Schooling preferences for familiar fish vary with group size in a wild guppy population

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SUMMARY

The ability of fish to recognize and preferentially associate with familiar conspecifics has been well documented in a series of laboratory experiments. In this paper we investigate the schooling preferences of wild female guppies, *Poecilia reticulata*, in the Upper Tunapuna River in Trinidad and confirm that they do indeed prefer to associate with familiar individuals. The guppies in this river occur in a series of pools that become isolated during the dry season. These fish interact solely with other individuals in their pool for periods of several months at a time and thus have ample opportunity to become accustomed to one another. Our study also reveals that the tendency of female guppies to school with familiar fish declines as the group size in which they naturally live increases. Preferences are strong when there are small numbers of females in a pool, but diminish thereafter. This indicates that the expression of familiarity is constrained by group size. The basis of recognition and the consequences of schooling preferences for familiar individuals are discussed.

1. INTRODUCTION

There are many advantages of living in groups (e.g., Hamilton 1971; Wittenberger & Hunt 1985; Krebs & Davies 1993). In fish, the antipredator (Neill & Cullen 1974; Magurran 1990a; Pitcher & Parrish 1993) and foraging (Pitcher *et al.* 1982; Clark & Mangel 1984; Krause 1993) benefits of group-living have been particularly well documented. It has recently become clear that individual members of groups can enhance their fitness further if they associate with familiar rather than unfamiliar conspecifics. This effect was highlighted by Chivers *et al.* (1995) who discovered that schools of familiar fathead minnows, *Pimephales promelas*, were more cohesive and increased their cooperative antipredator behaviour compared with schools comprised of unfamiliar individuals. An overview of recent studies giving evidence for partner choice by fish in the contexts of antipredator and foraging behaviour, as well as in kin selection is presented by Dugatkin & Wilson (1993), and further examples from many different taxa are described by Dugatkin & Sih (1995).

Recognition of familiar individuals may be beneficial, but under what conditions can it be achieved? The ability to discriminate familiar from unfamiliar conspecifics has been demonstrated in a wide variety of freshwater fish species (including *Lepomis macrochirus*, Brown & Colgan 1986; *Gasterosteus aculeatus*, Van Havre & FitzGerald 1988; and *Pimephales promelas*, Brown & Smith 1994). It is now known that schooling preferences for familiar individuals take time to emerge. For example, bluegill sunfish associate with familiar conspecifics after 3–7 days (Brown & Colgan 1986),

while the tendency of female guppies to school with their tank-mates develops gradually over a period of 12 days (Griffiths & Magurran 1997).

Time is one factor that mediates schooling preferences for certain individuals. The number of potential schooling partners may well be another. Most of the experiments to date have looked at partner choice among small groups of fish in the laboratory. In contrast, wild fish frequently occur in large aggregations or schools and it is possible that their ability to learn or remember the identities of particular individuals declines as there are more conspecifics to choose among. It may also be the case that the benefits of schooling with certain individuals diminish once groups reach a critical size and the ‘safety in numbers’ effects strengthen (Bertram 1978; Kiltie 1980; Kiltie & Terborgh 1983). On the other hand, if familiarity is based on recognition of a group characteristic, such as odour, then group size *per se* may play little role in determining schooling preferences. In order to test the hypothesis that the tendency of fish to school with familiar individuals will be inversely related to the size of group in which they naturally occur, we examined the behaviour of a wild population of guppies in Trinidad.

2. METHODS

(a) *Study species and field site*

Guppies occur widely in Trinidad. Part of their range includes intermittent rivers such as those found in the upper reaches of the Northern Range Mountains. In streams like the Upper Tunapuna, guppies can be confined to isolated pools for several months during the dry season. The number

of guppies within each pool in this stream is highly correlated during the dry season with surface area; pool sizes fall within the range of 1–10 m² (B. H. Seghers and A. E. Magurran, unpublished data). A guppy in the Upper Tunapuna will thus find itself interacting with a variable number of fish, depending on the size of pool in which it happens to be located. Our experiment examined the schooling preferences of female guppies. Although male guppies are brightly coloured and polymorphic, females are cryptic in appearance, and to the human eye at least, are not readily distinguishable. However, as female guppies have a higher schooling tendency than males, and invest more in anti-predator defence (Magurran & Seghers 1994a) they stand to make considerable gains from partner preferences even if they do find it more challenging to recognize one another.

