Loss of Shoaling Preference for Familiar Individuals in Captive-Reared Crimson Spotted Rainbowfish Melanotaenia duboulayi

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Loss of shoaling preference for familiar individuals in captive-reared crimson spotted rainbowfish *Melanotaenia duboulayi*

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**KEYWORDS**  
captive breeding, familiarity, hatchery, shoaling behavior

**ABSTRACT**

Captive-reared rainbowfish *Melanotaenia duboulayi* showed no preference for familiar individuals in an experiment examining shoaling preferences. Fortnightly re-examination of the shoaling preferences of the captive-reared population showed that the lack of preference for familiar individuals did not alter over an 8 week period. The same experiment performed on laboratory-reared offspring raised in isolated groups for 8 months since hatching also showed no preference for shoals consisting of familiar individuals. In contrast, trials performed on a wild population of *M. duboulayi* found a strong preference for familiar shoalmates, a result that is consistent with previous studies. The lack of shoaling preferences in captive-reared populations is probably the result of relaxed selection and inbreeding in the captive environment. The consequences of captive breeding for fish social behaviour are discussed with particular reference to hatchery production.

**INTRODUCTION**

The composition of shoals is often non-random and a variety of mechanisms result in self assorting. There is increasing evidence that familiarity with other individuals can be a strong incentive for shoalmate selection (Ward & Hart, 2003; Griffiths & Ward, 2006). The condition of ‘familiarity’ develops in guppies *Poecilia reticulate* Peters after a duration of 12 days of cohabitation, after this time the fish began to recognize individuals and preferentially associate with them (Griffiths & Magurran, 1997). Surprisingly, little is known about the development of familiarity in other fish species despite the fact that it is a widespread phenomenon. Fishes are capable of recognizing each other via both visual and chemosensory cues (Arnold, 2000). Three-spined sticklebacks *Gasterosteus aculeatus* L., for example, utilize indirect chemical cues emanating from conspecifics resulting from recent diet or the water chemistry that they have recently occupied making them more likely to associate with familiar individuals (Ward et al., 2005). In addition, it is likely that fishes can tell one another apart by subtle morphological variances such as size (Krause & Godin, 1994).

Shoaling with familiar individuals provides a number of benefits that are likely to have fitness consequences. Amongst the many reasons for shoaling with familiar conspecifics are the benefits accrued through individual experience with each shoal member (Brown & Laland, 2006; Couzin *et al.*,...
2006; Griffiths & Ward, 2006). Familiarity enables shoal members to predict how other members are likely to respond in a variety of circumstances (e.g. under predator attack). Shoals of fathead minnows *Pimephales promelas* Rafinesque comprised of familiar fish demonstrated greater shoal cohesion and partook in more predator avoidance behaviours than shoals of non-familiar individuals (Chivers et al., 1995). Groups of brown trout *Salmo trutta* L. comprised of familiar individuals foraged at a greater rate than groups of unfamiliar fish (Griffiths et al., 2004). Moreover, the spread of novel information through social groups is enhanced if the groups are comprised of familiar conspecifics (Swaney et al., 2001).

If shoaling with familiar fishes provides benefits to the shoal members, it could be assumed that most shoaling species would preferentially shoal with familiar individuals. Accordingly, a preference for shoaling with familiar fishes has been demonstrated in a variety of species including the crimson spotted rainbowfish *Melanotaenia duboulayi* (Castelnau) (Brown, 2002), *P. reticulata* (Magurran et al., 1994; Griffiths & Magurran, 1997) and *P. promelas* (Chivers et al., 1995). The adaptive benefits of shoaling with familiar individuals, however, may be 'titrated' against other considerations. If the ratio is large enough, European minnows *Phoxinus phoxinus* (L.) choose a larger shoal size consisting of strangers over a familiar shoal comprised of fewer individuals to gain safety in numbers (Barber & Wright, 2001). In addition, there may be circumstances where shoaling with familiar fishes does not provide an adaptive benefit (Gerlach & Lysiak, 2006; Gómez-Laplaza & Fuente, 2007). For example, in low-predation areas, the antipredator benefits associated with shoaling with familiar individuals may no longer be relevant. Thus, in these environments, natural selection may lead to the loss of preference for shoaling with familiar conspecifics and fish would associate with one another at random. Such differences in social preferences may also exist when comparing facultative v. obligative schooling species or populations.

