Differences in aggression between wild-type and domesticated fighting fish are context dependent

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We investigated differences in aggression between wild-type Betta splendens, Betta smaragdina, two domesticated strains of B. splendens selectively bred for cockfight-like contests and appearance, and Betta sp. mahachai, an unclassified Betta variety. We tested the fish for aggression once they reached sexual maturity as well as during adulthood. We used a combined mirror and video playback test and a mutual-viewing test to measure close-range gill extension and biting attempts as principal measures of aggression. As predicted, the short-finned domesticated strains were significantly more aggressive than wild-type B. splendens. However, the difference in aggression manifested itself only in the mutual-viewing test, which is the test that most closely resembled an actual fight. Betta splendens and the closely related B. smaragdina did not differ in aggression across the experimental contexts; however, B. sp. mahachai responded more aggressively to the combined mirror/video test than B. splendens. Four of the five groups of fighting fish tested responded significantly more aggressively to video images of an aggressive conspecific male than to blank screen controls, suggesting that video playback can be a useful tool for measuring aggression in fighting fish. Our results are relevant for improving our understanding of key aspects of territorial aggression in teleost fish as well as for developing the most reliable ways of measuring them.

Keywords: aggression; Betta spp.; domesticated strain; fighting fish; gill display; mutual viewing; video playback; wild-type

Huntingford (2004) suggested that, compared to the wild-type, domesticated fish might represent the aggressive, risk-taking end of the behavioural spectrum. This may be particularly the case for domesticated fighting fish. In Thailand, bettas have been bred for cockfight-like contests for hundreds of years (Smith 1965). Thai breeders claim that Betta splendens strains selectively bred for fighting by discarding losers and allowing winners to breed are significantly more aggressive than either the wild-type or domesticated veiltail variety (Smith 1965; Simpson 1968; Vierke 1975). The availability of these domesticated strains of fighting fish provides researchers with a unique opportunity to compare and contrast natural and exaggerated forms of aggressive displays (Kelley & Gorlick 1990). Surprisingly, few studies have taken advantage of this opportunity to uncover the key aspects and proximate cause of aggression in teleost fish by systematically comparing wild-type fighting fish with domesticated strains selectively bred for fighting contests. In this study we compared wild-type B. splendens, two B. splendens plakat strains bred for fighting and appearance, the closely related B. smaragdina and B. sp. mahachai, an unclassified fighting fish variety recently discovered in Thailand. We follow convention by referring to the domesticated short-finned variety of B. splendens in our study as ‘plakat’ (Polunas 2005) from the Thai for ‘biting fish’.

According to Thai breeders, success in fighting also depends on rearing conditions and training. Males selected for fighting contests are commonly reared in individual containers with limited visual access to other males. Social isolation affects aggression in B. splendens in

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two important ways. During development it has been linked to an irreversible increase in aggression (Ichihashi et al. 2004), whereas during adulthood it is associated with an initial decrease in aggression coupled with an increase after priming (Halperin et al. 1992; Halperin & Dunham 1993). We compared domesticated *B. splendens* males reared in social isolation with their siblings reared under more social conditions to test our prediction that social isolation rearing increases aggression.

Opercular display, which involves the erection of the gill covers and is often accompanied by the dropping of the branchiostegal membranes (Simpson 1968), is a valid indicator of fighting ability. The amount of time a male spends performing opercular display towards its mirror image or another male in a mutual-viewing test reliably predicts the outcome of actual combats in *B. splendens* (Evans 1985). The biological importance of opercular display, a display shared by various other perciform fish (Gorlick 1990), is underscored by evidence from various fields. For example, McDonald et al. (2004) recorded multiunit-evoked potentials from the optic tectum of *B. splendens* in response to a displaying conspecific male. Distinctive high-frequency bursts of neural activity in the optic tectum were linked to full opercular display by the stimulus male, offering clues about the way fighting fish males may pay particular attention to opercular display. This display is costly because it severely limits the ability of fish to ventilate their gills to extract oxygen from water, and is therefore likely to be an honest signal of aggression (Abrahams et al. 2005). In this study we focused on close-range opercular display to test our prediction that the fighter plakat strain is more aggressive than the wild-type *B. splendens* and to compare aggression among five fighting fish species and strains.

