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The influence of rearing experience on the behaviour of an endangered Mexican fish, *Skiffia multipunctata*

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Abstract

Reintroduction projects may fail because captive-reared animals do not possess the behavioural skills required for survival in the wild. Rearing captive-bred animals in semi-natural enclosures prior to release has been used to improve the survival of reintroduced endangered species, but it is unclear how rearing environment influences the development of behaviour. This study examined the effect of rearing conditions on the behaviour of the goodeid *Skiffia multipunctata*, an endangered species of Mexican fish. Under standard laboratory conditions, the courtship, aggression, boldness and foraging behaviour of fish raised in aquaria was compared to that of fish reared in outdoor ponds. We present initial behavioural descriptions for this species and show that laboratory-reared fish displayed increased courtship, aggression and curiosity towards a novel predator in comparison to their pond-reared counterparts. Laboratory-reared fish also commenced foraging on a novel food item (*Artemia*) more rapidly than fish reared outdoors. These findings suggest that captive rearing environments promote the development of behavioural tendencies, such as boldness and aggression, which could be detrimental to the survival of reintroduced individuals.

Keywords: Captive; Conservation; Environment; Goodeid; Reintroduction

1. Introduction

Captive breeding and the subsequent reintroduction of animals into the wild is becoming an increasingly important technique for the recovery of endangered populations (Snyder et al., 1996). However, reintroduction projects are often unsuccessful because captive-bred animals lack the behavioural skills necessary to survive in the wild (e.g., Beck et al., 1994; Lyles and May, 1987; Snyder et al., 1996; Price, 1999). Behavioural differences between wild and captive-bred animals can arise through a number of processes. For example, captive breeding often results in the inadvertent selection of traits that are favourable in captivity, such as tameness and resistance to stress (Kohane and Parsons, 1988), or from the relaxation of selective forces that are common in nature, such as predation. In addition, artificial rearing environments can often deprive animals of ontogenetic experiences required for the appropriate development of behaviour.

Behavioural deficiencies that arise as a result of inappropriate rearing experiences are more easily rectified than those occurring through selection; therefore, it is intuitive that research efforts should focus on the importance of the developmental environment. Studies of the black-footed ferret (*Mustela nigripis*) provide a good

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example of the importance of rearing experience on subsequent reintroduction success. Miller et al. (1994) compared the post-release survival of black-footed ferrets (*M. nigripes*) that were raised either in standard cages or semi-natural enclosures, with that of wild animals that had been translocated (moved to a new location). Although the translocated group showed the highest survival rates, ferrets that had been reared in a semi-natural environment showed enhanced survival compared to those raised in standard cages (Miller et al., 1994).

Captive breeding is an extremely expensive method of conserving endangered species (Beck et al., 1994); finding ways in which to improve the success of reintroduction should therefore be a conservation priority. However, surprisingly few conservation studies have specifically investigated the relationship between rearing environment and those behaviours that are likely to be fundamental to post-release survival. In the wild, animals rely on a variety of behaviours for their survival, such as foraging skills and predator recognition. Furthermore, wild animals frequently interact with heterospecifics, for example, through competition for resources or predator-prey encounters. Yet little is known of the effects of heterospecifics on the development of behaviours such as foraging, competitive interactions and predator recognition.

