<0.001 \). Fish in both natural and artificial shoals are therefore more likely to enter the feeder together than might be expected if each individual was foraging independently, suggesting that the guppies were foraging socially.

In natural shoals, the majority of fish first entered the feeder alone (using a rule that individuals only followed if they entered the feeder within 5 seconds of
another fish), rather than following closely behind another fish (Wilcoxon signed
ranks test: \( z = -2.501, N=10, P=0.036 \) figure 4), but this was not the case in artificial
shoals \( z = -2.014, N=10, P=0.123 \), figure 4). Using a 10 or 20 second rule, however,
there was no difference in the number of individuals who first entered the feeder alone
and the number that first entered the feeder by following another individual
(Wilcoxon signed ranks test: 10 seconds: natural shoals: \( z = -1.869, N=10, P=0.093 \);
artificial shoals: \( z = -1.841, N=10, P=0.099 \); 20 seconds: natural shoals: \( z = -1.279, N=10, P=0.201 \);
artificial shoals: \( z = -1.236, N=10, P=0.216 \), figure 4). Using all rules,
there were elevated numbers of individuals that solved the task by following in the
natural compared to the artificial shoals (5 seconds: Mann Whitney U: \( z = -2.282 \),
\( N_{\text{natural}} = N_{\text{artificial}} = 10, P=0.039 \), 10 seconds: Mann Whitney U: \( z = -2.868 \),
\( N_{\text{natural}} = N_{\text{artificial}} = 10, P=0.008 \), 20 seconds: Mann Whitney U: \( z = -3.032 \),
\( N_{\text{natural}} = N_{\text{artificial}} = 10, P=0.008 \). As a proportion of the total number of individuals that
successfully solved the task, there was a non-significant trend towards a higher
proportion of individuals following in natural shoals. (20 seconds: Mann Whitney U:
\( z = -1.810, N_{\text{natural}} = 10 N_{\text{artificial}} = 6, P=0.073 \).

*Do individuals follow close associates when first entering the feeder?*

We confined our analysis within shoal following patterns to natural shoals for
two reasons. Firstly, in natural shoals, individuals may be predicted to follow familiar
associates, and secondly, because successful foraging (and thus the opportunity to
learn by following) occurred in only half of the artificial shoals. We found no
evidence for higher associations in observed lead-follow pairs (using the 20 second
rule, see methods for definition) than would be expected if individuals followed others
at random (Table 1b; Fisher’s omnibus test to combine $P$-values across shoals:

$F_{20}=17.205, P=0.639$).

Do individuals forage with close associates?

Previous work has suggested that individual guppies that associate in one context may also associate in another (Croft et al. 2006). We investigated whether social associations before the foraging trial were significant predictors of associations during the 30 minute foraging period. We again defined a pair of fish as being associated during foraging when they entered the feeder within 20 seconds of one another. If individuals forage with close associates more frequently than with distant associates, we would predict a positive correlation between the social associations and foraging associations. We used Mantel tests for matrix correlations, and found no evidence to suggest that associations during foraging could be predicted by the social structure before the task (Table 1c, $p$-values combined using Fisher’s omnibus test:

$F_{20}=27.93, P=0.111$).

DISCUSSION

Foraging Success, Boldness, Familiarity and Social Conformity

This study demonstrates for the first time that naturally occurring fish have a foraging advantage over randomly composed shoals. In natural shoals, more individuals successfully fed from the feeder than in artificial shoals, thus benefiting from enhanced foraging success. Whilst a number of factors may have influenced the success of natural shoals, our results suggest three plausible explanations: (1) a risk perception hypothesis – differences in the perception of danger, resulting in greater
risk-prone or bold behaviour in the natural shoals and more risk-averse or shy
behaviour in the artificial shoals; (2) a *social learning hypothesis* – elevated levels of
following and reduced effects of conformity in natural compared to artificial shoals;
and (3) a *time trade-off hypothesis* – differences in the prioritisation of foraging and
establishing social ties, resulting in reduced foraging motivation in artificial compared
to natural shoals.

