



Diet, familiarity and shoaling decisions in guppies

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Animals are known to derive benefits from associating with familiar individuals, and familiarity is important in the structuring of animal groups. In fish, individuals are known to shoal preferentially with others they have previously spent time with (familiar individuals). One mechanism used in fish shoaling decisions may be local olfactory cues; individuals prefer to shoal with conspecifics that have experienced a similar recent environment to themselves. We investigated the role of diet-based cues in the social decisions of domestic guppies, *Poecilia reticulata*. Diet strongly affected shoaling decisions. Fish fed a bloodworm diet preferred to shoal with familiar individuals and with those that had been fed a similar diet. Flake food-fed fish, on the other hand, preferentially associated with fish fed on bloodworm, and showed a strong preference for unfamiliar fish when both shoals were fed flake food. These results suggest that several factors may interact to influence shoaling decisions. Bloodworm-fed fish also strongly preferred the olfactory cues of their own diet to flake food odour whereas flake food-fed fish showed no preference for either odour type. Fish on bloodworm diets, however, grew faster. We suggest potential explanations for the flake food-fed individuals' preference for unfamiliar fish.

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The role of familiarity in structuring interactions between animals has received much attention in the recent literature. Associating with familiar individuals is known to have a number of benefits, including enhanced predator escape responses and increased foraging efficiency (reviewed in Krause & Ruxton 2002; Griffiths 2003; Ward & Hart 2003). Familiarity also stabilizes dominance hierarchies, resulting in decreased aggression (sea trout, *Salmo trutta*: Hojesjo et al. 1998), and reduces aggression between territorial neighbours (e.g. turnstones, *Arenaria interpres*: Whitfield 1986; for a review in birds, see Temeles 1994).

In a number of fish species, including guppies, *Poecilia reticulata* (Magurran et al. 1994), bluegill sunfish, *Lepomis macrochirus* (Brown & Colgan 1986), three-spined sticklebacks, *Gasterosteus aculeatus* (Van Havre & FitzGerald 1988) and fathead minnows, *Pimephales promelas* (Brown & Smith 1994), individuals preferentially associate with,

or avoid, conspecifics based on past interactions. Griffiths (2003) distinguished between familiarity (condition-independent recognition) and condition-dependent recognition. Condition-dependent recognition is defined as occurring when individuals are distinguished on the basis of past experiences (e.g. associates during predator inspection behaviour) in association with specific cues (such as size, colour or spatial position), and can be acquired over a very short time frame (Griffiths 2003; Ward & Hart 2003). 'True' familiarity uses experience alone (i.e. does not seem to be associated with specific phenotypic or behavioural cues; Griffiths 2003), and requires multiple interactions between individuals over an extended period of time. In guppies, for example, preferences for familiar individuals are not detected until after 12 days of association (Griffiths & Magurran 1997a). Once developed, familiarity may persist over a period of weeks, even in the absence of reinforcement (Chivers et al. 1995; Bhat & Magurran 2006).

However, there is a discrepancy between the shoaling preferences observed in laboratory settings, and the cohesiveness of fish shoals in the wild (Griffiths 2003). For example, although kin-biased behaviour (an example of

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condition-dependent recognition) is common in the laboratory, there is little evidence for kin-based association patterns in the field (Griffiths 2003). For preferences based on true familiarity, the number of conspecifics with which an individual fish can potentially interact may be larger than the number it can recognize. In guppies, the upper limit to the number of fish with which individuals can become familiar is around 40 (Griffiths & Magurran 1997b), yet in many species, the potential number of associates may be much larger (Hoare et al. 2000; Ward et al. 2002). Furthermore, fish shoals tend to be unstable (Krause et al. 2000; Croft et al. 2003a; Griffiths 2003), and individuals may rapidly move between shoals, which persist for only a few seconds (Croft et al. 2003b). As individuals within a shoal may not remain with the same conspecifics for the extended period needed for familiarity to develop, other mechanisms may be important in determining shoaling preferences.

