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Captive breeding promotes aggression in an endangered Mexican fish

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ABSTRACT

We compared the behaviour of wild and captive-bred butterfly splitfins (*Ameca splendens*), an endangered freshwater fish, to investigate whether captive breeding results in the behavioural divergence of wild and captive individuals. In a first experiment, we examined whether the captive environment allows for the similar expression of behaviours observed in wild fish. The foraging, courtship and aggressive behaviour of fish in their natural habitat (in Mexico) was compared with that of their counterparts that have been bred at London Zoo, UK, for 40 years. These *in situ* observations revealed that wild fish were preoccupied with searching for food whereas captive fish engaged more in aggressive interactions. In a subsequent laboratory experiment we compared the behaviour of wild-caught and captive-bred fish under standard conditions in two novel habitats: structured (enriched) and unstructured (bare) aquaria. Overall, captive-bred butterfly splitfins displayed higher levels of aggression than wild-caught fish. The relationship between aggression and habitat structure was influenced by density; captive-bred males were more aggressive when observed in structured habitats than unstructured ones, but only when they were stocked at a high density. We also found an effect of tank structure on foraging behaviour, with individuals spending more time foraging in unstructured tanks than structured tanks. There was no effect of captive breeding or habitat structure on courtship behaviour.

Our findings suggest that captive environments can promote the development of aggressive behaviour which may affect the suitability of captive-bred fishes for reintroduction into the wild.

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1. Introduction

Captive breeding is a conservation strategy that is widely used for the recovery and reintroduction of endangered species. However, captive-bred animals often exhibit deficiencies in foraging, predator avoidance and reproductive behaviour, which can present major difficulties when attempting to restore wild populations (reviewed by Beck et al., 1994; Brown and Laland, 2001; Fleming, 1994; Griffith et al., 1989; Snyder et al., 1996). Behavioural differences among wild and captive-bred animals can arise through both intentional and unintentional processes. Intensive rearing practices often aim to maximise production by selecting on preferred traits, such as enhanced growth rate, which can indirectly affect correlated traits such as aggression (Price, 1988). Adaptation to the captive environment (domestication) can also promote

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behavioural traits that are advantageous in captivity but maladaptive in the wild, such as tameness and a reduced response to stress (Kohane and Parsons, 1988). Furthermore, captive environments often differ substantially from wild habitats causing behavioural differences to arise as a result of differential experience (Price, 1999).

Captive animals exist in a very different environment to that of their free-living counterparts (see reviews by Huntingford, 2004; Price, 1999). Animals living in captivity do not need to devote a large proportion of their time to searching for food because they have a regular supply of good quality food available to them. Mate choice is sometimes absent in intensively managed animals so that reproduction occurs without the need to attract mates or compete with rivals, whereas in other systems it goes unchecked by counterbalancing natural selection. Captive animals are also protected from their predators (except humans) and therefore do not need to trade-off predation risk with other activities such as foraging and courtship behaviour (Huntingford, 2004). The need to maximise production and/or minimise space requirements means that captive animals are often stocked at very high densities. A number of studies of commercial species have demonstrated that intensive rearing practices can result in a divergence of behavioural traits in captive and wild animals (for a salmonid example, see Gross, 1998). However, the extent of this divergence has rarely been assessed for less intensive captive breeding programs, such as those implemented at zoos and conservation organisations.

Zoos are becoming increasingly involved in captive breeding projects for endangered freshwater fishes (Andrews and Kaufman, 1994). One particularly vulnerable group is the Goodeinae, a clade endemic to freshwater habitats in the High Plateau (900–3000 m above sea level) of Central Mexico. Of the 36 species described (Webb et al., 2004), thirteen are on the IUCN (2006) red list of threatened animals, ten are in the critically endangered, endangered, or vulnerable categories, one is extinct, and two are listed as extinct in the wild (one of which is the butterfly splitfin) (but see De la Vega-Salazar et al., 2003). The main threats to the family are habitat loss due to urbanisation and an increasing demand for water for human consumption, and pollution arising from agricultural and industrial processes (Lyons et al., 1998). Large-scale introductions of carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), and tilapia (*Oreochromis* spp.) may also have contributed to the decline of goodeids (De la Vega-Salazar and Macías Garcia, 2005).