Multiple fish stocks around the world are now artificially bolstered (or indeed entirely maintained) by the addition of fishes reared in hatcheries. Nevertheless, the success rate of such releases is frequently very low, and it is widely recognized that fishes reared in captivity display a multitude of behavioural and physiological deficits owing to the unnatural environments in which they are reared (Brown & Laland, 2001; Brown & Day, 2002). Captive rearing can lead to inbreeding and relaxed selection in a few generations (Brown & Day, 2002; Huntingford, 2004; Porta et al., 2007). A lack of predators, high-density social environment and abundant food in captivity may annul many of the potential gains to be made by preferentially shoaling with familiar individuals. If this is in fact the case, then there will be serious consequences for conservation and fisheries releases that rely on captive-reared fishes.

In the present study, *M. duboulayi* was utilized to determine if captive rearing over multiple generations influences shoalmate preferences. The shoaling preferences of a captive stock derived from only 15 individuals collected in 1990 was compared with individuals captured from the wild. Given the lack of selective pressure for schooling behaviour in captivity, it was hypothesized that the captive stock would have lost its preferences to shoal with familiar individuals. The preference for familiar individuals in the captive stock was re-examined over a period of 8 weeks to investigate the effects of temporal social reinforcement. In addition, fish bred from the captive stock were reared in isolated groups since hatching, and tested at 8 months of age to determine if preferences for familiarity could be enforced by artificially maintaining long-term, stable social groups.

**MATERIALS AND METHODS**

The effect of familiarity on shoaling preferences was investigated using shoals of *M. duboulayi*. This species is an Australian native fish found in shoals in a variety of freshwater habitats across eastern Australia (Allen & Cross, 1982). Wild *M. duboulayi* acclimatize readily to life in captivity and are simple to breed and maintain making them ideal model organisms (Brown, 2003).
Captive-reared, adult fish of c. 3 years of age \( (n = 130) \) were obtained from the Environmental Protection Agency (EPA), Sydney, Australia, where they were housed in large aquaria \( (2000 \text{ mm} \times 500 \text{ mm} \times 800 \text{ mm}) \). This stock was derived from an original wild population of 15 fish collected from the wild in 1990 and had been bred at the EPA for many generations. Upon arrival at Macquarie University, Sydney, Australia, the fish were distributed into 10 shoals comprised of 13 fish \( (12 \text{ females and one male}) \) and housed in isolated aquaria containing a filter, heater and river gravel. Each aquarium measured \( 920 \text{ mm} \times 350 \text{ mm} \times 400 \text{ mm} \) and contained aged water maintained at 23° C (range ± 1° C). The water pH varied from 7.0 to 7.4. Lighting was provided by overhead fluorescent bulbs providing 12 h of light per day. The fish were fed daily on commercial flake food (Tetramin; www.tetra-fish.com). The fish were housed together in this arrangement for 2 weeks before experimentation and for the duration of the experiment to ensure familiarity was established with shoalmates.

The EPA fish were bred and their offspring reared in the laboratory in small stable groups to determine if preferences for familiarity could be established in the captive stock by enforcing close social relationships. These ‘laboratory-reared’ fish were separated into two isolated groups immediately after hatching and grown out in four isolated aquaria identical to that of their parents until 8 months of age when they were subjected to testing. Each group contained 12 females and a single male.

To provide a comparison with the captive populations, wild fish were caught from the Orara River, New South Wales \( (30°15'26" \text{ S; 153°00'42" E}) \). Collection was done at two high predation locations c. 50 m apart, separated by two runs and a pool. Although it is possible that fish could move between these locations, the distance was judged to be sufficient to afford a degree of social isolation. Twenty-four females and two males from each location were collected and transported to Macquarie University. The wild populations were housed in the same conditions as the captive-reared population. The fish from each location was split between two separate aquaria thus maintaining shoal sizes equal to those of the captive-reared shoals. The wild fish were maintained in these four isolated aquaria for 2 weeks after collection to ensure there was no residual stress from capture that could bias the results. This period of time also reduced the possibility that the fish utilized chemical cues based on the location of capture or prey preferences to recognize individuals. Wild fish were gradually weaned off a diet of live blood worms \( (\text{Chironomonos} \text{ spp.}) \) and onto standard flake food during the first week in captivity.