It is well known that individuals associate on the basis of species (Keenleyside 1955), size (Krause et al. 1998; Ward & Krause 2001), parasite load (Dugatkin et al. 1994; Krause & Godin 1994a) and colour (McRobert & Bradner 1998). Decisions may also be made in a behavioural context, such as predator inspection behaviour (Milinski et al. 1990; Dugatkin & Alfieri 1991; Croft et al. 2006) or competitive ability (Metcalf & Thomson 1995). Recent work suggests a further cue that may be important: shoaling decisions may be based on the recognition of local olfactory cues (Olsen et al. 2003; Ward et al. 2004, 2005). Arctic charr, *Salvelinus arcticus*, preferentially associate with individuals fed on the same recent diet (Olsen et al. 2003), and when offered a choice between unfamiliar individuals that have recently experienced a similar environment (microhabitat) to themselves and unfamiliar individuals that have experienced a different environment, sticklebacks show a strong preference for conspecifics from a similar environment (Ward et al. 2004), even when exposed to that environment for only 24 h (Ward et al. 2005). Hypothesized benefits of such an association include the recognition of individuals that exploit the environment in a similar way, or those that possess knowledge of the local environment (Ward et al. 2004, 2005). This has been termed 'general recognition', in contrast to the specific recognition of individuals based on past social experience, and allows individuals to discriminate between potential shoalmates without the need for individual recognition. How widely such general recognition is used across fish species, and in which contexts, remains unknown.

We investigated the role of diet in shaping shoaling preferences in domestic guppies. Guppies have been used extensively in work on familiarity and shoaling (e.g. Griffiths & Magurran 1997a, b, 1998, 1999; Croft et al. 2004), and much is known about the benefits of association with familiar individuals (Lachlan et al. 1998; Swaney et al. 2001). Preferences based on environmental (habitat) cues are thought to occur through olfactory detection of cues arising from the fish themselves (Ward et al. 2004), and guppies are known to use olfactory cues from conspecifics in both shoal and mate choice decisions (Griffiths & Magurran 1999; Shoehet & Watt 2004); they thus have the

necessary capacity to detect olfactory cues resulting from environmental or dietary sources. Based on previous work (Ward et al. 2004, 2005) we hypothesized that fish will preferentially associate with familiar individuals and with those fed on a similar recent diet. In addition, we investigated the mechanisms underlying the shoaling preferences we observed, in terms of the attractiveness and quality of the different diets.

METHODS

Study Species and Holding Conditions

Wild populations of guppies occupy a range of habitats, including both fresh and brackish water, and feed on a variety of foodstuffs. Under laboratory conditions, they will feed on a range of commercially available tropical fish foods. We used domestic guppies (Neil Hardy Aquatica, London, U.K.) as they are individually identifiable without the need for marking. Individual identification was essential for the third part of this investigation, where we measured the effect of diet on growth rate, and was used during other parts to ensure fish were tested only once (see Controls). Domestic fish have been used to investigate the benefits of associating with familiars (Lachlan et al. 1998; Swaney et al. 2001).

To investigate the effect of habitat and diet cues on shoaling preferences, we divided 320 size-matched (38 ± 4 mm) female guppies haphazardly into groups of 10 and allocated them to 32 holding aquaria (550×200 mm and 200 mm high), furnished with a thin layer of gravel and a foam filter, and filled to a depth of 140 mm. We used groups of 10 to ensure that the groups were small enough for the fish to develop familiarity as it is traditionally understood (the limit for guppies is around 40 individuals; see Introduction).

The 32 aquaria were divided into eight blocks of four. Within each block, the fish received one of two treatments, with two aquaria allocated to each treatment. Two aquaria were allocated a bloodworm (*Chironomus* spp.) diet (fresh-frozen bloodworm; BWA and BWB) and two a flake food diet (FFA and FFB). Flake food was chosen as it contained a mixture of foodstuffs, to represent the generalist foraging strategy of guppies, while bloodworm represents a high protein diet but lacks other foods eaten by guppies in the wild. The fish were fed daily ad libitum on their allocated diet. They remained in the aquaria for 14 days before choice trials began. In all aquaria, water temperature was held constant at 25°C, under a light:dark regime of 12:12 h. No visual or chemical communication was possible between the holding aquaria.

