1 **Using a robotic fish to investigate individual differences in social**

2 **responsiveness in the guppy**

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15 *Running title: Social responsiveness towards Robofish*

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Abstract

Responding towards the actions of others is one of the most important behavioral traits whenever animals of the same species interact. Mutual influences among interacting individuals may modulate the social responsiveness seen and thus makes it often difficult to study the level and variation of individuality in responsiveness. Here, biomimetic robots (BRs) that are accepted as conspecifics but controlled by the experimenter can be a useful tool. Studying the interactions of live animals with BRs allows pinpointing the live animal's level of responsiveness by removing confounding mutuality. In this paper, we show that live guppies (*Poecilia reticulata*) exhibit consistent differences among each other in their responsiveness when interacting with a biomimetic fish robot – 'Robofish' – and a live companion. It has been repeatedly suggested that social responsiveness correlates with other individual behavioral traits like risk-taking behavior ('boldness') or activity level. We tested this assumption in a second experiment. Interestingly, our detailed analysis of individual differences in social responsiveness using the Robofish, suggests that responsiveness is an independent trait, not part of a larger behavioral syndrome formed by boldness and activity.

Keywords: biomimetic robots, fish-inspired robots, *Poecilia reticulata*, robotic fish, transfer entropy, social responsiveness, animal personality, animal temperament, behavioral syndromes

Introduction

Synchronized behaviors such as collective movements depend on the capability of involved subjects to respond to the actions of their social partners [1-6]. Such a responsiveness towards the social environment has been termed either 'sociability' ([7], the tendency to approach rather than to avoid conspecifics, see also [2, 8]), 'social competence' ([9], as an adaptive response in a social context) or 'social responsiveness' ([10], a tendency to respond to past or present reputation/action of conspecifics).

While there is some discussion regarding terminology (see [11]), assessing any response of an individual towards its social environment inevitably requires the presentation

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43   of social cues from conspecifics. The use of live conspecifics for this purpose typically is

44   problematic as they often interact with the focal individual and thereby introduce confounding

45   variation into the experimental design (see e.g. [12-15]). Thus, experimenters tried to control

46   for or standardize the possible mutual interactions among subjects. Some studies used pre-

47   trained live "demonstrators" to interact with naïve individuals [16-21], while other approaches

48   modified binary classical shoaling assays [22] in a way that individuals, although spatially

49   separated, can decide whether to associate or not with a visible conspecific in an adjacent

50   compartment [8, 23]. More recently, video playbacks or computer animations have been used

51   to create and even manipulate social stimuli [24-31]. Similarly, others have presented live

52   animals with spatially-separated artificial models of conspecifics [32-35]. However, realistic

53   tests of social responsiveness towards movement patterns of social partners, as for example

54   found in collectively moving shoals of fishes, herds of ungulates or flocking birds, require a

55   spatial scale [36].

56   The need to provide a spatial scale while still be able to control for or standardize

57   social interactions inspired experimenters to look for the interactions of live animals towards

58   moving replicas (see [37-42]). Recently, sticklebacks have been found to differ consistently

59   from each other in their attraction towards a dummy school that circulates at a constant speed

60   [42], a technique that has been also used previously to investigate shoaling tendencies in blind

61   cave tetras (*Astyanax mexicanus*, [40]). Even more sophisticated experiments became feasible

62   through the development of biomimetic robots [36, 43, 44]. Biomimetic robots, that are

63   accepted as conspecifics by live animals have several advantages over previous approaches,

64   such as the ability to completely standardize the behavior of the interacting robot, to set its

65   parameters to either resemble those of focal fish or show a sharp contrast with them, as well

66   as to allow the possibility to create interactive scenarios that nevertheless follow controlled

67   rules that can be adapted intentionally [45-48].

68   Using a biomimetic robot (hereafter called 'Robofish') that is accepted as a

69   conspecific by live Trinidadian guppies (*Poecilia reticulata*; see [45]), we ask in the current

70   study (a) whether animals differ in their social responsiveness and whether this difference can

71   be measured with a biomimetic robot; (b) whether among-individual differences in social

72   responsiveness towards moving (robotic) conspecifics are linked to other behavioral traits that

73   have been established to differ among live animals ('personality traits' see [7]).

74   In our first experiment (question a), we specifically predicted that an individual's level

75   of responsiveness towards a live conspecific should resemble that towards moving Robofish.

76   As our Robofish, although accepted as conspecific, is steered in an non-interactive open-loop

77   mode to omit mutual influences on the live fish's behavior, we do not expect that interactions

78   with Robofish resemble fully that of live fish interactions but predict that interaction patterns

79   should be similar. We measured several interaction parameters (inter-individual distance,

80   velocity cross-correlations, Transfer Entropy) of focal fish with live partners and, in a

81   subsequent test with Robofish partners and calculated behavioral repeatability, a measure for

82   consistent individual differences [49]. If tests with Robofish are able to depict an individual's

83   responsiveness, among-individual differences should be consistently detectable also when the

84   same focal fish is tested with a live partner.

85   It is known that behaviors often form correlated suits, so-called behavioral syndromes

86   [50]. For example, it is known from studies on sticklebacks that individuals with increased

87   tendencies to take risks (behavioral trait 'boldness' and/or 'exploration behavior') lead more

88   often and are less attracted by others, while those with lower tendencies to take risks are more

89   likely to follow others [8, 15]. As similar relations are possible in regard to the social

90   responsiveness of an individual (question b), we predicted in our second experiment that focal

91   fish that were highly responsive to Robofish's actions should be less risk-taking and

92   explorative while those that did not respond strongly to Robofish should be more risk taking

93   and explorative.

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## Methods

Study organism and maintenance

97   We used wild-type guppies (*Poecilia reticulata*) for our experiments that have been bred in

98   the laboratory for several generations and originated from wild-caught individuals. Test fish

99   came from large, randomly outbred single-species stocks maintained at the animal care

100   facilities at the Faculty of Life Sciences, Humboldt University of Berlin. We provided a

101   natural 12:12h light:dark regime and maintained water temperature at 26°C. Fish were fed

102   twice daily *ad libitum* with commercially available flake food (TetraMin™) and once a week

103   with frozen *Artemia* shrimps.

