Aggression in captivity and the implication for interspecific aggression between once sympatric species of Mexican goodeid

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Abstract. Many species of fish show increased aggression in captivity, but studies normally focus on intraspecific aggression rather than interspecific interactions. Ameca splendens, a goodeid originally from Mexico, exhibits increased levels of intra-specific agonistic behaviour in captivity, and this increases further with density. This study compared agonistic interactions occurring in single species aquaria (A. splendens) with those in aquaria shared with Skiffia francesae. S. francesae are extinct in the wild, but were once sympatric with A. splendens. To improve captive rearing conditions and chances of successful reintroduction of S. francesae, programs must consider possible future co-habitation. Given the heightened aggression of captive bred A. splendens there is potential for negative effects on S. francesae in mixed species aquaria. This study undertook to reveal whether there was a direct quantifiable inter-specific aggression between captive-bred A. splendens and S. francesae. Results indicate that A. splendens males do not target S. francesae, regardless of density and instead focused their aggression towards other A. splendens males. Suggesting that higher levels of intraspecific aggression in captive A. splendens do not translate into increased levels of interspecific aggression, and specifically that S. francesae are unlikely to be directly impacted by captive A. splendens, at least in the short term.

Keywords: Ameca splendens, Skiffia francesae, behaviour, captive-bred; environmental enrichment.

Resumen. Agresión en captividad e implicancias para la agresión interespecífica entre antiguas especies simpátricas de goodeidos mexicanos. Muchas especies de peces en cautiverio muestran alto grado de agresividad y muchos estudios se han enfocado principalmente en el comportamiento agresivo intraespecífico en lugar de aquel interespecífico. Ameca splendens, un goodeido originario de México, exhibe niveles elevados de agresividad en cautiverio, los que aumentan con la densidad. En este estudio se comparó interacciones agresivas mostradas por A. splendens en ausencia de otras especies con aquellas interacciones agresivas mostradas en presencia de Skiffia francesae especie originalmente simpática, pero actualmente extinta en estado silvestre. Intentos potenciales de reintroducción pueden ser mejorados mediante enriquecimiento del medio, incluyendo la exposición a otras especies con las que eventualmente la especie reintroducida compartirá el hábitat natural. Debido al elevado grado de agresividad de A. splendens en condiciones de cautiverio, existe un impacto potencial negativo que podría perjudicar a S. francesae en cultivos mixtos. En este estudio se cuantificó la agresión de A. splendens sobre S. francesae y se evaluó la influencia de la densidad sobre este comportamiento. No se registraron ataques de A. splendens sobre S. francesae, incluso a elevadas densidades, cuando las tasas de agresividad hacia machos conspecíficos fueron altas. Esto sugirió que niveles altos de agresión intraespecífica mostrados por A. splendens en condiciones de cautiverio, no resultaron en un incremento de agresividad interespecífica. Por lo tanto, A. splendens no impactaría negativamente a S. francesae, al menos en el corto plazo, lo que permitiría la convivencia en cautiverio de ambas especies.

Palabras clave: Ameca splendens, Skiffia francesae, comportamiento, cautiverio, enriquecimiento ambiental.
Introduction

Captive breeding has been shown to promote increased aggression in some species of fish (Kelley et al. 2006, Salonen & Peuhkuri 2006), but most studies focus on the potential detrimental effects this may have on the exhibiting species (Ruzzante 1994, Huntingford 2004, Salonen & Peuhkuri 2006). There is less focus on the potential impact the increased levels may have on other species, which would be of particular importance for those species that may be housed in mixed-species aquaria together.

