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To breathe or fight? Siamese fighting fish differ when facing a real opponent or mirror image

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1 **To breathe or fight? Siamese fighting fish differ when facing a real opponent or mirror**
2 **image.**

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25 **Abstract**

26 Displays are a feature of animal contest behaviour and have been interpreted as a means of
27 gathering information on opponent fighting ability, as well as signalling aggressive
28 motivation. In fish, contest displays often include frontal and lateral elements, which in the
29 latter involves contestants showing their flanks to an opponent. Previous work in a range of
30 fish species has demonstrated population-level lateralization of these displays, preferentially
31 showing one side to their opponent. Mirrors are commonly used in place of a real opponent to
32 study aggression in fish, yet they may disrupt the normal pattern of display behaviour. Here,
33 using Siamese fighting fish, *Betta splendens*, we compare the aggressive behaviour of males
34 to a mirror image and real opponent behind a transparent barrier. As this species is a
35 facultative air-breather, we also quantify surface breathing, providing insights into underlying
36 fight motivation. Consistent with previous work, we found evidence of population-level
37 lateralization, with a bias to present the left side and use the left eye when facing a real
38 opponent. Contrary to expectations, there were no differences in the aggressive displays to a
39 mirror and real opponent, with positive correlations between the behaviour in the two
40 scenarios. However, there were important differences in surface breathing, which was more
41 frequent and of longer duration in the mirror treatment. The reasons for these differences are
42 discussed in relation to the repertoire of contest behaviour and motivation when facing a real
43 opponent.

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45 **Keywords:** aggression, contests, lateralization, mirrors, surface breathing.

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49 **1. Introduction**

50 Although there is considerable taxonomic variation in contest behaviour (Arnott and Elwood
51 2009a; Hardy and Briffa 2013), displays typically precede and intersperse with escalated
52 fighting behaviour. These displays are usually interpreted as providing a means of gathering
53 information on the size and fighting ability, termed resource holding potential (RHP), of an
54 opponent (Parker 1974). However, evidence to support this interpretation is often lacking
55 (Taylor and Elwood 2003; Arnott and Elwood 2009a; Elwood and Arnott 2012, 2013; Fawcett
56 and Mowles 2013). An alternative view is that they serve to signal information about the
57 aggressive motivation of each contestant (Camerlink et al. 2015).

58 The preflight display phases are typically dynamic processes involving the contestants
59 interacting in a particular pattern. There are numerous examples across diverse taxa, and for
60 invertebrates these include; the preflight cheliped displays of hermit crabs (Elwood et al.
61 2006), the waving of the enlarged claw during fiddler crab contests (Backwell et al. 2000) and
62 of legs in spiders (Elwood and Prenter 2013). Commonly cited vertebrate examples include;
63 the stereotyped displays of lizards (Van Dyk and Evans 2008) and frogs (Reichert and
64 Gerhardt 2014), the mutual vocal displays occurring between male red deer (Clutton-Brock
65 and Albon 1979) and fallow deer (Jennings et al. 2012), as well as the conspicuous lateral
66 visual displays termed parallel walks occurring in these ungulates (Jennings and Gammell
67 2013).

68 Aggressive displays have been studied extensively in fish species, which lend
69 themselves to laboratory and field studies (e.g. Enquist et al. 1990). The contest displays of
70 many fish comprise frontal displays and lateral displays, which in the latter involves
71 contestants showing their flanks to an opponent. During lateral displays, fish can align in two
72 ways, with their heads either facing in the same direction (head to head) or in opposite

73 directions (head to tail). Moreover, a range of fish species has been shown to exhibit
74 population-level lateralization, preferentially showing one side to their opponent (Bisazza and
75 de Santi 2003; Reddon and Balshine 2010). For example, competing convict cichlids,
76 *Amatitlania nigrofasciata*, more commonly show their right than their left flank (Arnott et al.
77 2011; Elwood et al. 2014). When both contestants show their right side the head to tail
78 configuration results and this is more common than the head to head configuration (Arnott et
79 al. 2011). Such population-level lateralization of displays thus provides some predictability
80 and enables coordination of these agonistic interactions (Ghirlanda et al. 2009), potentially
81 facilitating a mutual assessment process (Arnott et al. 2011). Additionally, it may also act to
82 reduce the likelihood of injury should one fish escalate the contest (Rogers 1989; Bisazza et
83 al. 2000; Arnott et al. 2011). The importance of coordinating displays in fish, such as head to
84 tail positioning, can be examined by comparing mirror images to live opponents behind a
85 transparent partition (Elwood et al. 2014), because with a mirror the fish can never align in
86 the head to tail configuration and this key feature of the mutual display is lost.