The aim of this study is to examine the effects of rearing environment on the courtship, anti-predator behaviour and foraging ability of the goodeid fish, *S. multipunctata*, a species of high conservation interest. We then relate these findings to potential behavioural divergence between wild and captive-bred fishes, and discuss how this could influence reintroduction success. The Goodeinae (Family Goodeidae) is a clade of live-bearing freshwater fish endemic to the High Plateau of Central Mexico. The 36 species of goodeid that are currently recognised (Webb et al., 2004) occupy a variety of habitats ranging from warm springs and canals to large lakes and rivers (Miller and Fitzsimmons, 1971). Goodeines are viviparous and the young receive nutrition from the female via a unique placenta-like structure known as the trophotaeniae (Turner, 1933, 1937). Although little is known of their behaviour, elaborate courtship displays and female choice have been reported in several species (e.g., *Xenotoca* sp., Fitzsimmons, 1972; *Girardinichthys multiradiatus*, Macías Garcia, 1991). Members of the *Skiffia* genus (which comprises four species) are highly sexually dimorphic; the anterior rays of the male's dorsal fin are shortened so that the fin appears to be bi-lobed (Mendoza, 1965). Male courtship is complex and involves a loop, or figure-of-eight dance, similar to that observed in other goodeid species (Fitzsimmons, 1972, 1976; Macías Garcia, 1991). A combination of habitat loss, increased pollution and the introduction of non-native species (De la Vega-Salazar et al., 2003) have been implicated in the reduction of many species of goodeid. Indeed, 13 species of goodeid are included on the IUCN (2003) Red List of Threatened Species. In the case of the *Skiffia* species, (*S. lermae*, *S. multipunctata*, *S. bilineata*), the natural range has decreased in recent years (De la Vega-Salazar et al., 2003); the remaining species (*S. francesae*) is extinct in the wild, but is currently undergoing captive breeding (e.g., in Chester Zoo, UK).

Skiffia multipunctata was chosen for this experiment because it is endangered, but also because of the availability of captive stock and because it is the closest relative of *S. francesae*. Juvenile *S. multipunctata* were randomly assigned to one of three rearing groups: (1) semi-natural rearing with heterospecifics, (2) semi-natural rearing in single-species groups, and (3) standard rearing conditions in the laboratory. When the fish reached sexual maturity, we assessed the courtship behaviour, boldness and foraging ability of adult males towards a novel fish. The use of males, which are individually recognisable by their unique colour patterns, allowed the behaviour of the same fish to be observed in each context. We anticipated that captive feeding practices (e.g., discrete amounts of food presented in the same location) would result in increased levels of aggression in laboratory-raised fish, whereas fish reared in semi-natural ponds (with experience with a variety of food types) would display enhanced foraging skills. Because captive fish live in a static environment and are infrequently exposed to new stimuli, we anticipated that they would display increased curiosity toward an unfamiliar fish model. Courtship behaviour was not predicted to vary among the treatment groups, as both rearing groups would have the experience of courting and competing for females.

2. Methods

2.1. Origin and maintenance of fish

Skiffia multipunctata is endemic to the Lerma Basin in the states of Jalisco and Michoacan in Mexico. However, in recent years *S. multipunctata* has been reported at only a few localities, including a small lake (area = 44230 m^2, depth = 1.4 m) in Orandino, Jalisco (19°57′44″ N, 102°19′61″ W). Water flows into the lake from a small spring; outflow is controlled by sluice gates which when raised, allow water to be released through a concrete channel. The lake contains two enclosures used for rearing carp (*Cyprinus carpio*) and two small reed beds. Other fish species inhabiting the lake include four goodeids (*Chapalichthys pardalis*, *Goodea atripinnis*, *Allophorus robustus*, *Zoogoneticus quitzeoensis*), three poeciliids (*Poeciliopsis infans* (native), *Poecilia sphenops* and *Xiphophorus helleri* (both introduced)) and a minnow (*Notropis* sp.). Herons and snakes (*Thamnophis* sp.), which are potential goodeid predators (Macías Garcia et al., 1998), have also been observed at the site.

