

# Species and population differences in social recognition between fishes: a role for ecology?

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The social organization of animals is reliant on recognition. However, the precision and specificity with which an individual animal recognizes another in a social context, and the sensory mechanisms that it employs, may vary both within and between species. Differences in the ecology and in the mating systems of species may drive the evolution of different recognition abilities, ranging from individual-specific recognition to more general forms of recognition. We examined social recognition in two important model species in behavioral ecology, the guppy (*Poecilia reticulata*) and the three-spined stickleback (*Gasterosteus aculeatus*). We found that guppies were capable of individual recognition of conspecifics, as well as being able to differentiate between groups of conspecific based on cues relating to resource use and habitat use. By contrast, sticklebacks showed no ability to recognize individuals in a social context after prior interactions. Nonetheless, two out of three populations of sticklebacks demonstrated general recognition abilities, based on cues relating to resource use. We discuss the potential relationship between social recognition mechanisms and the ecological and life-history parameters of species and populations. *Key words*: chemo-sensory, fission–fusion, groups, head, shoaling, social organization, social recognition. [*Behav Ecol*]

When one animal detects another, it can assess the available cues and compare them with a recognition template. Depending on the quality of the cues and the sophistication of the template, identification may be made to some level in a hierarchy ranging from basic discrimination between threat and nonthreat, or between conspecifics and heterospecifics, through to specific individual recognition.

Animals that live in social groups must be able at the very least to discriminate between their own and other species. Beyond this, the specificity and extent of the recognition mechanisms used vary from species to species. Patterns of social organization in many animal taxa suggest that social animals are not equally attracted to all conspecifics. In these cases, animals are clearly capable of discriminating between subsets of the population, or even between individuals (see Krause et al. 2008); and this ability to discriminate in turn mediates their choice of group mates. Shoaling fish, like many other social animals, often demonstrate an association preference for an unrelated individual or individuals, outside of a mate choice context. “Familiarity,” as this phenomenon is known, acts to enhance the benefits of grouping, further reducing the per-capita risk of predation (Chivers et al. 1995; Griffiths et al. 2004) and improving foraging performance (Ward and Hart 2005; Webster and Hart 2007).

Social recognition underlies familiarity, but there are different mechanisms by which this may be achieved. A fish encountering another individual may recognize its specific individual identity, or it may simply distinguish some other, more general cue, enabling it to differentiate between subsets of the population. In human terms, this might, for example, be the difference between recognizing a particular individual

person by their voice and recognizing the likely origins of a person based on their regional accent or the language that they use. Specific individual recognition is gradually learned over a period of time (Griffiths and Magurran 1997a), and there is an upper limit to the number of different individuals that can be memorized (Griffiths and Magurran 1997b). Contrastingly, the more general form of recognition does not require individuals to have any prior experience of each other. This may occur when association decisions are mediated by chemical cues relating to recent habitat use and/or diet (Ward et al. 2004, 2005). These alternative mechanisms are not mutually exclusive, although the extent to which each is used may vary between populations and species according to their ecology.

The distinction between these two forms of recognition is important. The ability to recognize specific individuals has profound implications for the evolution of complex social behaviors including tit-for-tat and reciprocal altruism (see Sachs et al. 2004). Research interest into the implications of individual recognition in fishes increased following the publication of a number of laboratory studies suggesting that individuals engaged in predator inspection behavior could recognize and recall defectors (Milinski, Kulling, and Kettler 1990; Milinski, Pfluger, et al. 1990; Dugatkin and Alfieri 1991; but see Thomas et al. 2008). It was assumed by many researchers that recognition of individuals, a phenomenon predominantly described in laboratory studies, might also operate extensively in free-ranging populations, where it could underpin these complex interactions. In fact, the evidence for the operation of individual recognition in free-ranging fish populations is equivocal (e.g., evidence for Klimley and Holloway [1999], Griffiths and Magurran [1997a], Ward et al. [2002]; no evidence for Helfman [1984], Hoare et al. [2000], Godin et al. [2003], and Magurran and Queiroz [2003]). There remains the possibility that for many species, individual recognition observed in the laboratory might be an artifact of experimental designs, which enforce prolonged interaction between individuals and which prevent the diluting effects on social