87 Nevertheless, mirrors are frequently used instead of a live opponent in studies on
88 aggression in fish (Cantalupo et al. 1996; Earley et al. 2000; Wilson et al. 2011; Balzarini et
89 al. 2014). Mirror images might be a good choice of stimuli when repeated measures design
90 require several bouts and opponent variability can be avoided. However, the validity of using
91 mirrors for studying contest behaviour has been questioned. For example, mirror images fail
92 to elicit the same brain gene expression (Desjardins and Fernald 2010) or the same hormonal
93 responses (Oliveira et al. 2005) as live opponents. Furthermore, Elwood et al. (2014) recently
94 compared the displays of convict cichlids to a mirror and a real opponent, finding a lower
95 frequency of displays to a mirror but with individual displays of greater duration. This slower
96 pace of the interaction to a mirror suggested that social responses from opponents are a key

97 component necessary to elicit the normal repertoire of contest behaviour, as has also been
98 suggested for lizards (Ord and Evans 2002). The presence of an appropriately responding
99 opponent during aggressive displays thus appears to be a key driver necessary for the
100 interaction to progress.

101 Although only focussing on displays in their study, Elwood et al. (2014) predicted that
102 the disruption of normal display behaviour caused by the mirror may lead fish to abandon
103 displays in favour of other activities, including other forms of aggression such as biting. This
104 is one focus of the current study. In addition, Elwood et al. (2014) confirmed previous
105 findings of population-level lateralization in the convict cichlid (Arnott et al. 2011), with a
106 right-sided lateral display bias that was evident to both a real opponent and when facing a
107 mirror. With Siamese fighting fish, *Betta splendens*, interacting with real opponents and when
108 facing a mirror image there was evidence of population-level right side bias (Bisazza and de
109 Santi 2003), but this was not found in other studies with a mirror (Cantalupo et al. 1996;
110 Takeuchi et al. 2010). A recent study demonstrated a population-level right side preference to
111 real opponents during early reproductive stages but not at late stages (Forsatkar et al. 2015).
112 Indeed, some individuals switched from a right side bias to a left side bias after spawning thus
113 illustrating the confusion about laterality in this species. Here we use *B. splendens* in mirror
114 and real opponent tests to examine population-level lateralization. Further, we test the
115 predictions of Elwood et al. (2014) concerning the utility of a mirror image in eliciting normal
116 contest behaviour and, in particular, if the motivation to compete is the same to a mirror as to
117 a real opponent. *B. splendens* offers a widely used model for studies on aggression (e.g.
118 Simpson 1968). Their pre-fight displays consist of raised opercula and spread fins during
119 frontal displays and lateral displays (Castro et al. 2006). The frontal displays impair
120 respiration and are physiologically demanding such that contestants will interrupt the display

121 sequence at intervals to engage in surface breathing (Regan et al. 2015).

