Can we measure fish sociability with the mirror test?

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Many fish species are social and spend most of their life in shoals, but sociability can vary greatly among species, populations, and even individuals. Sociability has been largely studied by measuring the time spent by a focal fish in proximity to one or more conspecifics. To control for the behaviour of stimulus fish, the conspecific has often been substituted by a mirror, assuming that the subject perceive its mirror image as a conspecific. The reliability of the mirror test has recently been questioned, both at the behavioural and molecular level, because of the discrepancy in fish response when exposed to a mirror image and a live conspecific. In this study we compared the sociability scores of a social fish, the guppy (*Poecilia reticulata*), obtained using live fish or a mirror as stimuli, in order to assess reliability of the mirror test. We found that the sociability score assessed with the standard mirror test did not significantly correlate with the one assessed with live stimuli. Yet, we observed a positive correlation between the scores of the two tests when the mirror test was performed in a more naturalist context in which the minimum distance between stimulus fish and mirror was controlled and a hidden conspecific provided fish odour to the testing tank. Our findings provide evidence for the validity of the mirror test as a measure of sociability but suggest that some cautions should be taken in the experimental design.

Many marine and freshwater fish spend most of their life in social groups and form shoals of different size (Krause, Hoare, et al., 2000; T. J. Pitcher & Parrish, 1993). Shoaling acts as an efficient anti-predator behaviour because it increases the efficiency in detecting predators and dilutes the individual risk of being predated (Magurran, 1990). Shoaling also provides other benefits, such as faster location of food sources (T. J. Pitcher, Magurran, & Winfield, 1982) and protection against harassing males (Dadda, Pilastro, & Bisazza, 2005; Pilastro, Benetton, & Bisazza, 2003). On the other hand, living in shoals increases competition for resources (Krause & Ruxton, 2002) as well as risk of parasite transmission (Richards, van Oosterhout, & Cable, 2010). As a consequence of the trade-off between costs and benefits, sociability and shoaling tendency vary greatly among species, populations, individuals, and contexts, and the causes and the results of this variability have consistently attracted the attention of evolutionary biologists (for a review see Krause & Ruxton, 2002).

Although sociability can be measured in the wild (e.g. Croft, Krause, & James, 2004), experiments performed in the laboratory allow to control the effect of confounding factors and to manipulate the context in which this behaviour is expressed (Krause, Butlin, Peuhkuri, & Pritchard, 2000). The classical method adopted in laboratory experiments consists in inserting a focal fish in a novel tank with a group of conspecifics confined behind a transparent partition. Time spent by the focal fish in proximity to the conspecifics is usually taken as a measure of its sociability (see Brown & Irving, 2013; Budaev, 1997; Cote, Fogarty, & Sih, 2012; Cote, Fogarty, Weinersmith, Brodin, & Sih, 2010; Morrell, Hunt, Croft, & Krause, 2007; Ward, Hart, & Krause, 2004) since more sociable individuals should be more prone to join groups than asocial individuals. An alternative version of the same test consists in using a single fish as stimulus (Grossman et al., 2011; e.g. Smith & Blumstein, 2010). In this case, however, the result of the test is likely to be influenced by the behaviour and other individual characteristics of the stimulus fish. The use of a shoal as stimulus is therefore

74 preferred, as it provides a stimulus that should reflect the average behaviour and characteristics of
75 the species (Cote et al., 2012).

76 It is thought that individual fish do not recognize their mirror image (Tinbergen, 1951). Therefore,
77 in social species such as the guppy, *Poecilia reticulata*, and the mosquitofish, *Gambusia holbrooki*,
78 the mirror image has often used as social stimulus (A. D. Dugatkin, 1988; Milinski, 1987) and time
79 spent close to a mirror has been used as a measure of sociability (Budaev, 1997; De Santi, Sovrano,
80 Bisazza, & Vallortigara, 2001; Jason A. Moretz, Martins, & Robison, 2006). Theoretically, the use of
81 the mirror has several advantages over the use of live conspecifics (see Rowland, 1999). For
82 instance, the use of the mirror allows to control for the variability in behaviour of live stimuli.
83 Moreover, live stimuli may respond differently to different subjects (e.g. being attracted or being
84 aggressive) on the basis of behaviour and chemical cues (Rowland, 1999). Finally, live stimuli need
85 to be well accustomed to the testing tank prior of the experiment to avoid fear, freezing and other
86 responses to the novel environment, which may affect the behaviour of the focal fish (Lucon-
87 Xiccato, Dadda, Bisazza, & Manser, 2016, Lucon-Xiccato et al. submitted). Conversely, the mirror
88 provides a stimulus image that 'behaves' in a standardize way and do not suffer the problems of
89 habituation to the tank.