The experimental arena dimensions were identical to that of the housing aquaria and the water depth was maintained at 300 mm. They were equipped with filters at either end to facilitate water movement, water temperature was maintained at 23° C and light was provided by a single fluorescent light suspended above. Two clear perspex partitions were added to divide the tank into three compartments (Fig. 1). The central compartment was 320 mm wide \( (\text{c. 10 body lengths}) \) and divided into two halves (familiar and unfamiliar sectors) by a dotted line marked on the aquarium. Thus, the distance between the two end compartments was small enough to ensure adequate sampling of both shoals would readily take place, but distant enough to ensure that an independent choice could be expressed. Narrow gaps on either side of the perspex were sufficient to allow steady water flow between the sections but did not permit the fish to pass through them. Water flow between the compartments was tested by applying food dye to each end and the movement of the dye tracked. A clear, plastic cylinder of diameter 115 mm was placed in the middle of the centre compartment of the aquarium. Strings were attached to the cylinder allowing it to be lifted remotely.

A ‘target shoal’ consisting of five individuals was established in each housing aquaria. The individuals in the target shoals were randomly selected and fin clipped to distinguish them from the rest of the fish in the aquaria which were used as test subjects. Target-shoal membership remained constant throughout the experiment and the mean standard length \( (LS) \) of the target-shoal fish did not differ between each of
the housing aquaria (ANOVA, $F_{9,40}, P > 0.05$). The same target shoals where used as stimuli for each of the test subjects drawn from a given housing aquarium. For example, all seven test subjects from housing aquarium 1 were exposed to a familiar target shoal from housing aquarium 1 and an unfamiliar target shoal from housing aquarium 5. This ensured that the familiar and unfamiliar individuals in the target shoals at each end of the test arena did not vary for test subjects drawn from the same home aquarium. The target shoals, however, vary between test subjects drawn from different housing aquaria. For example, all seven test subjects from housing aquarium 2 were exposed to a familiar target shoal from housing aquarium 2 and an unfamiliar target shoal from housing aquarium 6. Thus, all subjects had to choose between target shoals consisting of familiar or unfamiliar individuals. Preferences for familiar or unfamiliar target shoals did not vary between housing aquaria (ANOVA, $F_{9,58}, P > 0.05$). Target shoals from two visually isolated aquaria were placed in the compartments at either end of the experimental arena (Fig. 1). The end at which each target shoal (familiar or unfamiliar) was located varied from trial to trial to eliminate side biases. In half of the cases the familiar target shoal was on the left, and in the remaining trials it was on the right. Water in the test tank was changed periodically throughout the experiment. The remaining females from each aquarium ($n = 7$ fish $\times$ 10 aquaria) were utilized as test subjects and tested one at a time. The test subject was initially confined to the clear cylinder in the middle of the central compartment, where it remained for 2 min settling time and could view the shoals at either end of the experimental arena. The cylinder was then gently raised and the subject fish released. Over a 5 min period, the time the subject fish spent in either half of the centre compartment (either familiar or unfamiliar sector) was recorded. At the end of the trial, the subject fish was removed and the next fish placed in the cylinder.

**FIG. 1.** Experimental arena used for examining shoaling preferences. The familiar target shoal of *Melanotaenia duboulayi* is illustrated on the right, the unfamiliar target shoal is shown on the left and the test subject in the centre compartment.

In the case of the captive-reared stock, the procedure was repeated once fortnightly using the same test subjects until four trials had been performed. This was intended to highlight any potential reinforcement of familiarity preferences over the 8 week period and to counteract the possibility that familiarity preferences were retained from previous encounters with shoalmates at the EPA (potentially confounding the results).
In total, 70 captive-reared EPA fish, 35 wild fish and 30 laboratory-reared fish were tested. Only females were used in the experiments because the inclusion of males in the experimental arena induced courtship behaviour. *Melanotaenia duboulayi* show strong shoaling behavior especially in unfamiliar environments, thus a preference to school with familiar fish would be illustrated by a strong tendency to remain close to the familiar shoal. In this instance, a central neutral zone in the experimental arena was not chosen, because the fish rarely utilize this space, merely passing through it to inspect each target shoal (Brown, 2002). The time the test fish spent shoaling with the familiar and unfamiliar shoals was evaluated using two-tailed *t*-tests. No transformation was necessary before analysis.