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structure of immigration into and emigration from the shoal, factors that in nature would erode group stability and prevent the learning of individual identities. As a consequence, the importance of individual recognition and its implications for complex social interactions may have previously been overstated. To further confound this issue, recent research has identified a more parsimonious mechanism of general recognition operating in fishes, one that is based on resource specific cues (e.g., Griffiths and Ward 2006; Ward et al. 2007; Webster et al. 2007).

Where does this leave our understanding of recognition in shoaling fishes? Some species are subject to ecological conditions that might well favor the evolution of the capacity for individual recognition: periods of prolonged interaction with a limited group of conspecifics, providing the opportunity to learn identities. In other species, which inhabit open areas and occur in high population densities, the encounter rate with conspecifics may be sufficient to promote reliance on general forms of recognition. In this study, we compared the mechanisms of recognition used by two species that differ markedly in their ecology and the nature of their social environments. We selected two well-established model organisms, the Trinidadian guppy (*Poecilia reticulata*) and the three-spine stickleback (*Gasterosteus aculeatus*).

Guppies occur naturally in streams in Trinidad. During the rainy season, their habitat typically consists of pools linked by riffles, but in the dry season, small groups of guppies may be trapped within lentic pools for several months at a time (see Magurran 2005). Guppy dispersion may therefore be constrained, and individual fish in small, stable groups may interact repeatedly with each other. The promiscuous mating system of the species may also place a premium on individual recognition, because guppies that are able to direct their reproductive effort more effectively (i.e., to avoid repeated courtship and mating with a particular individual or individuals) are likely to be selected for.

Sticklebacks are, like guppies, a model study organism in animal behavior; however, the ecology and life histories of the two species are very different. Sticklebacks tolerate a wide variety of aquatic habitats ranging from fresh to saltwater, including riverine, lacustrine, and marine environments across the northern hemisphere (see Bell and Foster 1994). Differences in turbidity and flow regimes between these environments may favor the use of different sensory modalities. Sticklebacks in many populations may potentially encounter large numbers of conspecifics each day and so are not necessarily constrained to repeated interactions with the same individuals.

Numerous studies have reported the development of familiarity in both species (see Griffiths 2003; Ward and Hart 2003; Ward and Hart 2005). But although both species appear to express the same behavior with respect to showing a preference for familiar individuals, less is known about the mechanisms that underpin this behavior in each species. Similarly, little study has been made of the possibility that disparities in the environmental conditions experienced by different populations of the same species could influence the recognition mechanisms used. In this study, we hypothesized that the mechanisms underlying social recognition would differ between these two species because of the constraints imposed by their very different ecology and life histories. Specifically, we predicted that although the guppies would be capable of using both individual and general (e.g., habitat-mediated) recognition, the sticklebacks would only use the latter form. We further hypothesized that the mechanisms underlying social recognition would differ between populations of the sticklebacks that experience different environmental conditions, for the reasons described in the previous paragraph.

## MATERIALS AND METHODS

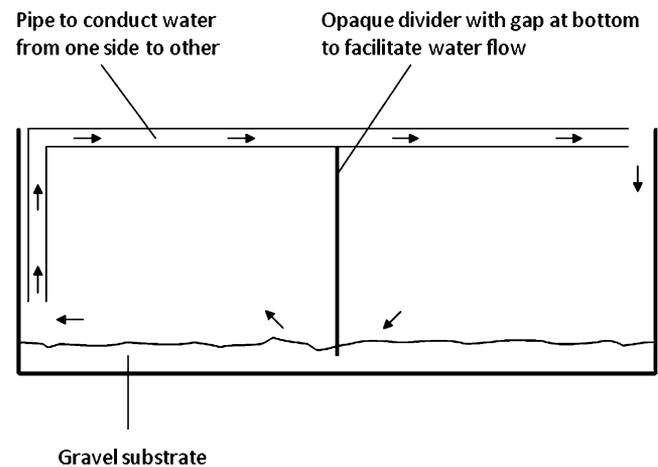
### Part 1: species differences in social recognition