90 In territorial species, modified versions of the mirror test described above have been used to study
91 intraspecific aggressiveness (Balzarini, Taborsky, Wanner, Koch, & Frommen, 2014; Desjardins &
92 Fernald, 2010; Elwood, Stoilova, McDonnell, Earley, & Arnott, 2014; J. A. Moretz, Martins, &
93 Robison, 2007; Oliveira et al., 2016; Scherer, Buck, & Schuett, 2016). In recent years, some of
94 these studies on aggressive behaviours have raised concerns on the validity of the mirror test. For
95 example, in some species of cichlid aggressive behaviours induced by a real opponent are
96 substantially different from those induced by an opponent simulated by the mirror (Balzarini et al.,
97 2014; Elwood et al., 2014). Moreover, hormonal response, brain activation and gene expression

98 triggered by a real opponent were found to notably differ when compared to those triggered by a
99 mirror-simulated opponent (Desjardins & Fernald, 2010; Oliveira, Carneiro, & Canario, 2005;
100 Oliveira et al., 2016).

101 Regarding the use of the mirror in social contexts, during cooperative predator inspection the
102 mirror test has been shown to provide a reliable measure of how fish would behave with a live
103 social companion (L. A. Dugatkin & Alfieri, 1991). Yet, the validity of the mirror test for measuring
104 sociability is still unclear (T. E. Pitcher, 1979). Indirect findings have suggested that some
105 components of fish' social behaviour towards their mirror image, such as number of looks at the
106 mirror image**,** positively correlate with shoaling tendency, whereas others mirror-directed
107 behaviours, such as number of contacts, correlate with other behavioural traits not associable to
108 sociability, such as fear avoidance and activity (Budaev, 1997).

109 In this study, we investigated whether sociability measured with the mirror test is a reliable
110 measure of sociability measured with live conspecific stimuli and whether the mirror test can be
111 improved to provide a more naturalistic context. For this purpose, we used the guppy, a species
112 commonly adopted to study shoaling behaviour and social interactions (Croft et al., 2004; Seghers,
113 1974). In their natural habitat guppies are highly social and actively associate with conspecifics
114 forming dyads or shoals of different size (Croft et al., 2003; Griffiths & Magurran, 1998). Female
115 guppies, in particular, display strong sociability toward live conspecifics or their mirror image
116 (Budaev, 1997; Morrell et al., 2007). We conducted a series of experiments in which we measured
117 the sociability of an individual using both the mirror test and live conspecifics. We assumed that
118 the sociability expressed towards conspecifics in our experimental set up represented the closest
119 measure of 'true' sociability; thus, we expected that guppies were more attracted to conspecifics
120 than towards mirror images, and we used the strength of the correlation between the sociability
121 measured in the two experimental conditions as a validation of the mirror test results.

In experiment 1, we sequentially measured sociability of focal female guppies towards a group of 6 females and the mirror image, and correlated the two scores. The setting of the mirror test followed previous studies (Bisazza, Dadda, & Cantalupo, 2005; De Santi et al., 2001; Sovrano & Andrew, 2006; Sovrano, Rainoildi, Bisazza, & Vallortigara, 1999). In experiment 2, we tested an improved version of the mirror test aimed at mimicking more closely a natural situation. We reasoned that some possible limits are intrinsic of the mirror test, such as the symmetrical response of the mirror image and the perfect and immediate feedback (Rowland, 1999), but other factors, such as the distance between subject and stimulus image and the absence of conspecific's odour (Oliveira et al., 2016) could be fixed. The first improvement (experiment 2a) consisted in limiting the minimum distance between the focal fish and the mirror to the inter-individual distance usually occurring in shoaling fish (T. J. Pitcher & Parrish, 1993). This also prevents the focal fish from swimming convulsively along the mirror (Carson & Merchant, 2005; Weetman, Atkinson, & Chubb, 1998). The second improvement consisted in adding the odour of a conspecific to the stimulus tank (experiment 2b), as guppies use olfactory cues from conspecifics in shoal choice decisions (Griffiths & Magurran, 1999). We expected that this 'more realistic' setting of the mirror test would improve its effectiveness in simulating a live conspecific and thus increase the strength of the correlation between the two sociability measures.