Butterfly splitfins (*Ameca splendens*) are sexually dimorphic goodeid fish with males exhibiting larger fins and deeper bodies than females (max. male total length = 8 cm, female = 12 cm; a photograph and other information about butterfly splitfins can be found at www.fishbase.org). Mature males possess bright yellow terminal bands and black sub-terminal bands on the caudal fin and (to a lesser extent) the dorsal and anal fins (Miller and Fitzsimmons, 1971). Although the behaviour of butterfly splitfins has not been formally described, elaborate courtship displays and female choice have been reported in several other goodeid species (*Xenotoca* spp. Fitzsimmons, 1972; e.g. *Girardinichthys multiradiatus* Macías Garcia, 1991).

In 1960 and 1961, 12 butterfly splitfins, *Ameca splendens,* (six males and six females) were collected from the wild and bought to London Zoo, UK, as part of a captive breeding program. Although the exact location of this collection is unknown, the fish are thought to have originated from either the Teuchitlán River or further downstream, where the Teuchitlán River runs into the Ameca River. The captive population has been allowed to interbreed freely and we estimate that approximately 80 generations have been produced since the program commenced. In Mexico, butterfly splitfins begin to reproduce at about 6 months of age and produce a brood approximately every 2 months except during the winter. In captivity in the UK, we estimate that the generation time will be longer with around 2 cohorts being produced every year. Although the allelic diversity of captive-bred butterfly splitfins is lower than wild fish, there is no evidence to suggest that the genetic origin of the wild and captive-bred fish is different, or that the captive population is suffering from inbreeding depression (Bailey et al., submitted for publication).

Although butterfly splitfins have been declared extinct in the wild since 1996 (IUCN, 2006), in 1997 a population was rediscovered living in springs at a water park (El Rincon) near the town of Ameca (A. Moyaho, unpubl. data). The springs lie at the headwaters of the Teuchitlán River, the river from which all historical records of butterfly splitfins originate (Miller and Fitzsimmons, 1971). The water park represents a good approximation to the original ecology of butterfly splitfins. Only a few exotic species have been introduced (see Section 2) and butterfly splitfins in this habitat face less disturbance than any remaining fish would in the De la Vega Dam (erstwhile Teuchitlán River). The restricted historical distribution of this species suggests that the origin of wild and rediscovered populations is likely to be similar; even if fish for the captive population were collected at the furthest point of their distribution (i.e. below the junction of the Teuchitlán and Ameca Rivers; (Miller and Fitzsimmons, 1971)), the distance between the collection locations of wild and rediscovered fish is only 15 km.

The aim of this study was to investigate whether captive breeding of butterfly splitfins results in behavioural differences between wild and captive fish. We focus on the behaviour of male butterfly splitfins because individual fish can be readily distinguished through their yellow and black colour bands (which females do not possess). First, the foraging, courtship and aggressive behaviours of fish observed in the wild were compared with their counterparts maintained in captivity at London Zoo. It was anticipated that *in situ* observations would provide an indication of whether the captive environment allows for the similar expression of behaviours that are observed in wild fish. Second, the aggressive behaviour and habitat use of wild and captive-bred fish was compared under standard novel conditions for structured (enriched) and unstructured (bare) aquaria, in order to assess the importance of the environment on behaviour. The effect of stocking density on the relationship between captive breeding and aggression was subsequently examined by observing captive-bred fish at a higher density.

2. Methods

We performed two sets of behavioural observations on wild and captive-bred butterfly splitfins. The first set of observations (Section 2.1) were performed *in situ* in the wild (Mexico) and in captivity (London Zoo) and were designed to assess whether the captive-breeding environment at London Zoo aquarium allows for the similar expression of behaviours observed in wild fish. The second set of observations (Section 2.2) compared the behaviour of wild-caught and captive-bred fish under standard conditions in order to assess whether there were behavioural differences between wild and captive-bred fish when observed in the same environment. During these observations (Section 2.2) we also investigate how tank structure (enriched or bare) might contribute towards any behavioural differences observed between wild and captive-bred fish.

2.1. *In situ observations*

At the El Rincon water park, near the town of Ameca in Mexico (20° 45′ N, 103° 50′ W), butterfly splitfins inhabit a series of semi-natural springs: a deep, isolated bathing pool (surface area = 255 m^2, 1.6 m deep) and three interconnected shallow lagoons (areas = 418 m^2, 2180 m^2, 6730 m^2; depths = 0.5 m, 1.0 m, 1.5 m, respectively) that are joined to a bathing spring (area = 128 m^2, 1.0 m deep). Observations were conducted from a concrete walkway running parallel to the smallest lagoon, allowing the observer to easily measure the behaviour of individual fish. Preliminary observations performed in the other lagoons revealed that the behaviour of fish observed from the walkway was representative of fish observed in other areas. The substrate of the smallest lagoon was relatively homogenous, consisting of pebbles and small amounts of algae. In this lagoon, butterfly splitfins are sympatric with mollies *Poecilia sphenops* (introduced), *Poeciliopsis infans*, the blackfin goodea *Goodea atripinnis*, tilapia *Oreochromis* spp., and bluegill sunfish *Lepomis macrochirus*.