The Upper Tunapuna is a low predation system (Magurran & Seghers 1994b) where guppies co-occur with the cyprinodont, *Rivulus hartii*. The swamp eel, *Synbranchus marmoratus*, is also present. Like many other low predation habitats in Trinidad, this guppy population is female biased. At the time of our study there was a 2:1 sex ratio in favour of females in the Upper Tunapuna (B. H. Seghers and A. E. Magurran, unpublished data).

The study was conducted in March 1996. Seven discrete pools, containing from eight to 194 adult females (B. H. Seghers and A. E. Magurran, unpublished data), were selected for the investigation. Some of the pools in the 20-pool section of the river contained too few fish for our purposes. Other pools were contiguous and we could not precisely determine the effective group size. By choosing isolated pools we could be fairly certain that the female guppies in them had coexisted for about three months during the dry season. Laboratory tests indicate that familiarity is acquired over a matter of days (Griffiths & Magurran 1997) and therefore had ample time to develop in these isolated pools. For this reason we designated females in a given pool as 'familiar'. The study was comprised of two parts: an investigation of group size on schooling preference (where group size refers to the total number of females in a pool) and a control to examine the effects of the batch marks used to distinguish familiar from unfamiliar individuals.

(b) Experiment

A small natural pool (110 cm max. length × 60 cm max. width × 5 cm max. depth), isolated from others in the system, was used as the observation arena. No other fish, of any species, occurred in this pool. Four equally sized familiar females, randomly chosen from one of the seven groups and four equally sized unfamiliar female guppies, from a different pool, were gently transferred to the observation pool. Unfamiliar fish (i.e. ones that were unknown to the test females, though not necessarily from each other) were given a small alcian blue mark on their caudal peduncle. This meant that they could be easily distinguished from the familiar females. Marked fish were allowed to settle for 1 h before the familiar fish were introduced. All fish were then given a further 20 min settling time before observations were made. Two familiar fish were observed for 15 min in succession. The time that each focal fish spent schooling with a familiar or an unfamiliar nearest neighbour was measured. Total schooling time was also recorded. For the purposes of this experiment a focal fish was defined as schooling if it was within three body lengths of another female. At the end of the trial the total length of each fish was measured. Observations were made on at least four and in some cases six or eight individuals from each of the seven pools investigated (figure 1). No fish was tested more than once.

(c) Control

The aim of the control experiment was to ascertain whether the marking procedure influenced schooling preferences. Eight familiar guppies, i.e. females originating from the same pool, were placed in the observation arena. Four randomly selected individuals were marked in the usual way. All fish were allowed to settle for 1 h before the schooling behaviour of marked and unmarked focal fish was recorded as described above. The trial was repeated four times, so that observations were made on eight individuals in total.

(d) Data analysis

Preferences for familiar and unfamiliar schooling partners were expressed as the percentage of total time schooling in order to account for variation in schooling tendency between groups. As the behaviour (time spent with familiar fish) of the two successive focal fish in each trial did not differ significantly ($F_{1,86} = 5.69 \times 10^{-12}$, $p > 0.99$, one-way ANOVA, mean and standard deviation for each focal fish 50.0, 50.0 and 15.51, 14.87 respectively), both data points were used in the following analysis. A two-way general linear model (glm) investigated the effect of familiarity and group size on partner choice during schooling. A glm was chosen as it allows analysis of non-orthogonal data (McCullagh & Nelder 1983).

