CHAPTER 8 Do female rainbowfish (*Melanotaenia spp*.) prefer to shoal with familiar individuals in the presence and absence of predators?

8.1 ABSTRACT

This is the first study investigating shoal-mate preference for familiar individuals conducted on Australian native fishes. Shoaling with familiar individuals may have many benefits including enhanced escape responses or increased foraging efficiency. This study describes the results of two complimentary experiments. The first utilised a simple binary choice experiment to determine if rainbowfish (*Melanotaenia spp*.) preferred to shoal with familiar individuals or strangers. The second experiment used a "free range" situation where familiar and unfamiliar individuals were free to intermingle and were then exposed to a predator threat. Like many other small species of fish, rainbowfish are capable of identifying and distinguishing between individuals and choose to associate with individuals that they are familiar with. However, rainbowfish do not significantly alter their preference for familiar individuals when under threat from a stationary predator model.

8.2 INTRODUCTION

When fish are separated from shoal mates and a predator appears, the most immediate response in many species is to rejoin a shoal and thereby find safety in numbers. Given a choice between a number of shoals, the fish must make a decision about which shoal to join by taking a number of things into account. Fish may assess shoals based on species composition (Parrish 1989; Krause and Godin 1994), shoal size (Hagar and Helfman 1991), the size distribution of shoal members (Pitcher *et al.* 1985, 1986, Ranta *et al*. 1992, Pitcher and Parish 1993), fish colouration (McRobert and Bradner 1998), the level of parasitism (Krause and Godin 1996, Barber *et al.* 1998) or the reproductive status of members within each shoal (Van Havre and FitzGerald 1988). The preference to shoal with conspecifics may be overridden by size selectivity (Ranta *et al.* 1992, Krause and Godin 1994).

The decision to join a shoal may be determined by the situation that the fish finds itself in and its motivational state. Individuals may join and leave schools as their motivation changes (Seghers 1981). A balance may be struck between the perceived level of predator threat and hunger level (Morgan 1988). If the fish is threatened the decision may be made more quickly (Hagar and Helfman 1991).

It has been shown previously that guppies (*Peocilia reticulata*), bluegills (*Lepomis macrochirus*), paradise fish (*Macropodus opercularis*), sticklebacks (*Gasterosteus aculeatus*) and fathead minnows (*Pimephales promelas*) choose to shoal with familiar individuals (Magurran *et al*. 1994, Griffiths and Magurran 1997a, Miklosi *et al*. 1992, Brown and Colgan 1986, FitzGerald and Morrissette 1992, Chivers *et al*. 1995). Schools of fathead minnows comprised of familiar fish show more effective anti-predator behaviours than schools comprised of unfamiliar individuals (Chivers *et al*. 1995). On the other hand, pumpkinseed fish (*Lepomis gibbosus*) and juvenile rockbass (*Ambloplites rupestris*) do not spend more time with familiar conspecifics (Brown and Colgan 1986), possibly because these species are either solitary or territorial.

In light of such variation between species, I was interested to determine whether rainbowfish (*Melanotaeniidae spp.*) could recognise, and prefer to shoal with, individuals that they were familiar with. Furthermore, I wanted to determine if rainbowfish become more or less choosy when under threat of predation. Given the existing evidence, which suggests that shoaling with familiar individuals has predator avoidance benefits, it might be expected that the preference to shoal with familiar individuals to increase under threat from predation. No studies of this sort have been conducted on Australian native fishes and only one study (Griffiths 1997) has considered if preferences for familiar fish change with increased risk.