104

The Robofish system

106   The Robofish system consists of a glass tank (88×88 cm), which is mounted onto an

107   aluminum rack. A two-wheeled robot can move freely on a transparent platform below the

108   tank (figure 1A-B). The robot carries a magnet, coupling its motion with a second magnet in

109   the tank above. The second magnet serves as the base for a three-dimensional 3D-printed fish

110   replica (standard length (SL) =30.0 mm; resembling a guppy female, see figure 1C). This kind

111   of replicas are accepted as conspecifics by live guppies (and other fishes), most likely through

112   the use of glass eyes and by swimming in a natural motion pattern [45, 51]. The entire system

113   is enclosed in a black, opaque canvas to minimize exposure to external disturbances. The tank

114   is illuminated from above with artificial light reproducing the daylight spectrum. On the floor,

115   a camera is facing upwards to track the robot. A second camera is fixed above the tank to

116   track both live fish and the replica. Two computers are used for system operation: one PC

117   tracks the robot, computes and sends motion commands to the unit over a wireless channel;

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118   the second PC records the video feed of the ceiling camera, which is subsequently tracked by

119   a custom-made software [52].
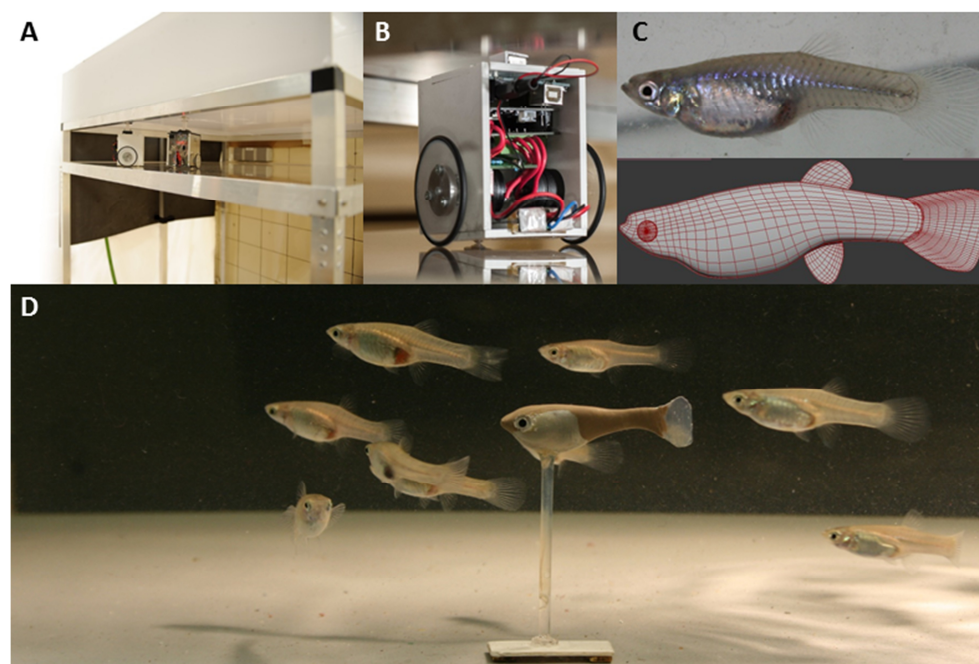
Figure 1: The Robofish system. (A) The robot unit is driving on a second level below the test arena. (B) Close-up of the robot unit. (C) A picture of a live guppy female served as template for the virtual 3D mesh that was printed on a 3D printer. (D) Guppy replica with a group of female guppies in the test arena.

Experiment 1

Experimental setup

To compare responses of live focal fish between tests with Robofish and with a live partner, each focal fish was tested once with Robofish and subsequently another time with a live model individual. This was done by testing one half of the focal fish first with Robofish and after two days with a live model fish, while the other half of the focal fish were first tested with a live model fish and after two days with Robofish. Focal fish were randomly assigned to start with the Robofish or live model treatment.

At the beginning of our experiment, we randomly selected adult fish from our stock tanks (females-only to reduce possible sex-specific differences) and marked them individually with VIE elastomeric color tags (see [53]). During this procedure, we also measured body length as

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136   standard length (from tip of snout to end of caudal peduncle) to the nearest millimeter (focal

137   fish: SL ± SEM = 30.1 mm ± 0.4 mm, *N*=30; live model fish: 30.5 mm ± 0.3 mm, *N*=30).

138   To initiate a trial, we transferred half of the test fish (*N*=15) into a Plexiglas cylinder

139   located at the upper left corner of the arena (see figure 2). The Robofish replica was also

140   located within the cylinder. After a habituation period of 2 minutes, robot and live fish were

141   released by lifting the cylinder with an automatic pulley system. When the live fish left the

142   cylinder (= one body length away from the cylinder's border), Robofish started swimming in

143   a natural stop-and-go pattern [45, 54] along a zigzag path to the opposite corner (figure 2).

144   After reaching this corner, the Robofish randomly swam to either the bottom left or the top

145   right corner in which it ultimately described a circular path for three rounds (figure 2).

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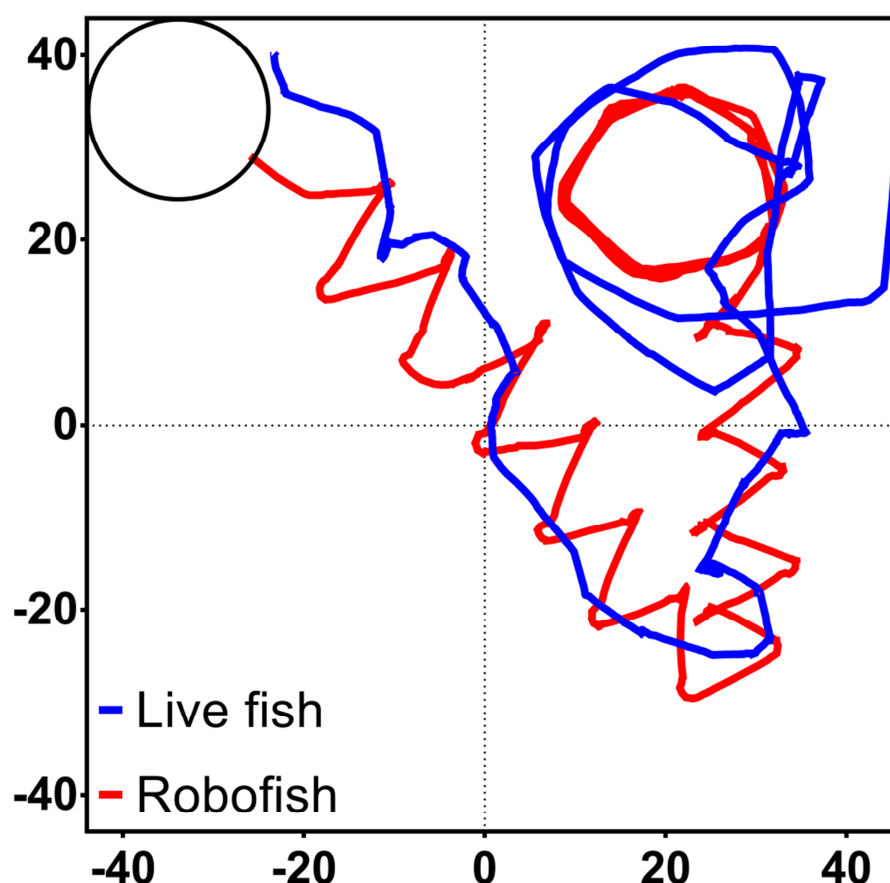
147   **Figure 2:** Example track of Robofish with live guppy in an 88cm x 88 cm test arena. After the live fish left the