Experiment 1: Shoaling Preferences

To investigate shoaling preferences, we carried out two shoaling preference experiments, using standard binary choice tests: one testing for preferences based on recent diet, and the other for preferences based on familiarity. The test aquaria measured 600×200 mm and were filled to a depth of 130 mm with fresh water. A mesh barrier

(hole dimensions 3 mm, approximately 7 perforations/cm²) at either end of the aquaria (positioned 120 mm from the end) provided compartments for the stimulus shoals, allowing both visual and olfactory communication between the focal fish and stimulus shoals. Observations of dye movement showed that olfactory cues could pass the barrier. Our design reflected those of previous studies where the role of olfactory cues in social decisions has been investigated (e.g. Ward et al. 2004). We marked a preference zone 120 mm from the barrier, which represents three to four body lengths of a typical adult female domestic guppy, a distance that falls within the range of inter-individual distances most commonly observed in shoaling fish in nature (Pitcher & Parrish 1993). Stimulus shoals were placed in each end compartment, and the focal fish was placed in a transparent, perforated cylinder (diameter 8 cm, 78 holes 3 mm in diameter), in the centre of the test aquaria, between the two preference zones. After the stimulus and focal fish had been placed in the test aquaria, we allowed a 10-min settling period, permitting odour cues to pass through the barrier. The cylinder was then lifted via a remote pulley mechanism, and the focal fish was allowed to swim freely in the test tank. As a measure of shoaling preference, we recorded the time spent within each preference zone (± 1 s) using a stopwatch.

Diet Cue Preferences

To test for preferences based on recent diet, we gave focal individuals (bloodworm-fed fish: $N = 25$; flake food-fed fish: $N = 26$) a choice between a shoal composed of four unfamiliar individuals that had experienced a similar recent diet to themselves, and a shoal composed of four unfamiliar individuals that had experienced a different recent diet to themselves. Within each block, fish from BWA and FFA were given a choice between shoals from BWB and FFB, and fish from BWB and FFB were given a choice between shoals from BWA and FFA.

Familiarity

To investigate whether the fish showed evidence of shoal choice based on prior association (familiarity), we carried out further binary choice tests using the same procedure as above. Fish were given a choice between a shoal composed of four individuals from the same holding aquarium, and a shoal composed of four individuals from the aquarium where the fish experienced the same diet cue (bloodworm-fed fish: $N = 26$; flake food-fed fish: $N = 25$). For example, fish from BWA were given a choice between familiar individuals from BWA and unfamiliar individuals from BWB.

Controls

All fish within each block were tested over a 4-day period after the 2-week familiarization period, such that fish from any particular aquarium were tested for both diet cue preferences and familiarity preferences on the same

day, and fish from all aquaria within a block were tested on each day. We alternated tests for familiarity and diet cue preferences, and returned all fish to their temporary aquarium between trials. Focal fish were identified from natural colour markings before trials began. Each fish was tested once, and no fish was tested for both diet cue preferences and familiarity preferences to ensure that they had no previous experience of the unfamiliar stimulus shoal. Fish used as stimulus fish were never used as focal fish. However, after testing, focal fish were used as stimulus fish in later tests, to vary the composition of the stimulus shoals, and reduce the total number of fish needed for the experiment. Stimulus shoals were chosen randomly from the six or more available fish (those not identified as test fish) in any given temporary aquarium. Thus, focal fish from any given aquarium were likely to have been offered stimulus shoals that differed in composition from the stimulus shoals offered to other fish from the same aquarium.

To control for any side biases during shoal choice tests, we alternated the side of the tank containing the familiar (diet cue or individual recognition) stimulus shoal. Before trials began, fish were moved from their holding aquarium to a temporary aquarium (200 × 300 mm and 210 mm high, water depth 100 mm) containing clean water, and on moving the fish from the temporary aquarium to the test aquarium, we took care not to transfer water or any extraneous material. This ensured that any cues originated from the fish, and not from water or other material transferred with the fish. To move fish between aquaria, we used small hand-held dip nets; the fish were out of the water for only a few seconds for each transfer. The water within the test aquarium was changed between each trial. Any trials in which the fish did not enter both preference zones within the first 5 min of the trial were excluded from the analysis to ensure that the fish sampled both shoals. In addition, we excluded those trials where aggression (chasing and biting attempts) was present in one or both stimulus shoals (two trials excluded), as this resulted in effectively different stimulus shoal sizes (as fish that were the subject of aggression lay motionless in the corners of the tank), and may have influenced the decision of the test fish. No physical damage occurred to the fish.

Experiment 2: Diet Choice Tests

To investigate whether the choices made by individuals in experiment 1 were based on general preferences for particular diets rather than specific preferences for particular individuals, we carried out a further experiment investigating diet choice. A subset of 160 fish from experiment 1 (blocks 5–8) were used in this second experiment. After experiment 1, fish were measured and returned to their holding aquaria, and their previous diets were maintained. Experiment 2 was carried out between 21 and 26 days after the fish had first been placed in the holding aquarium at the start of the study (i.e. the week after the familiarity and diet cue tests). Prior to testing, fish were not fed for 24 h to ensure they were motivated to feed.