3. RESULTS

The analysis confirmed the hypothesis that wild female guppies prefer to school with familiar individ-

Figure 1. Relationship between group size and schooling preferences for familiar females. The graph shows the mean time ($\pm 95\%$ confidence limits) that focal individuals spent schooling with familiar females. Schooling time with familiar fish is expressed as a percentage of total schooling time for that individual. The hatched line represents the point at which equal time is spent schooling with familiar and unfamiliar schooling partners. The total number of females per pool and the number of fish tested from that pool were: eight females in pool, $n = 4$ females tested; 20, $n = 6$; 36, $n = 8$; 62, $n = 6$; 63, $n = 6$; 29, $n = 6$; 194, $n = 8$.

uals ($F_{1,74} = 148.35$, $p < 0.001$). The interaction between familiarity and group size was highly significant ($F_{6,74} = 15.20$, $p < 0.001$; figure 1) indicating that schooling preferences for familiar fish decreased as group size increased. This suggests that the recognition ability of guppies is constrained by the number of individuals with whom they interact. Familiar and unfamiliar guppies did not differ in size ($F_{1,138} = 1.68$, $p = 0.198$), their mean total lengths being 27.5 mm ($\pm$ s.e. = 0.3) and 28.0 mm ($\pm$ s.e. = 0.3), respectively.

The control demonstrated that schooling decisions were not influenced by the marking procedure. Female guppies equally familiar with one another did not demonstrate a preference for schooling with either marked or unmarked fish ($F_{1,12} = 3.19$, $p = 0.099$). The total lengths of marked and unmarked fish did not differ either ($F_{1,14} = 0.01$, $p = 0.93$, one-way ANOVA). There is no reason to suspect, therefore, that the preferential association with familiar fish found in the main experiment can be attributed to preferences for the presence or absence of alcian blue marks.

4. DISCUSSION

This study reveals that wild female guppies prefer to school with familiar partners. Indeed, in the case of fish from the smaller groups, these preferences are even stronger than those documented previously for guppies in the laboratory (Magurran *et al.* 1994; Griffiths & Magurran 1997). Our results indicate that wild schools may not be random assemblages of conspecifics and that familiarity, based on past interactions, can influence the choice of schooling partner.

The investigation also demonstrates that schooling preferences for familiar individuals are mediated by group size. Females originating from small groups are able to recognize and preferentially associate with familiar conspecifics. However, as group size increases, the behaviour is progressively lost and females choose their neighbours at random. We cannot determine the precise point at which familiarity ceases to influence behaviour because the group sizes that were tested were those that nature had created, and not ones that we predetermined. Yet the trend is clear, and there is a pronounced interaction between familiarity and group size. Intriguingly, a previous study of schooling fidelity in wild fish (Helfman 1984) found little tendency towards associations among particular individuals. Helfman monitored the behaviour of 102 individually identifiable yellow perch, *Perca flavescens*, in a population in Cazenovia Lake, New York, which ranged from 107 to 445 fish.

Why do partner preferences for familiar fish diminish in larger groups? One possibility is that the choice of schoolmates is based on individual recognition. If this were the case then females in the largest pool would have to learn the identities of almost 200 individuals in order to recognize them all as schooling partners, a significant challenge indeed. Dunbar (1992, 1995) has shown that neural capacity limits the extent of social networks in higher vertebrates. A comparative study of 38 genera of primates (Dunbar 1992) revealed a

significant link between relative neocortex volume and size of the social group in which the animals typically live. Social insects such as ants also demonstrate a positive trend between group size and complexity of their neural apparatus (Jaffe & Perez 1989; Jaffe & Chacon 1995). The most social (those which form the largest colonies) of 13 species of Formicidae ants were found by Jaffe & Perez (1989) to have both the most complex chemical communication systems and the most highly developed corpora pedunculata and olfactory lobes. It seems plausible that social relationships based on individual recognition will be constrained by brain size in fish too. It may be that female guppies can recognize a certain number of individuals and that this number will remain constant irrespective of the group size in which they naturally occur. Thus, even in large pools, females might preferentially associate with certain individuals. We deliberately avoided the possibility of selecting subgroups of females by choosing fish, at random, from different sections of a pool. However, it would be interesting in future work to search for alliances among subsets of females that live in large aggregations. It would also be informative to use laboratory manipulations to further explore the relationship between group size and individual recognition.