148   start cylinder (upper left), Robofish moved in a natural stop-and-go pattern along a zigzagged path to the

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149   opposite corner. Upon arrival, Robofish moved to either the bottom left or the top right corner (here: top right)

150   and described a circular path.

151

152   The trial was then terminated and the test fish was transferred back to its holding tank. Each

153   trial was videotaped for subsequent analysis. A video recording following this protocol is

154   available as an online supplement (Video S1). To test whether the focal fish's response

155   towards Robofish can be linked to the response towards a live conspecific, the other half of

156   the focal fish was instead introduced into the start cylinder accompanied with a live

157   companion comparable in size to the Robofish replica (see above). Again, we lifted the

158   cylinder after 2 min of habituation and videotaped the trial for 2 min, starting when the last

159   fish left the cylinder. Trials involving only live fish were comparable in duration to Robofish

160   trials (live-live: 120s; Robofish: 124.1 s ± 1.9 s; mean ± SEM, variation in duration is due to

161   stop-and-go swimming pattern of Robofish). After two days the testing was repeated;

162   however, fish were now introduced to the opposite treatment. Thus, each focal fish ($N = 30$)

163   was tested with both the Robofish and a live companion ($N = 30$). To further randomize our

164   testing procedure, we performed Robofish and model fish trials in an alternating order at each

165   experimental day.

166   All video recordings were subjected to a custom-made software [52] to extract

167   position and orientation of both interaction partners over time. Based on the tracked positions,

168   we calculated several measures that characterize social interactions (see [19, 54-58]).

169   As a simple proxy for the social interaction among subjects, we calculated the inter-

170   individual distance (IID) between focal fish and companion (Robofish or live fish, body

171   centroids) for each trial [2]. It is strongly correlated with other distance-related measures, such

172   as the time fish spent within a specific range (not shown), and short distances between

173   subjects suggest strong interactions (e.g., at least one subject must follow the companion

174   closely).

175   As our major goal was to determine focal individual's responsiveness towards its

176   companions (Robofish or live model), we calculated subject-specific interaction measures for

177   each individual (focal, live model as well as Robofish) within a pair. Freely interacting live

178   fish respond rapidly to conspecifics' movements by adjusting their own movement patterns

179   [19, 54, 57-60]. To quantify this response in movement patterns, we calculated time-lagged

180   cross-correlations of velocity vectors (TLXC), which allow to distinguish how strongly

181   subject adjust their own movement towards that of the partner's movement [61]. For any

182   given time lag τ, TLXC indicates the strength of the correlation between the velocity vector of

183   the focal individual at time $t+\tau$ and the other companion individual at time t. A large positive

184   value implies that on average the focal individual responds by moving in the same direction as

185   its companion, whereas values close to zero correspond to a random response and negative

186   values indicate a movement in opposite direction. In a representative sample of our dataset, all

187   first extrema in the cross-correlation can be found for lags < 6 s. We thus restricted our

188   analysis to lag-times up to $\tau = 6$ s. We calculated the cross-correlation averaged over the

189   entire time lag window for both subjects within a pair. Subject-specific TLXCs were then

190   used to calculate a global correlation measure as the difference between focal fish's average

191   cross-correlation and companion's average cross-correlation (ΔTLXC; positive values: focal

192   fish followed on average; negative values: focal fish led on average). When interactions are

193   strong (e.g., when individuals are in close range), ΔTLXC values around zero indicate rapidly

194   switching leadership roles/high mutuality in social responsiveness. When interactions are

195   weak (e.g., at longer distances between fish), ΔTLXC values around zero indicate weak

196   overall social responsiveness (for more details on the calculation of TLXC please see our

197   Supplemental Information S1_Text1).

198   Transfer Entropy (TE) between velocity vectors of both individuals [62, 63]. $TE_{i \to j}$ is

199   an information theoretic, model-free measure of directed ('causal') coupling between two

200   time series i and j, and was shown to generalize Granger Causality to arbitrary nonlinear,

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201 stochastic processes [64]. For simplicity, we will use the short notation TE_i for the average

202 $TE_{i \rightarrow j}(\tau)$, the Transfer Entropy between individual i and j at time lag τ. As for TLXC, we

203 measured the average TE_i by averaging $TE_{i \rightarrow j}(\tau)$ over the entire time window (see SI_Text1)

204 to quantify responsiveness. As the baseline values of TE_i depend on various factors not

205 related to actual couplings, as e.g. activity of individuals, trajectory length and parameters of

206 the used entropy estimator [65], we calculated the difference between focal fish's average TE_i

207 and companion's average TE_j ($\Delta TE = TE_{j \rightarrow i}(\tau) - TE_{i \rightarrow j}(\tau)$), which may be interpreted as the

208 global net information flow with respect to velocity dynamics (see S1_Text1) between both

209 individuals with the sign indicating the direction of the information flow and the absolute

210 value indicating its strength. Positive values indicate that information is predominantly

211 transferred from the companion (j) to the focal fish (i) while negative ones indicate the

212 opposite. Values around zero suggest equal information transfer between subjects (when

213 interactions are strong) or no information transfer (when interactions are weak).