Several researchers have suggested that the perception of danger (resulting in
risk-averse or shy behaviour) may reduce foraging motivation (Warburton 2003),
information transmission, and learning (Dall et al. 1999) among animals. In our study,
fish in natural shoals approached the feeder more rapidly after it was placed in the
experimental pool, and showed a lower overall shoaling tendency (illustrated by the
predominance of smaller groups and lower association strength) than fish in artificial
shoals. A short latency to approach a novel object and low shoaling tendency are often
used as indicators of boldness when assessing behavioural syndromes in fish (e.g.
Budaev 1997, Ward et al. 2004, Brown et al. 2007). The ability (Sneddon 2003) and
opportunity (Dugatkin & Alfieri 2003) for fish to learn a novel task has previously
been shown to be enhanced by increased boldness: Bold fish tend to be more
successful, learning more rapidly compared with shy fish. Although we cannot
distinguish between behavioural types (e.g. differences in boldness or innovativeness,
Sih et al. 2004) or differences in anxiety, motivational state or curiosity, our findings
are consistent with the idea that associating with natural group-mates generates a
reduced perception of danger.
Familiarity with the physical environment (i.e. the habitat) has been shown to influence risk perception in fishes (Brown 2001). However, this is unlikely to have played a role in the current experiment as both natural and artificial shoals had spent equal amounts of time in captivity and in the test arenas. The reduced perception of danger may instead stem from the familiar social environment experienced by the natural shoals during the course of the experiment. In contrast, individuals in artificial shoals experience an unfamiliar social environment, in addition to the unfamiliar physical environment of the experimental pool. This may cause them to behave in a more risk-averse manner, resulting in lower foraging success than fish in natural shoals. It takes 12 days for familiarity to develop among members of guppy shoals (Griffiths & Magurran 1997b) and individuals in the artificial shoals (composed 36-40 hours before the experiment commenced) may have moved between groups frequently in order to begin the process of familiarisation, resulting in the observed higher and less variable levels of association in artificial compared to natural shoals.

The effect of the social environment is one factor that has previously been shown to influence individual performance in tests of boldness (Griffiths et al. 2004, Sih & Watters 2005). Bhat & Magurran (2006) found that individual guppies emerged more quickly from a refuge in the presence of a familiar partner than they did when paired with an unfamiliar partner, suggesting a role for familiarity in determining perception of risk or levels of boldness. Enhanced foraging success and improved learning performance are also linked to familiarity (Swaney et al. 2001, Griffiths 2003, Ward & Hart 2003, Griffiths et al. 2004, Ward & Hart 2005). Our work suggests that one underlying mechanism for the increased foraging success of familiar shoals may be reduced perception of risk.
A second potential explanation for the relative success of natural shoals is that they exhibited higher levels of social learning of the route to the food source. This hypothesis is supported by the observation that entries to the feeder were clustered in time, and that levels of following in natural shoals were somewhat elevated in comparison to artificial shoals. However, clustering was evident in both natural and artificial shoals, and the proportions of individuals first entering the feeder by following were similar. One factor known to influence the level of social learning is the ‘conformity effect’ (positive frequency-dependent social learning) mediated by shoaling patterns (Day et al. 2001; Brown & Laland 2001). We found that in natural shoals, groups within the shoal were smaller than those in artificial shoals. Day et al. (2001) found that although individuals in larger groups are on average generally more successful at locating food than individuals in smaller groups, individuals in smaller groups can find a resource more quickly when the resource is hidden. Day et al. (2001) attributed this to a greater reluctance on the part of individuals to leave larger than smaller groups. In our study, the feeder was opaque, thus, in order for a fish to enter and feed it needed to break visual contact with the rest of the shoal. Therefore, individuals in artificial shoals may have been more reluctant to leave their larger groups and enter the feeder than individuals in natural shoals. Alternatively, the more a shoal subdivides into smaller groups, the greater the chance that any single individual will find a hidden resource. This high level of subdivision may be due to lower levels of risk aversion linked to the familiar social environment.

A further hypothesis to explain the relative success of natural shoals is related to the prioritisation of different activities in the different shoal types. Individuals in artificial shoals may prioritise learning about each other (i.e. the process of
familiarisation) over immediate foraging, given the benefits associated with
familiarity (Griffiths 2003, Ward & Hart 2003). Larger group sizes, higher association
strengths and lower variation in associations in artificial shoals compared to natural
shoals support the idea that individuals are switching associations rapidly as part of
this process.

