1 Guppies prefer to follow large (robot) leaders irrespective

2 of own size

3 David Bierbach[1*], Hauke J. Mönck[2], Juliane Lukas[1], Marie Habedank[1], Pawel Romanczuk[3], Tim

4 Landgraf[2], Jens Krause[1,4]

5 *1* Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland

6 Fisheries, Berlin, Germany

7 *2* Freie Universität Berlin, FB Mathematik u. Informatik, Berlin, Germany

8 *3* Department of Biology, Institute for Theoretical Biology, Humboldt Universität zu Berlin, Berlin,

9 Germany

10 *4* Faculty of Life Sciences, Thaer-Institute, Humboldt University of Berlin, Berlin, Germany

11 *corresponding author: david.bierbach@gmx.de

12

13 Abstract

14 Body size is often assumed to determine how successful an individual can lead others with larger individuals

15 being more likely to lead than smaller ones. However, direct evidence for such a relation is scarce. Furthermore,

16 even if larger individuals are more likely to lead, body size correlates often with specific behavioral patterns

17 (e.g., swimming capacity) and it is thus unclear whether larger individuals are more often followed than smaller

18 ones because they are larger or because they behave in a certain way. To control for behavioral differences

19 among differentially-sized leaders, we used biomimetic robotic fish – Robofish – of different sizes. Robofish is

20 accepted as a conspecific by live guppies (*Poecilia reticulata*) and provides standardized behaviors irrespective

21 of its size. We specifically asked whether larger leaders are preferentially followed when behavior is controlled

22 for and whether the preferences of followers depend on their own body size or their risk taking behavior

23 ('boldness'). We found that live guppies followed larger Robofish leaders closer than smaller ones and this

24 pattern was independent of the followers' own body size as well as risk-taking behavior. This is the first study

25 that shows a 'bigger is better' pattern in leadership in shoaling fish that is fully independent of behavioral

26 differences between differentially-sized leaders and followers' own size and personality.

Bierbach et al.

Introduction

28   What defines how successfully an individual can lead others? In shoaling fish, those individuals that

29   occupy front or periphery positions within a shoal are assumed to have the greatest influence on the

30   group's movement direction, hence are capable of leading the other shoal members [1-4]. Often,

31   occupation of front or peripheral positions is related to motivational or phenotypical differences

32   among individuals [2, 5]. For example, individuals that go front are hungrier [6, 7], more risk-taking

33   ('bolder') [4, 8-11] or simply larger [5, 12] than the rest of the shoal. Mechanistically, those

34   individuals at front may swim faster [4, 12] or have larger repulsion areas [5, 13], both leading to an

35   assortment within the shoal. However, being at the front (i.e., taking the lead) is often not the only

36   factor determining leadership success. Using the golden shiner (*Notemigonus crysoleucas*), Reebs

37   ([14]) showed that a minority of informed large fish was capable of leading a shoal of small fish to a

38   food location but informed small fish had much lower success in leading a shoal of large fish even

39   when occupying the front positions in the shoal. Furthermore, when sticklebacks (*Gasterosteus*

40   *aculeatus*) were grouped with two partners of different personalities, they were more likely to follow

41   the partner of similar personality out of cover [8]. Thus, both body size as well as behavior may

42   determine leadership success in fishes. In addition, both body size and behavior often covary with

43   each other, for example larger fish can swim faster than small ones [1] or exhibit a certain personality

44   [15] and only recently Romenskyy et al. [13] concluded that "fish of different sizes cannot be

45   considered simply as particles of different physical size, since their behavior changes with their size".

46   It is thus unclear whether larger individuals are more often followed than smaller ones because they

47   are larger or because they behave in a certain way. Furthermore, we do not know whether following

48   depends on the followers' own size or behavior and how follower size and behavior may interact with

49   leader size. To answer these questions it is necessary to experimentally control for the leader's

50   behavior while simultaneously varying its body size (or vice versa).

51   In the current study, we used a biomimetic robotic fish – Robofish – that is accepted as a

52   conspecific by live fish (guppy, *Poecilia reticulata*, [16, 17]) to gain control over the behavior of the

53   leader. We asked (a) whether larger leaders are preferentially followed (as predicted by a "bigger is

Bierbach et al.

54   better" hypothesis) when behavior is controlled for and (b) whether the preferences of followers

55   depend on their own body size or their risk taking behavior ('boldness').

56

Methods

### *Study organism and maintenance*

59   We used Trinidadian guppies (*Poecilia reticulata*) that were descendants of wild-caught fish from the

60   Arima River in North Trinidad. Test fish came from large, randomly outbred single-species stocks

61   maintained at the animal care facilities at the Faculty of Life Sciences, Humboldt University of Berlin.