The test tank (Fig. 1) was based on a Y-maze design and followed that of Shohet & Watt (2004). It consisted of an opaque container (400 × 280 mm), filled to a depth of 85 mm with fresh clean water. Along one of the long sides of the tank, two test compartments (105 × 105 mm and 180 mm high) were separated from the rest of the tank by mesh (as above, hole diameter 3 mm). To ensure that no particles of food were present in the choice tank, which may have influenced decisions, we created extracts by filtering 1.5 litres of clean water through approximately 5 g of bloodworm, or an equivalent volume of flake food, using filter paper (grade 595, diameter 320 mm). Pilot trials indicated that this produced a filtrate with an odour detectable by the fish. Diet cue filtrate entered the tank through the test compartments at a rate of 100 ml/min. To ensure that the water level remained constant, water flowed out of the tank through an overflow behind the holding cylinder. On the base of the tank, we marked two choice lines to delimit preference zones for each diet cue. The choice line marked the point at which the fish could first see into the test compartments.

Two randomly selected focal fish, from the same holding aquarium, were placed in a transparent, perforated cylinder (identical to those used in experiment 1) for 3 min before the flow of the diet cues started. Bloodworm-fed fish ($N = 23$ pairs) and flake food-fed fish ($N = 30$ pairs) were tested alternately wherever possible. After the flow of cues started, the fish remained in the cylinder for a further 1 min to allow the cues to spread throughout the test tank (pilot trials with dye indicated that this was a suitable time period). In each trial, bloodworm extract flowed into one test compartment and flake food extract into the other. To control for any side biases by the fish, we systematically alternated the cues every two trials (i.e. after a bloodworm-fed pair and a flake food-fed pair had been tested). After the 4-min acclimatization period, the transparent cylinder was raised clear of the water so

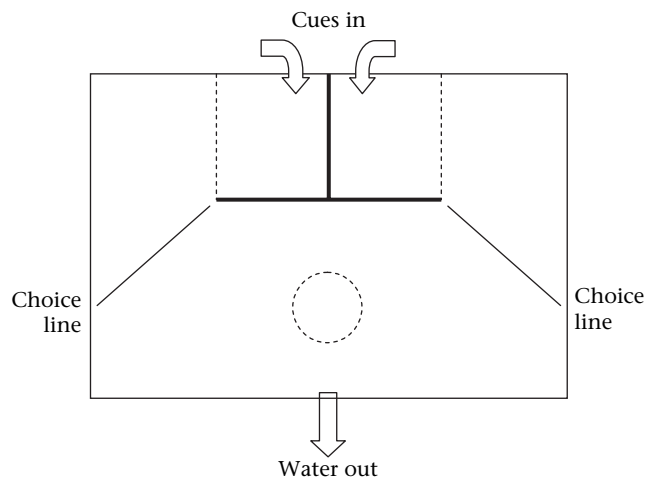


Figure 1. Schematic aerial view of the diet choice tank. Dashed lines indicate perforated walls, thick solid lines indicate solid opaque walls, which provided a barrier to water flow, and the thin solid lines indicate the choice lines. The area contained by the choice line and the perforated wall represents the bloodworm and flake food choice zones.

that it did not interfere with the flow of cues around the test tank, releasing the fish.

Two observers, positioned at the corners of the tank closest to the outflow of water, recorded the behaviour of the fish during a 5-min trial. Each observer followed one of the two stimulus fish. First, we recorded the initial preference of the fish (i.e. the preference zone that was entered first). Then, using a stopwatch, we recorded the time spent by each fish in each of the preference zones (± 1 s). A fish was regarded as entering or leaving the preference zone when its head crossed the choice line. No fish was tested more than once, and the water in the test tank was changed between trials. Two fish were used in the experiment as single fish showed signs of stress (freezing on the bottom) during pilot trials. The nonindependence of each member of a pair was recognized in the statistical analysis. Any pairs in which aggression (chasing, biting attempts) was noted were excluded from the analysis (six pairs excluded), as we could not be confident that movement into a preference zone represented a choice rather than an attempt to escape from the aggressor. No physical damage occurred to the fish; trials were terminated if aggression continued for more than 2 min after the fish were released (no aggression was observed before release).