#### Study animals

Adult female Trinidadian guppies, size matched to  $30 \pm 3$  mm, were obtained from a population held at the University of Leeds, UK, in October–December 2006. They were the descendants of guppies collected from a wild population in the Tacarigua river in the Northern mountain range of Trinidad in 2005 ( $10^{\circ}40.7'N$ ,  $61^{\circ}19.2'W$ ). Adult sticklebacks, size matched to  $35 \pm 5$  mm were collected in October 2006 from drainage ditches connecting to the Great Eau, Lincolnshire, UK ( $53^{\circ}25'N$ ,  $0^{\circ}12'E$ ).

#### Conditioning protocol

Fish were held for a period of 3 weeks in aquaria measuring  $45 \times 20 \times 20$  cm (see Figure 1). These were divided into two by an opaque divider to create two compartments, each measuring  $22.5 \times 20 \times 20$  cm. There was a gap at the bottom of the divider of 1 cm, which was covered by the addition of a 2-cm layer of gravel substrate that enabled water circulation between the compartments, but prevented fish from being able to see fish in the other compartments or from being able to cross into the other compartment. A 1.5-cm-diameter pipe conducted water between the compartments. This water movement was driven by an air pump so that approximately 100 ml of water was transferred between compartments each minute. At the beginning of each 3-week conditioning period, 48 guppies and 48 sticklebacks were divided equally between the six aquaria so that there were 16 conspecifics in each with eight fish in each compartment. Within each species set, fish in two of the three aquaria were fed exclusively on bloodworm for the 3-week period, whereas fish in the third aquarium were fed water fleas (*Daphnia* sp.). In addition, 5 ml of Blackwater Extract, an aquarium water supplement containing plant tannins, was added on a weekly basis to the tanks where fish were fed bloodworm in order to manipulate the habitat conditions experienced by the fish. After the 3-week conditioning period, fish were tested in the procedure described below. Once this was complete, a new batch of 48 fish was added, and the conditioning period was repeated with the one exception that now only one of the three aquaria was provisioned with bloodworm and Blackwater Extract, whereas fish in the other two



**Figure 1**  
Side view of the conditioning aquaria used in the species recognition experiments. This design allows fish to live in a common environment, in terms of water chemistry, while only being able to directly interact with half of the fish.

aquaria were fed *Daphnia*. Sticklebacks were held in water at 14 °C, guppies at 24 °C.

#### Experimental design and protocol

Experiments were conducted in a simple binary choice experiment. The test tank measured 45 × 20 × 20 cm and was divided into three compartments using clear, perforated plastic dividers such that there was a single, central compartment measuring 25 × 20 × 20 cm flanked by two outer compartments, each measuring 10 × 20 × 20 cm. To encourage the flow of water, and therefore chemical cues, from the outer compartments into the central compartment, water was siphoned from a single header tank by two 0.6-mm-diameter tubes, one tube to each of the outer compartments. The flow rate was controlled with a valve to a volume of 20 ml/min/tube. Excess water drained from a central hole drilled in the front wall of the aquarium. The walls of the aquarium, with the exception of the front wall of the central compartment, were screened with black plastic to minimize disturbance to the test fish. Each experiment began with the addition of five stimulus fish to each of the outer compartments. These were allowed to settle for a period of 3 min before a single fish (the “focal fish”) was added to the center of the central compartment in a cylinder constructed from clear, perforated plastic. The focal fish was allowed to settle for 5 min and to assimilate visual and olfactory cues arising from the stimulus fish before it was released by raising the holding cylinder. For the following 5 min, we recorded the amount of time spent by the focal fish within three body lengths of each of the two groups of stimulus fish. Lines were drawn on the outside of the aquarium to indicate a distance of three body lengths. We used the position of the tip of the fish’s snout to determine which zone the fish occupied. After the trial was completed, a further 5 min elapsed before a second focal fish was introduced and the procedure repeated. The stimulus fish were removed after three trials at which point the water was removed and the tank rinsed to remove any residual cues. Groups of stimulus fish were allocated haphazardly to the outer chambers to control for side bias. No fish were reused.