Recognition based on a group characteristic, such as a shared odour, removes the need to learn individual identities. Odour has already been implicated in partner choice in fish. For example, Brown & Smith (1994) found that olfaction plays a major role in the preference of fathead minnows for familiar shoalmates, and Quinn & Busack (1985) demonstrated the ability of juvenile salmon, *Oncorhynchus kisutch*, to preferentially associate with siblings on the basis of chemosensory cues. It is also known that condition-dependent recognition, that is, recognition based on traits such as body size or competitive ability or inspection tendency, is acquired more quickly (Milinski *et al.* 1990a, b; Ranta & Lindström 1990; Dugatkin & Alfieri 1991; Metcalfe & Thomson 1995) than recognition based on familiarity alone (Griffiths & Magurran 1997). However, if female guppies in the Upper Tunapuna could recognize fish from their own pool on the basis of one or more group characteristics, such as smell, then we would not have found the observed relationship between schooling preference and group size.

It could be argued that marked fish have a different schooling tendency to unmarked fish. We conducted a laboratory experiment to demonstrate that time spent schooling by marked and unmarked females does not differ ($F_{1,19} = 0.55$, $p = 0.467$). The procedure closely followed the 'bottle method' described by Magurran *et al.* (1994). Each of 20 Upper Tunapuna females (ten of which were marked 1 h previously, the other ten remaining unmarked) were allowed to choose between either an empty transparent bottle or an identical bottle containing four females. Total length (mm) of marked and unmarked fish did not differ ($F_{1,19} = 0.57$, $p = 0.462$).

Kinship is another factor that could explain why partner preferences vary with group size, and it is

possible that females in small pools are more closely related than those in large ones. This scenario cannot be excluded completely at this stage, though ongoing laboratory tests will tell us if relatedness influences schooling decisions in guppies. However, it is important to note that the flow between the pools in the Upper Tunapuna increases considerably during the wet season (May–December), and there is opportunity for fish movement during much of the year.

Many fish are now understood to have the cognitive ability to distinguish kin from non-kin, although work to date has concentrated on salmonids. For example, Quinn & Busack (1985) demonstrated that juvenile coho salmon, *Oncorhynchus kisutch*, prefer water conditioned by both familiar and unfamiliar siblings over non-siblings. Similarly, Brown & Brown (1992) found that both juvenile Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, can discriminate kin from non-kin, preferring water conditioned by kin in a two-choice tank. Recently, work by Warburton & Lees (1996) has demonstrated that kin discrimination may also be possible for domestic strains of guppy. Although it is clear that fish are able to distinguish relatives from unrelated conspecifics, and despite the apparent advantages to an individual of choosing to school with kin (Blaustein *et al.* 1987), there is little evidence to date to support the idea that wild schools are composed of related individuals (Avisé & Shapiro 1986; Naish *et al.* 1993).

Guppies in the Upper Tunapuna experience a low predation regime. We might expect the benefits of schooling with familiar individuals to be magnified in localities where there are many predators (Chivers *et al.* 1995). It is already well known that schooling tendency covaries with predation intensity in guppy populations (Seghers 1974; Magurran & Seghers 1994*a*) and it would be interesting to explore the idea that fish from high-risk sites are more adept at recognizing potential schooling partners. Such fish do, after all, have a greater predisposition to refine their antipredator behaviour as a consequence of early experience (Magurran 1990*b*; Huntingford & Wright 1993). However, as guppies in high predation sites are rarely restricted to isolated pools, they may encounter more individuals during their daily activities than our Upper Tunapuna fish did.

In the absence of a high threat of predation what other advantages might fish obtain from schooling with familiar individuals? An adaptive explanation for the behaviour of Upper Tunapuna females is that feeding benefits accrue from schooling with familiar poolmates. Familiar fish may be able to forage more efficiently than unfamiliar ones by avoiding exploited food patches and by knowing the competitive abilities of their foraging partners (Metcalf & Thomson 1995). If this were true, familiar individuals would gain direct feeding advantages and, in the case of females, the indirect benefits of increased fecundity, which is related to body mass (Reznick *et al.* 1990). An interesting proposition for future work would be to consider the extent to which individual feeding territories overlap and to investigate any differences in habitat utilization and schooling preferences between males and females.