The *S. multipunctata* used for this experiment were the first-generation, laboratory-born descendents of fish collected from Orandino, in June 2000. All holding aquaria ($50 \times 25 \times 30$ cm^3, filled to a depth of 25 cm) contained an air filter and a small amount of aquatic weed. Illumination was provided by two overhead fluorescent strips (75 and 20 W) set on a 12-h light/dark cycle. In March 2001, 18 pregnant females were isolated in individual birth units which were suspended in the holding aquaria (1–2 per tank). Post-partum females and their offspring (i.e., first-generation, laboratory-born fish) were maintained in the birth units and fed twice daily on a diet of commercially prepared flake food. All offspring born within a three-week period between April and May 2001 were collected. A total of 17 broods were produced comprising 6–42 individuals (mean ± SE = 19.3 ± 2.66). Each brood was divided equally among the three rearing treatments (i.e., in a brood of 21, seven fish would be allocated to each treatment): semi-natural rearing with heterospecifics (mixed species (MS) treatment), semi-natural rearing without heterospecifics (single species (SS) treatment), or laboratory-rearing without heterospecifics. Juveniles not assigned to a rearing group were returned to stock aquaria and played no further part in the experiment.

2.2. Semi-natural (pond) rearing environments

The semi-natural rearing environments consisted of three isolated outdoor concrete ponds, each subdivided with steel mesh partitions (1 mm mesh) into a further four pools ($345 \times 146 \times 65$ cm^3, filled to a depth of 45 cm). Each pool contained a similar assemblage of aquatic plants which were collected from the local area: *Nymphea odorata* (Mexican pond lily), *Nymphoides phallax*, *Scirpus* sp. (little reed), *Ceratophylum demersum*, *Myriophylum proserpinacoides* (parrot's feather), and the weeds *Potamogeton* sp. and *Naja* sp. *Elodea* sp. (non-native) was also present in the ponds. Half of the pools ($N = 6$) were each stocked with 65–70 individuals of *G. atripinnis* (a herbivore/planktivore) and 17 *Z. quitzeoensis* (a small benthivore, M. De la Vega-Salazar, pers. commun.), both of which are sympatric with *S. multipunctata* at Orandino. Ponds were allowed to stabilise for one month prior to the experiment, during which time the water clarity increased as the plants became established.

In May 2001, 20 juvenile *S. multipunctata* were introduced into each of the 12 pools containing either heterospecifics (MS treatment) or no other fish species (SS treatment). In order to acclimate young *S. multipunctata* to their new environment, fish in all ponds were initially placed in punctured, transparent plastic bags. Each bag was held open and afloat by a rim of polystyrene, allowing the fish to remain suspended in the water column. A small amount of aquatic weed (~10 cm, *Elodea* sp.) was added to each bag to provide substrate for food (micro invertebrates) and shelter. Fish in the outdoor ponds were carefully monitored after introduction and fed finely ground flake food for the first three days. After this time they were observed foraging off weed and material falling into the ponds and were fed no further. Juveniles were gently released from their bags one week after introduction.

The ponds were not aerated during the experimental period and large amounts of blanketing green algae formed over the surface during this time. The pools contained invertebrates such as dragonfly and damselfly nymphs (Odonata), gastropod molluscs, pond skaters and skimmers (Hemipterans: Gerridae), backswimmers (Coleoptera: *Notonecta* sp.), crayfish (*Cambarellus montezumae*) and detritivorous leeches (*Erpobdella punctata*). Daytime pond temperatures between May and June 2001 were variable, ranging from a minimum of 17 °C at 9 am to a maximum of 24.5 °C at 5 pm.

2.3. Laboratory-rearing environment

Twelve individuals were placed in each of six, visually isolated laboratory aquaria (conditions identical to those of stock tanks) and fed twice daily with flake food. Temperature was maintained at 21 ± 0.5 °C. Any offspring born during the rearing period (May 2001–April 2002) were removed and transferred to stock aquaria.