Information Transmission Within Shoals

Previous work has suggested that individuals benefit by learning more rapidly
from familiar than unfamiliar shoal mates (Lachlan et al. 1998, Swaney et al. 2001),
as fish in familiar groups are more likely to follow one another, leading to an increase
in social learning of novel tasks (Swaney et al. 2001). We found evidence that fish
followed one another into the feeder, and that association patterns were consistent
over time, allowing us to investigate the links between associations and following
patterns. However, despite our finding that association patterns before the foraging
trial could be used to predict associations after the foraging trial, associations during
foraging could not be predicted by previous association patterns. Neither could we
find any evidence that individuals specifically followed close associates. Thus, we
were unable to find any evidence that information was transmitted along strong ties in
natural shoals. However, to our knowledge this is the first time a social networks
approach has been used to study patterns of potential information transmission.

There may be several explanations for our findings. Firstly, individuals may
choose their social partners differently from the way they choose their foraging
partners. Individuals may, for example, benefit by foraging with those that they know
to be poor foragers (Metcalfe & Thompson 1995), rather than with those that are
preferred associates in other contexts (e.g. predator inspection, Croft et al. 2006).

Secondly, our method of establishing the social and foraging ties may have been inadequate, although it has been previously found to be sufficient to quantify the non-random structure of such groups (Croft et al. 2004 online supplementary material). Thirdly, our power to detect an effect may be reduced by the presence of random interactions creating ‘noise’ around the non-random preferred interactions.

A fourth possibility is that information may spread via local (Thorpe 1956) or stimulus (Spence 1937) enhancement, where the activity of an individual draws the attention of an observer towards a particular location or object. Information is therefore scrounged by naïve individuals at distance, rather than acquired through close dyadic transmission. Although individuals were unable to see shoal-mates while they were foraging, they may have been able to detect successful foragers after they emerged from the feeder (Lachlan et al. 1998), or the presence of individuals near the feeder may have facilitated movement towards it by naïve individuals. Individuals may therefore have learnt the task from any other individual in the shoal, rather than those with which they were strongly associated. Such exploitation of social information has been demonstrated experimentally in fish (Ward & Hart 2003). However, information is more likely to be scrounged from close by than from further away, and one might still expect patterns of foraging to be linked to association patterns.

Finally, we cannot rule out the possibility that the task used in this experiment was easily learned asocially by individual fish, and consequently did not require social information for its solution. The movement of odour cues may have facilitated this,
although they are likely to have been well contained (Laland, K. N. unpublished data), particularly without water movement to disperse them (Vogel 1994). Experiments using similar tasks, where individuals learn the route to a foraging resource, have provided evidence for social learning (Reader et al. 2003), and we found evidence that individuals entered the feeder in small groups, suggesting foraging was a social activity. Studies where inexperienced individuals are unlikely to learn the task themselves provide the most compelling evidence for social learning (Lefebvre & Palameta 1988), thus individuals within a shoal could be trained in a more complex task (e.g. Reader & Laland 2000, Stanley et al. in press), and the links between social structure and foraging patterns investigated.

Further work is clearly needed to demonstrate whether reduced risk perception, social learning or a further explanation underlies the improved foraging success of natural groups. Evidence is growing for variation in behavioural types across animal species (Sih et al. 2004), and the methodology available for assessing boldness and other traits is increasing, providing the opportunity to assess individual behavioural types in relation to social environment and performance in novel tasks.

Social network techniques provide the ideal opportunity for investigating the relationship between group structure, innovation and the diffusion of information or learned behaviours (Latora & Marchiori 2001). We demonstrate that differences exist between natural and artificial shoals in terms of their social structure, but although these differences may have influenced the effectiveness of information transmission, our investigation found no direct links between social structure and patterns of learning within shoals. Further investigation may reveal a more subtle relationship
between social structure and patterns of information transfer, and we hope our study
encourages this.

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The experiments presented here comply with the laws of the country in which they
were carried out.

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Table 1: Results of the Mantel tests for matrix comparisons and within-shoal foraging events, investigating a) correlations between social structure before and after the foraging trial, b) whether individuals first enter the feeder by following a close associate and c) correlations between social network structure and associations during the foraging trial, for the 10 natural shoals only. P-values for significant positive correlations are highlighted in bold, significant negative correlations are marked with an asterisk. Combined P values are the result of Fisher’s Omnibus tests (see text for details).