Observations were conducted in May 2001 between 1000 and 1500 h. Mean temperature (at 1500 h) during this period ranged from 26.6 °C to 28.6 °C. Only the behaviour of mature males was recorded, which were easily distinguishable from females by the presence of black and bright-yellow bands on the caudal fin (see above). During a 10-min observation period, the frequency of foraging events, courtship displays and aggressive encounters were dictated into a voice recorder. We focused on the frequency at which these behaviours were performed because adaptation to captivity is thought to cause quantitative, rather than qualitative changes in behaviour (Price, 1984). Foraging events were defined as occasions when fish were observed biting or nibbling at the water's surface or on the substrate. Courtship displays were characterised by the male quivering his body in a head down position and aggressive interactions were defined as all chases involving the test male (further details on the behaviour of butterfly splitfins are provided in Table 1). The trial was stopped if the focal fish swam out of view within 5 min of commencing the recording, and these data were excluded from the analysis. The behaviour of 20 males was recorded. Although we cannot rule out the possibility that some males were recorded more than once, we minimised this risk by selecting males from a wide area and noting down distinguishing size/colour patterns where possible.

Table 1 – Description of behaviours observed in wild-born and captive-bred butterfly splitfins (*Ameca splendens*)

Behaviour	Definition
Male courtship display	The male positions himself head down (45° to vertical) and perpendicular to the female and rapidly quivers the anterior part of his body. This behaviour (comparable to 'head flicking' reported in male *Xenotoca variata*, Fitzsimmons, 1972; Fitzsimmons, 1976) is similar to that observed in females, but is performed more vigorously. The male sometimes rotates his body through 180° to display the other flank. This behaviour does not escalate into the figure-of-eight-dance, or loop dance, reported in several goodeid species (Fitzsimmons, 1972; Macías Garcia et al., 1994).
Male aggressive interaction	Dominant males frequently chase subordinates and the aggressors often exhibit darkened colouration during such encounters. Submissive males sometimes remain motionless near the surface and change direction or swim backwards to avoid the dominant male. Subordinate males occasionally perform lateral head movements similar to the females' response to male courtship.

Observations of captive-bred butterfly splitfins were carried out at London Zoo Aquarium in July 2001. The fish were housed either in a glass display tank (volume = 132 l) or an opaque plastic (off-show) holding aquarium (volume = 445 l). Both tanks were maintained between 25.5 ± 0.5 °C and the densities and sex ratios of these tanks were similar. The environment in the display tank was highly structured and consisted of a layer of gravel, three large rocks, a log, and a large amount of aquatic weed. In contrast, the off-show tank contained only 2 bricks and a small plastic plant. Both groups were maintained on a mixed diet of tropical fish flakes, daphnia, bloodworm and *Artemia* nauplii. All observations at London Zoo were conducted between the hours 0800 and 1500, during which we recorded the behaviour of each male over a 10-min period (again, we focussed on males as they are individually recognisable). All behavioural interactions involving the focal male (foraging, courtship displays and aggression; see Table 1 for behavioural definitions) were dictated into a voice recorder as described above. Males in the display tank were highly territorial. The territory of each male was sketched (relative to the substrate), his colour patterns noted, and each individual was recorded only once. Males in the non-display tanks were less distinguishable and did not maintain territories, but the markings of each male were described in order to avoid observing the same fish more than once. A total of 32 individuals were observed, 14 in the display tank and 18 in the off-show tank.