Video playback, a technique that over the past decade has been increasingly used in research on aggression in fish (e.g. Rowland et al. 1995; Clement et al. 2005), has not yet been widely used in research on fighting fish. Recent reviews have outlined the pros and cons of the use of video stimuli in behavioural biology (Fleishman et al. 1998; McGregor 2000; Rosenthal 2000). The advantages of video images include the ability to manipulate them to control for behavioural variation between individuals, and to reduce the number of animals needed in an experiment (Trainor & Basolo 2000). However, some species have different spectral sensitivities, critical flicker-fusion thresholds, visual acuity or depth perception to those of humans, and may perceive video images differently to us (D’Eath 1998; Fleishman et al. 1998).

There is little information about aspects of the visual system of *Betta* species that are relevant to their suitability for video playback studies. We do know, however, that under optimal conditions of temperature and stimulus intensity the flicker-fusion rate of domesticated *B. splendens* is 30–35 which is not very different from our own (Douglas & Hawryshyn 1990). Allen & Nicoletto (1997) found that domesticated *B. splendens* males respond strongly to video animations of both long- and short-finned males. One way to validate video playback is to confirm that the subject animal responds to the video images in a comparable way as to the real stimulus (D’Eath 1998). Therefore, we compared aggressive display to video images with display to a mirror and to a live conspecific across a transparent barrier.

**METHODS**

**Study Species**

We studied the following fighting fish species and strains: *B. splendens* wild-type, *B. splendens* fighter plakat, *B. splendens* fancy plakat, *B. smaragdina* wild-type, and *B. sp. mahachai*. The fighter plakat is a short-finned domesticated strain of *B. splendens* that has been bred for cockfight-like contests. The fancy plakat is a short-finned domesticated strain bred for body type and coloration. It is derived from plakat strains bred for fighting and reflects its ancestry by being notably more aggressive than other strains of *B. splendens* bred for appearance, including the veiltail variety (Polumas 2005). *Betta smaragdina* is closely related to *B. splendens* (Rüber et al. 2004), and has also been domesticated for fighting and for the aquarium hobby market. *Betta* sp. mahachai was only recently discovered in the swamp forest habitat of the Mahachai area of Samutsakorn Province in Thailand and has not been recognized as a species. Whether this fish is indeed a new *Betta* species or a domesticated crossbred variety that was released into this habitat is unknown. All fish used in this research were bred and reared in our laboratory. The breeding stock of the fancy plakat was purchased from a local retailer and all other breeding stock was purchased from a well-established commercial breeder/exporter in Thailand.

**Rearing and Maintenance**

Spawn reared from the breeding stocks was moved to grow-out tanks (72 x 45 cm and 23 cm deep) at 30 days after hatching. After 60 days in the grow-out tanks males were individually housed in 1.5-litre clear plastic bottles. The bottles were separated by white corrugated plastic partitions to prevent continuous mutual viewing. The white plastic also reduced any ‘mirror’ effect of the reflection of the inner surface of the bottles. Every day we removed all partitions from selected rows, allowing the males in those rows a full day of mutual viewing. The partitions of the other rows were removed for 2 h only. The bottles were rotated through the rows on a daily basis. This regime allowed the males 2 h of exposure to their rotating left and right neighbours every day, and a full day of social exposure about every 8 days. It was maintained until testing and resumed after testing. All males had visual access to the daily activities in the laboratory at all times.

Ten males of each species/strain were randomly selected for testing and maintained among their male siblings until testing. During the test period the partitions between the bottles were removed for 2 h per day only.

To test the effect of social isolation on aggressive behaviour, we put 10 randomly selected males from the fancy plakat spawn into a different rearing and maintenance regime. These males were also housed in 1.5-litre bottles separated by white corrugated plastic partitions.
from day 90, but the bottles were stored in containers that restricted the males’ visual access to the laboratory. For this group of males the partitions between the bottles were removed for 30 min each day only. This regime remained in force throughout the study period. Although the relative social isolation was probably accompanied by a certain degree of stress, the social isolates were protected from the more severe stress that can accompany full sensory deprivation. They were allowed limited social exposure each day and had limited visual access to the laboratory. Other than the rearing condition, the maintenance conditions for these isolates did not differ from those of the other fish in this study.

All fish were fed daily with freshly hatched nauplii of *Artemia salina* (twice daily up to approximately 5.5 months after hatching, once daily thereafter). Tank water temperature was maintained at 25°C. The fish laboratory was illuminated by overhead fluorescent light with lights on at 0700 and off at 2100 hours.