We conducted a total of three treatments (see Figure 2):

1. Direct experience. Stimulus fish were taken from the same aquarium, one group from either side of the opaque barrier, so that they had a common environmental experience but the focal fish, taken from the same aquarium, had recent direct experience with only one of the two stimulus groups, for example, focal fish from D1, stimulus fish group 1 from D1, stimulus fish group 2 from D2 (see Figure 2).
2. Environmental cues I. One group of stimulus fish was taken from separate aquaria within each of the two different conditioning treatments, and the focal fish was taken from the third aquarium, so that it had no recent direct experience with any of the stimulus fish but

shared recent general environmental experience with one group, for example, focal fish from D1, stimulus fish group 1 from D3, stimulus fish group 2 from B1 (see Figure 2).

3. Environmental cues II. One group of stimulus fish was taken from each of two aquaria in the same environmental treatment, so that they had a common environmental experience. The focal fish was taken from the same aquarium as one of the two stimulus groups, although from a different compartment, so that it may have experienced differences in tank-specific cues relating to the two stimulus groups, for example, focal fish from D1, stimulus fish group 1 from D2, and stimulus fish group 2 from D3 (see Figure 2).

## Part 2: population differences in social recognition

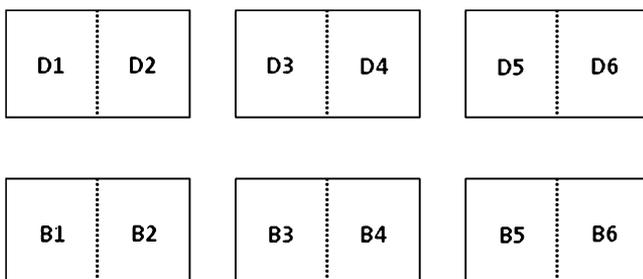
### Study animals and collection sites

Adult sticklebacks, size matched to 30 ± 5 mm, were collected in July 2006 from two sites in Silver Lake, Sackville, New Brunswick, Canada (46°55'N, 64°21'W). We selected the sites on the basis of the differing ecological conditions that occurred at each (see Table 1), predicting that these would influence the social recognition mechanisms of the fish that inhabited them. Site 1 was at the inflow; Site 2 was at the shoreline in the main body of the lake. The two sites were separated by 1.6 km.

### Experimental design and protocol

Fish collected from the two sites were subject to three separate behavioral assays. These were conducted using a flow channel measuring 71.5 × 38 cm with a water depth of 9.5 cm. Mesh barriers were used to create a central compartment measuring 34 × 38 × 20 cm (L × W × D) within the flow channel itself. We distributed a thin layer of sand as a substrate in this central compartment. Water flowed into the channel at two points, the top left and the top right corner, from two 15-l reservoir buckets at a rate of 180 ± 20 ml/min at each point. The water drained out of the flow channel at the bottom left and bottom right corner through holes drilled into the walls. This had the effect of creating two parallel currents within the channel. Baffles placed parallel to the current served to constrain the two streams. We repeatedly tested the flow patterns using waterborne dye and found that the dye streams remained separate for the full length of the flow channel with only a small amount of mixing in the center of the flow channel. We used this information to define three equally sized zones within the flow channel, with one neutral zone in the center and a zone for each stream. We marked these zones by placing thin strips of plastic in the sand substrate. The water used in the flow channel was not recirculated.