8.3 METHODS

8.3.1 Experiment 1: Binary Choice

Female rainbowfish (*M. duboulayi*) were collected using bait traps from two tributaries of the Mary River system 120 km north of Brisbane. Four traps were set within 5 meters of one another at each location and all the fish were captured within half an hour. Thirty fish were taken from each site. While it is possible that many of the fish captured from one location were part of the same shoal, it is most likely that they were derived from many shoals. To ensure that individuals from each site were only familiar with individuals from their own site two distant localities were chosen rather than sampling two pools in the same stretch of river. The sites were separated by approximately 50 km of stream. The fish were brought back to The University of Queensland and each population was isolated from the another in holding tanks for two months. The fish were fed once daily on flake food, pH was maintained at around 6.5 - 7, water temperature was 23°C and the light regime was approximately 12 hours light and dark. A glass tank measuring 120 x 30 cm and 25 cm deep was divided into three 40 cm compartments using two perspex partitions. The partitions had a number of holes drilled through them, allowing a flow of water to permeate through the tank. The middle compartment was further divided into three 13.3 cm (approximately equal to 2.5 body lengths) zones using a marker on the underside of the tank, one zone closest to each end compartment and a middle zone (Figure 8.1). The entire set up was surrounded by a white cloth partition and illuminated by twin, fluorescent, 18W light tubes suspended one meter above the tank. A video camera was placed overhead and all observations were made from video footage.

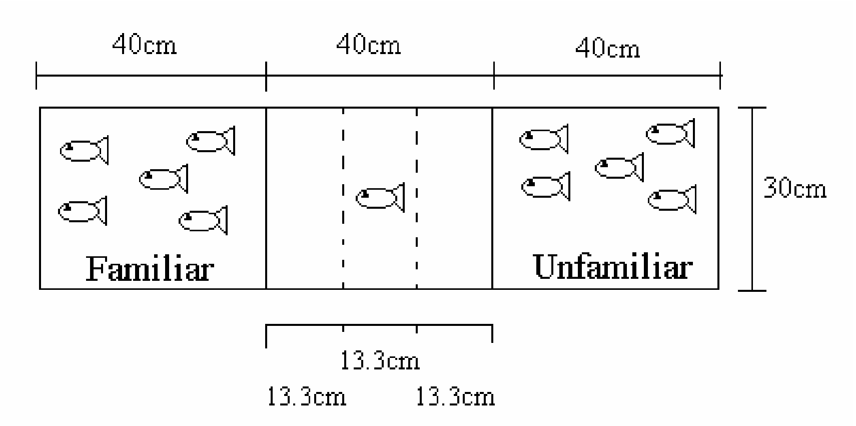

Figure 8.1. Diagram of the layout of the experimental tank in Experiment 1 (top view).

Only females were used in the experiment because they are considered to form the core of shoals (Griffiths and Magurran 1998) whereas males tend to be more solitary and aggressive. Five females from each of the two locations were matched for size and placed in each of the end compartments. The end at which the fish were placed was randomised between trials to eliminate any right-left bias. The same shoal of fish was used throughout the experiment. A chooser female was then picked randomly from one of the holding tanks and introduced to the middle third of the centre compartment in a clear plastic bottle. After 15 min the bottle was lifted away by a remote pulley device and the chooser was allowed to roam freely throughout the centre compartment. The chooser female had to move from the centre zone and visit both zones closest to the side compartments and then return to the centre zone before recording began. This ensured that she had sampled both shoals. The maximum length of each trial was 30 min. The amount of time (in sec) spent in either of the thirds closest to the side compartments was recorded and then transformed to a percentage score (the proportion of time spent shoaling with the stimulus shoals after the chooser female had satisfied the choice criterion). The percentage score enabled a comparison between females, as some satisfied the criterion more quickly than others. After testing, the standard length of each chooser was measured. The average standard length of the test females was 5.8 cm (± 0.25 cm SE.). No females were tested more than once and none of the chooser females were ever used as part of a shoal.

Of the 16 females tested only nine passed the choice criterion for recording. Of these, two fish were from one site and seven from the other. Owing to the low replicate size from one of the populations the data from the two sites were pooled. The percentage data were arc-sin transformed and checked for normality. A two-tailed paired t-test was then conducted. A regression analysis was performed on the fish length data to highlight any possible effects size may have had on shoal choice.