214 *Statistical analysis*

215   In order to see whether the magnitude of social interactions between live pairs and

216 Robofish pairs differed on average, we compared inter-individual distance (log-transformed),

217 velocity cross-correlations (average TLXC of a pair as well as ΔTLXC) and Transfer Entropy

218 (average TE of a pair as well as ΔTE) between live pairs and Robofish pairs using paired *t*-

219 tests. In a second analysis, we compared TLXC and TE between subjects within Robofish as

220 well as live fish pairs using paired samples *t*-tests. A correlational analysis between IIDs and

221 TLXCs as well as TEs can be found as supplemental information (SI_Text2).

222   In our third analysis, we asked whether focal fish's individual differences in

223 responsiveness towards Robofish are mirrored in their interactions with live companions. We

224 thus used univariate Linear Mixed Models (LMMs) with IID, TLXC (subject-specific and Δ

225 TLXC)), and TE (subject-specific and ΔTE) as dependent variables and included focal fish ID

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226 as a random factor to calculate the behavioral 'repeatability' [49]. The repeatability of a

227 behavior is defined as the proportion of the total behavioral variance (sum of variation that is

228 attributable to differences among individuals plus variation within individuals) towards the

229 amount of variation that is attributable to differences among individuals. As variance

230 estimates are inherently tied to the total variation present in the response variable, we first

231 mean-centered and scaled the variance of our response variables to 1 within each treatment

232 (e.g., z-transformation). No fixed factors were included in the LMM to obtain conservative

233 measures of among- and within-individual variation [49]. A significant repeatability estimate

234 is interpreted as evidence of consistent individual differences and we tested for significance

235 using likelihood ratio tests (see [66]).

236

Experiment 2

Experimental setup

239 The aim of our second experiment was to investigate a potential link between social

240 responsiveness and other already established personality traits. Here, we were also interested

241 in possible differences among the sexes and thus included males in our tests. To do so, male

242 (N=17, SL = 19.5 mm ± 0.4 mm SEM) and female guppies (N=25, SL=27.6 ± 0.6 mm) were

243 VIE tagged as described for experiment 1 and kept in 100-L tanks. After one week of

244 acclimatization, all fish were tested three times for their personality types including their

245 tendency to respond to Robofish.

246

Personality tests with Robofish

248 A test trial consisted of three consecutive parts. In the first part, focal fish were randomly

249 taken from the stock tank and introduced into an opaque plastic cylinder with a small opening.

250 The opening was closed with a sponge and fish were given 1 minute for habituation. Then, the

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251   sponge was removed and we scored the time each fish took to leave the cylinder as a measure

252   of boldness (smaller values indicate bolder personalities, see [67, 68]). Robofish was

253   positioned close to the opening at the outside of the cylinder so that the live fish could not see

254   the Robot from the inside but could not miss it once it left the cylinder. Once the focal fish

255   has left the cylinder, Robofish initiated the same zigzag sequence as described for experiment

256   1. However, this time Robofish did not move in a circular path, but was removed immediately

257   after reaching one corner. To test for focal fish's tendency to respond towards Robofish, we

258   calculated subject-specific as well as pair-wise parameters described for experiment 1 as

259   response variables. After Robofish was removed, focal fish were left in the tank and given 2

260   minutes for habituation. All fish resumed normal swimming within this 2 minutes and we

261   videotaped them for another 3 minutes to get a measure of general activity (mean

262   velocity,[69, 70]). Hence, each trial scored three different behavioral traits for which guppies

263   are assumed to differ consistently among each other. Video analysis and parameter calculation

264   followed the description provided for experiment 1.

265

266   *Statistical analysis*

267   To quantify how repeated testing or differences in sex and body size of the fish affected

268   average behavioral traits, we analyzed 'boldness' (emergence time in s; log transformed prior

269   to all statistical analysis), 'social responsiveness' (log-transformed IID, ΔTLXC and ΔTE; see

270   experiment 1) and 'general activity' (mean velocity in cm/s) as dependent variables in

271   separate LMMs with trial (three repeated test runs) and sex as fixed factors and focal fish's

272   body size (SL) as a covariate. Focal ID was included as random factor to account for repeated

273   tests.

274   To see whether focal fish differed consistently in any of the behavioral traits, we used

275   another set of LMMs with behavioral traits as dependent variables and Focal ID as a random

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276   factor. Similar to the analysis described for our first experiment, we first mean-centered and

277   scaled the variance of our response variables to 1 within each trial (e.g., z-transformation).

278   We further asked whether our recorded behavioral traits may form a larger behavioral

279   syndrome. We thus used Principle Component Analysis (PCA) with average values (z-

280   transformed values averaged over the three trials) of pair-wise parameters (log emergence

281   time, mean velocity, log IID, ΔTLXC and ΔTE) and extracted all components with

282   Eigenvalues > 1. The resulting component matrix was Varimax rotated to ease interpretation

283   of component loadings. Only loadings with values ≥ 0.8 were considered as important.

284

Results

286   **Experiment 1**

287   ***General response in pairs involving Robofish and only live fish***

288   On average, general coupling between subjects was weaker in Robofish pairs than in live fish

289   pairs. Distance between subjects was longer (paired *t*-test, IID: t_{29}=-2.353; *P*=0.022, figure

290   3a), velocity correlations were less pronounced (average TLXC of both subjects: t_{29}=-3.434;

291   *P*=0.002; figure 3b) and information transfer rates were lower (average TE of both subjects:

292   t_{29}=-3.434; *P*=0.002; figure 3c) when focal fish were paired with Robofish as compared to a

293   live companion.

294   Robofish's velocity vectors were not correlated with those of live focal fish as

295   indicated by velocity vector cross-correlations (TLXC) of Robofish around zero that were

296   significantly lower than those of the focal fish in Robofish pairs (t_{29}=-6.613; *P*<0.001; figure

297   3b). In live pairs, both fish adjusted their velocities towards each other as indicated by high

298   TLXCs that did not differ between subjects (t_{29}=-0.901; *P*=0.375; figure 3b). As a result,

299   ΔTLXC was significantly higher in Robofish pairs compared to live fish pairs (t_{29}=-4.031;

300   P<0.001; figure 3b).

301   Regarding TE, the lack of response of the Robofish led to a significant difference in

302   TE values between live focal fish and Robofish (t_{29}=-5.442; P<0.001; figure 3c) with a net

303   information flow towards focal fish (positive ΔTEs, figure 3c). This pattern was not seen in

304   live pairs where TEs did not differ between subjects (t_{29}=-1.259; P=0.218; figure 3c) as

305   information flow was shared equally (small ΔTEs, significantly different to that found in

306   Robofish pairs; t_{29}=-2.001; P=0.043, figure 3c).

307   Overall, our results indicate that focal fish in Robofish pairs were predominately

308   adjusting their own swimming behavior to that of Robofish and not vice versa, while focal

309   fish and live model companions within live pairs were mutually responding towards each

310   other. Thus, the general difference in interaction patterns between Robofish pairs and live fish

311   pairs are likely due to the mutual responses that we see in live fish pairs but, as intended, not