Given that in the aforementioned experiments we compared shoaling tendency with 6 stimuli fish versus one single mirror image, we conducted a third experiment in which we compared the sociability measured towards the mirror image and towards a single live conspecific, an alternative method to measure sociability in guppies (Smith & Blumstein, 2010).

METHODS

Experimental fish

Fish used in this study were descendants of wild-caught guppies from a high predation risk site (Lower Tacarigua river) in Trinidad. Guppies were maintained in large mixed-sex stock tanks (115L) at standard conditions. The temperature was maintained at 26 ± 1 °C and illumination set on a 12h:12h light/dark cycle. Fish were fed with brine shrimp nauplii (*Artemia salina*) and commercial food flakes (DuplarinS). We used 102 females randomly chosen from stock tanks.

Experiment 1

In experiment 1 we tested 30 females. Each female performed a sociability test with live conspecifics and a test with the mirror in a randomized order. Females were individually isolated in a 2-L tank without any visual or olfactory access to other fish for 24 h before the experiment. Between the two tests (live conspecifics and mirror) females were placed back in their 2-L isolation tank for a 24 h-interval.

The sociability test with live conspecifics consisted in a standard preference test in which we recorded the time spent by the focal fish near (i.e. within 6 cm from the partition wall) a shoal of conspecifics (see Cote et al., 2012; Cote et al., 2010; Ward et al., 2004). The experimental tank was a glass aquarium (48 x 20 cm, 30 cm high, and filled with 14 cm of well water) divided into three compartments by means of two transparent plastic partitions (figure 1a). The central, larger sector (24 x 20 cm) housed the subject during the experiment. One of the two lateral compartments (12 x 20 cm each) housed a stimulus shoal, while the other compartment was empty. Since the transparent partitions were provided with holes, they allowed both visual and olfactory contacts between the shoal and the focal individual. Two 15-W fluorescent lamps illuminated the lateral sectors, whereas the central sector received indirect light from the lateral compartments.

169 Illumination in the room was kept off allowing us to observe fish without disturbing them. We
170 used a stimulus shoal of 6 randomly chosen female guppies which were not familiar with the focal
171 fish (raised in a different stock tank). The stimulus shoal was randomly introduced to one of the
172 small compartments of the experimental tank 1 h before the experiment started. The stimulus
173 shoal was changed every 3 trials and the left/right location of the shoal was alternated between
174 trials to avoid lateral bias. For the test, the focal fish was introduced into the centre of the central
175 compartment and allowed to acclimatize for 2 min. A pilot experiment revealed that, with our set
176 up, after 2 min of acclimation guppies resumed normal behaviours, ceased freezing, and started to
177 associate with the stimuli. Two marks on the bottom of the tank 6 cm away from each transparent
178 partition virtually divided the central compartment in three areas: a central no-choice area, a
179 choice area for the stimulus shoal and a choice area for the empty lateral sector of the tank.
180 Following previous studies (Cote et al., 2012; Shohet & Watt, 2004), we recorded the position of
181 the subject across these three areas every 12 s for 10 min. The measurement was performed by
182 an experimenter sitting motionless beside the tank and behind a curtain.

183 In the mirror test social preference was measured as the time spent by the focal fish within 6 cm
184 from the mirror. The experimental tank was the same used for the sociability test with live
185 conspecifics (see above) with the exception that one of the two transparent partitions was
186 replaced with a one-way mirror (30 x 20 cm) whereas the other lateral sector was empty (figure
187 1b). To avoid side bias, the left/right location of the mirror was alternated between the trials. The
188 test procedure was the same adopted for the test with live conspecifics. With the modalities
189 described for experiment 1, we recorded each 12 s whether the focal fish was in the no-choice
190 area, in the choice area adjacent to the mirror or in the choice area adjacent to the empty
191 compartment.
192

193 *Experiment 2*

194 Experiment 2 tested whether two modifications of the mirror test (increased distance between
195 the subject and the mirror, and addiction of conspecifics' odour) might provide a more natural
196 context to the focal guppies and whether these modifications increased the correlation with
197 sociability scores observed with live conspecifics test. We performed this experiment in two
198 conditions in which we added sequentially the two modifications.
199 In the first condition (experiment 2a) we tested 24 females in a sociability test with live
200 conspecifics as described for experiment 1 and the mirror with the improvement regarding the
201 distance. In the apparatus described for experiment 1, we relocated the mirror 2 cm behind a
202 transparent plastic partition (figure 1b). This set up limited the fish to the minimum inter-
203 individual distance of two body lengths, that falls within the range of distance between two fish
204 occurring in natural shoal (T. J. Pitcher & Parrish, 1993).