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REFERENCES

- Avisé, J. C. & Shapiro, D. Y. 1986 Evaluating kinship of newly settled juveniles within social groups of the coral reef fish *Anthias squamipinnis*. *Evolution* **40**, 1051–1059.
- Bertram, B. C. R. 1978 Living in groups: Predators and prey. In *Behavioural ecology* (ed. J. R. Krebs & N. B. Davies), pp. 64–96. Oxford: Blackwell.
- Blaustein, A. R., Bekoff, M. & Daniels, T. J. 1987 Kin recognition in vertebrates (excluding primates): empirical evidence. In *Kin recognition in animals* (ed. D. J. C. Fletcher & C. D. Michener), pp. 287–331. London: Wiley Interscience.
- Brown, G. E. & Brown, J. A. 1992 Do rainbow trout and Atlantic salmon discriminate kin? *Can. J. Zool.* **70**, 1636–1640.
- Brown, J. A. & Colgan, P. W. 1986 Individual and species recognition in centrarchid fishes: evidence and hypotheses. *Behav. Ecol. Sociobiol.* **19**, 373–379.
- Brown, G. E. & Smith, R. J. F. 1994 Fathead minnows use chemical cues to discriminate natural shoalmates from unfamiliar conspecifics. *J. Chem. Ecol.* **20**, 3051–3061.
- Chivers, D. P., Brown, G. E. & Smith, R. J. F. 1995 Familiarity and shoal cohesion in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Can. J. Zool.* **73**, 955–960.
- Clark, C. W. & Mangel, M. 1984 Foraging and flocking strategies: information in an uncertain environment. *Am. Nat.* **123**, 626–641.
- Dugatkin, L. A. & Alfieri, M. 1991 Guppies and the TIT FOR TAT strategy: preference based on past interaction. *Behav. Ecol. Sociobiol.* **28**, 243–246.
- Dugatkin, L. A. & Wilson, D. S. 1993 Fish behaviour, partner choice experiments and cognitive ethology. *Rev. Fish Biol. Fish.* **3**, 368–372.
- Dugatkin, L. A. & Sih, A. 1995 Behavioral ecology and the study of partner choice. *Ethology* **99**, 265–277.
- Dunbar, R. I. M. 1992 Neocortex size as a constraint on group size in primates. *J. Hum. Evol.* **20**, 469–493.
- Dunbar, R. I. M. 1995 Neocortex size and group size in primates: a test of the hypothesis. *J. Hum. Evol.* **28**, 287–296.
- Griffiths, S. W. & Magurran, A. E. 1997 Familiarity in schooling fish: how long does it take to develop? *Anim. Behav.* (In the press.)
- Hamilton, W. D. 1971 Geometry for the selfish herd. *J. Theor. Biol.* **31**, 295–311.
- Helfman, G. S. 1984 School fidelity in fishes: the yellow perch pattern. *Anim. Behav.* **32**, 663–672.
- Huntingford, F. A. & Wright, P. J. 1993 The development of adaptive variation in predator avoidance in freshwater fishes. In *Behavioural ecology of fishes* (ed. F. A. Huntingford & P. Torricelli), pp. 45–61. Chur: Harwood Academic.
- Jaffe, K. & Chacon, G. 1995 Nonlinear trends in the evolution of the complexity of nervous systems, group size, and communication systems: a general feature in biology. *Behav. Brain Sci.* **18**, 386.
- Jaffe, K. & Perez, E. 1989 Comparative study of brain morphology in ants. *Brain Behav. Evol.* **33**, 25–33.