The experiments were reviewed and approved by the Committee on the Ethics of Animal Experiments (CEAE) at the University of Miyazaki. No special licence was required. After the experiments, the fish were kept as part of an ongoing longitudinal project on fighting fish behaviour and physiology.

**Materials**

**Video stimulus materials**

Aggressive display by one male of each species/strain in the study was recorded with a Canon DM-FV M20 digital video camera. The video materials were edited with iMovie (Apple Computer, Inc., Cupertino, CA, U.S.A.) on a Macintosh G3 computer. We prepared stimulus movies with conspecific display for *B. splendens*, *B. smaragdina* and *B. sp. mahachai* and an additional movie for both domesticated *B. splendens* strains, featuring display by a fighter plat-kat male. Each edited 15-min display stimulus movie contained 5 min of blank screen, followed by 5 min of aggressive display, followed by 5 min of blank screen (Allen & Nicoletto 1997). Care was taken to edit display movies of similar behavioural content and intensity, while maintaining continuity between sequences (Rosenthal 2000). Table 1 presents the duration (s) of fin and opercular display shown in each 5-min stimulus movie. With the exception of the *B. smaragdina* movie, which featured more fin display and less opercular display than the other three movies, the stimulus movies were similar in display content. The four stimulus movies were burned on to separate DVDs.

**Apparatus**

The test tank for the mirror/video test consisted of a glass tank (20 × 20 × 20 cm) covered on the back, left and bottom sides with white plastic. The right wall was partially covered with white plastic leaving an open space of 10.5 × 7 cm to fit a mirror as well as the 9.5 × 5.3-cm screen of a portable DVD player (Bluedot Model BDP-4860). The front wall of the test tank was left uncovered allowing video recording of the experiment. The test tank was positioned on wooden blocks, allowing the portable DVD player to slide under it so that the screen could be positioned flush against the tank wall. The tank contained a ceramic heater preset at 25°C and a thermometer, both positioned on the far left-hand side of the tank. When played back on the portable DVD player’s screen the stimulus male appeared close to life size.

**Procedures**

**Mirror/video test**

The mirror/video test consisted of a 15-min exposure to a mirror immediately followed by the presentation of the 15-min display stimulus movie described above. The actual stimulus presentation test therefore lasted 30 min. Switching to the DVD player from the mirror took about 25 s and we did this as quietly as possible so as not to disturb the subject. The fish had not been exposed to a mirror or video presentation prior to the first test and were thus initially naive to the experimental conditions. They were not used for other behavioural experiments during this study.

We tested the fish in random order. Before testing, the fish were netted from their home tank and rapidly transferred to the test tank. They were allowed to habituate overnight to the test tank. The test was started when the fish oriented towards the mirror. All mirror/video tests were recorded on video with the digital video camera described above.

**Mutual-viewing test**

The mutual-viewing test was conducted with two test tanks as described for the mirror/video test minus the white plastic covers. The two tanks were positioned flush against each other and a white opaque plastic sheet was inserted between the two adjacent walls to prevent mutual viewing until testing. Before the test the fish were netted, quickly transported from their home container to the test tank and allowed to habituate for up to 5 h. Considering the results we obtained with this test it appears that 3–5 h is sufficient habituation time for fighting fish in our laboratory to engage in territorial aggression as soon as mutual viewing in this arrangement is allowed. Pairs of males of the same species/strain were matched by their

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Table 1. Display stimulus movie scenarios

<table>
<thead>
<tr>
<th>Movie</th>
<th>Fin display Duration (s)</th>
<th>Fin display %*</th>
<th>Opercular display Duration (s)</th>
<th>Opercular display %*</th>
<th>Total display Duration (s)</th>
<th>Total display %*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. sp. mahachai</em></td>
<td>103.2</td>
<td>34</td>
<td>115.8</td>
<td>57</td>
<td>219.0</td>
<td>73</td>
</tr>
<tr>
<td><em>B. splendens</em></td>
<td>81.6</td>
<td>27</td>
<td>172.2</td>
<td>57</td>
<td>253.8</td>
<td>85</td>
</tr>
<tr>
<td><em>B. smaragdina</em></td>
<td>200.4</td>
<td>67</td>
<td>57.0</td>
<td>19</td>
<td>257.4</td>
<td>86</td>
</tr>
<tr>
<td>Fighter/fancy</td>
<td>95.4</td>
<td>32</td>
<td>114.9</td>
<td>38</td>
<td>210.3</td>
<td>70</td>
</tr>
</tbody>
</table>

*Percentage of total movie (300 s).*
mean total opercular display shown in the mirror/video tests. With the exception of one fighter plakat male (see below) all available males were tested only once in the mutual-viewing test. To start the test we removed the opaque partition and the ensuing aggressive interaction across the transparent sides of the adjacent tanks was then video recorded for 25 min.