**RESULTS**

Two captive-reared, one wild and five laboratory-reared fish spent the entire time on one side and were thus discarded from the data set as they had not adequately 'sampled' each of the target shoals. Captive-reared *M. duboulayi* showed no shoaling preference for familiar or unfamiliar shoalmates during the first test period (*t*-test, d.f. = 67, *P* > 0·05) spending 53·68 ± 2·71% (mean ± S.E.) of their time shoaling with the familiar fish. The preferences did not change significantly over the following 8 weeks (*t*-test, d.f. = 67, *P* > 0·05 for all trials and comparisons between trials), suggesting that the preference for familiar shoalmates in the captive-reared fish was not reinforced over time (Fig. 2). Similarly, the laboratory-reared fish (reared in isolated groups since hatching until 8 months of age) showed no preference for shoaling with familiar individuals (*t*-test, d.f. = 24, *P* > 0·05), spending 51·52 ± 7·46% (mean ± S.E.) of their time shoaling with the familiar shoal (Fig. 2). In contrast to the captive populations, wild caught *M. duboulayi* showed a significant preference for familiar shoalmates (*t*-test: d.f. = 33, *P* < 0·001) spending 71·33 ± 5·11% (mean ± S.E.) of their time shoaling with familiar individuals. When the wild, and captive-reared (first trial) and laboratory-reared populations were compared directly, it was shown that the wild fish spent significantly more time shoaling with familiar individuals than the captive fish (*t*-test, d.f. = 101, *P* = 0·001 and d.f. = 58, *P* < 0·05 for captive-reared and laboratory-reared offspring, respectively).

**DISCUSSION**

Despite the many benefits associated with shoaling with familiar individuals, captive-reared fish failed to express a preference for shoaling with familiar fish. Even after 10 weeks spent in stable, isolated groups no preference was observed. Similarly, captive-reared offspring, maintained in small social groups from hatching until 8 months of age, showed no preference for familiar shoalmates. In contrast, wild fish showed strong preferences for shoaling with familiar individuals.

Preference for shoaling with familiar shoalmates is a widespread phenomenon in schooling fishes. *Poecilia reticulata* (Magurran et al., 1994; Griffiths & Magurran, 1997), paradise fish *Macropodus opercularis* (L.) (Miklosi et al., 1992) and *P. promelas* (Chivers et al., 1995) all preferentially shoal with familiar rather than unfamiliar fish when given the choice. A preference for familiarity has also been demonstrated in several species of the *Melanotaenia* genus collected from a number of locations. Brown (2002), for example, found that wild-caught *Melanotaenia splendida splendida* (Peters) and *M. duboulayi* both prefer to associate with familiar shoalmates in the presence and absence of predators. The results of the examination of shoaling preferences of the wild-caught fish employed here are consistent with these observations.

The EPA population that had been held in captivity for the last 17 years showed no shoaling preferences for familiar individuals. It is unlikely that the captive-reared EPA fish had remembered shoalmates from their housing conditions before arrival at Macquarie University. Familiarity preferences in *P. reticulata*, for example, are retained after 5 weeks of separation (Bhat & Magurran, 2006). The failure to selectively shoal with familiar conspecifics even after 8 weeks of being maintained in relatively small shoals,
however, suggests that this possibility is remote. Moreover, the laboratory-reared offspring raised in isolated groups since hatching until 8 months of age also showed no preference for shoaling with familiar individuals.

![FIG. 2. Mean ± S.E. proportion of time spent by focal *Melanotaenia duboulayi* on side of experimental tank containing familiar conspecifics. Trials 1 to 4 represent repeated observations of the Environment Protection Agency (EPA) captive-reared population \( n = 70 \), followed by the first generation laboratory-reared population \( n = 30 \) and the wild population \( n = 35 \).](image)

It is possible that the original stock from which the captive-reared fish were collected in 1990 lacked a preference for familiar conspecifics, but none of the populations or species of *Melanotaenia* tested to date have shown this unusual trait. Moreover, such a position also assumes that preference for familiar fishes is heritable, to date, however, there are no data to support such a supposition. Nevertheless, the potential for population variation for shoaling preferences with conspecifics requires further investigation. The lack of preference for familiar individuals could also theoretically be generated by a complete loss of schooling behaviour in the captive stock. This is not the case here, however, as the captive fish still showed strong tendencies to stay close to either target shoal, but their choice of shoal was not influenced by the degree of familiarity. Indeed, three quarters of the captive EPA fish spent > 70% of their time shoaling with just a single target shoal. It is apparent, therefore, that the captive stock has either lost the ability to recognize individuals, or the benefits associated with shoaling with familiar individuals are no longer relevant in the captive environment.