The butterfly split-fin *Ameca splendens* (Miller & J Fitzsimons 1971), a freshwater livebearing fish from the Goodeidae family, provides an ideal subject for examining interspecific agonistic behaviours in captivity for several reasons. First *A. splendens* show increased aggression in captivity compared to *A. splendens* populations in the wild, and rates of aggressive behaviours increases with density (Kelley et al. 2006). Second like many livebearers *A. splendens* are easily maintained in aquaria and have a long history of use in laboratory studies, ranging from effects of radiation (Rackham & Woodhead 1984), to protein transport through the placenta (Schindler 2003). Third at a maximum of 12 cm in total length (López-López & Sedeño-Díaz 2009) *A. splendens* have potential to dominate many of the potential smaller Goodiedae species tank mates as larger fish have been shown to generally dominate smaller individuals in other species (Rowland 1989, Jaroenutsasinee & Jaroenutsasinee 2001). Additionally, *A. splendens* are considered extinct in the wild (IUCN 2010) and although there is evidence that they still exist in isolated areas (Bailey et al. 2007) captive bred populations remain important to ensuring the survival of the species and potential reintroduction programs.

The Golden skiffia *Skiffia francesae* (Kingston 1978) are also extinct in the wild. Existing only in captivity they are a good choice in tank mates for *A. splendens* in this study particularly as they were naturally sympatric occurring in the Ameca river system where both species were endemic (Kingston 1978). *S. francesae* only reach a 5 cm maximum length are smaller in comparison to *A.splendens* and potential targets for interspecific aggression displayed by *A. splendens*. More importantly however is that there are potential advantages for captive breeding and potential reintroduction programs of *S. francesae* to be gained from keeping with *A. splendens*. Any eventual reintroduction of *S. francesae* may be into habitats where *A. splendens* are present are also to be reintroduced.

The eventual goal of programs of captive breeding is to re-establish wild populations, but reintroduction from captive breeding has inherent problems, with failed reintroductions outnumbering successful ones by 2 to 1 (Fischer & Lindenmayer 2000). Captive reared stocks tend to express inadequate behavioural responses leading to reduced success of foraging, predator avoidance and courtship behaviours (Snyder et al. 1996). These behavioural deficiencies can lead to a reduction in post-release survival rates (Philippart 1995), where captive bred species seem less able to adapt to novel situations, and become less competitive compared to wild populations (Mathews et al. 2005). This is true for many fish species where, although much behaviour is innate, learning plays a large role in their adaptation to their environment (see Brown & Laland 2003, and references therein).

Environmental enrichment is a general term covering a wide variety of methods used to improve the biological functioning and fitness of captive animals and has been suggested as a way to increase post reintroduction success by allowing greater ranges of behaviour to be exhibited by captive species (Newberry 1995, Watters & Meehan 2007). Environmental enrichment methods include: increasing complexity in the physical environment of their enclosures and provide social enrichment opportunities for life-skills training with exposure to other animals as with (Newberry 1995). Indeed pre-conditioning captive species is commonly used to increase post-release survival in reintroduction programs (Brown & Day 2002), and it has been shown that prior exposure to predators and habitat complexity influences behavioural responses in fish (Brown & Wharburton 1997). Some fish have been shown to learn anti-predator responses (Mathis et al. 1996) and improved foraging success (Karplus et al. 2007) from heterospecifics. This suggests that mixed-species rearing in captivity may have an effect on the success in reintroduction attempts. The most pertinent example was a study of captive bred naive *Skiffia multipunctata*, a close relative to *S. francesae* by Kelley et al. (2005). Which found that *S. multipunctata* reared without heterospecifics developed behavioural deficiencies which were not present in *Skiffia multipunctata* that had been raised with heterospecifics.

However if intraspecific aggression due to captivity carries over to increased interspecific aggression the benefits of exposure to heterospecifics may be reduced to the captive
rearing efforts. Moreover any negative effects may be compounded by increasing the fish density inside the aquaria, especially as Kelley et al. (2006) showed that captive-bred male *A. splendens* become more aggressive in aquaria with higher fish densities. This study provides a first step towards potential mixed species rearing by asking whether the increased intraspecific aggression of *A. splendens* in captivity leads in to increased levels of interspecific aggression with *S. francesae* and if the interspecific aggression can be related to fish density.

**Methods**

**Subjects and husbandry**

All fish used in the study were captive bred individuals maintained in the St Andrews Aquarium, Scotland originating from individuals obtained from populations that had been maintained in captivity for multiple generations in the London Zoo.