Our first behavioral test assessed a general recognition and preference for conspecific chemical cues. Fifteen conspecifics from one of the two populations were allocated haphazardly to one of the reservoir buckets and allowed to acclimatize for 30 min. During this period, cues arising from these fish



**Figure 2**

Diagrammatic representation of environment and diet treatments showing the different compartments within tanks. D = *Daphnia*; B = bloodworm.

**Table 1**  
Treatments

	Site 1	Site 2
Water flow	12–21 cm/s	No flow
Visibility	>5 m	<10 cm
Substrate characteristics	Sand and mud	Thick, fine mud
Environmental complexity	Low; very few aquatic plants, very little logs/other debris	High; dense beds of aquatic plants, many submerged fallen branches

percolated through one side of the flow channel. The other reservoir bucket also had water flowing through it but had no fish, producing a “blank” plume. After this, a single focal fish from the same population as the stimulus fish was introduced to the center of the central compartment of the flow channel. It was allowed to acclimatize for 5 min, during which time it was observed to ensure the prerequisite that it visited both sides of the channel and therefore experienced both streams prior to the test commencing. Where this was the case, the focal fish was observed for a further 5 min, and the time spent in each of the three zones was recorded. Once this period had elapsed, the focal fish was removed, and a further 5 min passed before we added a second focal fish. After the completion of five trials, we replaced the stimulus fish and flushed the system out for 1 h before starting the process once more with the addition of new stimulus fish. We carried out 15 replicates for each site.

Our second behavioral assay examined the behavior of focal fish in the flow tank in response to chemical cues from site 1 conspecifics and site 2 conspecifics, which were presented simultaneously at different sides of the flow channel. We allocated 15 sticklebacks from each site to each of the reservoir buckets and performed experiments as detailed above. To ensure that the cues provided by each population arose from their habitat, as opposed to any laboratory artifact, we tested the fish immediately on return to the laboratory after capture. There was a maximum period of 2 h between capture and testing. We alternated between site 1 and site 2 focal fish, flushing the system after each trial as described above after five trials and switching the position of the stimulus fish in order to control for side bias. Again, we carried out 15 replicates for each site.

Our third behavioral test assessed the behavior of focal fish in response to conspecific chemical cues mediated by different habitat and diet experience in the laboratory. A total of 210 fish—105 from each site—were conditioned concurrently in batches of 15 fish for a period of 1 week to one of two habitat and diet treatments. These treatments were as previously described: In one, fish were fed with bloodworm, and their water was dosed with 5 ml of Blackwater Extract per 10 l of water; in the other, fish were fed with *Daphnia* sp. After this, in experiments, focal fish were presented simultaneously with plumes containing cues arising from stimulus conspecifics from their own site taken from each treatment. We carried out 14 replicates for each site, with seven focal fish from each of the habitat and diet treatments.

#### Data analysis

The data were log transformed to reduce heteroscedacity. Species and population data were analyzed using General Linear Models (GLM); binary choice experiments were analyzed by subtracting the time spent by each focal fish with stimulus shoal b from time spent with stimulus shoal a and comparing the resulting value with a null expectation of 0 using a one-sample *t*-test. We used a sequential Bonferroni technique (Benjamini and Hochberg 1994) to adjust  $\alpha$  levels where multiple tests were performed.

## RESULTS

### Species differences in social recognition mechanisms

Across all treatments, the response of guppies differed from that of sticklebacks (GLM:  $F_{1,89} = 5.1$ ,  $P = 0.026$ ).