205 In the second condition (experiment 2b) we tested other 24 females in a sociability test with live
206 conspecifics and in a mirror test with the two improvements. We integrated the distance between
207 subject and mirror adopted in experiment 2a with the odour of a live conspecific placed behind
208 the mirror. A donor of olfactory cues was a female guppy randomly chosen from stock tanks and
209 placed behind the mirror 1 h before the beginning of the test (figure 1b). The donor of olfactory
210 cues was changed every 3 trials. The two fish were in olfactory contact but visual contact was not
211 possible.
212 Other details of procedure and apparatus were identical to the ones described for experiment 1.
213

214 *Experiment 3*

215 In experiment 3 we investigated whether using a single live conspecific rather than a shoal (see
216 Smith & Blumstein, 2010) provides a sociability score that is more similar to the one provided by

217 the mirror test. We used 24 females that performed two tests in a randomized order: a sociability
218 test with a single-stimulus female and a mirror test. The test with the live conspecific was identical
219 to the one described in experiment 1, except the use of a single stimulus. We used the mirror test
220 with the higher correlation score found during the previous experiments, that is the one of
221 experiment 2b.
222
223 *Statistical analyses*
224 Sociability score was calculated as proportion of times the focal fish was observed within 6 cm
225 from the sector with the social stimulus (being it either live conspecifics or mirror) over the total
226 number of observations in which the focal fish was in one of the two choice areas. We thus
227 excluded the observations in which the focal fish was in the central, no-choice area. One female in
228 the experiment 3 avoided both its mirror image and the live conspecific, and was therefore
229 excluded from statistical analysis. Data were arcsine-square root transformed before the analysis
230 (Sokal & Rohlf, 1995). For each experiment, one-sample t-test was used to examine whether the
231 preference for the social stimulus was significantly greater than chance (50%). For each
232 experiment, we then run mixed-effects ANOVAs to compare the preference for the social stimulus
233 between the two tests. All models included test type (live stimulus or mirror) and test order (live
234 stimulus at first or viceversa) as fixed effects and female identity as random effect to account for
235 the non-independence of the data. Pearson correlation analysis was used to assess the
236 relationship between the sociability measured in the two tests (live stimulus or mirror) within each
237 experiment. This approach based on the null-hypothesis significance testing provides information
238 on the correlation between two variables. When the correlation between the two sociability
239 measures was not significant, we calculated a Bayes factor that expresses the relative probability
240 that the two variables are not actually correlated (Wetzels & Wagenmakers, 2012). Statistical

241 analyses were performed using SPSS statistics (version 21.0).