2.4. Behavioural observations

During one week of April 2002, large hand nets were used (during a 30-min period) to remove all fish in each outdoor pool. Numbers of fish of each species inhabiting each pool were counted (for pools containing heterospecifics) and all *S. multipunctata* were sorted into males, females and their offspring (recognisable through their size differences). Adult *S. multipunctata* were transferred to laboratory aquaria and all other fish were transferred to non-experimental ponds. At the end of each day, baited funnel traps were laid into the fished pools, so that any individuals that had escaped the nets could be caught and counted. Pools were observed for several days after they had been fished to ensure that all fish had been successfully removed or counted. Adult *S. multipunctata* were housed in the laboratory in visually isolated aquaria (ca. 1:1 sex ratio) in their treatment groups. These fish were fed a diet of commercially prepared flake food twice a day and were observed feeding on their day of capture. All pond-reared fish were allowed to acclimate to captive conditions for a minimum of one week. As the experience of being chased and caught by a net may have affected behaviour, fish that had undergone the laboratory-rearing treatment were also caught and transferred to new holding tanks.

Five identical tanks were used for the behavioural observations ($50 \times 25 \times 30$ cm^3, filled to a depth of 25 cm), each containing an air filter, a layer of gravel and a large rock anchoring small amounts of the aquatic weed *Valisneria* sp. (novel to fish from all three rearing treatments). Observations were conducted between 09:00 and 13:00. Two males and one female *S. multipunctata* were gently placed in each observation tank on the afternoon of the day before a trial. We chose fish of similar sizes and from the same treatment group but different ponds or tanks (i.e., fish were unfamiliar with one another). We anticipated that this would maximise the level of courtship behaviour observed as males often direct more courtship towards unfamiliar females (e.g., Kelley et al., 1999). The following morning, the fish in each observation tank were fed a small amount of flake food and left to settle for 30 min. Three, 10-min observations were performed, during which time courtship, boldness, and foraging skills were assessed (always in this order). Fish were allowed to settle for 1 h between each 10-min trial. Only the behaviour of males was recorded, as these are individually recognisable through their unique colour patterns. In each trial, the dominant of the two males (usually the larger and/or more brightly coloured fish) was observed, since these individuals displayed higher levels of courtship and aggressive behaviour than subordinate fish. The dominance status of the fish was assigned during the acclimatisation period and confirmed at the start of observations. In practice, dominance could be clearly determined within a few minutes of observing the fish. A sketch was made of each focal male so that it was possible to recognise the fish in subsequent trials. Thus, for each group of three fish, behavioural data were collected from a single individual (with the exception of foraging skills, see below). Each group was observed once in each context and both the focal and the non-focal fish were replaced with new, experimentally-naïve fish for each set of three trials. Observations were performed behind a blind to ensure minimal disturbance to the fish.

2.4.1. Courtship behaviour

During the 10-min observations of male courtship, the time that the focal male spent courting the female along with the frequency of courtship displays and the number of male-male chases were recorded. These behaviours are described in Table 1.

2.4.2. Boldness

The boldness of the test fish was assessed using a realistically painted pike model (total length = 13 cm, body depth = 2.5 cm) that was weighted to remain in mid water. Pike (*Esox* spp.) are native to North America and are not sympatric with Mexican goodeids; therefore, this model represented a novel threat. Prey fish respond more readily to predators with elliptical cross-sections than to those with, e.g., round cross sections (Webb, 1984) and preliminary observations confirmed that *S. multipunctata* performed anti-predator behaviour (e.g., predator inspection) towards our model. Observations began as soon the model had been gently placed in the front left corner of the observation tank, with its snout orientated away from the observer. During each 10-

Table 1
Description of behaviours observed in male *S. multipunctata*

Behaviour	Definition
Courtship	
Time spent courting	Male continuously pursues female in an attempt to display or mate. Includes time spent displaying and performing 'figure-of-eight' dance (see below)
Courtship displays	The male positions himself in front of, or slightly to one side of the female, and rapidly waggles his dorsal and anal fins in a manner that resembles the waving or flagging display of other goodeids (Maciás Garcia, 1991). The male continually adjusts his position whilst displaying, often positioning his body vertically (head or tail down) in front of the female. As the display escalates, the male executes frequent 180 °C turns, so that the resulting trajectory resembles a figure of 8. This display is analogous to the 'loop dance' performed by goodeids in the *Xenotoca* genus (Fitzsimmons, 1972, 1976). Display events are typically separated by a mating attempt, chasing off the other male, or by swimming away from the female
Frequency of male-male chases	Number of times the focal male pursues, or is pursued by the other male
Boldness	
Time spent in close proximity to model	Time spent in visual fixation with the model. Usually, the fish approaches the model from the front, and slowly swims around both sides of the body
Number of approaches	An approach toward the model is complete once the focal fish is orientated laterally to the model and has swum away