Experiment 3: Growth

To investigate whether the preferences observed in experiment 1 could be explained by differences in the quality of the diets offered to the fish (which may result in different growth rates), we divided a further 40 individuals between four aquaria, identical to those used for holding the fish in experiments 1 and 2. Before placing them in the aquaria, we recorded the natural colour markings of each fish (to allow for subsequent identification), and weighed it (± 0.001 g; the average of 3 weights was used). Fish in two of the aquaria were fed ad libitum on bloodworm and those in the remaining aquaria were fed ad libitum on flake food. After 2 weeks, the fish were weighed again.

Data Analysis

We calculated the proportion of the total time spent shoaling that was spent shoaling with each of the stimulus shoals (experiment 1), or swimming in the preference zone for each diet (experiment 2), and normalized the data using arcsine transformation. We used Student's t tests to compare (proportion of time spent with familiar shoal or diet) – (proportion of time spent with unfamiliar shoal or diet) against zero. To investigate initial preferences in the diet choice experiment, we used binomial tests, comparing to an expected probability of 0.5, representing random choice. A repeated measures ANOVA was used to investigate differences in growth between diets. Alpha levels were adjusted across all tests following the Benjamini & Hochberg's (1995) method for false discovery rate (FDR) control: qualitative significance of the results was not altered (Table 1). All statistical tests are two tailed.

Table 1. Results of Benjamini & Hochberg's (1995) False Discovery Rate (FDR) control applied to the statistical tests in this study

Test number*	Test	Observed <i>P</i>	Rank of <i>P</i> †	FDR alpha level
Experiment 1: Shoaling preferences				
1	Diet cue preference, BW	0.015	9	0.0321
2	Familiarity preference, BW	0.034	11	0.0393
3	Diet cue preference, FF	0.009	7	0.0250
4	Familiarity preference, FF	0.015	10	0.0357
Experiment 2: Diet choice				
5	Body size	0.056	12	0.0429
6	Both fish choose, BW	0.001	2	0.0071
7	Both fish choose, FF	0.001	3	0.0107
8	Identical choice, BW	0.004	6	0.0214
9	Identical choice, FF	0.013	8	0.0286
10	First choice, BW	0.001	4	0.0143
11	First choice, FF	0.832	14	0.0500
12	Time spent with familiar diet, BW	0.002	5	0.0179
13	Time spent with familiar diet, FF	0.079	13	0.0464
Experiment 3: Growth				
14	Diet*Time interaction	0.001	1	0.0036

Observed *P* values that remain significant after correction are highlighted in bold. BW: bloodworm-fed fish; FF: flake food-fed fish.

*Test number refers to the order of statistical tests in the text.

†Observed *P* values are ranked in descending order of significance.

RESULTS

Experiment 1: Shoaling Preferences

Bloodworm-fed fish showed significant preferences for shoaling with bloodworm-fed fish over flake food-fed fish and for shoaling with familiar over unfamiliar individuals (*t* test: diet cues: $t_{23} = 2.636$, $P = 0.015$; familiarity: $t_{24} = 2.250$, $P = 0.035$; Fig. 2). Flake food-fed fish showed significant preferences for shoaling with bloodworm-fed fish over other flake food-fed fish, and for unfamiliar fish over familiar fish (*t* test: diet cues: $t_{22} = -2.885$, $P = 0.009$; familiarity: $t_{21} = -2.665$, $P = 0.015$; Fig. 2).

Experiment 2: Diet Choice Tests

At the start of experiment 2, there was no difference in body length between bloodworm-fed fish and flake food-fed fish ($t_{157} = 1.924$, $P = 0.056$). We looked first at whether the behaviour of the two fish was independent, predicting that the fish would influence each other's behaviour. In the majority of cases, both fish entered the preference zones (bloodworm-fed fish: 17/18 pairs; binomial test: $P < 0.001$; flake food-fed fish: 19/22 pairs; $P = 0.001$), and in a significant majority of these cases, the fish made the same decision as to which zone to enter first (bloodworm-fed fish: 14/17; binomial test: $P = 0.013$; flake food-fed fish: 16/19; $P = 0.004$). Thus, the behaviour of the two fish was unlikely to be independent (i.e. they followed one another in the tank). We therefore used only the preference of the first fish in our investigation