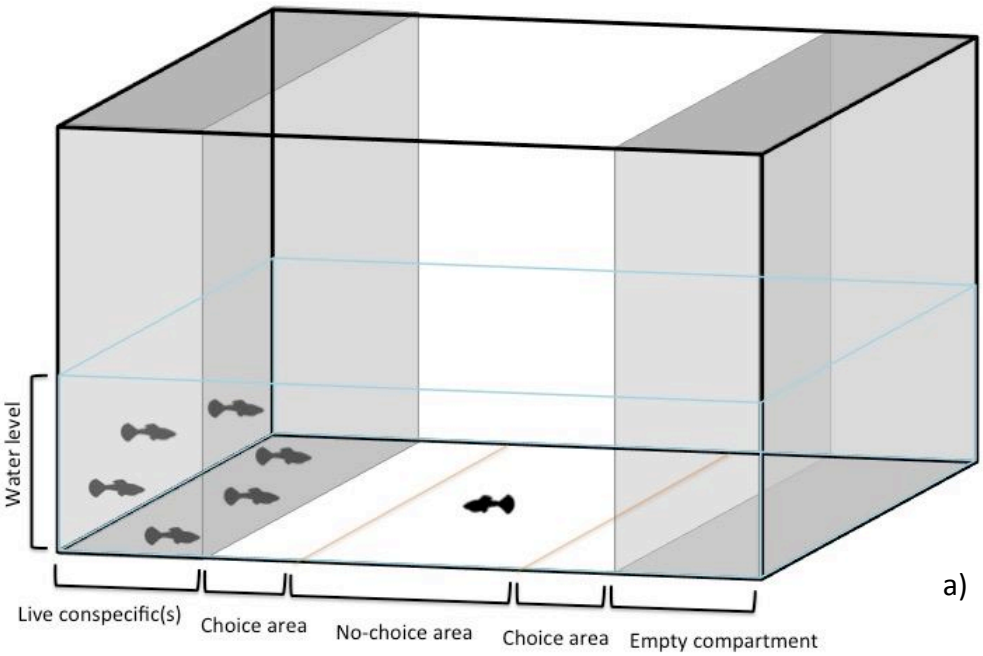
242

243 *Ethical Note*

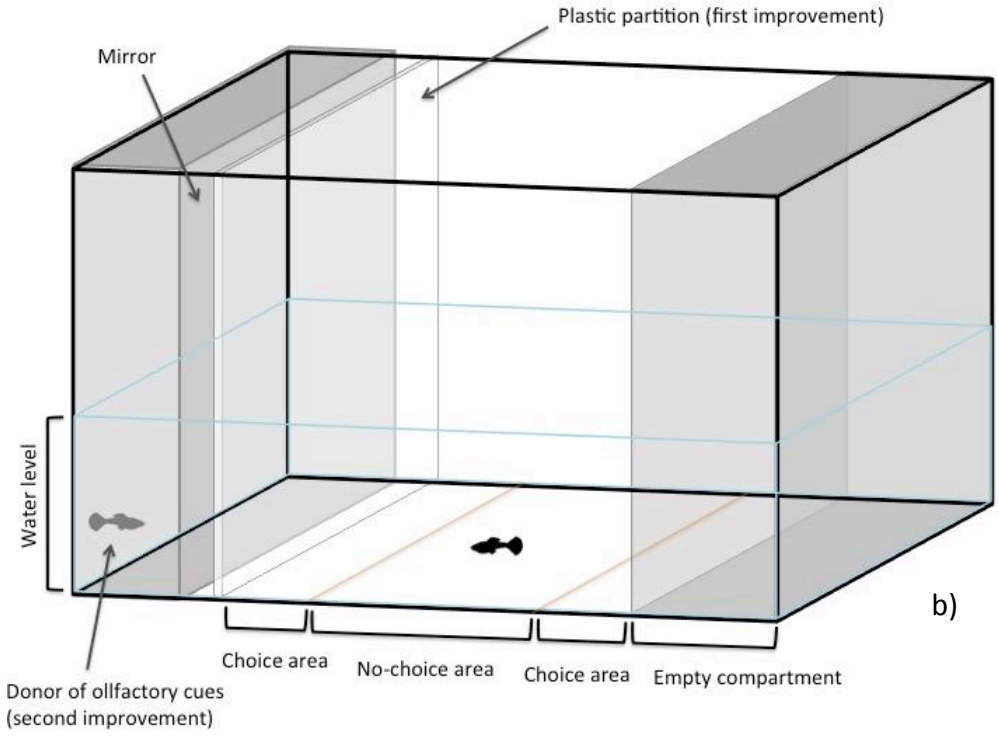
244 This research was approved by the University of Padova Ethical Committee (protocol number:
245 32/2015). Fish used were descendants of wild-caught fish, so no transport of the experimental fish
246 was necessary. Behavioural tests did not involve any invasive manipulations and were performed
247 mimicking, as best as possible, natural conditions (e.g. a gravel substrate, full spectrum lighting
248 and aeration providing some water flow in the tank). After the study all fish were returned to
249 stock tanks.

250 Figure 1 – Three-dimensional view of the experimental apparatuses: a) apparatus adopted for tests with
251 live conspecifics (see text for details); b) experimental apparatus used for mirror test. In experiment 2a, the
252 mirror was positioned 2 cm behind a plastic partition, whereas in the experiment 2b a live female guppy
253 was added behind the mirror to provide olfactory cues.

254



a)



b)

RESULTS

Experiment 1

Time spent close to the social stimulus, either live conspecifics or mirror, was significantly greater than expected by chance (live conspecifics, *t* test: $t_{1,29}$=17.994, *P*<0.001; mirror, *t* test: $t_{1,29}$=15.179, *P*<0.001; figure 2). Preference for the social stimulus did not differ between live conspecifics and mirror, and there was not significant effect of test order (table 1). Preference for live conspecifics did not significantly correlate with preference for mirror image (Pearson's correlation: *r*= 0.155, *P*=0.415; figure 3). Bayesian analysis revealed that the absence of correlation was 5.08 times more likely that the presence of correlation.

Experiment 2

Experiment 2a: preference for the social stimulus, either live conspecifics or mirror, was significantly greater than expected by chance (live conspecifics, *t* test: $t_{1,23}$=12.821, *P*<0.001; mirror, *t* test: $t_{1,23}$=7.130, *P*<0.001; figure 2). Preference for live conspecifics was significantly greater than preference for mirror image, but there was not significant effect of test order (table 1 and figure 2). Preference for live conspecifics was significantly correlated with preference for mirror image (Pearson's correlation: *r*= 0.471, *P*=0.020; figure 3).