62   We provided a natural 12:12h light:dark regime and maintained water temperature at 26°C. Fish were

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

64   frozen *Artemia* shrimps.

65

The Robofish system

67   The Robofish is a three-dimensional (3D)-printed guppy-like replica that is attached to a magnetic

68   base. The magnetic base aligns with a wheeled robot that is driving below the actual test tank (88×88

69   cm) on a transparent second level. Hence the replica can be moved directly by the robot (Figure 1).

70   The entire system is enclosed in a black, opaque canvas to minimize exposure to external disturbances.

71   The tank is illuminated from above with artificial light reproducing the daylight spectrum. On the

72   floor, a camera is facing upwards to track the robot's movements through the transparent second level.

73   A second camera is fixed above the tank to track both live fish and replicas. Two computers are used

74   for system operation: one PC tracks the robot, computes and sends motion commands to the robot over

75   a wireless channel. The second PC records the video feed of the second camera which is afterwards

76   tracked by custom-made software [18]

Bierbach et al.
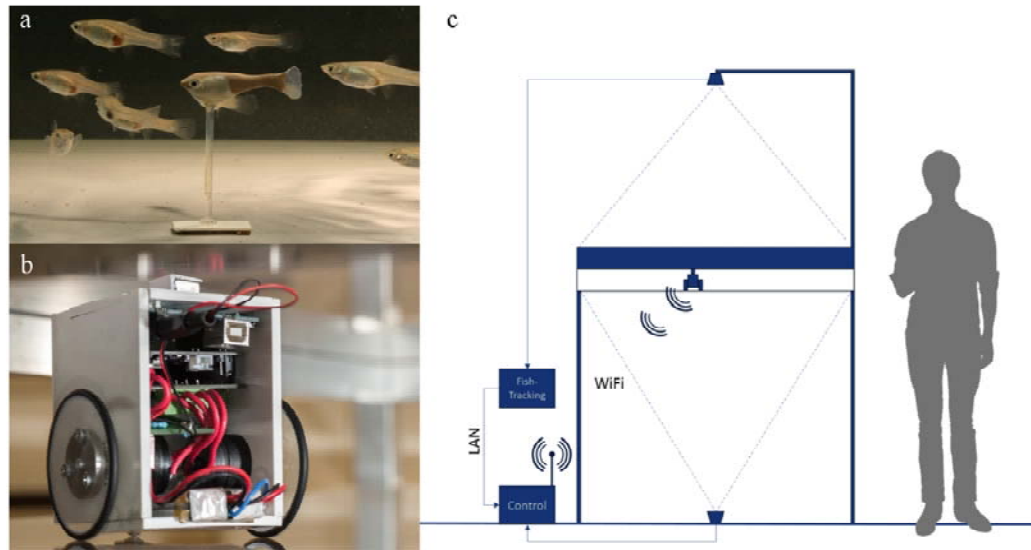
Figure 1: The Robofish system. (a) Guppy-like replica (3D printed and colored) with a group of female guppies in the test arena. (b) Close-up of the robot unit. The robot unit is driving on a second level below the test arena (c).

Experimental setup

To provide live guppies with differently-sized Robofish leaders, we used three replicas that differed only in body size (r1=20 mm standard length (SL); r2=25 mm SL, r3=30 mm SL, see Figure S1). As we used transparent screws to attach the replica to its magnet foot, all replicas regardless of size kept the same distance to the water surface (1 cm, at 10 cm water level). Similarly, we used live test fish (only females to avoid sex effects) that were preselected into three body size classes: 20 mm (ranging from 18.0 to 21.9, mean=20.1, $n=$ 24), 25 mm [23.7 to 27.2, mean= 25.2 $n=$ 33], 30 mm [28.0 to 32.0, mean= 30.0 $n=$33]. Thus, we had a balanced two-way design with the factors leader size (three levels) and live fish size (three levels).

To initiate a trial, we transferred our test fish into an opaque PVC cylinder located at the lower left corner of the test tank. The PVC cylinder had an opening (diameter 3 cm) which was closed with a sponge. We removed the sponge after 1 minute of acclimation and we noted the time each fish took to leave the cylinder as a proxy for its risk taking tendency ('boldness') which might correlate with

95   following tendencies [10, 19, 20]. We started the Robofish's movement when the live fish left the

96   cylinder (= one body length away from the cylinder's border). The Robofish moved along a zigzag

97   path to the opposite corner and then counter-clockwise to its start position (see Figure 2 for an

98   example track as well as video in the supplement SI_video_1). This round was repeated for a second

99   time and a trial took about 60 s in total. Each trial was videotaped for subsequent tracking and the test

100   fish was transferred back to its holding tank. Based on the tracked positions, we calculated the inter-

101   individual distance (IID) between focal fish and Robofish, which has been shown to reflect a live

102   fish's tendency to follow the moving Robofish [20].