2.2. Comparison of behaviour of wild-caught and captive-bred males in structured and unstructured environments (density = 0.16 fish/l)

Wild butterfly splitfins (20 males and 20 females) were collected from the El Rincon water park near Ameca in May 2001 and returned to the aquarium at UNAM, Mexico City. Fish were housed in mixed sex groups (ca. 1:1) at a density of 0.16–0.19 fish/l and fed commercially prepared flake food twice daily. All holding tanks (dimensions = 50 × 25 × 30 cm, filled to a depth of 25 cm) contained an air filter and were illuminated by two aquarium slim-line lights (75 and 20 W) on a 12 h light/dark cycle. Fish were allowed to acclimate to captive conditions for 10 days prior to commencing trials, after which they displayed similar behaviour to butterfly splitfins that had been maintained in captivity for one year. Two larger tanks (dimensions = 100 × 25 × 25 cm, filled to a depth of 22 cm), each containing an air filter and lit with two 39 W fluorescent slim-line bulbs (65 cm from the water surface), were provided for observations. Water temperature in the aquaria during the study period was 21 ± 0.5 °C. A series of markings were drawn on the front of the glass so that each tank was split into six equal-sized areas labelled a–f. One of the tanks (hereafter referred to as the structured environment) contained gravel, some aquatic weed, and a small shelter constructed of two red tiles and a stone. The habitat in the structured tank was sketched in order to aid future reconstruction (see below). The other observation tank (unstructured environment) was left bare.

On the evening prior to observations, two males (distinguishable by their colouration) and three females of similar size to each other were placed in each observation tank. The following day, all fish were fed to satiation and each male was roughly sketched to aid future recognition. Behavioural descriptions for each male were made over a period of 10 min using a voice recorder (see above). In addition, the position of the male within the tank (section a, b, c, etc.) was recorded every 30 seconds using the markings drawn on the front of the glass. This made it possible to estimate the position of the focal males in both tanks. Females were used only to elicit courtship behaviour in males and their behaviour was not recorded. We assumed that all females provided equivalent mating stimulation for males; we did not observe any heightened male interest towards particular females which often occurs when females are close to parturition. Once all four males had been observed, both sets of fish (males and females) were gently transferred to the other tank, and left to acclimatise overnight. A reciprocal design was used so that half the males were observed first in the structured tank and vice versa.

The following morning males were identified using the sketches made on the previous day and the behaviour of each male was observed in his new environment as described above. This procedure was repeated until the behaviour of 20 males had been recorded. No male was recorded more than once. At the end of each paired trial, the total length of each fish was measured to the nearest mm and all fish were returned to stock aquaria, where they were maintained as breeding stock.

Captive-bred butterfly splitfins originating from London and Whipsnade Zoos were transported from London to St. Andrews University in Scotland in July 2001. The fish from Whipsnade Zoo were derived from the London stock and were used to avoid removing a large proportion of mature males from the London population. At Whipsnade Zoo, butterfly splitfins were maintained in similar conditions to those in London. At St. Andrews University, goodeids were housed in aquaria (61 × 30 × 38 cm, filled to a depth of 33 cm) containing a layer of gravel and an air filter and were maintained in mixed sex groups (ca. 1:1) at a density of 0.15–0.17 fish/l. The water temperature was the same as that used for experimental observations in Mexico (21 ± 0.5 °C), allowing us to compare the behaviour of wild-caught and captive-bred fish under as similar conditions as possible.

Observations were conducted in October 2001 in tanks of the same dimensions, temperature and lighting conditions as those used for wild fish. Although there are seasonal differences in the time at which we conducted the observations in Mexico (May) and St. Andrews (October), we attempted to minimise these by ensuring that captive fish were acclimatised to laboratory conditions before observations commenced. Using the sketch made previously, the habitat structure (gravel, aquatic weed, and a small shelter) was made as similar as possible to that provided for the observations of wild fish in Mexico. As in Mexico, fish were fed a mixed diet of dried flake food and blood worm before observations commenced. The behaviour of 16 mature males was recorded as described above; females were used only to elicit male courtship behaviour. At the end of the trials, the total length of each fish was recorded to the nearest mm and the stock was donated to St. Andrews Aquarium.

2.3. Behaviour of captive-bred males at high density (= 0.26 fish/l)

Trials conducted to compare the behaviour of captive-bred fish in a structured and unstructured tank were repeated using 12 males and five females. The aggressive behaviour of eight individually recognisable males was recorded in both environments, exactly as described above.

2.4. Statistical analyses

As the *in situ* observations were conducted under very different environmental conditions we did not perform statistical test on these data but report the percentage of fish observed that performed a particular behaviour. For the experimental trials, we used general linear mixed models (GLMM) to investigate the influence of fish origin (wild-caught or captive-bred), tank structure (structured or unstructured) and the interaction between these factors on fish behaviour. These models allow the incorporation of both fixed and random factors and can be used to test the effects of nested factors and repeated measures designs. We specified 'individual' as the subject term ($n = 36$) and 'tank' as the repeated measures factor (2-levels: structured or unstructured). We entered 'tank', 'origin' and the tank*origin interaction as fixed factors and individuals nested within origin as a random factor in the model. We tested the effect of different covariance structures in the model using Akaike's information criteria (AIC) and selected the model with the lowest AIC score.