Experiment 2b: preference for the social stimulus, either live conspecifics or mirror, was significantly greater than chance (live conspecifics, *t* test: $t_{1,23}$=13.648, *P*<0.001; mirror *t* test: $t_{1,23}$=9.936, *P*<0.001; figure 2). As in experiment 2a, preference for live conspecifics was significantly greater than preference for mirror image (table 1; figure 2) and was positively correlated with preference for live conspecifics (Pearson's correlation: *r*= 0.486, *P*=0.016; figure 3).

Comparison between experiment 2a and experiment 2b: preference for the mirror image did not significantly differ between experiment 2a and 2b (*t* test: $t_{1,46}$=-1.730; *P*=0.090). However, adding

290 the odour of a live conspecific as additional factor did not enhance the score of the correlation
291 (figure 3). An ANCOVA analysis showed that slopes of the relationship between the preference for
292 the live conspecifics and the preference for the mirror did not significantly differ between
293 experiment 2a and 2b (ANCOVA: $F_{1,48}$=0.005; P=0.944, figure 3).

294 *Experiment 3*

295 Preference for the social stimulus was significantly greater than chance (live conspecifics, *t* test:
296 $t_{1,22}$=17.303, P<0.001; mirror, *t* test: $t_{1,22}$=10.931, P<0.001; figure 2). Preference for live
297 conspecifics was significantly greater than preference for mirror image (table 1; figure 2). In
298 contrast with experiment 2, social preference towards a single live conspecific did not significantly
299 correlate with social preference for the mirror although this relationship approach statistically
300 significance (Pearson's correlation: r= 0.388, P=0.067; figure 3). Bayesian analysis revealed that the
301 absence of correlation was 1.19 times more likely that the presence of correlation.

Table 1 – Results from the ANOVAs testing the effect of test type (live stimulus or mirror image) and of test order on sociability.

		Df	F statistic	*p*-value
Experiment 1	Test type	1,29	0.943	0.340
	Test order	1,28	0.413	0.526
	female ID	28,29	1.540	0.127
Experiment 2a	Test type	1,23	39.492	<0.001
	Test order	1,22	1.838	0.189
	female ID	22,23	2.673	0.012
Experiment 2b	Test type	1,23	19.926	<0.001
	Test order	1,22	0.995	0.329
	female ID	22,23	2.880	0.007
Experiment 3	Test type	1,22	16.143	0.001
	Test order	1,21	1.668	0.211
	female ID	21,22	2.184	0.038

FIGURE LEGENDS

Figure 2 – Sociability (mean ± s.e.m) measured with the two types of test (live conspecific(s) and mirror) in each experiment.