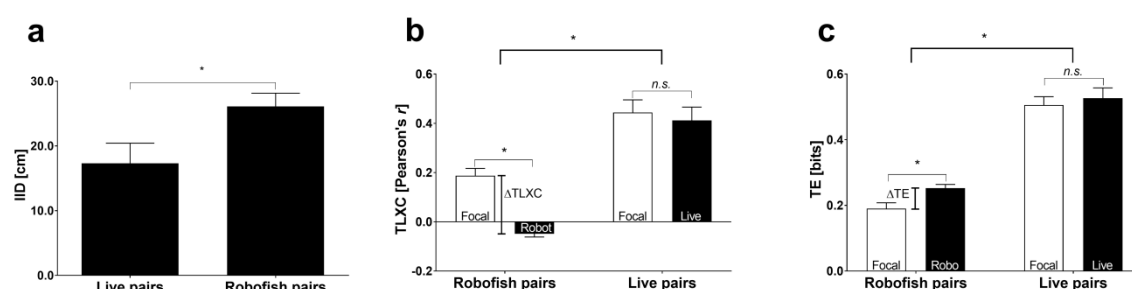312   in Robofish pairs.

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315   Figure 3: Average differences between Robofish pairs and live fish pairs in inter-individual distances (IID, a),

316   time-lagged cross-correlation of velocity vectors (TLXC, b) and Transfer Entropy (TE, c). Shown are means ±

317   SEM. Asterisks indicate significant differences in t-tests (see main text).

318

Individual differences in social responsiveness

We hypothesized that a live fish's reaction towards Robofish should reflect its social responsiveness, similar as to when tested with another live companion. Although there are general differences among response towards Robofish and a live companion, we found that focal individuals differed consistently across treatments with regard to all subject-specific interaction parameters as well as inter-individual distances (IID) and Robofish's TE and ΔTE (table 1a). Only companions' TLXC and ΔTLXC were not repeatable. This is most probably due to the discrepancy between high TLXC values in live companions (highly responsive) and the low TLXC values of Robofish (non-responsive; see figure 3b). Interestingly, focal individuals' influence on companions' TE and ΔTE seem to be strong enough to even detect consistent individual differences here.

Table 1: Behavioral repeatability of subject-specific and pair-wise interaction parameters. Shown are repeatability values obtained from LMMs on treatment-centered and normalized parameters along with 95% credibility intervals and significance levels from likelihood ratio tests. A significant repeatability indicates consistent individual differences. Significant repeatability values are in bold type face.

Parameter	Repeatability	95%CI		*P*
Experiment 1				
Inter-Individual Distance (IID)	**0.44**	**0.35**	**0.54**	**0.007**
Cross-correlation, focal fish (TLXC$_{focal}$)	**0.40**	**0.29**	**0.51**	**0.015**
Cross-correlation, companion (TLXC$_{companion}$)	0.00	n.a.	n.a.	0.934
Difference in Cross-Correlations (ΔTLXC)	0.09	0.00	0.80	0.767
Transfer Entropy, from focal fish (TE$_{focal}$)	**0.33**	**0.22**	**0.48**	**0.045**
Transfer Entropy, from companion (TE$_{companion}$)	**0.30**	**0.18**	**0.48**	**0.049**
Difference in Transfer Entropy (ΔTE)	**0.45**	**0.36**	**0.54**	**0.005**
Experiment 2				
emergence time (boldness)	**0.39**	**0.30**	**0.48**	**0.004**
mean velocity (general activity)	**0.31**	**0.22**	**0.42**	**0.012**
Inter-Individual Distance (IID)	**0.31**	**0.22**	**0.42**	**0.012**
Cross-correlation, focal fish (TLXC$_{focal}$)	**0.31**	**0.21**	**0.42**	**0.013**
Cross-correlation, Robofish (TLXC$_{Robofish}$)	0.10	0.02	0.37	0.320

Difference in Cross-Correlations (ΔTLXC)	0.32	0.23	0.43	0.011
Transfer Entropy, from focal fish (TE$_{focal}$)	0.31	0.21	0.42	0.013
Transfer Entropy, from Robofish (TE$_{Robofish}$)	0.33	0.24	0.44	0.009
Difference in Transfer Entropy (ΔTE)	0.18	0.08	0.34	0.032

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Experiment 2:

Is Social Responsiveness part of a larger behavioral syndrome?

On average, boldness scores were not affected by repeated testing, i.e. there was no effect of

habituation ($F_{2,82}$=0.092, P=0.911; figure 4a). However, focal fish decreased their general

activity significantly over time (sig. effect of factor 'trial' in LMM: $F_{2,82}$=9.111, P<0.001,

figure 4b). A similar effect was observed for social responsiveness parameters (IID:

$F_{2,82}$=30.908, P<0.001, figure 4c; ΔTLXC: $F_{2,82}$=11.737, P<0.001, figure 4d; ΔTE:

$F_{2,82}$=5.683, P=0.005, figure 4e) and focal fish adjusted their velocities to a lesser extent and

kept longer distances towards Robofish with repeated testing. Interestingly, net information

flow towards focal fish (ΔTE) increased with repeated testing. Body length of the test fish had

no significant effect in either model (not shown), while males received more information from

Robofish than females (ΔTE: sig. effect of factor 'sex': $F_{1,39}$=9.626, P=0.004).

Our repeatability analysis found that focal fish differed consistently in all three

behavioral traits (Table 1, figure 4, right side). This repeatability indicates that focal fish

largely maintained their individual differences in boldness and activity as well as