103

104   ***Statistical analysis***

105   We initially log10 transformed both recorded continuous variables (IID, time to leave start box) to

106   match a Gaussian distribution. We then used the IID as dependent variable in a ANOVA (unianova

107   package in SPSS 25) with 'leader size', 'live fish size' and their interaction term as fixed factors and

108   'time to leave start box (log10)' as covariate. In order to test whether differently sized live fish differ

109   in their risk aversion tendency, we further correlated live fish body size with time to leave shelter

110   using Spearman's rank order correlation.

111

112   **Results**

113   Regardless of own size (non-significant factor 'live fish size' $F_{2,78}$= 1.52; p=0.23), live guppies

114   followed larger Robofish replicas significantly closer than smaller ones (significant effect of factor

115   'leader size' $F_{2,78}$= 4.49; p=0.014, figure 2). There was no size assortative pattern detectable (i.e.,

116   smaller live fish did not follow smaller replicas closer than larger ones and *vice versa*) as suggested by

117   a non-significant interaction term 'leader size × live fish size' ($F_{4,78}$= 0.49; p=0.74). Also, the time

118   each fish took to leave the start box had no significant influence on its following behavior ($F_{1,78}$=0.90;

119   p=0.35).

Bierbach et al.

120   There was no significant correlation between live fish's body size and their tendency to leave

121 the start box (Spearman's r=0.186, p=0.08).
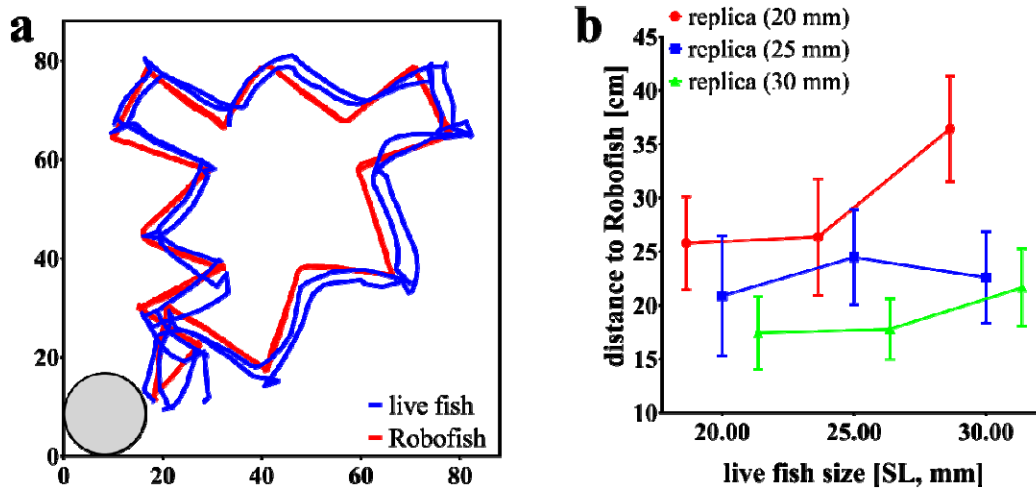
122

123 **Figure 2:** Following behavior of live guppies towards differently sized Robofish replicas. (a) Example track of a

124 trial with Robofish. Fish were introduced into the start box (grey circle, lower left corner) and released into the

125 tank after 1 minute. Robofish then moved on a predefined zig-zag trajectory through the tank until it reached its

126 start position. This movement was repeated a second time and a trial lasted about 60s in total. (b) Inter-individual

127 distance (IID) between live fish and Robofish replicas. Shown are means (± S.E.M) for each size combination.

128

Discussion

130 Live guppies followed larger Robofish leaders closer than smaller ones and this pattern was

131 independent of the followers' own body size as well as risk-taking behavior. While keeping the

132 leaders' behavior constant through the use of a biomimetic robot, this is the first study that showed a

133 'bigger is better' pattern in leadership in shoaling fish that is fully independent of behavioral

134 differences between differentially-sized leaders.

135   Body size is often inevitably linked to specific behavioral patterns [15] and it is thus

136 experimentally difficult to disentangle what cue (body size or linked behavior) is used by individuals

137 that have to choose among differently-sized conspecifics. While researchers from the field of sexual

138 selection make use of video animations in binary choice tests to decouple behavior from body size and

139 keep either one constant while varying the other (see [21-24]), the study of collective movement has

140 largely relied on the use of live stimuli (but see [25] for a working Virtual Reality set-up). We

141 addressed this issue by using a biomimetic robot that is accepted by live fish as a conspecific and

142 provided the same behavioral cues while varying only body size. With this technique, we show that

143 the leading success of larger individuals is determined by their large body size and does not require

144 differences in swimming speed, boldness or occupied spatial position.