The *A. splendens* were housed in a large, densely populated, mono-specific display tank with over a hundred and fifty individuals. Prior to the trials a random selection of 43 fish were transferred to a smaller (150cm X 160cm X 55cm deep) holding tank where they were kept as trial candidates, at a density of 0.03 fish per litre. The holding tank was also used as the holding tank for focal males and other fish used in the observations during their ‘rest’ periods between treatments. The holding tank allowed easier recognition and capture of specific fish and the recapture of focal males could be done without prolonging the capture process and causing unnecessary capture stress.

The St Andrews *S. francesae* population consisted of thirty adult fish and they were housed in a separate tank in the breeding room. During capture and relocation fish were handled gently and kept in a constant water temperature. Treatment tanks and holding tanks were maintained at 24°C. Water chemistry was monitored and water changes in all holding tanks were maintained at 24°C. The tanks were in a room with lighting on a 12 hour day/night light cycle with a physical barrier between the two tanks.

Prior to the observational trials ‘focal’ *A.splendens* males, which would be the only fish whose behaviours were recorded, were distinguished based on individual markings on their caudal fins and photographs taken prior to the experimental stage to ensure easy identification during the study. A total of eight focal males were used during the experiments, each of which were returned to the holding tank for at least five days between trials and were never used in consecutive trials.

The observational tanks were stocked with fish before each trial according to the pre-set experimental treatments (Table I) with one focal male, and randomly selected individuals were added to the tanks one at a time to reach the density, gender and species composition required for a particular treatment. Treatments were classified according to a combination of fish density and presence or absence of *S. francesae*, such that there were four treatments: low density without *S. francesae*, low density with *S. francesae*, high density without *S. francesae* and high density with *S. francesae*. Table I summarizes the numbers of fish and total density used in each treatment observational trial included one focal male. Treatments were assigned to the different tanks randomly to eliminate any systematic effects of the tank or tank position on our response variables.

Fish used were between 3.5 and 5 cm (TL) with *A.splendens* individuals selected to correspond with the same size range in mature *S. francesae*. In doing so we attempted to reduce any behavioural bias that may arise due to size. In practice the actual size of the males was on average 4.5 (± 0.6 se) cm in length and the largest *S. francesae* available only reached 3.5 cm, with an average of 3 (± 0.5 se) cm, all sizes are recorded as total length.

**Experimental protocol**

All experiments were conducted during June and July, and observational trials were begun at 2:00 pm. At the start of the experiment trial subjects were released into one of the observation tanks as per the treatment design in Table I and fish were allowed to settle for an hour before feeding. The fish were then left to acclimate for 23 hours before observations.
Table I. The four treatments used were defined by the density of *Ameca splendens*, and the presence or absence of six *Skiffia francesae* in the observation trials.

<table>
<thead>
<tr>
<th>Low density <em>A. splendens</em> only</th>
<th>High density <em>A. splendens</em> only</th>
<th>Low density hetero-specific</th>
<th>High density hetero-specific</th>
</tr>
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<tr>
<td>A focal <em>A. splendens</em> male and just one other male and a female, at a density of 0.05 fish per litre. (3 fish total)</td>
<td>Focal <em>A. splendens</em> male, three other males and three females, with a density of 0.11 fish per litre. (7 fish total)</td>
<td>The same <em>A. splendens</em> composition (as in low density single species treatment) and an additional six <em>S. francesae</em> with a mixed gender ratio. A combined density of 0.15 fish per litre. (9 fish total)</td>
<td>Same as the high density <em>A. splendens</em> tank with an additional six <em>S. francesae</em> of mixed gender ratio. A combined density of 0.21 fish per litre. (13 fish total)</td>
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trials began. After the acclimation phase, fish were fed thirty minutes prior to observations commencing. Ten minute observation trials were then run and repeated a total of three times with 20 minute breaks between observational trials.

During each observational trial the frequency and duration of behaviours of the focal male were recorded using a digital recorder, and Event Logger software from Pisces Conservation was used to record the duration of recorded behaviours. All aggressive interactions involving the focal male were recorded, whether he was the aggressor or the target, and the sex and species of the other participant in the interaction was also recorded. During the trials any aggressive actions involving *S. francesae* by any individual were recorded.