To examine the influence of fish density on aggression we compared the behaviour of fish observed in structured and unstructured environments at high (0.26 fish/l) and low densities (0.16 fish/l). These data were analysed using a repeated measures ANOVA with tank as the within-subjects (repeated measures) factor and density as the between-subjects factor. All dependent variables were tested for normality and homogeneity of variance prior to running the parametric models. The preference of focal males for tank areas a–e was compared using a Friedman test for both structured and unstructured aquaria. All tests were two-tailed and performed using SPSS v14.0 statistical software.

3. Results

3.1. In situ observations

We noted high levels of aggression in butterfly splitfins observed in the wild and in the display aquarium at London Zoo (80% and 100% of fish observed performed aggressive behaviours, respectively; Table 2). In the wild, males performed a median of one chase (IQR = 1.75) during the 10 min observation period whilst captive-bred males in the display tank performed a median of 33.5 (IQR = 23) chases per 10-min trial. Males in the display tank at London Zoo aquarium were highly territorial and were observed defending territories of approximately 2 body lengths in size. In contrast, lower levels of aggression (39%) and no territoriality was observed in the off-show tank (Table 2). Foraging behaviours were observed frequently in wild fish (70%) but less often in captive-bred fish in the off-show tank (39%) and rarely in captive-bred fish in the display tank (7%; Table 2). Wild fish were often observed darting towards anything falling into the water (e.g. insects) whereas captive fish attempted to feed whenever there was a disturbance behind their tank (from where they were usually fed). Low levels of courtship behaviour were observed in wild and captive-bred fish (15% and 7%, respectively) while no courtship was observed in captive-bred fish housed in the off-show tank (Table 2).

Table 2 – The percentage of wild and captive-bred male butterfly splitfins observed performing aggressive, foraging and courtship behaviours

	Wild	Captive	
		Display	Off-show
Aggression	80	100	39
Foraging	70	7	39
Courtship	15	7	0
n	20	14	18

Captive-bred fish were observed either in a public display tank or an off-show holding aquaria. The number of fish observed in each category is shown (*n*).

3.2. Comparison of behaviour of wild-caught and captive-bred males in structured and unstructured environments (density = 0.16 fish/l)

Logarithmic transformations ($\log x + 1$) improved the distribution of the behavioural data (frequency of aggressive interactions, courtship and foraging) and we confirmed that these data met the assumptions of homogeneity of variance. The general linear mixed models revealed a significant effect of fish origin on aggressive behaviour and an effect of tank environment on foraging frequency (Table 3). The estimates of the fixed effects provided by the model revealed that the level of aggression observed in captive-bred fish (mean ± s.e. = 12.06 ± 1.69 aggressive interactions) was greater than that observed in wild-caught fish (mean ± s.e. = 3.65 ± 0.82; Fig. 1a). There was no significant effect of tank structure or the origin*tank interaction on aggressive behaviour (Table 3). The removal of the interaction term from the model resulted in a significant effect of tank structure on aggressive behaviour

Table 3 – Results of general linear mixed models (GLMM) testing the effects of origin (wild or captive-bred), tank (structured or unstructured) and the origin*tank interaction on aggression, courtship and foraging behaviour observed in male butterfly splitfins

Behaviour	Fixed effect	Numerator df	Denominator df	F	P
Aggressive frequency	Origin	1	34	16.958	**<0.0001**
	Tank	1	34	3.643	0.065*
	Origin*tank	1	34	1.452	0.237
Courtship frequency	Origin	1	34	0.946	0.338
	Tank	1	34	1.821	0.186
	Origin*tank	1	34	0.991	0.327
Foraging frequency	Origin	1	34	3.173	0.084
	Tank	1	34	6.810	**0.013**
	Origin*tank	1	34	0.772	0.386

The origin and tank factors were fixed effects whereas individual (*n* = 36) was treated as a random factor nested within origin. Significant effects are shown in bold.

* The effect of tank on aggressive frequency is significant following the removal of the (non-significant) interaction term ($F_{1,35} = 4.17$, $P = 0.049$).