responsiveness when interacting with Robofish over the course of the repeated testing.
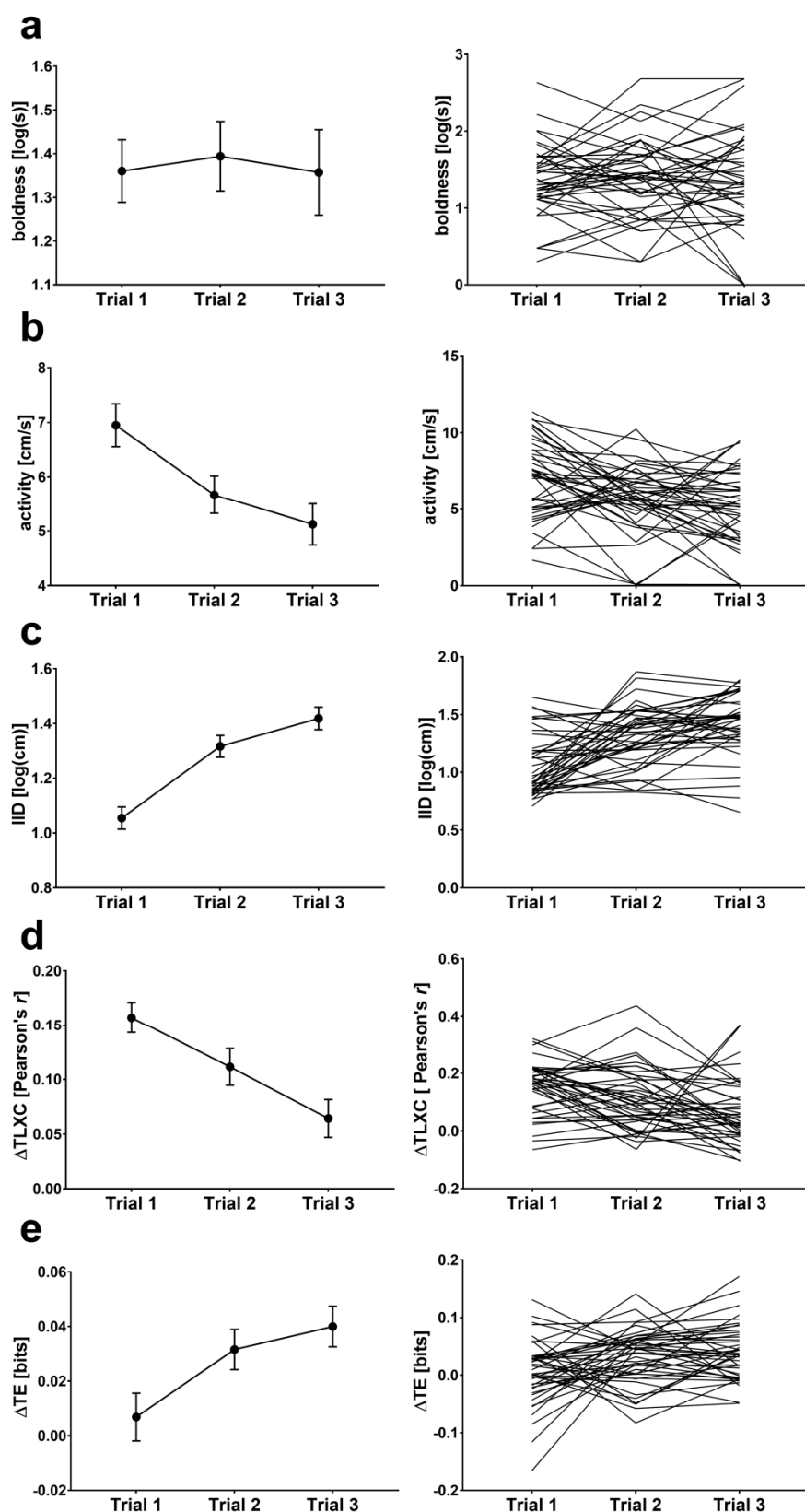
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Figure 4: Behavioral traits in the guppy. (left) Average values for behavioral traits across the repeated testing. Shown are means ± SEM. Please note that except for 'boldness', trial 1 was always significantly different from

357   trial 2 and 3 (*post-hoc* LSD tests). (right) Individual values for focal fish. Each line shows a focal individual's

358   behavioral expression over the repeated testing.

359

360   Principle component analysis (PCA) found 3 components with Eigenvalues >1 that explained

361   84.6% of the total variance in the data. Boldness and activity loaded strongest on component 2

362   (PC2, 24.5% variance explained; figure 5), which suggests that bolder fish (i.e., those that left

363   the start cylinder quicker) were also more active (negative relation). IID and ΔTLXC loaded

364   strongest on PC1 (39.5% variance explained), and, similar to the results from experiment 1,

365   ΔTLXCs were high in pairs where focal fish were also close to Robofish (see SI_Text2) while

366   ΔTLXCs decreased when IIDs increased (different signs in component loadings, see figure 5).

367   ΔTE loaded independent of other variables on PC3 (20.6% variance explained). To sum up,

368   our proxies for social responsiveness (IID, ΔTLXC, ΔTE), although repeatable, were not

369   correlated with boldness or activity and, thus, do not seem to be part of a larger boldness-
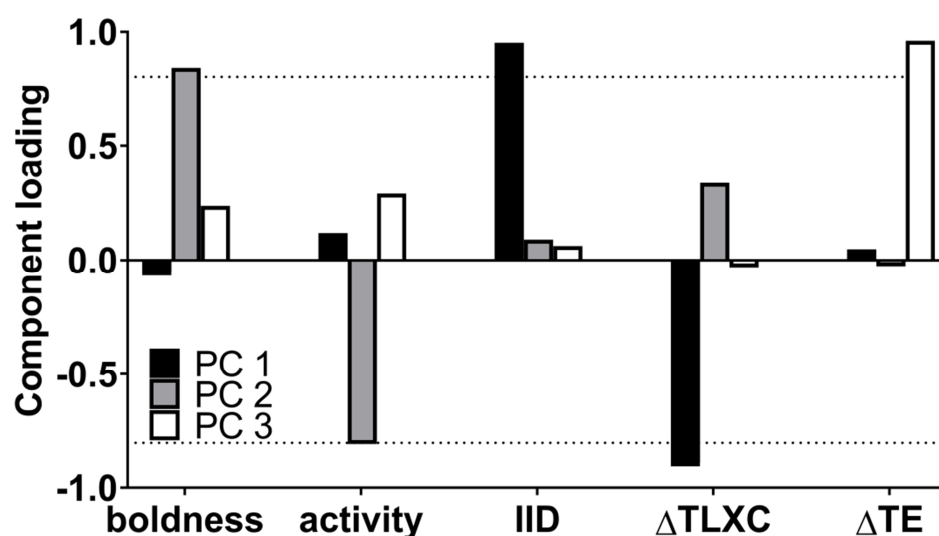
370   activity syndrome.

371

372   Figure 5: Results from Principle Component Analysis (PCA) on pair-wise behavioral traits. Shown are

373   component loadings for the first three PCs with Eigenvalues >1. Dashed line at 0.8 indicates important loadings.

374

Discussion

Our results show that guppies were consistent in their individual responses between a live companion and a robotic companion – the Robofish (first experiment). Furthermore, these individual differences are maintained over repeated testing with Robofish even though habituation to the test tank is detectable (second experiment). In addition, guppies differed consistently in their boldness and general activity with a similar habituation to the test tank found for the latter. While our boldness and activity measures were correlated (e.g., seem to form a behavioral syndrome with bolder individuals being also more active), social responsiveness towards Robofish was not correlated with boldness and activity measures and thus independent of the boldness-activity behavioral syndrome.