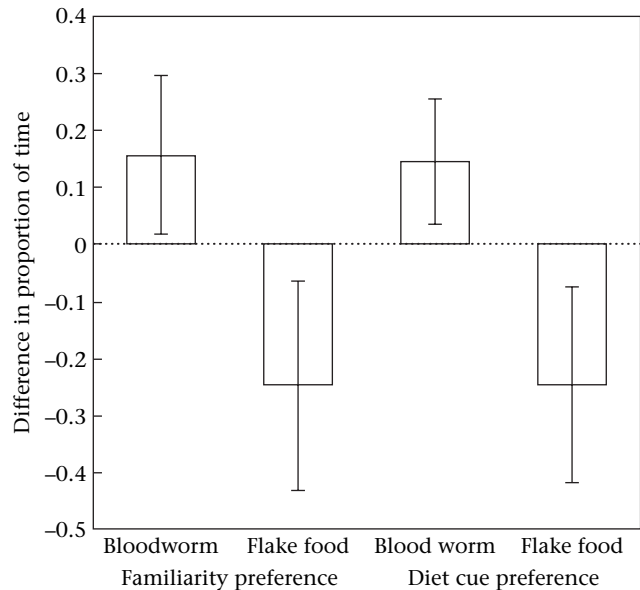


Figure 2. Results of the shoal choice experiment, showing the mean ± 2 SE difference between the proportion of time spent shoaling with the familiar shoal or diet cue (bloodworm or flake food) and the proportion of time spent with the unfamiliar shoal or diet cue. Zero indicates no preference, positive values indicate more time spent with the familiar shoal or cue, and negative values indicate more time spent with the unfamiliar shoal or cue. The X axis categories (bloodworm or flake food) refer to the diet of the test fish before the experiment.

of initial preferences. Our analysis includes trials where either one or both fish made a decision.

Bloodworm-fed fish initially chose to enter the bloodworm zone (16/18 fish chose bloodworm first; binomial test: $P = 0.001$; Fig. 3a), whereas flake food-fed fish showed no initial preference for either zone (12/22 fish chose bloodworm first; binomial test: $P = 0.832$; Fig. 3a). Over the trial, bloodworm-fed fish showed a significant preference for the bloodworm extract, spending a greater proportion of time swimming in the bloodworm preference zone than the flake food preference zone; flake food-fed fish showed no preference for flake food over bloodworm (*t* test: bloodworm: $t_{16} = 3.589$, $P = 0.002$; flake food: $t_{21} = 1.868$, $P = 0.079$; Fig. 3b).

Experiment 3: Growth

Table 2 shows the results of the repeated measures ANOVA. We used weight as the response variable, time as the within-subject factor and diet as the between-subjects factor. There was a significant interaction between diet and time: fish on both diets increased in mass, but this increase was greater in bloodworm-fed fish (Fig. 4).

DISCUSSION

Our results suggest that diet has a strong role to play in shoaling decisions. Diet affected familiarity preferences. In