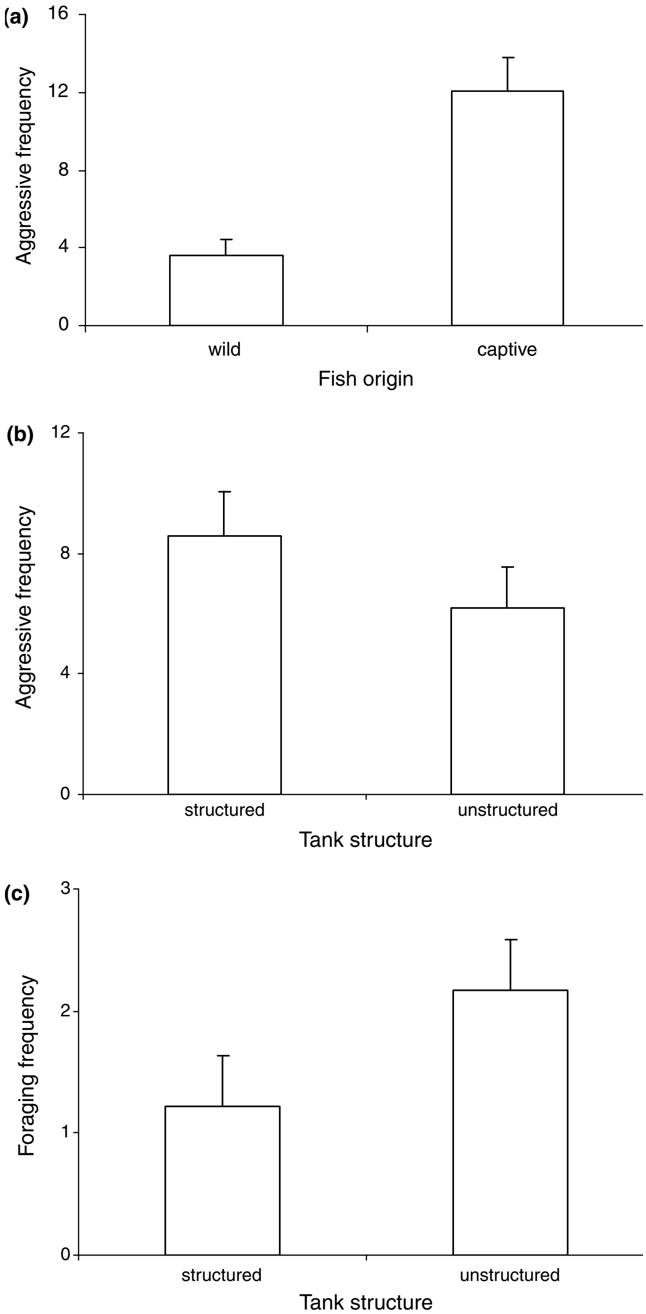

Fig. 1 – Aggressive behaviour in wild-caught and captive-bred butterfly splitfins (a). Aggressive behaviour was influenced by tank structure with males displaying higher levels of aggression in structured than in unstructured tanks (b). A comparison of foraging frequency between the two tank types revealed that males foraged more often in unstructured tanks than in structured ones. All graphs show means + s.e.; *n* = 20 and 16 for wild-caught and captive-bred males, respectively.

($F_{1,35} = 4.17$, $P = 0.049$), with males displaying higher levels of aggression in the structured tanks (mean ± s.e. = 8.58 ± 1.46) than the unstructured ones (mean ± s.e. = 6.19 ± 1.37; Fig. 1b).

The models also revealed that foraging behaviour was more frequent in the unstructured tanks (mean ± s.e. = 2.17 ± 0.41 foraging events) than in the structured ones (mean ± s.e. = 1.22 ± 0.42; Fig 1c). There was no effect of fish origin or the origin*tank interaction on foraging behaviour (Table 3) and removal of the interaction term from the model had no effect on the significance of the other factors. We found no effect of fish origin, tank structure or the interaction between these factors on courtship behaviour (Table 3).

There was no significant correlation between male body length and aggressive behaviour in wild ($r = 0.08$, $n = 19$, $P = 0.75$) or captive goodeids ($r = -0.02$, $n = 16$, $P = 0.94$). Indeed, wild-born fish were significantly larger than their captive-born counterparts (mean lengths ± SE: wild fish = 54.1 ± 1.8 mm; captive fish: 47.6 ± 1.0 mm; $t_{33} = 3.39$, $P = 0.002$). The mean size asymmetry between the two focal males in each trial did not differ between observations of wild (mean length difference = 2.78 ± 0.57 mm) and captive fish (mean length difference = 3.88 ± 0.10 mm) ($t_{15} = -0.99$, $P = 0.34$) and we detected no significant correlation between male size asymmetry and the level of aggression displayed by the larger male in either group (wild: $r = 0.26$, $n = 9$, $P = 0.50$; captive: $r = 0.49$, $n = 8$, $P = 0.22$).