In our first experiment, we aimed to validate our approach as biologically meaningful (see [71]) by showing that interactions of live fish with our robot (a) contain elements that are typical of pair-wise interactions in live fish and (b) that they reflect individual differences in guppies' social responsiveness. Indeed, guppies readily followed the moving Robofish and mean distances between subjects largely overlapped with those recorded for live fish pairs, although live fish were on average closer to each other. In regard to our measures of social responsiveness (velocity cross-correlations [TLXC] and transfer entropy [TE]), focal guppies tested with Robofish showed comparable albeit weaker response patterns as when tested with live companions. Nevertheless, live guppies paired with Robofish adjusted their velocities in accordance to Robofish (positive TLXC of live fish in Robofish pairs) especially when in close range (SI_Text2). Similarly, our measure of information transfer (TE) found live guppies to integrate directional information received from Robofish into their own movement patterns (information transfer from Robot to live fish) which was greatest when subjects were close together (SI_text2).

19

399   The major difference between interactions of live fish pairs and pairs involving

400   Robofish was the strikingly high mutual responsiveness of individuals in live pairs (no

401   significant differences in TLXC and TE within live pairs). In contrast, Robofish's (intended)

402   unresponsiveness was well detectable (zero TLXC, low information flow from live fish to

403   Robofish) leading to significant differences in net (Δ) TLXC and net (Δ)TE compared to live

404   fish pairs. These differences may have further caused the greater inter-individual distances

405   among subjects in Robofish pairs. Despite these differences, focal fish maintained individual

406   differences (sig. repeatability) in individual interaction parameters across both test situations

407   (i.e., with a live companion and a Robofish partner). We are, thus, confident that reactions

408   towards Robofish provide a consistent and reliable measure for social responsiveness in live

409   guppies (see similar validations for Zebrafish's (*Danio rerio*) responses towards biomimetic

410   robots [32] and experiments with sticklebacks and circulating replica shoals [42]).

411   While individual differences in behavior seem to be an inevitable outcome of complex

412   development and arise even in the absence of genetic and/or environmental variation [70],

413   many personality traits form larger correlated suits, so-called 'behavioral syndromes' [72]. In

414   our second experiment, we show that guppies differed consistently in boldness, activity, as

415   well as social responsiveness towards Robofish. However, responsiveness towards Robofish

416   was not correlated with boldness and activity and thus most likely not part of a larger

417   behavioral syndrome formed around a boldness-activity axis. The correlation between

418   boldness and activity is well described for poeciliid fishes including the guppy [69, 73-76]. As

419   a point of caution, we note that we might overestimate correlations between boldness and

420   activity as we measured both traits in short succession at the same day (see [77] for a

421   discussion).

422   In many species, bolder individuals are more likely to initiate exploration of new

423   environments and thus are assumed to have a greater tendency to lead others [8, 15, 21, 78-

424   82]. In sticklebacks, bolder individuals are often less socially attracted ('less sociable') and

20

425   less responsive to their current social partners [8, 23]. Both traits are assumed to result in

426   lower tendencies to follow other group members [15], yet this does not hold true for our

427   results as responsiveness towards Robofish was not correlated with boldness. We do not have

428   a compelling explanation for this discrepancy but might argue that our study design did not

429   allow any kind of mutual feedback, which is known to modulate leader-follower interactions

430   [81, 83] and is an inevitable property of any tests involving multiple live animals [36]. One

431   possibility to investigate the effect of mutual but still controllable feedback would be to use

432   interactive robots (closed-loop mode) that respond to live subjects [45, 48].

433

Acknowledgments

438

Ethics

440   The experiments reported herein comply with the current German laws approved by LaGeSo

441   Berlin (Reg 0117/16 to D.B.).

442

Author contributions

444   D.B., T.L., M.W. and J.K. conceived the study; D.B., J.L. and H.N. performed the

445   experiments; D.B. and P.R. analyzed the data; D.B., T.L. and H.N. developed the Robofish

446   system; D.B. wrote the manuscript. All authors contributed to and approved the submitted

447   version of the manuscript.

448

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Competing interests

The authors declare no competing interests.

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657 # Supplemental Information

658 **Using a robotic fish to investigate individual differences in social responsiveness in the guppy**

659 David Bierbach[*], Tim Landgraf[*], Pawel Romanczuk, Juliane Lukas, Hai Nguyen, Max Wolf, Jens Krause

660 *Authors contributed equally*

661 *SI_TEXT1: Calculation of interaction parameters*

662 Average time-delayed cross-correlation (TLXC):

663 We calculated first the time-delayed normalized cross correlation function for different values

664 of the lagtime τ: $C(\tau) = < v(t)v_f(t+\tau) >_t$ with v_f being the velocity of the focal individual and

665 $<...>_t$ indicating a time average. Then we calculated **TLXC** as the average $C(\tau)$ over a finite

666 range of timelags (0-6s): **TLXC** $= < C(\tau) >$

667 Average Transfer Entropy (TE):

668 The transfer entropy is calculated based on velocity vectors as input variables. First we

669 calculate the transfer entropy for a given timelag using:

670

$$TE(\tau) = \sum_t p(\mathbf{v}_f^{(t+\tau)}, \mathbf{v}_f^{(t)}, \mathbf{v}^{(t)}) \frac{p(\mathbf{v}_f^{(t+\tau)} | \mathbf{v}_f^{(t)}, \mathbf{v}^{(t)})}{p(\mathbf{v}_f^{(t+\tau)} | \mathbf{v}_f^{(t)})}$$

673 with $v_f^{(t+\tau)}$ and $v_f^{(t)}$ being the velocity vectors of the focal individual at time $t+\tau$ (future) and t

674 (past), respectively, and $v^{(t)}$ being the velocity of the other individual at time t. Here,

675 $p(\mathbf{v}_f^{(t+\tau)}, \mathbf{v}_f^{(t)}, \mathbf{v}^{(t)})$

676 is the overall probability of observing the corresponding values of the three velocity vectors,

677 $p(\mathbf{v}_f^{(t+\tau)} | \mathbf{v}_f^{(t)}, \mathbf{v}^{(t)})$

678 is the probability to observe future velocity vector of the focal individual $v_f^{(t+\tau)}$ conditioned on

679 the past velocity of the focal individual as well as the other individual, whereas

680 $p(\mathbf{v}_f^{(t+\tau)} | \mathbf{v}_f^{(t)})$

681 is the probability to observe $v_f^{(t+\tau)}$ conditioned only on the past velocity of the focal

682 individual. For efficient estimation of the transfer entropy we used the k-nearest neighbor

683 estimator [1-3]. The averaged TE was then obtained by averaging the TE(τ) over a range of

684 lagtimes τ (0-6s).