The response of guppies did not vary with treatment (GLM:  $F_{2,44} = 0.7$ ,  $P = 0.5$  see Figure 3). In all three treatments, focal guppies spent significantly more time with one of the stimulus shoals. In the direct experience treatment, focal fish spent more time with the shoal that they had interacted with than

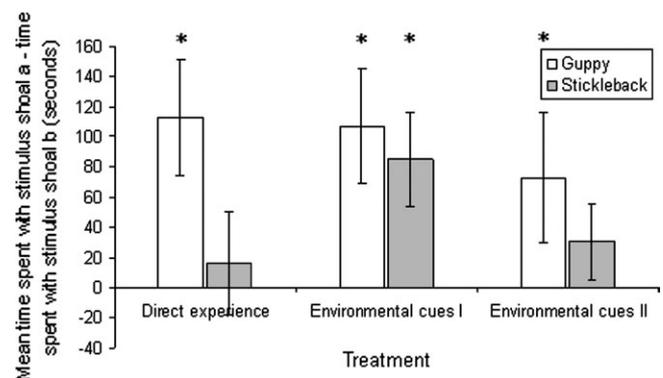
with the shoal that they had not (One-sample *T*-Test:  $t_{14} = 3.8$ ,  $P < 0.01$ ). In the first environmental cues treatment, focal fish spent more time with the stimulus shoal that had experienced the same habitat and diet regime as themselves than with the alternative shoal (One-sample *T*-Test:  $t_{14} = 3.1$ ,  $P < 0.01$ ). In the second environmental cues treatment, focal fish spent more time with the stimulus shoal taken from the other side of a divided aquarium, over a stimulus shoal taken from a separate aquarium (One-sample *T*-Test:  $t_{14} = 2.3$ ,  $P = 0.03$ ).

The response of sticklebacks varied marginally across treatments (GLM:  $F_{2,44} = 3.4$ ,  $P = 0.04$  see Figure 3). Focal sticklebacks showed no difference in time spent with either stimulus shoal in the direct experience treatment (One-sample *T*-Test:  $t_{14} = 0.5$ ,  $P = 0.62$ ). In the first environmental cues treatment, focal fish spent more time with the stimulus shoal that had experienced the same habitat and diet regime as themselves than with the alternative shoal (One-sample *T*-Test:  $t_{14} = 3.4$ ,  $P < 0.01$ ). In the second environmental cues treatment, focal fish showed no difference in time spent with either stimulus shoal (One-sample *T*-Test:  $t_{14} = 1.3$ ,  $P = 0.19$ ).

### Population differences in social recognition mechanisms

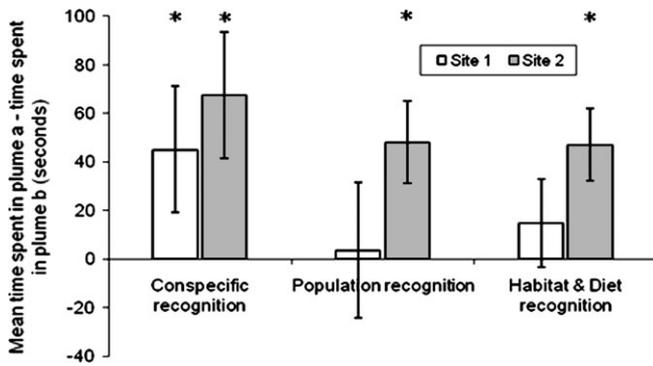
Across all treatments, the response of sticklebacks from site 1 differed from that of sticklebacks from site 2 (GLM:  $F_{1,89} = 4.8$ ,  $P = 0.03$ ).