min trial, the boldness of the focal fish was assessed by recording the time spent inspecting the model predator, the number of approaches, and the time taken to resume courtship or foraging behaviour (see Table 1 for definitions).

2.4.3. Foraging skills

To assess foraging skills, water containing 10 large adult brine shrimp (*Artemia* sp.) was gently poured into the front of the observation tank. The time taken for any of the three fish to first bite a shrimp was recorded for a period of 10-min.

At the end of trials, the standard length (snout to base of caudal fin, in mm) of each fish (focal and non-focal) was measured and all fish were placed in post-observation aquaria where they played no further part in the experiment. The behaviour of 25 males from each treatment group was recorded.

2.4.4. Data analysis

The frequency of courtship behaviour (time spent displaying and number of displays) observed in pond-reared fish was very low (no data were collected for $\geqslant 15$ individuals in each of these groups). In every trial, courting males performed courtship displays; these behaviours were therefore combined and are referred to as the proportion of males observed performing courtship behaviour. Contingency table analysis (Zar, 1999) was used to test for differences in the proportion of individuals that performed courtship behaviour among the rearing groups. Post hoc multiple comparisons were performed using a technique analogous to the Tukey test (Zar, 1999). The remaining data could not be normalised; differences in behaviour among the three rearing treatments were therefore analysed using Kruskal–Wallis tests followed by Dunn's non-parametric test for post hoc multiple comparisons (Zar, 1999). All tests were two-tailed and performed using SPSS 11.0 statistical software.

3. Results

3.1. Pond statistics

During the rearing period, *G. atripinnis* and *Z. quitzeoensis* escaped through the mesh into two of the ponds that previously contained only *S. multipunctata*. *S. multipunctata* reared in these ponds were therefore excluded from the analysis. Ponds from which the heterospecifics had escaped contained similar numbers of fish to the other pools; these data therefore remained in the analysis. Over the rearing period, the mean number of *G. atripinnis* present per pond decreased from 65/70 to 43.7 (±7.9 SE, $n = 6$). The population of *Z. quitzeoensis* increased in these pools from approximately 17 individuals per pond initially, to a mean of 151.5 fish (±19.3 SE fish/pond, $n = 6$) one year later. Of the 20 juvenile *S. multipunctata* placed in each pond at the start of the experiment, a mean of 17.5 fish (±6.9 SE, $n = 4$) survived to adulthood per single species pond, compared with 9.7 (±1.6 SE, $n = 6$) individuals remaining per multi species pond. The sex ratios in the single-species ponds (1:1.3) were similar to those observed in the multi-species pools (1:0.7). There were significantly more juvenile *S. multipunctata* in the single-species pools (mean ± SE = 106 ± 5.2 fish, $n = 4$) than in the heterospecific pools (mean ± SE = 1.0 ± 0.82 fish, $n = 6$; $t_3 = 20.0$, $P < 0.001$). Two of the laboratory-reared *S. multipunctata* died before reaching adulthood.

The length of focal males differed among treatments (one-way ANOVA: $F_{2,62} = 12.6$, $P < 0.001$; Tukey test, $P < 0.05$). Fish raised in outdoor ponds in SS treatments were significantly larger (mean ± SE = 35.6 ± 0.8 mm, $n = 23$) than those reared in the laboratory (mean ± SE = 29.9 ± 0.6 mm) and MS ponds ($n = 21$; MS group, mean ± SE = 32.76 ± 0.99, $n = 21$). The standard length of focal fish relative to the (subordinate) male and female remained consistent across rearing regimes ($F_{2,62} = 0.09$, $P = 0.194$; $F_{2,62} = 3.04$, $P = 0.055$, respectively).