122 We recorded the frequency, total duration and the median duration of left lateral
123 displays, right lateral displays, frontal displays, and surface breathing, as well as attempted
124 bite frequency. The frequency and median duration should relate positively and negatively to
125 vigour whereas total duration is the product of frequency and median duration and is the more
126 commonly used measure of displays. Using this information we examine four key predictions.
127 First, consistent with the findings of Elwood et al. (2014), we predict there will be evidence of
128 population-level lateralization when displaying to both a mirror and real opponent. Second,
129 we predict the mirror will elicit a decreased frequency of displays and longer duration of
130 individual displays than when facing a real opponent. Third, we predict if the mirror impairs
131 the normal sequence of displays, individuals may switch to more escalated aggression
132 revealed by increased levels of attempted biting in the mirror treatment. Fourth, we examine
133 the frequency and duration of surface breathing to gain insights regarding the effectiveness of
134 mirrors compared to real opponents for eliciting agonistic displays. Because breathing rate has
135 been linked to the vigour of displaying (Regan et al. 2015), we predict that should the display
136 vigour differ between real and mirror opponents, there will be more surface breathing in the
137 condition with higher vigour. However, there are two other aspects that might influence
138 surface breathing. First, if the mirror offers a substandard stimulus resulting in a lower level
139 of aggressive motivation then this might cause the fish to switch to breathing more frequently
140 in the way that male newts breathe more readily when courting an unresponsive female
141 (Halliday and Sweatman 1976). Note that the vigour of the displays might not necessarily
142 differ with motivation because animals might not signal future intentions about persistence in
143 contests (Dawkins and Krebs 1978). Another way that surface breathing might differ between
144 real and mirror opponents is that fish might take their cues to breathe by the breathing

145 attempts of the opponent. With a mirror the apparent opponent will not be the first to go to the
146 surface and will not be the first to resume displaying following surface breathing. This might
147 disrupt the pattern of breathing when compared to a real opponent. Using the information on
148 levels of surface breathing, coupled with information on contest behaviour, should enable us
149 to disentangle which of the above scenarios is correct.

150

151 **2. Methods**

152 *2.1. Animals*

153 Thirty male, *B. splendens* were obtained from a local supplier (Grosvenor Tropicals, Belfast,
154 U.K.) in batches of six size-matched subjects and housed in individual glass tanks measuring
155 30 x 20 cm and 20 cm high, with approximately 2 cm depth of gravel. A controlled artificial
156 12:12 h light:dark cycle was in place, tanks filled with 8 litres of treated tap water, aerated for
157 30 minutes per day, and maintained at a temperature of 21-23°C. Fish were fed daily with
158 flake food, and on the day of experimental trials were fed after observations had concluded.

159

160 *2.2. Experimental protocol*

161 Tanks were aligned end to end with opaque partitions visually isolating the fish outside of test
162 sessions (as per Elwood et al. 2014). For at least 7 days prior to the onset of experimental
163 trials the fish were maintained in these individual tanks, thus ameliorating any behavioural
164 effects of prior winning or losing experiences (reviewed in Hsu et al. 2006). Each fish was
165 tested twice, once displaying against a mirror and once against a real opponent, in a random
166 order and with a gap of 10-15 min between tests. When observations against the mirror were
167 conducted the opaque partition between the two tanks was removed and immediately replaced
168 with a 20 x 20 cm mirror and the focal fish was filmed for 15 minutes. When a real opponent

169 was used the opaque partition was removed from between the two tanks and the focal fish was
170 filmed for 15 minutes. During filming, the laboratory was isolated from disturbance. Each
171 focal fish was exposed to the stimulus fish in the tank to the right, except for the last in the
172 row of tanks, which was moved so that it could see the first fish as the stimulus 24 h after
173 moving. Thus all focal fish/stimulus fish combinations were novel and pseudoreplication was
174 avoided.

175

176 *2.3. Behavioural Measures*

177 Of the 30 tested male subjects, 10 were omitted from further analyses due to a lack of display
178 behaviour by the focal or stimulus fish in one or both situations. Results are therefore based
179 on recordings from 20 focal fish. The films were observed and behavioural displays recorded
180 using Observer v. 3.0 software (Noldus Technology, Wageningen, The Netherlands). The
181 following activities were recorded; left lateral display, right lateral display, frontal display,
182 bite attempt and surface breathing. A left lateral display was recorded if the fish was
183 displaying its left lateral side at 45 degrees or less to the glass at the end of the tank closest to
184 the mirror/opponent, while a right lateral display if the right side was shown at 45 degrees or
185 less, and a frontal display was recorded if the fish was head on to the glass (>45 degrees) (as
186 per Elwood et al. 2014). An attempted bite was recorded when a subject made open-mouthed
187 contact with the glass/mirror, directed towards the real or perceived opponent. Surface
188 breathing was recorded when a subject ceased displaying and began gulping air at the surface.