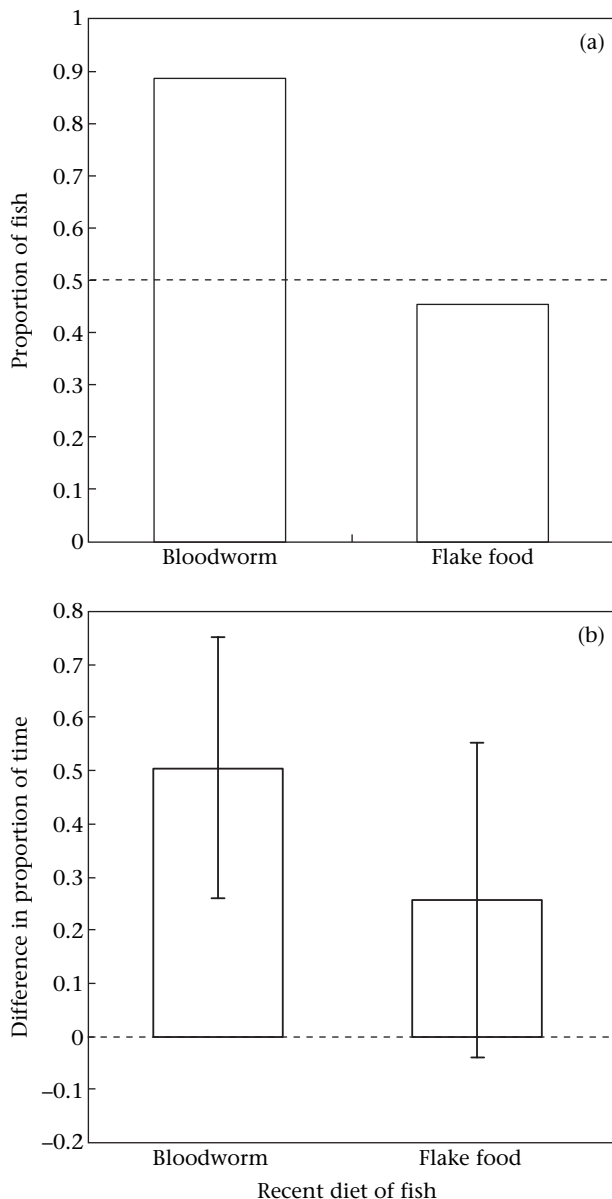


Figure 3. Results of the diet choice experiment for fish fed on bloodworm or flake food: (a) the proportion of leading fish entering the bloodworm zone (see Fig. 1) first and (b) the mean \pm 2 SE difference between the proportion of time spent with the familiar diet and the proportion of time spent with the unfamiliar diet. Zero indicates no preference, positive values indicate more time spent with the familiar diet cue.

accordance with our expectations, and in line with numerous other studies on the importance of familiarity in shoaling decisions (Krause & Ruxton 2002; Griffiths 2003; Ward & Hart 2003), fish fed on a bloodworm diet preferred familiar shoals to unfamiliar ones. In contrast, fish fed on a diet of flake food preferred unfamiliar fish. While other studies have shown no preference for either familiar or unfamiliar fish (Godin et al. 2003), to our knowledge, this is the first study to show a preference against familiar fish of the same sex in a social context, where there are no differences in shoal size.

Table 2. Results of the repeated measures ANOVA investigating the effect of diet on growth rates

Source	Type III sum of squares	df	F	P
Time	0.0634	1	92.030	<0.001
Time*Diet	0.0085	1	12.346	0.001
Error	0.0255	37		

Fish fed on both bloodworm and flake food diets showed a significant preference for stimulus shoals fed on bloodworm. Whereas bloodworm-fed fish preferred familiar food odour in the diet choice test, flake food-fed fish showed no preference for either diet. However, increased growth rate in fish fed a bloodworm diet may explain the preference of flake food-fed fish for those fed on bloodworm. Although the mass of fish did not differ significantly after the 2-week period (Table 2, Fig. 4), associated differences in body condition may be detectable by the fish. Food-deprived zebrafish, *Danio rerio*, prefer shoals of well-fed individuals to shoals of food-deprived individuals. While we do not know the mechanism for this, it suggests that they can associate cues such as body weight and size with the feeding history of individuals (i.e. in the recognition of food-deprived conspecifics; Krause et al. 1999), particularly when feeding rate or diet has differed over a longer time period.

A preference for well-fed conspecifics may also underlie the preference of bloodworm-fed fish for those fed on a similar diet, with individuals preferentially associating with shoalmates that grew faster and were in better condition. However, well-fed zebrafish showed no significant preference based on nutritional state (Krause et al. 1999). An alternative explanation is that fish benefit by associating with those that exploit the environment in a similar way to themselves, for example, if they are able to acquire information on the habitat, or associate with individuals that behave in a similar manner to themselves,

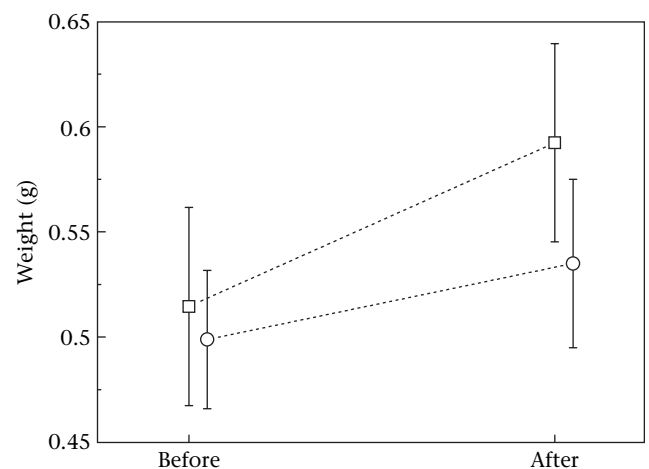


Figure 4. Results of the growth experiment, showing mean \pm 2 SE weight before and after 2 weeks on either a bloodworm (\square) or flake food (\circ) diet.

minimizing behavioural oddity (Ward et al. 2004, 2005). Fish in our experiments were able to use both visual and olfactory cues in their shoal choice decisions: by investigating visual and olfactory cues separately, the roles of dietary cues from the fish and different body condition between individuals could be teased apart.