Male goodeids showed no habitat preferences in the structured tank (Friedman tests: wild: $\chi^2 = 3.47$, df = 5, $P = 0.63$; captive: $\chi^2 = 8.03$, $df = 5$, $P = 0.16$), but both wild ($\chi^2 = 12.4$, df=5, $P = 0.03$) and captive fish $\chi^2 = 10.7$, df = 5, $P = 0.059$ tended to prefer one side (the right) of the unstructured tank.

3.3. *Behaviour of captive-bred males at high density (= 0.26 fish/l)*

The repeated measures ANOVA revealed a significant effect of tank structure ($F_{1,22} = 23.14$, $P < 0.001$), no effect of fish density ($F_{1,22} = 0.21$, $P = 0.654$) and a significant tank structure*density interaction ($F_{1,22} = 18.03$, $P < 0.001$) on male aggressive behaviour. Specifically, males were more aggressive in the structured environment than the unstructured one, but only when they were observed at a high density (paired *t*-test: $t_8 = 4.17$, $P = 0.004$; Fig. 2). Thus there was no difference in

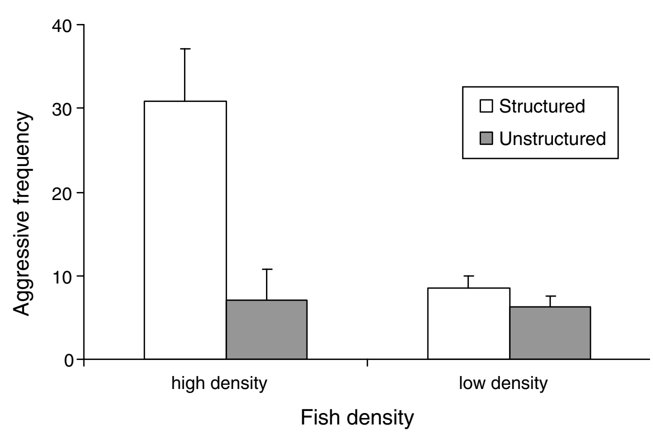

Fig. 2 – The relationship between fish density, tank structure and mean aggression. Males were more aggressive in the structured tank than in the unstructured tank, but only when observed at high density (0.26 fish/l vs. 0.16 fish/l). Graphs shows mean + s.e. *n* = 16 and 8 for fish observed at low density and high density, respectively.

male aggressive behaviour between the two tank environments when fish were observed at a lower density (= 0.16 fish/l; $t_{16} = 0.61$, $P = 0.55$; Fig. 2).

4. Discussion

The findings of this study suggest that captive breeding can result in the behavioural divergence of wild and captive-bred endangered fish. The first part of the study, which compared the behaviour of wild butterfly splitfins with that of their captive-bred counterparts, revealed differences in the frequency at which aggressive, courtship and foraging behaviours were performed. Although many factors (e.g. genotype × environment interactions) are likely to have contributed to the behavioural differences observed between wild-caught and captive-bred butterfly splitfins *in situ* (see below), such comparisons provide a useful estimation of whether captive conditions allow the expression of behaviour patterns similar to those observed in wild fish. This is an important component of captive-breeding programs as conservation management must entail preservation of a species' behavioural patterns as well as its genetic diversity (Shepherdson, 1994). Ensuring that the captive environment allows for the appropriate expression of behaviour is also a welfare issue and zoo managers need to ensure that animals humanely adapt to their artificial environment (Price, 1984).

The second part of this study revealed that captive-bred fish displayed higher levels of aggression than their wild-caught counterparts. Unfortunately, we did not evaluate the behaviour of first-generation captive-born fish; we are therefore unable to ascertain whether the heightened aggression in captive-bred fish is attributable to rearing experience, the effects of over 40 years of captive breeding, or a combination of these processes. Nevertheless, the comparison of the behaviour of wild and captive-bred species under standard conditions has recently been advocated as a simple method for assessing a species' suitability for reintroduction (Mathews et al., 2005).

A number of studies have demonstrated that rearing environment can promote aggressive behaviour in fishes (reviewed by Ruzzante, 1994). For example, rainbow trout (*Oncorhynchus mykiss*) grown in structurally enriched hatchery tanks or stream environments have increased competitive ability and achieve higher dominance ranks than fish reared in conventional hatchery tanks (Berejikian et al., 2001). Rearing environment can also affect the development of neurological processes, which are an important determinant of behaviour; hatchery reared rainbow trout for example, have smaller brains than their wild reared counterparts (Marchetti and Nevitt, 2003).