8.3.2 Experiment 2: Free range

Rainbowfish (*M. splendida splendida*) where captured using bait traps in the North Johnstone River (50 km south-west of Cairns) and air freighted to The University of Queensland. The females were separated out and divided into three groups that were kept in isolation from one another. One group contained 14 individuals (familiar fish) and the other two groups contained seven fish each (unfamiliar fish). The familiar fish were marked using a fluorescent polymer (Frederick 1997). Marking did not have any noticeable affect on shoaling behaviour. Each group was housed separately under identical conditions as outlined in Experiment 1.

After a one month separation, two familiar fish and two unfamiliar fish (one from each tank of seven fish) were selected at random and placed into a test tank. The test tank measured 90 x 35 cm and the water depth was maintained at 25 cm. A video camera and light were mounted overhead as with Experiment 1. Following a 15 min settling period the locations (X, Y coordinates) of all four fish were recorded every 30 sec for 15 min. After 15 min a realistic predator model of a mouth almighty (*Glossamia aprion*) (standard length 20 cm, see Brown and Warburton 1997 for details) was gently lowered into the tank via a remote pulley system. Mouth almighty is a widespread and common predator of rainbowfish in the North Johnstone River. The location of the rainbowfish was then monitored for a further 15 min. The mean number of times that a familiar or unfamiliar fish was the nearest neighbour of the familiar fish was calculated from video footage. These data were then used to calculate the mean proportion of time the familiar fish spent as the nearest neighbour of each of the other fish. The data were arcsin transformed and the difference in preference between control and predator periods were examined using a one-way ANOVA. Preference for shoaling with familiar individuals was tested by simple t-tests. It was expected that if familiar fish chose their nearest neighbour at random, the would spend 33% of their time with a familiar individual.

8.4 RESULTS

8.4.1 Experiment 1: Binary choice

As indicated above, nine out of the 16 fish tested failed to satisfy the choice criterion. Many of the fish simply moved to one end of the tank and stayed there for the remainder of the trial. Those fish that did satisfy the criterion spent, on average, 69% of their time in the zone closest to fish from the same location (Figure 8.2), representing a significant preference for familiar fish (F = 11.210, df = 8, *P* = 0.004). There was no correlation between fish size and the amount of time spent close to the familiar fish (R^2 = 0.031, n = 9, *P* = 0.649).

8.4.2 Experiment 2: Free range

There was no significant difference between the control and predator periods in the proportion of time familiar fish spent closest to one another (F = 0.005, n = 7, df = 1, *P* = 0.946). Data for the two periods were then combined to test for a significant preference for familiar individuals. Familiar fish spent 48% of their time closest to one another which differs significantly from the expected value of 33% (t = -3.051, df = 13, *P* = 0.005) (Figure 8.3). When the predator was introduced the fish initially spent a large proportion of their time at the end of the tank furthermost from the model. After some time they began to approach the model, typically as a single school. The behaviour observed was typical of that shown by most predator-experienced rainbowfish towards predator models (see Brown and Warburton 1997, 1999)