Sticklebacks from site 1 showed a marginally significant preference for a plume containing conspecific chemical cues over the alternative, a blank plume (One-sample *T*-Test:  $t_{14} = 2.2$ ,  $P = 0.045$ ; see Figure 4); however, there was no difference between the time that they spent in a plume containing chemical cues from conspecifics of their own population versus a plume containing cues from conspecifics from the alternative population (One-sample *T*-Test:  $t_{14} = 0.2$ ,  $P = 0.86$ ), or between time spent in a plume containing conspecifics with the same recent habitat and diet experience versus time spent in plume containing conspecifics with the alternative habitat and diet treatment (One-sample *T*-Test:  $t_{13} = 0.9$ ,  $P = 0.37$ ). In contrast, sticklebacks from site 2 showed significant preferences in all three treatments, preferring 1) conspecifics chemical cues over a blank plume (One-sample *T*-Test:  $t_{14} = 2.5$ ,  $P = 0.024$ ; see Figure 4); 2) the chemical cues of conspecifics from their population over those of conspecifics from site 1 (One-sample *T*-Test:  $t_{14} = 3.1$ ,  $P < 0.01$ ); and 3) the chemical cues of conspecifics from the same habitat and diet regime as themselves over the alternative (One-sample *T*-Test:  $t_{13} = 3.3$ ,  $P < 0.01$ ).



**Figure 3**

The mean difference ( $\pm$ SD) in the time spent shoaling with two stimulus shoals as a function of treatment in guppies and sticklebacks. Asterisk indicates significant departure from null expectation of zero.



**Figure 4**  
The mean difference ( $\pm$ SD) in the time spent in one of two odor plumes as a function of treatment in two populations of sticklebacks. Site 1 is the river population, site 2 is the lake population. Asterisk indicates significant departure from null expectation of zero.

## DISCUSSION

Social recognition varies within as well as between species. Guppies were able to recognize specific individuals, as well as being able to distinguish general environment-related cues deriving from conspecifics. Contrastingly, sticklebacks showed no ability to recognize particular individuals when environmental cues were controlled for. Of the three populations of sticklebacks tested, two of them manifested the ability to differentiate between plumes of conspecific cues, whereas the third population showed no preference.

The ability to respond to a suite of different conspecific cues, including both a general form of recognition and highly specific, individual recognition, may be most appropriate in systems where individuals are constrained to repeatedly interact with the same group of individuals. Earlier experiments by Griffiths and Magurran (1997b) reported an upper limit of approximately 40 separate individual identities that could be learned by adult guppies. In more open populations, free-ranging fishes that do not defend a territory or live within a narrowly defined home range may encounter several hundreds or thousands of conspecifics daily, which is likely to reduce the adaptive benefits of recognizing particular individuals. From this arises the prediction that populations of individuals constrained within small habitat areas, such as isolated pools, or areas where population density is sparse, or where the opportunity or benefit of moving between groups is low, may be more likely to be capable of specific individual recognition than conspecific populations occupying larger and more open habitats. Despite this, the Tacarigua river, from which our guppy population came, is less prone to drying up and forming isolated pools than many other guppy habitats in Trinidad, so it would be interesting to compare social recognition abilities and mechanisms between guppy populations taken from ecologically different habitats. Furthermore, it would also be useful to examine closely related species in this context to consider the evolution of social recognition against the background of phylogenetic distance. Differences in social recognition capabilities and mechanisms may have implications for the social organization and social network structures in such populations and the spread of information, genes and diseases (Croft et al. 2006; Krause et al. 2008) as well as the likelihood of reciprocity in larger populations (Boyd and Richerson 1988).

In the present study, we found no evidence that sticklebacks are able to identify specific individuals. Nonetheless, several published studies have reported this ability in fish in the

laboratory, including sticklebacks (e.g., Milinski, Kulling, and Kettler 1990; Milinski, Pfluger, et al. 1990). Although these studies are at variance with our own findings, we do not rule out that individual recognition may occur. Firstly, all recognition pathways have two steps: Initially the cue must be detected; then, subsequently, there must be a behavioral response. Therefore, we cannot rule out that sticklebacks in our experiments were able to differentiate between the cues of individual conspecifics but simply failed to modify their response in accordance with this. Secondly, it is possible that it only becomes important for sticklebacks to respond differentially to particular conspecifics in contexts other than this basic social example—individual recognition may be manifest in mate choice decisions (e.g., Aeschlimann et al. 2003), in kin recognition (Frommen and Bakker 2004; Frommen et al. 2007), in territorial disputes (Waas and Colgan 1994), during predator inspection (Milinski, Kulling, and Kettler 1990; Milinski, Pfluger, et al. 1990), or its expression may be mediated by social status (Gomez-Laplaza and Fuente, 2007).