3.2. Behavioural analyses

There were significant differences in the proportion of fish performing courtship behaviour among the three rearing treatments ($\chi^2 = 25.37$, $P < 0.001$). A greater proportion of laboratory-raised males performed courtship behaviour (86%) than either group of pond-reared fish, and SS treatment fish were more likely to engage in courtship (43%) than those raised in MS ponds (10%; post hoc test: $P < 0.05$). However, MS treatment fish that courted spent a greater percentage of their time courting (median = 45.2%, $n = 2$, IQR = 17.2%) than SS group fish (median = 33.7%, $n = 10$, IQR = 44.2%). Laboratory-raised fish that courted spent a median of 62% of their time courting ($n = 18$, IQR = 54.9%). Laboratory-born fish were also involved in significantly more male–male chases than MS pond-raised fish ($H_2 = 16.1$, $P < 0.001$, Dunn's post hoc test: $P < 0.05$, Fig. 1(a)). Chasing behaviour was observed more frequently in laboratory (86%) and SS pond-reared fish (74%) than MS pond-reared fish (43%). Of those fish that displayed chasing behaviour, laboratory-reared fish performed more chases (median = 15.5 chases, $n = 18$, IQR = 18.25) than SS (median = 8 chases, $n = 17$, IQR = 9) or MS reared groups (median = 4, $n = 9$, IQR = 6).

The level of boldness observed in focal males also differed among the treatment groups. Laboratory-reared *S. multipunctata* spent more time in close prox-