Our aim with the second experiment was to investigate whether the preference for associating with bloodworm-fed fish, by fish from both diet treatments (experiment 1), could simply be explained by a general preference for the odour of bloodworm as a food resource. We observed that flake food-fed fish showed no preference for either diet, whereas bloodworm-fed fish preferred bloodworm. These results suggest that a general preference for bloodworm cannot explain the preference of flake food-fed fish for bloodworm-fed fish as shoalmates in experiment 1. Two factors other than dietary preference may have influenced the results of experiment 2. First, the concentration of odour cues may have differed between the food types and fish may prefer a stronger cue to a weaker one. However, fish from both treatments were exposed to the same concentrations and the lack of a preference in the flake food-fed fish supports the hypothesis that a general preference for bloodworm cannot explain the results of experiment 1. Second, experience with a particular diet may be necessary for the fish to develop a preference for that diet. While this hypothesis may explain the results of experiment 2, it once again supports the hypothesis that the preference of flake food-fed fish for bloodworm-fed fish in experiment 1 cannot be explained by dietary preference for bloodworm.

The finding that flake food-fed fish avoided associations with familiar individuals warrants further discussion. What could be the potential benefits of this? Mating with novel and therefore unfamiliar partners is common (and occurs in guppies; Hughes et al. 1999; Kelley et al. 1999), and the benefits are well understood (e.g. Jennions & Petrie 2000). The benefits of associating with unfamiliar individuals of the same sex are less clear. The only difference between the bloodworm- and flake food-fed fish was their diet; there were no other differences in holding conditions, or in the size of the fish, suggesting that familiarity preferences are mediated by diet. One potential explanation is that by associating with novel shoalmates, individuals may attempt to gain access to alternative resources. This hypothesis is supported by the results of the diet cues and growth experiments.

One can imagine that if environmental conditions are poor, preferences for unfamiliar individuals, as observed in the flake food-fed fish, could be adaptive, as it could allow individuals to seek out novel food resources or improved shelter, for example. Associating with well-fed conspecifics may benefit individuals in two ways. First, being well fed may indicate good food-finding abilities and thus it could be beneficial for a deprived fish to join a well-fed group (Krause et al. 1999). Second, competition for food may be reduced, as other nutritionally deprived individuals are more likely to be competitors for food (e.g. they may have higher motivation to compete) than well-fed individuals (Krause et al. 1999). For example, dominance status increases with nutritional deprivation in rainbow trout, *Oncorhynchus mykiss* (Johnsson et al.

1996). Flake food-fed fish showed no preference for associating with either cue in the diet choice trials, indicating that they may be willing to switch to a new diet: this could be adaptive, as it could lead to faster growth rates. Individuals would thus associate with familiar individuals when conditions were good, but switch to unfamiliar conspecifics when conditions are poor (a win-stay, lose-shift strategy; e.g. Nowak & Sigmund 1993).

There may be a trade-off between the benefits of associating with familiar individuals and the benefits of associating with better-fed, unfamiliar individuals (which will carry a cost, as individuals associating with unfamiliar individuals will lose the benefits associated with familiarity). The resolution of trade-offs can be complex (Houston et al. 2003; Morrell 2004), and shoaling priorities may also be influenced by other factors, including predation risk (Ashley et al. 1993), whether foraging is a priority (Hoare et al. 2004), competitive ability (Metcalfe & Thomson 1995) and dominance status (Gomez-Laplaza 2005).

Individuals may face a trade-off when there are different benefits to associating with two stimulus shoals, and they may need to prioritize which of the cues they use to make their decision (Krause & Godin 1994b; Wong & Rosenthal 2005). Such conflicts have been investigated in the context of species, shoal size and the sizes of the individuals within shoals (Ashley et al. 1993; Krause & Godin 1994b; Hoare et al. 2004; Wong & Rosenthal 2005). In climbing perch, *Anabas testudineus*, a preference for large, unfamiliar shoals over smaller familiar shoals has been recorded (Binoy & Thomas 2004). Our work suggests that preferences for familiar fish can be overridden by diet and individuals may trade off familiarity with nutritional state. The question of whether familiarity or other cues takes precedence in shoal choice decisions warrants further investigation, and provides interesting questions for future research.

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