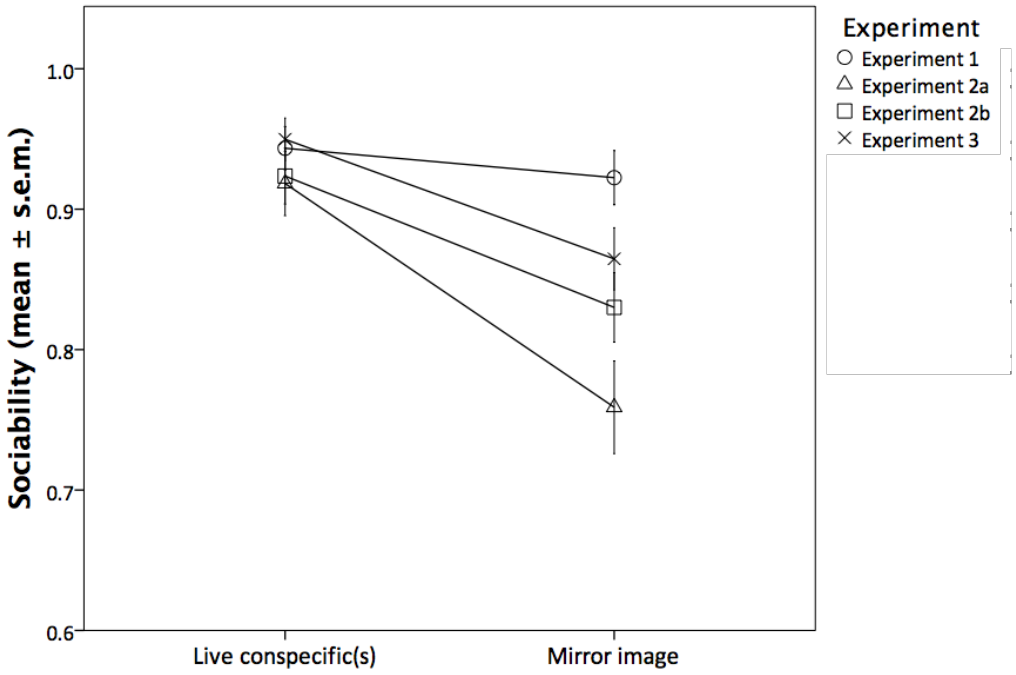
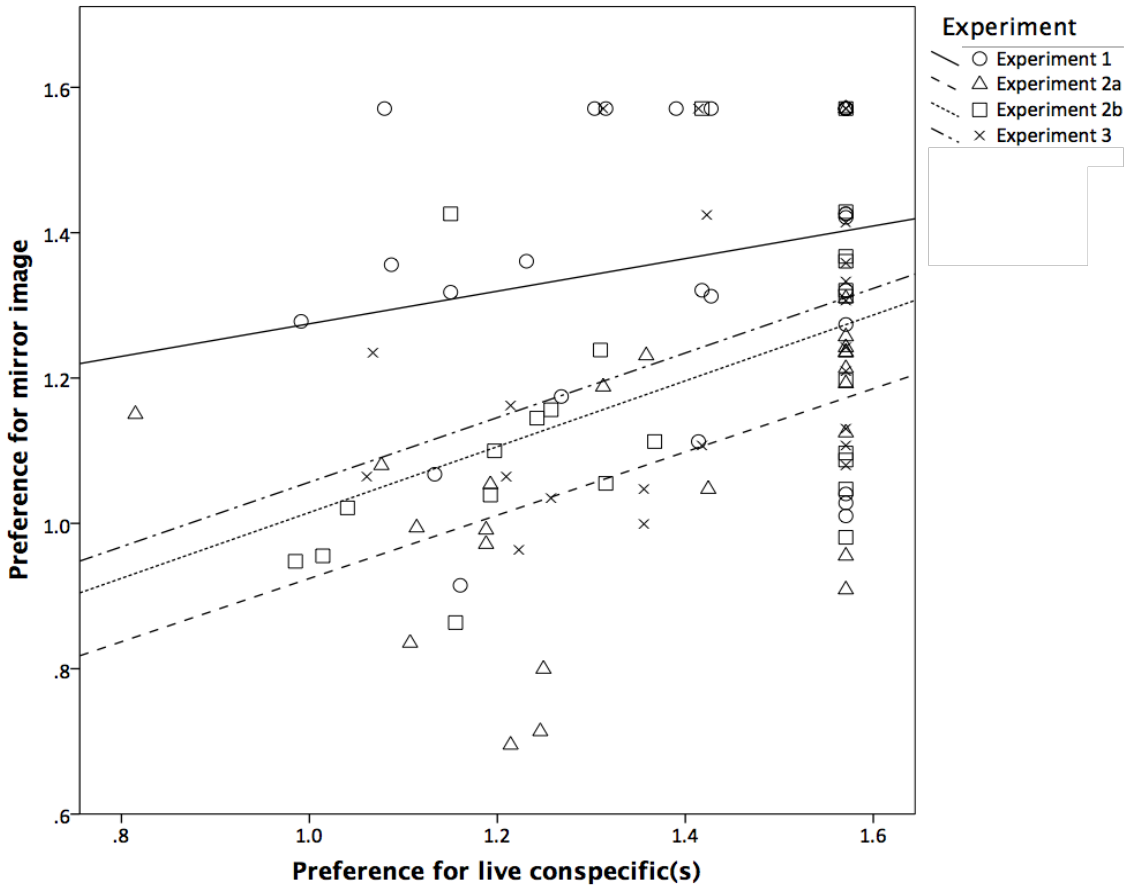


Figure 3 – Correlations of sociability measured with the mirror and with live conspecific(s) in each experiment.



DISCUSSION

We investigated whether the mirror test can be used as reliable measure of sociability in a social fish, the guppy. For this purpose, we correlated the preference for the mirror image with preference for a shoal, a common method to measure sociability in fish. Our findings suggest that the mirror test provides a reliable measure of sociability only once improved to provide a more naturalistic context.