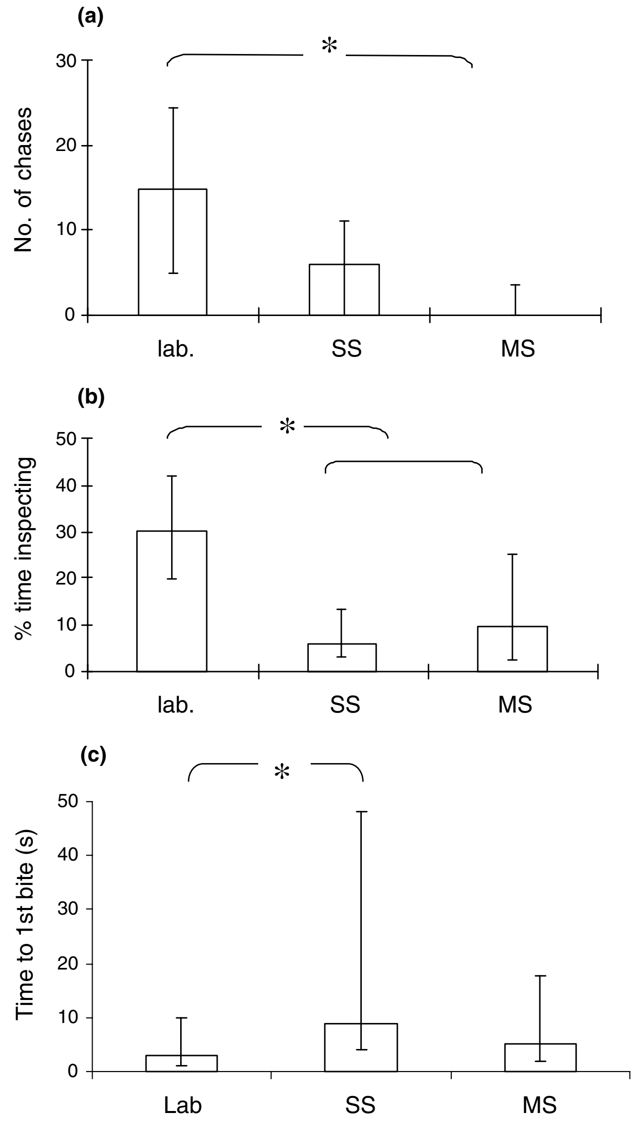

Fig. 1. Number of chases (a), percentage of time spent in close proximity to a novel model fish (b), and time to commence foraging (c) by *S. multipunctata* raised in the laboratory (lab.), or in single species (SS), or multi species (MS) outdoor ponds. Fish raised in the laboratory were more aggressive ($H_2 = 16.1$, $P < 0.001$), spent more time near the model ($H_2 = 17.5$, $P < 0.001$) and were quicker to begin feeding ($H_2 = 6.56$, $P = 0.038$) than pond-reared fish. Bars indicate median and IQR, $n = 21$, 23, 21 for lab-reared, SS and MS groups, respectively, and * indicates significant difference at $P < 0.05$ (post hoc tests).

imity to the model than either group of pond-reared conspecifics ($H_2 = 17.5$, $P < 0.001$, Dunn's test: $P < 0.05$, Fig. 1(b)). There was no difference in the frequency of approaches towards the model or the time to resume feeding/courtship behaviour between the groups ($H_2 = 1.67$, $P = 0.43$; $H_2 = 1.95$, $P = 0.38$, respectively). There was a significant effect of treatment on time to first bite ($H_2 = 6.56$, $P = 0.038$); laboratory-reared fish began foraging on *Artemia* more rapidly than those reared in single species groups outdoors (post hoc test: $P < 0.05$, Fig. 1(c)).

4. Discussion

The results presented here suggest that rearing environment may promote the expression of particular behavioural traits such as increased courtship and aggression, boldness towards a novel predator model, and foraging ability. If the behavioural differences observed here translate to how *S. multipunctata* would perform if reintroduced into the wild, we predict that laboratory-reared fish, which showed high levels of all the above-mentioned behaviours, would have a reduced probability of survival. For example, a large number of studies have demonstrated that conspicuous activities associated with courtship, such as elaborate displays and territorial fights, can lead to a higher risk of predation (reviewed in Lima and Dill, 1990; Magnhagen, 1991; Sih, 1994). Furthermore, our finding that laboratory-reared fish approached the model predator more closely than their pond-reared counterparts suggests that they will be more susceptible to predation when subsequently released into the wild. Captive-rearing conditions may therefore promote the development of risk adverse behaviours that increase predation mortality of reintroduced species. If *S. multipunctata* is to be reintroduced, we suggest that fish for release should be raised in semi-natural ponds that mimic conditions that they would encounter in the wild.

Populations of *S. multipunctata* showed enhanced levels of survival and reproduction in ponds that did not contain the other goodeid species *G. atripinnis* and *Z. quitzeoensis*. At the end of the rearing period, *Z. quitzeoensis* was the predominant species in mixed species pools, despite being initially stocked in lower numbers than *G. atripinnis*. There were differences in body length among the rearing treatments; fish reared in single species ponds were the largest and those reared in the laboratory were the smallest. The behavioural observations revealed that *S. multipunctata* that had been raised in the laboratory displayed higher levels of courtship and aggression, and acted more boldly toward a novel fish than those reared in the outdoor ponds. These differences are most likely to result from environmental effects as sibling groups were divided equally among the rearing treatments.

These results indicate that the presence of heterospecifics had an overall negative effect on survival and reproduction of *S. multipunctata*. Conditions in the ponds also appeared to be unfavourable to *G. atripinnis*, although a large increase in the number of *Z. quitzeoensis* was observed. Stomach content analyses (M. De la Vega Salazar, pers. commun.) have revealed that *Z. quitzeoensis* feeds on small invertebrates and behavioural observations (C. Macías Garcia, unpublished) indicate that these are browsed off the substrate and plant surfaces. It is likely that the large area of mesh that separated the pools provided a suitable substrate for the type of prey that this species feeds on. In their natural habitat in Mexico, *S. multipunctata* live with a variety of native and non-native heterospecifics (poeciliid and goodeid species, see Section 2). Recent research has shown that habitat alterations, caused by introduced species such as carp (*Cyprinus carpio*) and tilapia (*Oreochromis* sp.), have been partly responsible for the decline of goodeids (De la Vega-Salazar et al., 2003). In addition, translocation and cultivation of the larger goodeids such as *G. atripinnis* for human consumption may also adversely affect other species of goodeid. Further research is required to investigate how *S. multipunctata* interacts with both native and introduced species in order to understand the factors that have contributed towards its decline.