- Kiltie, R. A. 1980 Application of search theory to the analysis of prey aggregation as an antipredator tactic. *J. Theor. Biol.* **87**, 201–206.
- Kiltie, R. A. & Terborgh, J. 1983 Observations on the behavior of rain forest peccaries in Peru: why do white-lipped peccaries form herds? *Z. Tierpsychol.* **62**, 241–255.
- Krause, J. 1993 The influence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. *J. Fish Biol.* **43**, 775–780.
- Krebs, J. R. & Davies, N. B. 1993 *An introduction to behavioural ecology*. Oxford: Blackwell.
- Magurran, A. E. 1990a The adaptive significance of schooling as an anti-predator defence in fish. *Ann. Zool. Fennici* **27**, 51–66.
- Magurran, A. E. 1990b The inheritance and development of minnow anti-predator behaviour. *Anim. Behav.* **39**, 834–842.
- Magurran, A. E. & Seghers, B. H. 1994a Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour* **128**, 121–134.
- Magurran, A. E. & Seghers, B. H. 1994b Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B* **255**, 31–36.
- Magurran, A. E., Seghers, B. H., Shaw, P. W. & Carvalho, G. R. 1994 Schooling preferences for familiar fish in the guppy, *Poecilia reticulata*. *J. Fish Biol.* **45**, 401–406.
- McCullagh, P. & Nelder, J. A. 1983 *Generalized linear models. Monographs on statistics and applied probability*. Cambridge University Press.
- Metcalf, N. B. & Thomson, B. C. 1995 Fish recognize and prefer to shoal with poor competitors. *Proc. R. Soc. Lond. B* **259**, 207–210.
- Milinski, M., Külling, D. & Kettler, R. 1990a Tit for tat: sticklebacks 'trusting' a cooperating partner. *Behav. Ecol.* **1**, 7–11.
- Milinski, M., Pflüger, D., Külling, D. & Kettler, R. 1990b Do sticklebacks cooperate repeatedly in reciprocal pairs? *Behav. Ecol. Sociobiol.* **27**, 17–21.
- Naish, K.-A., Carvalho, G. R. & Pitcher, T. J. 1993 The genetic structure and microdistribution of shoals of *Phoxinus phoxinus*, the European minnow. *J. Fish Biol.* **43**, 75–89.
- Neill, S. R. St J. & Cullen, J. M. 1974 Experiments on whether schooling by their prey affects the hunting behaviour of cephalopods and fish predators. *J. Zool. Lond.* **172**, 549–569.
- Pitcher, T. J., Magurran, A. E. & Winfield, I. J. 1982 Fish in larger shoals find food faster. *Behav. Ecol. Sociobiol.* **10**, 149–151.
- Pitcher, T. J. & Parrish, J. K. 1993 Functions of shoaling behaviour in teleosts. In *Behaviour of Teleost Fishes* (ed. T. J. Pitcher), pp. 363–439. London: Chapman & Hall.
- Quinn, T. P. & Busack, C. A. 1985 Chemosensory recognition of siblings in juvenile salmon (*Oncorhynchus kisutch*). *Anim. Behav.* **33**, 51–56.
- Ranta, E. & Lindström, K. 1990 Assortative schooling in three-spined sticklebacks? *Ann. Zool. Fennici* **27**, 67–75.
- Reznick, D. N., Bryga, H. & Endler, J. A. 1990 Experimentally induced life-history evolution in a natural population. *Nature, Lond.* **36**, 357–359.
- Seghers, B. H. 1974 Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution* **28**, 486–489.
- Van Havre, N. & FitzGerald, G. J. 1988 Shoaling and kin recognition in the three-spine stickleback (*Gasterosteus aculeatus* L.). *Biol. Behav.* **13**, 190–201.
- Warburton, K. & Lees, N. 1996 Species discrimination in guppies: learned responses to visual cues *Anim. Behav.* **52**, 371–378.
- Wittenberger, J. F. & Hunt Jr, G. L. 1985 The adaptive significance of coloniality in birds. In *Avian Biology*, vol. 8 (ed. D. S. Farner, J. R. King & K. C. Parkes), pp. 1–78. New York: Academic Press.

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