Conversely, it may be the case that many fish, especially shoaling species, cannot perform individual recognition but are capable only of recognizing general cues, such as population-specific or habitat- and diet-specific chemical cues (Ward et al., 2004, 2005, 2007; Behrmann-Godel et al. 2006; Matsumura et al. 2007). Indeed Ward et al. (2005) demonstrated that though tank mates housed together for 2 weeks initially showed a preference for shoaling with one another over shoaling with fish from another tank, when some were moved to another, chemically different tank these shoaling preferences disappeared rapidly. These results strongly suggest that tank-specific chemical labels, rather than individual-specific recognition might be the underlying mechanism of recognition observed in sticklebacks in the laboratory. This would help to explain published studies showing no increase in preference for familiars in threatening situations (Griffiths 1997; Brown 2002).

In part 2 of our experiment, we found that sticklebacks living in two geographically close but ecologically distinct populations differed in their response to conspecific chemical cues. This intriguing finding raises the possibility that animal populations could show phenotypic plasticity of their sensory biases in response to local ecological conditions. This is most likely to occur in species, like sticklebacks, which are relatively short lived. Sticklebacks that lived in turbid and complex waters (site 2) were able to recognize chemical cues arising from members of their own population and conspecifics who had the same recent habitat and diet experience, whereas fish from clear, uncluttered waters (site 1) apparently could not. Again, we cannot exclude the possibility that the fish from site 1 could differentiate between the cues but did not modify their behavior on this basis; however, other studies performed on these same populations in foraging and in predator recognition contexts suggest that the chemosensory abilities of site 1 fish are poor relative to fish from site 2 (Ward AJW, unpublished data). The potential also exists that our findings could be explained by different levels of stress experienced by members of each population; however, we saw no evidence to support this: Fish from both sites showed no freezing or darting behaviors indicative of stress responses during the trials.

For the first time in studies of this kind, our experiments control for the confounding influence of tank-specific environmental cues. We know that the chemical profiles expressed by fish are a complex mix of intrinsic (condition, hormonal, and genetic) and extrinsic (habitat, diet) factors. Where fish are maintained in separate aquaria for extended periods, it is likely that they gradually develop a tank-specific chemical label. In many experiments examining familiarity, fish are presented with a choice of conspecifics from their own tank and fish from

a separate tank. If and when fish show a preference for fish from their own tank, this is often taken to mean that they are capable of individually recognizing these individuals. If chemical cues are available, however, the simpler possibility exists that the fish are simply recognizing a basic tank-specific chemical cue produced by their erstwhile tank mates. In the present study, guppies were capable of precisely this and showed a preference for fish that had occupied a separate, physically and visually isolated compartment of their own aquarium. Only if fish in such tests are able to discriminate on the basis of visual cues alone, or if, as here, the fish have been taken from a common environment, can specific, individual recognition be said to have occurred. This is not to say that individual recognition can only occur on the basis of visual cues, but rather that where it is posited to be based on chemical cues, these cues must be more specific than simple habitat cues, that is, they must relate to the individual's unique chemical signature.

Fish social recognition is clearly a complex area. Species and even populations are capable of different levels of recognition, which may relate to their proximate ecology. Clearly, it is important that future studies control carefully for the different cues that experimental fish may use. Furthermore, it would be interesting to examine the effects on the social organization of wild fish populations of different recognition mechanisms and abilities—do fish that are capable of more specific recognition form more cohesive shoals, or do they form more persistent association patterns? Finally, it would be extremely useful to separate the components of the recognition pathway because the failure to respond to cues is not the same as a failure to detect them. In order to determine whether this is the case, it would be useful to conduct electro-olfactograms to inform us whether they show a stronger response to particular individuals.

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