In the first experiment we sequentially measured sociability towards the mirror image and a shoal of conspecifics. Time spent close either the mirror image or live conspecifics was, as expected, significantly greater than expected by chance, suggesting that guppies were attracted by the social stimuli. Although mean sociability scores did not differ between the test with live conspecifics and with the mirror image, we failed to find a significant correlation between the measures in the two conditions and the absence of correlation was 'substantially' (Jeffreys, 1988) also supported by the Bayesian analysis. Thus, guppies responded somewhat differently to the two social stimuli. This result suggests that the mirror test based on standard procedures may not be a good proxy of 'true' sociability (i.e. that expressed towards a group of live conspecifics). This result parallels those obtained in cichlids for aggressiveness (e.g. Balzarini et al., 2014; Elwood et al., 2014). The discrepancy could be attributed to some differences between the characteristics of real fish and mirror image that are important in recognition and social response to conspecifics (Arnott, Ashton, & Elwood, 2011; Rowland, 1999). Among the others, the mirror image is often seen by the focal fish at an unnatural closer distance than a real conspecific would be. Furthermore, the standard mirror test lacks the chemical cues typical of the interactions with other real fish. To evaluate the influence of this experimental set up on fish behaviour, in our second experiment we removed these limitations of the mirror test to set a more 'naturalistic' context.

The first modification (experiment 2a) was aimed at keeping the stimulus fish at a minimum

distance from the mirror image similar to that occurring among shoaling fish (T. J. Pitcher & Parrish, 1993). Once a transparent partition limited the minimum distance at which the stimulus fish could approach its mirror image, sociability towards the mirror was more strongly correlated with sociability towards conspecifics as compared to the standard mirror test. This better matching between the two sociability measures when the mirror test was modified occurred despite the lower average sociability score observed in the mirror test after the addition of the spacing partition. This reduction possibly occurred because the unnatural behaviour of swimming convulsively along the mirror by the subject was removed. These results suggest that the predictive value of the mirror test may be enhanced with this simple adjustment of the experimental set up.

Guppies use olfactory cues to enable conspecific detection (Griffiths & Magurran, 1999; Shohet & Watt, 2004). In our second modified mirror test (experiment 2b) focal fish had also access to the holding water of a live conspecific placed behind the mirror and was therefore exposed to olfactory cues of a conspecific during the trial. Although the correlation score between preference for live conspecifics and for the mirror was slightly higher with the odour improvement of experiment 2b, this increase was not statistically significant. Together with the results of experiment 2a, this suggests that a realistic distance between the focal fish and its mirror reflection is the essential factor to elicit a reliable shoaling response of the focal fish. Overall, our results suggest that, although the mirror test has proven useful in a number of studies ranging from aggression to schooling, caution should be exercised when interpreting a response towards a mirror as identical to that towards a conspecific, at least when individual variation in sociability (e.g. differences in personality) is investigated.

In the third experiment we correlated the proximity preference for a single live conspecific and for the mirror image in order to evaluate whether the reliability of the mirror test is enhanced when

the number of social stimuli is the same in the two tests. Contrary to our expectation, we did not detect a significant correlation between sociability measured towards a mirror image and towards a live conspecific, although the correlation value was close to the threshold for statistical significance and graphical inspection of figure 3, suggested that the slope of the correlation was similar in these three experiments. A possible explanation for this finding is that the stimulus fish, being alone in the stimulus chamber, may also be attracted to the focal fish. If shoaling tendency of the focal fish is influenced by that of the stimulus, stimulus fish shoaling tendency will affect the result of the test, causing the observed weaker matching with sociability measured in the mirror test.

Conclusions

In summary, our results indicate that the mirror test may be effectively used to measure sociability provided that some limitations of the experimental setting are removed. In particular, we found that a transparent spacer between the mirror and the subject is very effectively in obtaining a sociability score that is approximate the one with live conspecifics. The addition of the odour of a conspecific further improved the matching between the sociability towards the mirror image as compared to that towards a group of conspecifics, although not significantly so. Considering that the addition of a conspecific's chemical can easily be obtained, we suggest that this may be the preferable experimental setting for measuring sociability with the mirror test at least in guppies. It would be interesting to test whether these or other specifically tailored modifications of the mirror test will improve the detection of inter-individual differences in sociability also in other fish species.

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HIGHLIGHTS:

- Sociability can vary greatly among species, populations and even individuals
- Quantifying the attraction to a shoal is used as a method to measure sociability
- Mirror test is also used to measure sociability but its validity is questioned
- Time spent close to a shoal is compared to that spent close to a mirror image
- The validity of the mirror test increases setting a more ‘naturalistic’ context

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