As fighting fish are sensitive to even minor changes in experimental conditions (Halperin et al. 1992), we standardized testing and maintenance conditions. All tests were conducted during the afternoon hours. As for the holding tanks, water temperature of the test tanks was maintained at 25°C. Mean pH of the water in the test tanks was 6.99 ± 0.02 during the first mirror/video test, 7.17 ± 0.02 during the second mirror/video test and 7.11 ± 0.03 during the mutual-viewing test. Periodic checks of the holding tanks showed that pH values were within the same range as in the test tanks.

Test schedule and age at testing

We conducted two mirror/video tests separated by 6 months. The mutual-viewing test was carried out 12 months after the first mirror/video test. To test species and strain differences, we initially selected 50 males for testing (10 per species/strain). During the scoring of the videos of the first mirror/video test (see below), however, we found that four males did not display during the test. These males (one B. sp. mahachai, two wild-type B. splendens and one fancy plakat) were removed from further analyses, leaving 46 males for the first mirror/video test. During the 6 months separating the first and second round of mirror/video tests some males died of natural causes, leaving 36 males for the second mirror/video test and 34 for the mutual-viewing test (see the Results for numbers per species/strain for each analysis). One fighter plakat male was tested twice in the mutual-viewing test to complement the even sample required for this dyadic test; however, only the first test of this male was scored and used for the analysis. The average ages at testing (days from hatching) ± SE were: mirror/video test 1: 166.7 ± 1.1 days; mirror/video test 2: 415.5 ± 5.02 days; mutual-viewing test: 471.1 ± 5.8 days.

For the part of our study testing the effects of rearing condition, 10 fancy plakat males were tested during the first round of testing and eight were available for both the second mirror/video test and the mutual-viewing test. The average ages at testing for the males reared and maintained in social isolation were as follows: mirror/video test 1: 187.7 ± 2.2 days; mirror/video test 2: 386.3 ± 1.6 days; mutual-viewing test: 399 days.

Video scoring

Video recordings of the test sessions were viewed in random order for scoring by P.V. using ODLog (Macropod Software, Armidale, NSW, Australia). The following mutually exclusive behaviours were scored: (1) fin display: erection of the dorsal, caudal and anal fins (Simpson 1968); this was often accompanied by full or partial erection of the pelvic fins but scoring of fin display was not made contingent on this behavioural element; (2) opercular display: erection of the gill covers (branchiostegal display, Simpson 1968); (3) attempted bites directed at the stimulus wall or opponent in the adjacent tank; (4) approach/no display: the fish was oriented towards the stimulus wall/opponent and swam towards it or was idle; (5) withdraw/no display: the fish was oriented in the opposite direction to the stimulus wall/opponent and moved away from it or was idle. In addition, the position of the fish in the test tank was noted for each of these behaviour patterns with the use of lines drawn on the glass of the video monitor. We scored fish as being within or more than one fish length of the stimulus wall.

Preliminary Analyses

Response to video

We first confirmed that fighting fish males respond aggressively to video of a displaying conspecific male. For this we compared aggressive display observed before and after control conditions (blank screen) with display observed during the video presentation of a displaying conspecific male. We conducted a two-way ANOVA, with video presentation (control 1, display video, control 2) as the within-subjects factor and species/strain as the between-subjects factor. We found a significant main effect for video presentation ($F_{2,88} = 35.36, P < 0.0001$) but not for species/strains ($F_{4,88} = 1.02, P = 0.410$) nor for the interaction ($F_{8,88} = 1.50, P = 0.168$). Bonferroni tests showed significant differences between the two controls and the display video for B. splendens, B. smaragdina and the fancy plakat (Fig. 1). In addition, the fighter plakat displayed significantly more often during the display video than during control 2 (Fig. 1).

Longitudinal measures

We tested the relation between the two sets of data from the mirror/video tests 1 and 2 with Pearson partial correlation, controlling for group. We focused on aggressive display within one fish length of the stimulus wall (cf. McGregor et al. 2001). Time spent within one body length of an opponent behind glass predicts dominance in later physical fights (Bronstein 1994). Furthermore, time spent in close-range opercular display is a reliable indicator of a male’s ability to win an actual fight (Simpson 1968; Evans 1985).