189

190 *2.4. Ethical note*

191 This study was carried out in accordance with the guidelines for the treatment of animals in
192 behavioural research (ASAB 2012). Moreover, our experimental set-up prevented physical

193 contact between fish, eliminating the risk of injury as a result of aggression, and staged
194 interactions were brief (as recommended by Huntingford 1984). Following discussions with
195 the local Home Office veterinary inspector it was deemed that there was no likelihood of fish
196 being harmed by the procedure and thus no licence was required. Further, the maintenance of
197 fish in individual tanks without transferring for each test ensured there was minimal handling,
198 disturbance, or opportunity to succumb to harm from conspecifics. Following the
199 experimental phase fish were returned unharmed to the local supplier.

200

201 *2.5. Statistical Analyses*

202 From behavioural observations, we obtained the frequency, total duration and median duration
203 of each of the following activities; left lateral display, right lateral display, frontal display,
204 surface breathing. In addition, we obtained the frequency of attempted bites. The data were
205 not normally distributed and the nonparametric Wilcoxon matched-pairs signed ranks test for
206 non-independent data was used to compare responses to real opponents and mirror images.
207 The same test was used to compare the frequency, total duration, and median durations of left
208 and right lateral displays. Spearman rank correlations were used to examine relations between
209 displays to mirrors and real opponents for each display component, as well as relations
210 between surface breathing and agonistic activities. Although we used multiple tests,
211 Bonferroni corrections were not applied given they have been criticised (Nakagawa 2004) for
212 exacerbating the problem of low statistical power for behavioural studies, where the risk of
213 Type II errors is generally greater than the risk of Type I errors. Moreover, we were interested
214 in comparing overall patterns of behaviour between the two scenarios, therefore necessitating
215 the need for multiple comparisons. Finally, any significant results we reported are also
216 biologically plausible in light of previous findings (e.g. Elwood et al. 2014). All analyses were

217 carried out using StatView (SAS Institute Inc., Cary, NC, U.S.A.).

218

219 **3. Results**

220 *3.1. Lateralization when displaying to a mirror and real opponent.*

221 With a real opponent there was a greater total duration of left side display compared to the
222 right side and a greater median duration of individual left lateral displays but not for the
223 frequency (Table 1). By contrast there was no lateral bias when displaying to a mirror (Table
224 1).

225

226 *3.2. Comparison of displays and surface breathing to a mirror and real opponent.*

227 There were no differences between a mirror image and live opponent in the frequencies,
228 median durations and total durations of aggressive displays or frequency of biting (Table 2).
229 However, surface breathing was greater to a mirror in terms of frequency, median duration
230 and total duration (Figure 1, Table 2).

231

232 *3.3. Correlations between surface breathing and agonistic activities.*

233 When facing a real opponent the frequency of surface breaths was positively related to
234 various indicators of display vigour, in terms of the frequency of bite attempts, left lateral
235 displays, right lateral displays and frontal displays (Table 3). Further, the frequency of breaths
236 was negatively related to both the median duration of left lateral displays and the median
237 duration of frontal displays but did not relate to the total duration of left and right lateral
238 displays or frontal displays (Table 3).

239 When facing a mirror the frequency of breathing events was positively related only to
240 the frequency of biting events and negatively with the median duration of frontal displays but

241 not to any other measure (Table 3).

242

243 *3.4. Correlations for display components between mirror images and real opponents*

244 The frequency of bites were positively correlated between the two conditions (Table 4). There
245 was a non-significant tendency for positive correlations between conditions for the frequency
246 of left lateral displays and right lateral displays but not for frontal displays. There were
247 positive correlations between the two conditions for the total duration of left lateral display
248 and for the right lateral display with the median durations of these displays also being
249 positively correlated between conditions. Other measures were not significantly related (Table
250 4).

