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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.

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 (Metcalfe & 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; Shohet & 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