Both close-range opercular display (mirror condition: $r_{42} = 0.62, P < 0.0001$; video condition: $r_{42} = 0.35, P = 0.021$) and bite attempts (mirror condition: $r_{42} = 0.60, P < 0.0001$; video condition: $r_{42} = 0.26, P = 0.085$) were moderately to highly stable within individuals across time. In contrast, close-range fin display was not stable over time (mirror condition: $r_{42} = 0.07, P = 0.636$; video condition: $r_{42} = 0.10, P = 0.506$). We combined the data of the developmentally stable behaviours from both tests for subsequent analyses and excluded fin display.

Aggression scores

To obtain single aggression scores for the mirror/video test we carried out principal component analysis (PCA) on
the two developmentally stable aggressive behaviours: opercular display within one fish length of the stimulus wall opponent in (1) the mirror condition and (2) the video condition, and attempted bites to the stimulus wall opponent in (1) and (2). The PCA generated two components (Table 2). The first principal component (PC1; eigenvalue = 1.82) primarily consisted of opercular display in the mirror condition and bite attempts in both the mirror and video condition. As opercular extension in the video condition also loaded on to PC1, this component reflects a comprehensive aggressive response. The second principal component (PC2; eigenvalue = 1.06) comprised primarily opercular display in the video condition and as such represents a selective response to the video component of the combined mirror/video test.

In the mutual-viewing test opponents represent non-independent pairs and the scores of the opponents were significantly correlated (Pearson partial correlation, controlling for group: opercular extension: $r_{15} = 0.93$, $P < 0.0001$; attempted bites: $r_{15} = 0.64$, $P = 0.003$). We decided to average the behaviour of the males in an opponent pair and conducted subsequent statistical analyses on these averages. To obtain a single aggression score for the mutual-viewing test we also carried out PCA on close-range opercular display and bite attempts. The one principal component generated by the analysis (eigenvalue = 1.48) explained 73.85% of the variance.

Data were inspected for normality and unequal variances before each analysis. Nonparametric tests were substituted for parametric tests when appropriate. We used two-tailed tests with alpha set at 0.05. For analyses we used GraphPad Prism (GraphPad Software, Inc., San Diego, CA, U.S.A.) and SPSS (SPSS, Inc., Chicago, IL, U.S.A.).

## RESULTS

### Mirror/Video Test

#### Species and strain comparison

Comprehensive aggressive behaviour in the combined mirror/video test (PC1 score) differed significantly between the groups (Kruskal–Wallis test: $H_4 = 18.25$, $P = 0.001$). Planned comparisons using Dunn’s multiple comparison test, comparing $B. splendens$ ($N = 8$) to the other species and strains, showed that, contrary to our prediction, the domesticated $B. splendens$ strains (fighter, $N = 10$; fancy, $N = 9$) did not behave more aggressively than wild-type $B. splendens$ (Fig. 2). However, $B. sp$. mahachai males ($N = 9$) were significantly more aggressive than wild-type $B. splendens$ ($P < 0.05$).

The species and strains did not differ on the PC2 score reflecting selective opercular responding to the video stimulation (Kruskal–Wallis test: $H_4 = 8.46$, $P = 0.076$).

#### Social versus isolate rearing

Males of the fancy plakat strain of $B. splendens$ reared in social isolation ($N = 10$) responded significantly more aggressively to the mirror and video stimulation than their siblings from the same spawn ($N = 9$) that were socially reared (unpaired $t$ test with Welch’s correction: PC1 score: $t_{18} = 2.50$, $P = 0.032$; Fig. 3; PC2 score: $t_{11} = 3.52$, $P = 0.005$).

### Mutual-viewing Test

#### Species and strain comparison

At the time of the mutual-viewing tests only four fancy plakat males remained. As the factor scores of males of the two domesticated $B. splendens$ strains for the
mutual-viewing test (fighter, \(N = 9\); fancy, \(N = 4\)) did not differ significantly (unpaired \(t\) test: \(t_{11} = 1.24, P = 0.24\)), the two groups were combined into one group (plakat) for the present analysis. The three wild-type groups and domesticated strain differed significantly in aggressive behaviour observed during the dyadic interactions (ANOVA: \(F_{3,29} = 5.29, P = 0.005\)). Dunnett’s planned comparisons showed that the domesticated strain (\(N = 13\)) was significantly more aggressive than the wild-type \(B. splendens\) (\(N = 6\); \(P < 0.05\); Fig. 4). No differences were found between \(B. splendens\) and \(B. smaragdina\) (\(N = 8\)), nor between \(B. splendens\) and \(B. sp. mahachai\) (\(N = 6\)).