251

252 **4. Discussion**

253 We found evidence of population-level lateralization for lateral displays when facing a real
254 opponent, with a bias to present the left side and use the left eye, in terms of total duration and
255 the median duration of the individual aggressive displays but not for frequency. Preferential
256 use of the left side or left eye during aggressive encounters has been reported for many
257 vertebrates (Vallortigara and Rogers 2005). However, variable results have been reported for
258 different fish species, with some reporting a left side / eye use bias (Sovrano et al. 1999;
259 Ariyomo and Watt 2013) and others reporting a right side bias (Bisazza and de Santi 2003;
260 Arnott et al. 2011; Elwood et al. 2014). Further, the Siamese fighting fish of the present study
261 only showed a significant population bias when facing real opponents and not with a mirror
262 image. Similarly, Ariyomo and Watt (2013) reported greater left-eye preference in male
263 zebrafish when viewing a real opponent compared to a mirror image. However, previous
264 findings in Siamese fighting fish are conflicting, with Bisazza and de Santi (2003) finding a

265 population-level bias with a mirror, whereas Cantalupo et al. (1996) and Takeuchi et al.
266 (2010) did not. Moreover, Elwood et al. (2014) found evidence of population-level
267 lateralization of convict cichlids in both the mirror and real opponent scenarios. This led to
268 the suggestion that the side-bias was a feature of the individual, rather than supporting a
269 previous suggestion (Arnott et al. 2011) that laterality provides a means for individuals to
270 coordinate their actions and cooperate in information exchange by aligning in a predictable
271 posture. The present findings add to recent and mounting evidence identifying influences of
272 lateralization on animal contest behaviour in a range of species (domestic cows, Phillips et al.
273 2015; fallow deer, Jennings 2012, 2014a, b; flies, Benelli et al. 2015a, b; Romano et al. 2015;
274 mosquitoes, Benelli et al. 2015c; Przewalski horses, Austin and Rogers 2014).

275 Previously, we reported distinct differences in the displays of convict cichlids to a
276 mirror compared to a real opponent (Elwood et al. 2014). In particular, the displays of cichlids
277 to a mirror appeared to be slower, with lower frequencies and longer individual displays to the
278 mirror image, explained by the apparent opponent in the mirror never making the first move
279 and thus failing to cause the focal fish to switch display (Elwood et al. 2014). In the present
280 study, however, we found no difference between aggressive displays to mirrors and to real
281 opponents. Further, we had speculated that Siamese fighting fish might respond to a mirror
282 image by escalating to biting more frequently or for longer because the image would not
283 cooperate as might a real opponent, but that was not the case. Thus, Siamese fighting fish
284 appear to display to mirror images in a similar manner to that of real opponents despite the
285 mirror image never making the first move or lining up in a head to tail configuration. It seems
286 that the displays of these fish are organised more in line with the endogenous motivation of
287 the focal fish rather than with the specific actions of the “opponent”. This idea of the
288 endogenous motivation being a major factor in controlling the display actions is supported by

289 our finding of positive correlations between aggressive activities to mirror and real opponents.
290 Such correlations have also been noted by others (Dore et al. 1978; Balzarini et al. 2014;
291 Elwood et al. 2014). Cichlids also fight mirrors and live rivals according to their own
292 endogenous motivation under normal conditions, but switch tactics to fight according to
293 opponent size when injected with isotocin (Reddon et al. 2012).

294 Despite the lack of differences in aggressive displays between the mirror and real
295 opponent conditions, there were distinct differences in surface breathing. Fish observing a
296 mirror image went to the surface substantially more often and each breathing event was of
297 longer duration resulting in a greater total duration spent surface breathing compared to when
298 confronting a real opponent.

299 Surface breathing is a means of getting additional oxygen that cannot be supplied in
300 the normal manner from the water via the gills. The number of breaths taken has been shown
301 here to be related to the vigour of displays in terms of positive relations with frequency,
302 particularly the biting frequency (see also Alton et al. 2013; Regan et al. 2015), and negatively
303 with the median duration, so we agree that breathing is typically affected, at least in part, by
304 oxygen requirement. However, we found no difference in the frequency or vigour of displays
305 between the mirror and real opponents and thus display vigour cannot account for the marked
306 difference in surface breathing frequency between these situations. The duration of each
307 surface breathing event was also greater to a mirror than to a real opponent. However, when
308 we examined correlations between median duration of breathing and other behavioural
309 measures no significant relations were found. Note that a longer time at the surface might not
310 necessarily mean that more oxygen is taken up at each visit. One possibility allowing for
311 differences in metabolic demand is that mirrors might induce a higher degree of fear
312 (Desjardins and Ferdinand 2010) and that fear might increase metabolic demand. However,

313 this hypothesis requires experimental investigation.