<table>
<thead>
<tr>
<th>Shoal</th>
<th>Correlation coefficient</th>
<th>P</th>
<th>Correlation coefficient</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.751</td>
<td><strong>0.003</strong></td>
<td>0.828</td>
<td>-0.17421</td>
</tr>
<tr>
<td>2</td>
<td>-0.092</td>
<td>0.683</td>
<td>0.848</td>
<td>-0.19205</td>
</tr>
<tr>
<td>3</td>
<td>0.075</td>
<td>0.357</td>
<td>0.852</td>
<td>-0.23796</td>
</tr>
<tr>
<td>4</td>
<td>0.221</td>
<td>0.123</td>
<td>0.811</td>
<td>-0.32566</td>
</tr>
<tr>
<td>5</td>
<td>0.018</td>
<td>0.462</td>
<td>0.432</td>
<td>0.188126</td>
</tr>
<tr>
<td>6</td>
<td>0.088</td>
<td>0.336</td>
<td>0.243</td>
<td>0.305839</td>
</tr>
<tr>
<td>7</td>
<td>0.606</td>
<td><strong>0.017</strong></td>
<td>0.195</td>
<td>-0.00192</td>
</tr>
<tr>
<td>8</td>
<td>0.627</td>
<td><strong>0.002</strong></td>
<td>0.249</td>
<td>0.495813</td>
</tr>
<tr>
<td>9</td>
<td>0.696</td>
<td><strong>0.007</strong></td>
<td>0.211</td>
<td>-0.03941</td>
</tr>
<tr>
<td>10</td>
<td>0.530</td>
<td><strong>0.005</strong></td>
<td>0.352</td>
<td>0.429002</td>
</tr>
<tr>
<td>Combined P</td>
<td>&lt;<strong>0.001</strong></td>
<td>0.639</td>
<td></td>
<td>0.111</td>
</tr>
</tbody>
</table>
**Figure legends**

**Figure 1:** Percentage frequency distributions of guppy groups in natural (open circles) and artificial (filled circles) shoals. Values are the median percentage frequency of individuals in groups of each size across all replicate trials. Error bars represent interquartile range. Solid lines are used as a visual aid only, linking values for each shoal type.

**Figure 2:** Comparing median values for network measures (association strength; AS) between natural and artificial shoals. a) shoal AS ($z=-3.628$, $N_{\text{natural}}=N_{\text{artificial}}=10$, $P<0.001$) and b) coefficient of variation in AS ($z=-3.175$, $N_{\text{natural}}=N_{\text{artificial}}=10$, $P=0.001$). Error bars indicate the inter-quartile range.

**Figure 3:** Results of the foraging trial comparing natural and artificial shoals. a) median time to approach the feeder ($z=-2.117$, $N_{\text{natural}}=N_{\text{artificial}}=10$, $P=0.035$) and b) the median number of fish feeding in each shoal ($z=-3.752$, $N_{\text{natural}}=N_{\text{artificial}}=10$, $P<0.001$). Error bars indicate the inter-quartile range.

**Figure 4:** Median number of fish in a shoal that first entered the feeder alone (open bars) as opposed to following a demonstrator (closed bars), for both a 5 second and 20 second following rule, in natural (5 secs: $z=-2.501$, $N=10$, $P=0.036$; 20 secs: $z=-1.279$, $N=10$, $P=0.201$) and artificial (5 secs: $z=-2.041$, $N=10$, $P=0.123$; 20 secs: $z=-1.236$, $N=10$, $P=0.216$) shoals. Error bars indicate the inter-quartile range.
Figure 1:
Figure 2

(a) Median shoal association index

(b) Median coefficient of variation

Shoal type

Natural Artificial
Figure 3

a)

![Bar chart showing median time to approach for Natural and Artificial shoals.](image)

b)

![Bar chart showing median number of fish feeding for Natural and Artificial shoals.](image)
Figure 4

The figure shows a bar chart comparing the number of leaders and followers in natural and artificial shoals under different time intervals. The chart displays the number of leaders and followers in natural shoals (5 secs and 20 secs) and artificial shoals (5 secs and 20 secs). The bars indicate the mean number of leaders and followers with error bars representing the standard deviation.