685

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695　　*SI_TEXT2: Additional results for Experiment 1*

696　　Measures of social responsiveness: Velocity vector cross-correlations (TLXC) and

697　　inter-individual distances (IID)

698　　We found that focal fish's (albeit weaker) responses to Robofish resembled those shown

699　　towards live conspecifics: Focal fish adjusted their movement patterns (velocity vectors)

700　　rapidly to those of their respective partners (both live companions and Robofish) when in

701　　close range and this response became weaker with increasing distance among subjects (figure

702　　S1a,b). This was indicated by a significantly negative correlation between inter-individual

703　　distance (IID) and focal fish's time-lagged velocity vector cross-correlation (TLXC) both in

704　　Robofish pairs (focal fish: $r_{pearson}$=-0.82; N=30, P<0.001; figure S1a) as well as in live fish

705　　pairs (focal: $r_{pearson}$=-0.79; N=30, P<0.001; figure S1b).

706　　　　　Regarding the companions, live model fish responded with a similar adjustment of

707　　own velocity vectors towards the focal fish and cross-correlations decreased similarly with

708　　increasing distance among subjects (companion: $r_{pearson}$ =-0.68; N=30, P<0.001; figure S1b).

709　　However, the non-interactive Robofish did not adjusted its movement towards the focal fish at

710　　any distance (no correlation detectable for Robofish's TLXC; $r_{pearson}$=0.2; N=30, P=0.32;
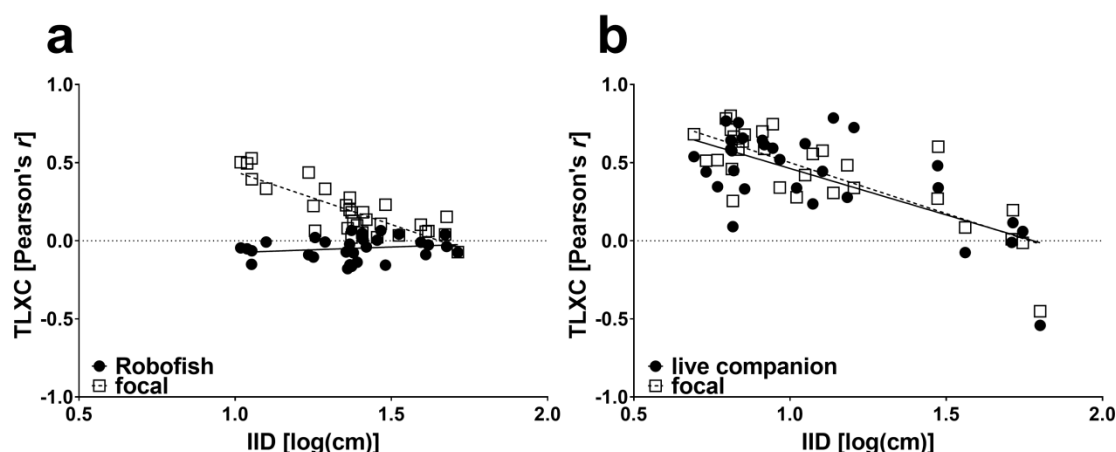
711　　figure S1a).

712

713

714　　Figure S1: Time-lagged cross-correlation of individual velocity vectors (TLXC) and inter-individual distances

715　　(IID). (a) In Robofish pairs, only live fish's TLXCs were correlated with inter-individual distance (IID) but not

716　　Robofish's TLXC. (b) In live fish pairs, both subjects showed a correlation between TLXC and IID.

717

718

719   Measures of social responsiveness: Transfer Entropy (TE) and inter-individual

720   distance (IID)

721   As for velocity cross-correlations, levels of information transfer towards focal fish in

722   Robofish pairs became higher the closer the subjects were (sig. negative correlation between

723   IID and Robofish's TE in Robofish pairs; r_{pearson}=-0.584; N=30, P<0.001; figure S2a), while

724   the TE from live fish to Robofish did not correlate with distance between subjects (no

725   correlation detectable for live fish's TE_i with IID in Robofish pairs; r_{pearson}=-0.350; N=30,

726   P=0.058; figure S2a).

727   Interestingly, TE for both subjects in live pairs was independent of inter-individual

728   distance (IID) (no correlations; focal: r_{pearson}=0.08; N=30, P=0.658; companion: r_{pearson}=0.02;

729   N=30, P=0.91; figure S2b).
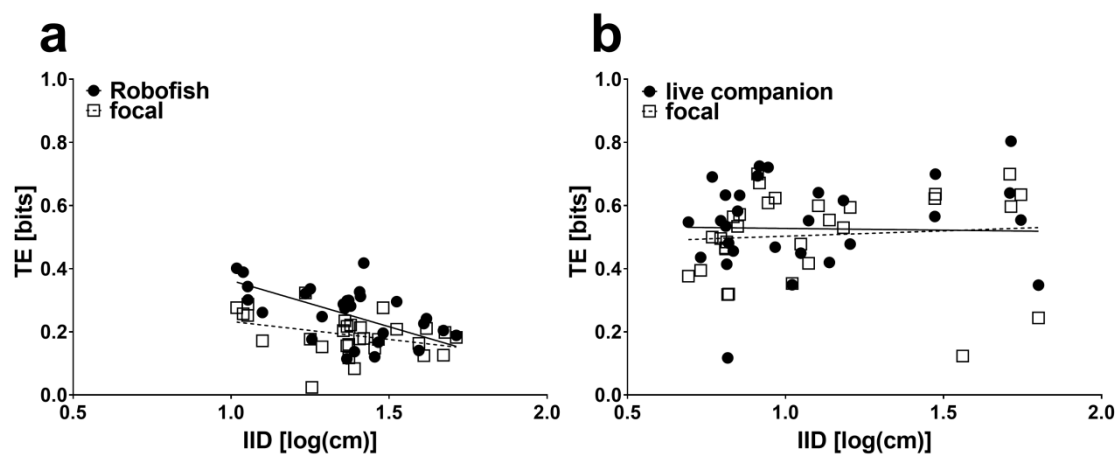

730

731   Figure S2: Transfer Entropy (TE) and inter-individual distances (IID). (a) In Robofish pairs, only Robofish's TE

732   were correlated with inter-individual distance (IID) but not live fish's TE. (b) In live fish pairs, both subjects

733   showed no correlation between TE and IID.

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