Skiffia multipunctata that were raised in outdoor ponds in single species groups were significantly larger than those reared in mixed species pools, and those reared in the laboratory. A number of factors may have contributed towards this effect, in particular differences among the initial stocking densities. The laboratory-reared fish were stocked at a higher density than those in the single, or mixed species outdoor ponds (initial stocking densities: SS ponds = 0.009 fish/litre, MS ponds = 0.045 fish/litre, laboratory aquaria = 0.38 fish/litre). Although tank-raised *S. multipunctata* were fed ad libitum, high-density conditions are known to have a negative effect on growth rate in fishes (Wootton, 1990). However, behavioural interactions could also contribute towards differences in fish size; for example, heightened competition in mixed species pools could cause these fish to have a lower growth rate (e.g., less time available for foraging) than fish reared in single species groups.

The results from this study indicate that the laboratory-reared fish spent more time courting, performed more courtship displays, were more aggressive and were more adept at foraging on a novel food item than their pond-reared counterparts. These findings are unlikely to be explained solely by differences in body size between laboratory- and pond-reared fish, as previous studies of *S. multipunctata* have found no relationship between body size and courtship (Pearson's correlation: $r = 0.06$, $n = 30$, $P = 0.75$) or aggression ($r = 0.23$, $n = 30$, $P = 0.21$) (J.L. Kelley, unpublished data). The enhanced courtship and aggression of laboratory-reared males may be a result of high stocking density; laboratory-reared males encountered females more frequently than those in the ponds, and experienced continuous competition from other males, which may have caused them to elevate these behaviours. Rearing conditions may also have facilitated aggressive behaviour, as laboratory-reared fish were unable to escape dominants. Several studies have demonstrated that rearing density can affect social interactions, which are further influenced by food availability. For example, steelhead trout fry reared in hatchery tanks at relatively low densities with limited food were more aggressive than those reared at high densities with food fed ad libitum (Berejikian et al., 1996). In the present study, fish reared in the laboratory were not food deprived, but the method of food presentation (flakes sprinkled at the front centre of the tank, twice a day) may have favoured aggressive behaviour. Ryer and Olla (1995) found that localising food in rearing tanks increased the frequency of agonistic behaviour in juvenile chum salmon (*Oncorhynchus keta*), compared to those whose food was scattered and could not be monopolised.

Laboratory-reared fish spent more time in close proximity to the novel model fish than those that were reared outdoors. Indoor tanks provide a less stimulating environment than the outdoors and may have caused these fish to show enhanced curiosity toward a novel object. The tanks in which the behaviour of *S. multipunctata* was compared were structured with materials (gravel, rocks and vegetation) that were novel to fish in all three rearing treatments. However, other factors (e.g., tank size, illumination, water temperature, feeding regime) would have been more familiar to the laboratory-raised fish than those reared in the outdoor pools, which may have favoured particular traits in the laboratory-reared fish. For example, the laboratory-reared fish may have been advantaged in the foraging trials because they were accustomed to receiving food at the front of their tank. It would be interesting to extend this study to determine whether the behavioural tendencies (e.g., enhanced courtship) of laboratory-reared fish are maintained in other contexts. In Ryer and Olla (1995) study, differences in the frequency of agonistic encounters caused by food distribution did not persist when the fish were tested in a novel environment. In contrast, Evans and Magurran (1999) found that in guppies (*Poecilia reticulata*), experimentally induced changes in male courtship were maintained when the fish were subsequently moved to novel surroundings.

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