314 If the increased breathing when confronted by a mirror is not due to a higher oxygen
315 requirement, it might be due to a lower motivation to display to the mirror. That is the mirror
316 image might provide an inappropriate, lower value stimulus for agonistic behaviour, which
317 then allows for the behaviour to be interrupted more frequently and for longer by a
318 subordinate activity (*sensu* McFarland and Sibly 1975; Halliday and Sweatman 1976).

319 Moreover, other researchers have previously suggested that air breathing can take the form of
320 a ‘displacement’ activity (Dore et al. 1978). Fight motivation might be reduced in the mirror
321 treatment but not result in reduced contest vigour compared to when facing a real opponent
322 because animals should not disclose future intentions in contests (Dawkins and Krebs 1978).
323 One way to clearly establish if this is the case would be to probe fight motivation using a
324 novel startling stimulus during the interaction. This causes an animal to temporarily break off
325 from the fight, with the time taken to resume the contest providing a measure of fight
326 motivation, with the technique having now been successfully used for a number of species
327 (e.g. hermit crabs; Elwood et al. 1998; fish, Arnott et al. 2009b, c, 2010; cuttlefish, Schnell et
328 al. 2015).

329 Another explanation for the increased frequency and duration of surface breathing
330 events in the mirror treatment is that breathing of the opponents under normal circumstances
331 is typically coordinated, with the fish engaging in near simultaneous air breathing (Simpson
332 1968). The mirror image cannot be the first to restart display, as might happen with a real
333 opponent causing the focal fish to respond. This might account for the increased duration of
334 surface breathing bouts in the mirror treatment. However, it is more difficult to use a similar
335 argument for the increased frequency of breathing. The mirror image cannot be the first to
336 initiate breathing and thus fewer breathing events should occur, the opposite to our findings.

337 Conversely, in real contests should a focal animal show intention of going to the surface this
338 might be inhibited because the opponent continues to display. A mirror image will not
339 continue to display and thus might enable more frequent breathing.

340 The idea that the focal animal should attempt to match the display behaviour of the
341 opponent is predicted by some contest theory models (e.g. ‘war of attrition without
342 assessment, Mesterton-Gibbons et al. 1996; ‘energetic war of attrition’, Payne and Pagel
343 1996, 1997) such that surface breathing is constrained to times of absolute need by both
344 opponents. Indeed, it is possible that with real opponents each attempts to prevent the other
345 from taking sufficient breaths and thus inflicts costs in terms of anaerobic respiration and
346 build-up of lactate. However, anaerobic capacity appears not to affect display vigour whereas
347 enhanced capacity for aerobic metabolism is linked to vigour and outcome (Regan et al.
348 2015). The idea of opponents influencing each other’s surface breathing is further supported
349 by breathing being better correlated with fight behaviour when facing the real opponent
350 compared to the mirror treatment.

351

352 **5. Conclusions**

353 The lateralization of displays showed a left side bias but only when facing a real opponent and
354 not with a mirror image. Despite this difference in lateralization, fish did not differ in their
355 levels of aggressive displays to mirror images and real opponents. They did, however, differ
356 in surface breathing, with far more and longer breathing acts when facing a mirror image.

357 This could not be due to increased oxygen requirement when facing a mirror and the
358 increased frequency may be explained by a lower motivation to display to a mirror. The
359 increased duration might be due to the mirror image never being the first to resume displaying
360 and thus the image does not induce the focal fish to stop breathing and return to aggressive

361 display. This study adds to mounting evidence of differences in behaviour when fish face a
362 mirror image compared to real opponent beyond a transparent barrier, therefore questioning
363 the utility of using mirrors. We accept, however, that even a live fish beyond a barrier is not
364 the same as interactions between unrestrained opponents.

365

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369

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527

528 **Figure captions**

529 **Figure 1.** Comparison of the frequency (a), total duration (b) and median duration (c) of
530 surface breathing when the focal fish (n=20) displayed to a real opponent or mirror image.