Social versus isolate rearing

Contrary to the result obtained in the mirror/video test, the fancy plakat isolates (\(N = 8\)) did not differ from their socially reared siblings (\(N = 4\); \(t\) test: \(t_{10} = 1.78, P = 0.11\)) but the trend was towards a stronger response in the socially reared fancy plakat males. This nonsignificant result was replicated when the isolates were compared to the combined plakat group (\(N = 13\); see above; \(t\) test: \(t_{19} = 1.86, P = 0.079\)).

DISCUSSION

Our results are relevant both for improving our understanding of the key aspects of territorial aggression in teleost fish and for developing the most reliable ways for measuring them. We were able to confirm our prediction that domesticated \(B. splendens\) males of the short-finned plakat variety are significantly more aggressive than wild-type \(B. splendens\). However, our results also showed that this difference depends on the nature of the aggressive stimulus. The difference in aggression between wild-type \(B. splendens\) and the plakat strains manifested itself only in the mutual-viewing test in which males were confronted with an interactive conspecific male. Neither its own mirror image nor a video image of an aggressive conspecific male was sufficient to demonstrate reliably the selective breeding-enhanced aggressiveness in the domesticated \(B. splendens\) plakat strain.

We believe that our findings underscore the importance of visual cues in fighting fish territorial aggression. Evidence of the key role of visual perception in fighting fish aggression and reproductive behaviour comes from various sources. For example, Robertson & Sale (1974) showed that \(B. splendens\) males can discriminate between the sexes solely by the use of visual stimuli. Eavesdropping studies show that domesticated \(B. splendens\) males and females extract information from viewing interactions between conspecifics (e.g. Oliveira et al. 1998; Doutrelant & McGregor 2000; McGregor et al. 2001). In addition, aggression intensity of domesticated \(B. splendens\) in mutual-viewing tests can vary as a function of watching other males fight (Matos & McGregor 2002) or being watched by other males (Dzieweczynski et al. 2005, 2006).

The three types of stimuli we used each presented different visual contexts and elicited different levels of
aggressive behaviour. In the mirror/video test the mirror image presented a perfect correlation with the subjects’ behaviour, whereas the video images of a conspecific male could correspond with the subjects’ actions only by chance. While faced with a live opponent in the mutual-viewing test, males of each of the species and strains in our study demonstrated the reciprocal nature of fighting fish territorial aggression as contesting pairs showed significantly correlated aggressive behaviour. Furthermore, being confronted visually, but not physically, with a live interactive opponent effectively elicited the predicted higher mean levels of aggression in the plakat males compared to the wild-type males. The *B. splendens* plakat males thus showed the most intense aggression in an experimental context that, from a visual perception perspective, most closely resembled an actual fight.

Providing partial confirmation for our prediction, the fancy plakat males reared and maintained in relative social isolation showed significantly more intense aggression in the combined mirror/video test than their socially reared plakat siblings. In contrast, they did not differ from their socially reared siblings in the mutual-viewing test. The main difference between the two groups seems to be that the isolates showed similar levels of aggression in each of the three experimental settings, whereas their socially reared siblings showed relatively less aggression in response to the video presentation, that is, compared to the mirror and live conspecific male. Being deprived of regular social stimulation thus resulted in high levels of aggression in the isolates, independent of the type of visual stimulus provided. In contrast, the socially reared plakat males were most aggressive when presented with a stimulus object that, from a visual perspective, either provided perfect correspondence (mirror image of self) or close correspondence (live conspecific interacting across a transparent barrier) to their own behaviour.