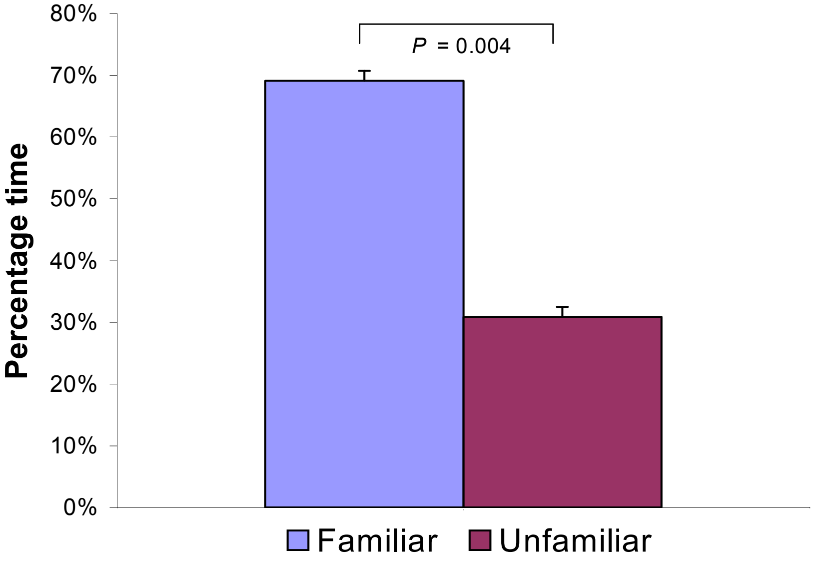

Figure 8.2. Experiment 1. The mean percentage of time (± se) the choosing individual spent in the zones closest to the familiar and unfamiliar shoals. The choosing females spent significantly more time in the zone closest to the shoal containing familiar individuals.

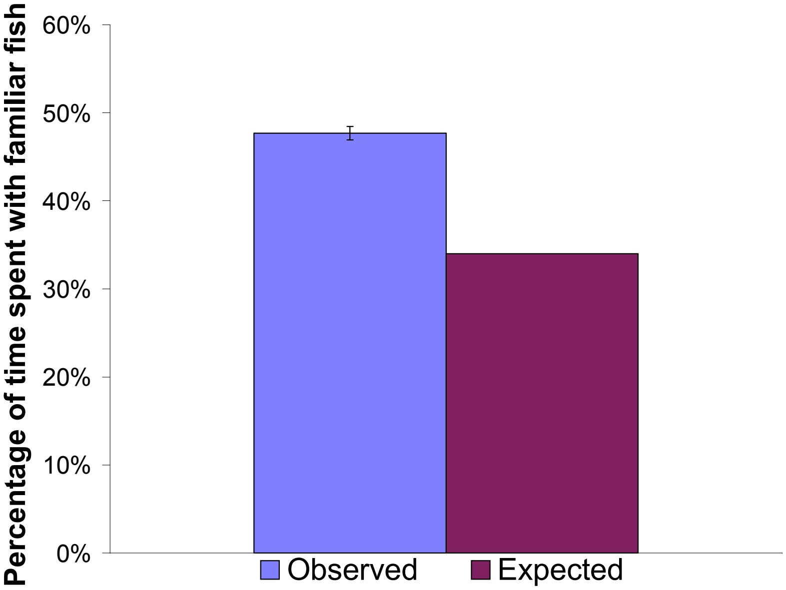

Figure 8.3. Experiment 2. The mean percentage of counts (± se) when a familiar fish was the nearest neighbour. Counts are lumped for the control and predator present periods. Familiar individuals were the nearest neighbour significantly more often than expected by chance.

8.5 DISCUSSION

It is clear from this study that rainbowfish, like other similar sized fishes from America and Europe, show strong preferences for shoaling with familiar individuals. However this preference does not change under the threat of predation. Individual recognition has been demonstrated in a number of studies conducted on fish (Myerburg and Riggio 1985, Brown and Colgan 1986, Van Havre and FitzGerald 1988, Miklosi *et al.* 1992, Brown and Smith 1994) and could potentially allow a fish to associate certain individuals with certain tasks based on prior experience. Shoals of fish often disband at night and reform at dawn (e.g. Helfman 1981) and it appears that schools are often made up of the same individuals day after day (Helfman 1984, McFarland and Hillis 1982). Owing to the apparent fitness benefits associated with it (see Chivers *et al.* 1995), familiarity with shoal mates may be one of the benefits of repeatedly shoaling with the same individuals.