Observed aggressive behaviours were recorded as one of two types, charge or chase and the duration of each behaviour recorded. In addition expressions of foraging and courtship behaviour were also recorded. A description of each the different behaviours is detailed Table II.

There was a 20 minute break between each observation trial. A total of 96 trials were run, with 8 focal males each observed during 12 different trials (three trials per the four treatments). Each of the focal males was returned to the holding tank between treatments for a minimum of four days, and were not used in consecutive trials.

**Statistical analyses**

All instances of the specific behaviours were analysed as variables, including the total number of both chases and charges combined as a count of frequency of aggression. The variables failed the assumptions of normality (Shapiro Wilks test) and for equality of variances (Levene’s test) and were thus analysed using non-parametric methods.

Differences in variables between treatment and density groups were analysed using the Kruskall Wallis test with a post hoc Man Whitney U test.

Kendall’s concordance test was used to determine if there were any consistent correlations between males and the behavioural variables.

**Results**

There was a significant increase in levels of aggression with increasing fish density in the tank (Fig. 1, $\chi^2 = 38.3, df = 3, p <0.001$). The aggression interactions were primarily between *A. splendens*, and there were low levels of agonistic behaviour directed at *S. francesae*. The numbers of aggressive behaviours targeted at *S. francesae* were not significantly different ($\chi^2 = 0.3, df = 1, p=0.56$) between the two densities (Fig. 1).

Separating all the exhibited aggressive behaviours into sustained ‘charge’ and short ‘charge’ types; it appears that the duration and types of aggressive behaviour displayed by the focal males differed with density. The number of charges significantly increase in high density ($\chi^2 = 23.8, df = 1, p-value <0.001$), however the mean number of charges did not differ between densities ($\chi^2 = 2.9, df = 1, p-value = 0.08$) (Fig. 2).

Small and large larvae performed small and reverse movements along the water column. ZCM values for small larvae changed from 17.2 m during the day to 23.2 m during nighttime. Larger larvae migrated from 16.3 to 22 m (Table 2). The thermocline was shallower than during wintertime, and both small and large larvae were positioned in and above it (Fig. 6).

The low frequency of *A. splendens* - *S. francesae* aggression (charges and chases combined) by the focal male show that *A. splendens*
Table II. Description of the recorded behaviours observed in the focal male *Ameca splendens* based on Kelley et al. (2006).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
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<tr>
<td><strong>Agonistic</strong></td>
<td>Male swims directly at an opponent with rapid beats of caudal fin, dorsal fin is compressed. The chase is sustained and the aggressor will move several body lengths.</td>
</tr>
<tr>
<td>Chase</td>
<td>Male bursts at an opponent but forward movement is no more than two fish body lengths. Aggressor rapidly returns to starting point of charge or stops and displays at submissive male. The charge it is not sustained, the male only using one to two beats of caudal fin and his dorsal fin not compressed, often it is fully erect. Some elements of displaying are often incorporated. Both agonistic behaviours may include biting, but this was rare in both cases.</td>
</tr>
<tr>
<td>Charge</td>
<td>Male positions himself in front of or slightly to the side of a female, often with his head down in a 45 degree angle and displays by fanning them out. As the display escalates he will commence rapid continuous wiggles of his caudal and dorsal fins As per Kelley (2006). Courtship normally ended as female swam away or the male stopped.</td>
</tr>
<tr>
<td>Courtship</td>
<td>Forage was counted if the male successfully bit a physical object at either the surface or substrate.</td>
</tr>
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</table>

are not targeting *S. francesae* (Fig.1). Even in low density treatments where the *S. francesae* were the most abundant fish. *S. francesae* targeted aggression by focal *A. splendens* males did not increase with density ($\chi^2 = 0.35$, df = 1, p-value > 0.05). Of the total number of agonistic interactions there were only three occasions where any *S. francesae* was chased and the majority of charges were non-contact. There were no instances where *S. francesae* performed any aggressive behaviour towards any *A. splendens*, and besides the limited incidents of aggression by *A. splendens* to *S. francesae* there were no other behavioural interactions between the two species observed.