Sexual maturity in domesticated *B. splendens* is estimated to occur around 110–120 days after hatching (Kirankumar & Pandian 2002; Reyes-Bustamante & Ortega-Salas 2002). Somatic growth is completed around day 200, and the maximum linear body length is attained around day 210 (Kirankumar & Pandian 2002). This is the approximate age of many of the domesticated *B. splendens* males available in pet stores, because at this age male bettas show maximum fin length and bright colours which significantly increase their sales potential. As only domesticated males and not females have significant sales potential, large-scale commercial breeders seek ways to increase their yield of male fish. For example, normal females can be sex reversed upon hormone administration (synthetic or natural androgens) during the sexually labile period of development, around 40 days from the first day of feeding (Kirankumar & Pandian 2002). Some of the males sold in large pet stores are probably sex-reversed males. When they reach sexual maturity, sex-reversed males show significant deficiencies in their reproductive behaviour (Kirankumar & Pandian 2002). As the aggressive behaviour of domesticated fighting fish may be related to their reproductive behaviour (Kavumpurath & Pandian 1992; Dziewcezynski et al. 2006), pet shop males may not be the best subjects for studies on fighting fish aggression. In fact, up to 15% of some samples of the domesticated veiltail variety do not show aggressive behaviour towards a same-sex conspecific (Bronstein 1994).

In the present study we used wild-type and domesticated fighting fish males reared and kept in our laboratory under standardized conditions, and tested them at about a month after reaching sexual maturity and again during adulthood. Our results complement findings in cichlids suggesting that certain aspects of aggressive behaviour in territorial teleosts are developmentally labile whereas others are stable (e.g. Francis 1990; Budav et al. 1999). Our study shows that individual differences in opercular display and biting intensity appear to be stable, at least as of sexual maturation, in both wild-type and domesticated *B. splendens*, as well as in the closely related *B. smaragdina* and the unclassified *B. sp. mahachai*. In contrast, fin display proved to be developmentally unstable in the fighting fish in our study. Taken together, these findings confirm the importance of studying detailed aspects of aggressive behaviour and their development, as opposed to taking a wholesale approach to aggression. Furthermore, these findings underscore the need for more longitudinal developmental research to gain a better insight into the proximate cause of teleost territorial aggression.

When compared to the closely related *B. smaragdina*, the wild-type *B. splendens* males in our study showed similar levels of aggression in each experimental condition. In contrast, *B. sp. mahachai* males showed significantly higher levels of aggression than wild-type *B. splendens* males in the combined mirror/video test but not in the mutual-viewing test. The difference primarily stems from response to the mirror, as *B. sp. mahachai* did not respond much to the video and was the sole group not to show significantly more aggression during the display video than during the video controls. More comparative research on *B. sp. mahachai* and wild-type *B. splendens* aggression can help determine whether one is more aggressive than the other and whether *B. sp. mahachai* is a separate species or a crossbred variety.

In our study, fighting fish males of two different species and two domesticated strains responded aggressively to video images of a displaying conspecific male. We believe that this research shows that video playback can be a useful tool to investigate specific aspects of the aggressive display of fighting fish. In his landmark paper on the aggressive display of *B. splendens*, Simpson (1968) convincingly showed that to appreciate fully the aggressive display of fighting fish we need to focus on its reciprocal nature. Mirror image stimulation cannot reproduce the signal exchange associated with males visually and/or physically confronting each other. In contrast, interactive video techniques are now technically feasible and could be useful for looking at the signal value of opercular display or other important display elements (McGregor 2000). Furthermore, as video playback allows an interactive stimulus to be kept constant across subjects, the technique appears especially well suited for the study of individual differences in fighting fish aggression, which, compared to research in other teleost fish, remains an understudied area.

During the past six decades aspects of the aggressive display behaviour (e.g. Simpson 1968; Bronstein 1994;
Polnau & Ma 2001), reproductive behaviour (e.g. Bronstein 1994; Jaroensutasinee & Jaroensutasinee 2001a, b), social information-gathering ability (Douretlant & McGregor 2000; Douretlant et al. 2001), physiology (Ganzer et al. 1984; Leitz 1987; Haller & Wittenberger 1988) and neuro-anatomy (Marino-Neto & Sabbatini 1988; Gorlick 1990) of fighting fish have been extensively studied. Surprisingly, few studies have set out to test the age-old claim of Thai breeders that domesticated plakat males show significantly more stamina and vigour in their aggressive display and fighting behaviour than either the wild-type B. splendens or domesticated veiltail variety (cf. Smith 1965; Simpson 1968). Although the small sample size of our study warrants some caution in interpreting our results, we believe that our preliminary study suggests that differences in aggression between wild-type B. splendens and fighter plakat males can be experimentally shown. As such we believe that future comparative studies of wild-type and fighter plakat B. splendens can serve as a model system for manipulations that explore proximal causes of aggressiveness in fish.

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References