The majority of the fish in Experiment 1 and those used in Experiment 2 were from predator sympatric environments and, contrary to expectations, their preference for familiar individuals did not increase with the introduction of a predator. Similarly, Griffiths (1997) found no difference in the amount of time European minnows spent shoaling with familiar individuals under increased threat from a predator model. Helfman (1984) suggests that populations that suffer from high predation pressure are more likely to show the higher levels of school fidelity. When faced with a predator, knowing how shoal mates are likely to react has obvious benefits. For example, fish prefer to inspect predators in the company of individuals who are known to be less likely to defect during an inspection visit (Milinski *et al.* 1990a, b, Dugatkin and Alfieri 1991). It could be that the rainbowfish tested in Experiment 2 and the population of minnows tested by Griffiths (1997) showed risk hypersensitivity (Helfman and Winkelman 1997), that is, they maintain high levels of avoidance irrespective of the level of risk. This latter hypothesis is not unexpected given the high level of predation pressure faced by the populations of fish tested so far. In future it would be interesting to compare populations originating from areas with differing levels of predation pressure. While Chivers *et al*. (1995) clearly demonstrated that schools comprised of familiar fish show more effective anti-predator behaviours than schools comprised of unfamiliar individuals, the data from the current study suggest there may be benefits for shoaling with familiar individuals other than those associated with predator avoidance.

Could familiarity provide a mechanism for kin recognition? FitzGerald and Morrissette (1992) found that stickleback fry chose to shoal with kin rather than non-kin when confronted by a predator (but see Peuhkuri and Seppa 1998). However, FitzGerald and Morrissette (1992) could not rule out the possibility that fry had learned to recognise one another early in development through olfactory cues. It may be possible to dispense with the notion that kin prefer to school with one another, since it is likely that in many cases simple familiarity could easily be invoked rather than kin recognition. Familiarity may provide a simple model to explain observations of non-random associations forming within shoals (Magurran *et al*. 1994).

Griffiths and Magurran (1998) found that female guppies showed significant preferences for shoaling with familiar individuals while males did not. They suggest that females form the core of natural shoals and males trade off shoaling with familiar conspecifics for greater mobility while searching for mating opportunities. A similar situation is almost certainly occurring in rainbowfish populations. Female rainbowfish resemble one another very closely. Since only females were used during these experiments, the data suggest that fish must rely on very subtle behavioural or morphological cues to discriminate between individuals. This may also be the case with guppies (Dugatkin and Alfieri 1991, Warburton and Lees 1996) although guppies do tend to be more variable in colouration. Such visual cues are likely to be involved in the development of dominance hierarchies (Barnard and Burk 1979) and the recognition of predators (Brown and Warburton 1997). However, we cannot rule out the importance of chemical recognition (see Olsen *et al*. 1998).

The study of familiarity is relatively new and a number of opportunities present themselves for further study. It is unknown, for example, how long it takes for familiarity to build up between individuals in a group of rainbowfish or how long it is maintained, particularly in the wild. Griffiths and Magurran (1997b) found that familiarity takes around 12 days to establish in guppies, but this figure is likely to vary between species and may be population and context dependent. It is assumed that rainbowfish, like many other similar sized fish, probably remain within a single pool for the majority of their lifetimes. Within that pool several large schools of fish may be found at any point in time, but individuals may occasionally move between groups. A level of familiarity may be maintained between many individuals within a large pool if fish are constantly receiving punctuated reinforcement as they move between shoals and interact with different individuals. It is likely, therefore, that all fish within the same pool are relatively familiar with one another although individuals are likely to be more familiar with the members of the shoal they are currently associated with. Similarly, it would be interesting to discover at what point mechanisms of familiarity break down as group size increases. In a recent paper Griffiths and Magurran (1997a) found that the tendency for female guppies to school with familiar fish decreases as group size increases. It may well be that the optimal number of individuals essential for familiarity to occur may play a role in determining the size of shoals observed in the wild.

It would also be interesting to investigate how familiarity is used by animals in dynamic environments. Do animals associate with certain individuals for certain tasks? It appears that fish are capable of associating individuals with the probability that they will continue to aid in the inspection of a predator rather than defecting and remaining behind (Milinski *et al*. 1990a, b, Dugatkin and Alfieri 1991). It also seems likely that hungry fish can recognise and preferentially associate with poor foragers, thus providing a foraging benefit to fish that associate with familiar conspecifics (Van Havre and FitzGerald 1988; Metcalf and Thomson 1995). We could predict, therefore, that familiarity provides a mechanism that allows the dynamic selection of shoaling partners, enabling fish to shoal with different individuals as their internal state and external circumstances dictate.

References

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