Figure. 1. Box plots showing median number of total aggressive interactions (chases + charges) between the focal male and other *Ameca splendens* individuals when *Skiffia francesae* were absent (LowNoS, HighNoS) and present (LowPlusS, HighPlusS) for two densities levels, compared to aggression targeted at *Skiffia francesae* at both densities (lowSkiffia and HighSkiffia). Central horizontal lines represent the median frequency. Interquartile range, max-min and
potential outliers are shown.

Figure 2. Median frequency (central horizontal line in each boxplot) of charges made by the focal males, charge frequency increases in treatments with higher densities. The graph also shows interquartile range, max-min and potential outliers.

Male and female *A. splendens* were both involved in aggressive behaviour, and at low densities there was no significant effect of gender, however at high densities of male *A. splendens* females were significantly less commonly involved in levels of aggression. No strong concordance could be found between focal males and any of the measured variables and removed the possibility of detecting any consistent levels of aggression for males across treatment or density.

**Discussion**

Increasing aggression within species in captivity is well known in fish, and in particular in fish from the livebearer group (Farr & Herrnkind 1974, Martin 1975, Smith 2007) and this study provides further evidence for this, as well as the effect of density on *A. splendens* aggression in captivity, which Kelley et al. (2006) first showed. The findings of this study also suggest that changes in density of conspecifics have a qualitative effect on the agonistic behaviours in this species. As density increases ‘chase’ type exhibitions of aggression become less common, end sooner, and are replaced by shorter ‘charges’. This change in type of aggression is similar to that of *Gambusia affinis*, another livebearer, which exhibited more male-male displays than chases in higher density conditions regardless of female density (Smith 2007). This is likely due to increased territoriality as the density of competitors increase, as has been shown for other captive species such as Atlantic salmon (Blanchet et al. 2006) and mosquitofish (Smith 2007). Related to the increase in conspecific competitors, rates of aggression may increase with an increase in the audience effect. Matos et al. (2003) showed that the presence of a male audience increases the aggressiveness of dominant males and something of this nature may play a role in *A. splendens* behaviour.

Even with the greater levels of aggression in high tank densities the *S. francesae* were subject only to low levels of aggression by *A. splendens*. This may be partly explained by the tendency of *S. francesae* to occupy higher positions in the water column and by the focus of *A. splendens* on aggression amongst conspecifics. Indeed using *A. splendens* to provide *S. francesae* with experience of a more complex ecological community prior to reintroduction to the wild could provide, as suggested in general by Brown & Laland (2001), useful ‘life skills training’ for these endangered fish, and reduce the negative effects of exposure to hetero-specifics, if and when they are reintroduced.
to the wild.

This study does not explore the indirect effects of keeping the two species in captivity, or the potential for longer term effects, and there is the potential for *S. francesae* to be negatively affected by the presence and aggression of *A. splendens*. Competition for example has been found to reduce the survival of species, such as Atlantic Salmon (*Salmo salar*), when reintroduced to fish communities containing species it has historically coexisted with (Ward, et al 2007). Foraging of one species may be affected by competition by another in systems where there is inadequate food supply. Courtship and harassment of female *S. francesae* by *A. splendens* and harassment of gravid individuals is another potential issue, for example interspecific courtship and harassment of female *Skiffia bilineata* by male guppies is known to occur (Valero et al. 2008). Further research will be needed to ensure there is no long term danger in maintaining the two species together in captivity.

It may also be possible for the presence of *S. francesae* to have a negative impact on *A. splendens*. Potential problems may arise due to an increase of the total fish density, causing indirect stress on less dominant *A. splendens* individuals as rates of aggression increase among dominant members. That said, even with a higher fish density of 0.21 fish per litre the rates of aggression between *A. splendens* males did not differ significantly from the rates of aggression in the *A. splendens* only tank with a density of 0.15 fish per litre, which ties in to previous studies which suggest that effects of fish density on behaviour can be species-specific (Saxby et al. 2010).

In conclusion this study shows that, at least in the short term, *A. splendens* does not pose a direct threat to *S. francesae* and they may potentially be maintained in aquaria together. Additionally this study showed that increased intraspecific aggression due to captivity does not necessarily equate to increased levels of interspecific aggression in *Ameca splendens*.

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