There are a number of proximate and ultimate explanations for the loss of shoaling with familiar individuals observed in the captive stock. First, the preference for familiar shoalmates may be shaped by experience during ontogeny. The raising of fishes in hatcheries has the potential to cause changes in the behaviour, physiology, genetics and even the brain structure of fishes over relatively short-time frames (Brown & Day, 2002; Marchetti & Nevitt, 2003; Huntingford, 2004). One of the ways in which these changes occur is through an individual's experiences in the context of the contemporary environment (i.e., learning; Brown & Laland, 2006). The environment in which the captive-reared populations were raised is
vastly less stimulating than the habitats fishes occupy in the wild. Various important aspects such as food availability, social environment and pressure from predation differ greatly between the captive and the wild environments. Thus, many of the proposed benefits of shoaling with familiar fishes, such as enhanced predator avoidance and foraging, may no longer be relevant because predators are lacking entirely and food is abundant in captivity. If there is no benefit to be gained from shoaling with familiar individuals, or indeed shoaling in general, then it is likely that trait will be lost from the individual's behavioural repertoire as a result of natural selection. This hypothesis assumes that keeping track of familiar individuals has a cost perhaps in the form of excessive cognitive investment. Future investigations will determine if, and how quickly, preferences for familiar individuals during ontogeny are lost in captivity.

Second, contrasting selection regimes between the captive and the wild environments operating over several generations may lead also to substantial heritable differences in shoaling behaviour. When captive-reared fishes are released from many of the selective forces mediating wild fish populations, there could potentially be relaxed selection for or against particular traits. This may occur through differential mortality, the survival of deleterious phenotypes in the captive environment, or by novel selective forces operating within the hatchery environment (Huntingford, 2004). Over several generations, this has the potential to drastically alter physical and behavioural traits. This is feasible even without the deliberate selection to enhance productivity (artificial selection). In the present context, the preference for shoaling with familiar individuals may have been lost over successive generations in captivity.

Last, the lack of preference for familiar individuals may be symptomatic of low heterozygosity in the captive population. The EPA population was derived from 15 wild fish, from which offspring have been bred in captivity for 17 years. Given that *Melanotaenia* spp. reach maturity in < 12 months (Allen & Cross, 1982), a conservative estimate of 10 generations may precede the experimental population. A substantial loss in genetic variation can occur in captive stocks after just a single generation due to the inclusion of related fishes in the broodstock and a low effective breeding population size (Porta et al., 2007). This reduced genetic diversity between individuals may make them indistinguishable from one another at a morphological and biochemical level, thereby inhibiting recognition mechanisms. This is particularly pertinent given the important role of major histocompatibility complex (MHC) in recognition and mate choice in fishes (Winberg & Olsen, 1992; Olsen et al., 1998; Milinski et al., 2005). For example, in the Lake Eacham rainbowfish *Melanotaenia eachamensis* Allen & Cross, females prefer shoaling with female relatives but avoid shoaling with related males (Arnold, 2000). Moreover, whilst outbred adult *G. aculeatus* can recognize unfamiliar kin, inbred adults cannot (Frommen et al., 2007).

In conclusion, as a consequence of experience, artificial selection regimes, inbreeding or a combination of these factors that stem from captive rearing, the social behaviour of captive-reared populations can become greatly distorted. It likely that captive-reared fishes will miss out on the many fitness benefits accrued through preferentially associating with familiar individuals, such as enhanced social learning, predator avoidance and foraging behaviour. This deficit in social behaviour could contribute to high post-release mortality rates so frequently observed in hatchery fishes (Brown & Laland, 2001; Brown & Day, 2002). The results presented here raise concern about the general social behaviour of captive-reared fishes which may have a number of other as yet unforeseen consequences for reintroduction programmes.
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References