531 **Table 1.** Summary of results from Wilcoxon matched-pairs signed ranks tests, examining
 532 lateralization of lateral displays to a mirror image and real opponent (n=20 focal fish). Median
 533 values presented, and durations presented in seconds. Significant P values (P<0.05) are in
 534 bold.

Display measure	Left side	Right side	Z statistic	P value
Real opponent				
Total duration	198.55	184.35	-2.43	0.015
Median duration	3.69	3.01	-2.60	0.010
Frequency	62.50	61.50	-0.02	0.983
Mirror image				
Total duration	235.10	220.35	1.57	0.117
Median duration	3.31	3.19	-0.49	0.627
Frequency	67.50	60.00	-1.46	0.144

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536

537 **Table 2.** Summary of results from Wilcoxon matched-pairs signed ranks tests, comparing
538 aggressive displays and surface breathing to a mirror and real opponent (n=20 focal fish).
539 Median values presented, and durations presented in seconds. Significant P values (P<0.05)
540 are in bold.
541

Display measure	Mirror image	Real opponent	Z statistic	P value
Frequency				
Left lateral	67.50	62.50	-1.35	0.179
Right lateral	60.00	61.50	-0.50	0.614
Frontal	91.50	88.00	-0.49	0.624
Bites	0.00	2.50	-1.07	0.286
Surface breaths	18.00	10.50	-3.14	0.002
Total duration				
Left lateral	235.10	198.55	-0.67	0.502
Right lateral	220.35	184.35	-0.78	0.433
Frontal	259.25	395.75	-1.31	0.191
Surface breaths	38.20	24.95	-2.95	0.003
Median duration				
Left lateral	3.31	3.69	-0.86	0.391
Right lateral	3.19	3.01	-0.85	0.398
Frontal	2.43	3.77	-0.97	0.332
Surface breaths	2.29	2.07	-2.093	0.036

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546 **Table 3.** Spearman Rank correlations are shown for the frequency of surface breaths and
 547 display components to a real opponent and a mirror image (n=20 focal fish). Correlations for
 548 the median duration of breathing events and other display components are also shown for the
 549 two conditions. Significant P values (P<0.05) are in bold.
 550

Display measure	Frequency of surface breaths				Median duration of surface breaths				
	Real opponent		Mirror image		Real opponent		Mirror image		
	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	
Frequency									
Bites	0.556	0.018	0.577	0.018	-0.085	0.71	0.09	0.70	
Left lateral	0.706	0.002	0.297	0.198	-0.17	0.45	-0.13	0.56	
Right lateral	0.874	0.003	0.274	0.236	-0.13	0.56	-0.21	0.36	
Frontal	0.648	0.005	0.178	0.444	0.11	0.64	-0.23	0.31	
Total duration									
Left lateral	0.137	0.56	0.058	0.81	0.17	0.45	-0.09	0.70	
Right lateral	0.279	0.229	0.37	0.109	0.314	0.17	0.05	0.82	
Frontal	0.143	0.54	0.072	0.76	-0.12	0.60	-0.35	0.12	
Median duration									
Left lateral	-0.531	0.019	-0.130	0.56	0.33	0.15	0.07	0.75	
Right lateral	-0.247	0.273	0.021	0.94	0.44	0.54	0.32	0.17	
Frontal	-0.502	0.027	-0.493	0.03	0.23	0.32	-0.11	0.91	

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552

553 **Table 4.** Correlations between specific display components to a mirror and those to real
 554 opponents (n=20 focal fish). Significant P values (P<0.05) are in bold.

Display measure	Spearman r_s	P value
Frequency		
Bites	0.676	0.003
Left lateral	0.445	0.052
Right lateral	0.409	0.075
Frontal	0.243	0.290
Surface breaths	0.379	0.10
Total duration		
Left lateral	0.507	0.027
Right lateral	0.531	0.021
Frontal	0.397	0.084
Surface breaths	0.322	0.160
Median duration		
Left lateral	0.605	0.008
Right lateral	0.770	0.001
Frontal	-0.053	0.820
Surface breaths	0.170	0.459

555

Figure 1.