Selective processes operating in captivity can favour aggressive behaviour if conditions that promote competition (e.g. feeding regime) allow more aggressive individuals to gain better access to food (Ruzzante, 1994). In the high density display tank at London Zoo, the fish had become accustomed to receiving their food in one particular part of their tank. Larger, dominant fish vigorously defended the space immediately around this area and would therefore receive a disproportionate amount of food. Under these conditions it is feasible that aggression would be selected for inadvertently. The higher levels of aggression observed in captive-bred males could also arise through selection on correlated traits. For example, studies of domesticated salmonids have revealed that intentional selection for fast growth rates may inadvertently affect aggressive behaviour, depending on the hatchery environment (Ruzzante, 1994). Furthermore, selection for enhanced growth can affect endocrine regulation systems, which in turn, could influence the expression of other behaviours such as aggression and risk-sensitive behaviours (Fleming et al., 2002). In the present study, we did not examine the growth rates of captive-bred and wild fish but it would be interesting to know if the (less intensive) selective processes operating in captive breeding programs can affect the expression of correlated traits.

Our study revealed that male butterfly splitfins were more aggressive in the structured environment than in the unstructured one. This finding contrasts with previous studies on zebrafish (Basquill and Grant, 1998) and brown trout (Sundbaum and Naslund, 1998) that have reported a decrease in aggression with increasing habitat complexity. In these studies the habitat structure allowed individuals to become visually isolated, therefore decreasing the overall level of aggression among individuals. The habitat structures in this study (aquatic vegetation and a shelter) were centrally located and therefore easily monopolised by aggressive individuals. Furthermore, refuges are likely to be a valuable resource for wild-caught fish; a study on perch (*Perca fluviatilis*) for example found that the presence of a shelter led to increased aggression in young wild fish (Mikheev et al., 2005). It may also be that a structured habitat facilitates the establishment of territorial boundaries based on visual landmarks, which would be largely unavailable in unstructured habitats (Sundbaum and Naslund, 1998).

Our subsequent investigations of the effect of density and habitat structure on aggression revealed that captive-bred males displayed higher levels of aggression in the structured environment than in the unstructured one, but only when they were observed at a high population density (= 0.26 fish/l vs. 0.16 fish/l). Studies on fishes in general have revealed that aggression tends to increase with population density (Ruzzante, 1994), but few studies have considered the effects of both density and habitat on aggression. However, one such recent study on Atlantic salmon revealed that aggression was positively correlated with population density, and also that higher levels of aggression were observed in preferred habitats than in non-preferred habitats (Blanchet et al., 2006). Unfortunately, we did not collect data on the aggressive behaviour of wild-caught fish observed at high densities, so the influence of captive-breeding on this effect is unknown. Nonetheless, revealing the relationship between fish stocking density, habitat structure and aggression in captive-bred fish is an important aspect of managing their welfare.

When we compared the foraging behaviour of wild and captive-bred butterfly splitfins under standard laboratory conditions, we found that males foraged more frequently in the unstructured tank than in the structured one. One possible explanation for this is that particulate matter, representing potential food items, is highly conspicuous in an unstructured environment. We found no differences in habitat use by wild and captive-bred butterfly splitfins, but both of these groups preferred the right side of the unstructured tank.

The filter was located in this area of the tank and the fish may have been attracted to the bubbles or used the filter as a shelter in this otherwise bare environment.

The findings from this study suggest that the captive rearing of endangered fishes can promote the expression of particular behavioural traits, such as increased aggression. This could cause problems if captive-bred individuals are released into the wild. In the case of the butterfly splitfin, aggressive males may have problems reproducing if attacks are directed towards females (Wolf et al., 2000), or they may be at greater risk of predation through reduced vigilance and increased conspicuousness. Aggression is often correlated with other traits such as boldness; in sticklebacks (*Gasterosteus aculeatus*) for example, aggressive individuals take greater risks to acquire resources and consequently have higher growth rates (Ward et al., 2004). An essential next step in the conservation of butterfly splitfins and other endangered species is to identify the behavioural traits that are correlated with aggression (i.e. through the construction of 'behavioural syndromes' Sih et al., 2004a; Sih et al., 2004b). This would permit an investigation into the effects of captive breeding on multiple traits and allow individuals to be ranked according to their suitability for release into the wild (Mathews et al., 2005).

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