145   It was previously found that large guppy females prefer to shoal with similar-sized (large) but

146 not smaller conspecifics while small guppy females did not shoal assortatively but also preferred to

147 shoal with larger females [26]. Small individuals can benefit from associating with larger conspecifics

148 if the larger ones take away the attention of harassing males [27, 28] or predators [29]. Large

149 individuals can benefit from associating with other large individuals to minimize the oddity effect

150 during predation [30]. However, also indirect benefits might play a role as larger body size might often

151 be an indicator of fitness (longer survival, better foraging abilities, higher dominance rank) and it thus

152 might be beneficial for followers (regardless of own size) to associate with those successful

153 phenotypes.

154   We do not know whether live fish may have an intrinsic preference for large body size (as

155 assumed in mate choice contexts, [31]) or larger leaders are simply better visible than smaller ones and

156 thus elicit stronger (retinal) stimulation which is translated into a stronger following response (see [32]

157 for an example of a visual field reconstruction in shoaling fish).

158   In contrast to the study by Reebs [14] we found no evidence that larger individuals follow less

159 than smaller ones. This can be due to species-specific difference as Reebs [14] used the obligate

160 shoaling golden shiner while we used the facultative shoaling guppy. As shoal membership in fishes is

161 highly dynamic and individuals may maximize their fitness by switching frequently between groups of

162 varying size and composition in response to changes in their physiological stage and the external

163 environment [33], we argue that following behavior can be indeed independent of own size [2]. Also,

164 we found no evidence that follower's risk-taking behavior affected their tendencies to follow

Bierbach et al.

165   differentially-sized leaders. This result is in contrast to studies on sticklebacks where shyer individuals

166   are better followers and are less likely to initiate leading themselves [8]. Besides possible species-

167   specific differences, we argue that reinforcing feedbacks due to mutual influences among live animals

168   may have led to the observed personality-dependent following behavior in sticklebacks [8, 34].

169   Our study shows a preference of shoaling fish to follow larger over smaller leaders. We argue

170   that fish, irrespective of their own size have an inherent preference to follow larger leaders, as doing so

171   provides either benefit for the follower or larger leaders are more visible and thus easier to follow.

172

173   **Acknowledgments**

179

References

180

181 1. Bumann D., Krause J. 1993 Front Individuals Lead in Shoals of Three-Spined Sticklebacks
182   (*Gasterosteus aculeatus*) and Juvenile Roach (*Rutilus rutilus*). *Behaviour* **125**(3), 189-198. (doi:
183   10.1163/156853993X00236).

184 2. Krause J., Hoare D., Krause S., Hemelrijk C.K., Rubenstein D.I. 2000 Leadership in fish shoals.
185   *Fish and Fisheries* **1**(1), 82-89. (doi:10.1111/j.1467-2979.2000.tb00001.x).

186 3. Lopez U., Gautrais J., Couzin I.D., Theraulaz G. 2012 From behavioural analyses to models of
187   collective motion in fish schools. *Interface Focus*.

188 4. Jolles J.W., Boogert N.J., Sridhar V.H., Couzin I.D., Manica A. 2017 Consistent Individual
189   Differences Drive Collective Behavior and Group Functioning of Schooling Fish. *Current*
190   *Biology* **27**(18), 2862-2868.e2867. (doi:10.1016/j.cub.2017.08.004).

191 5. Hemelrijk C.K., Kunz H. 2005 Density distribution and size sorting in fish schools: an
192   individual-based model. *Behavioral Ecology* **16**(1), 178-187. (doi:10.1093/beheco/arh149).

193 6. Krause J., Bumann D., Todt D. 1992 Relationship between the position preference and
194   nutritional state of individuals in schools of juvenile roach (Rutilus rutilus). *Behavioral Ecology*
195   *and Sociobiology* **30**(3-4), 177-180. (doi:10.1007/bf00166700).

196 7. McLean S., Persson A., Norin T., Killen S.S. 2018 Metabolic Costs of Feeding Predictively
197   Alter the Spatial Distribution of Individuals in Fish Schools. *Current Biology* **28**(7), 1144-
198   1149.e1144. (doi:https://doi.org/10.1016/j.cub.2018.02.043).

199 8. Nakayama S., Harcourt J.L., Johnstone R.A., Manica A. 2016 Who directs group movement?
200   Leader effort versus follower preference in stickleback fish of different personality. *Biology*
201   *Letters* **12**(5).

202 9. Nakayama S., Harcourt J.L., Johnstone R.A., Manica A. 2012 Initiative, Personality and
203   Leadership in Pairs of Foraging Fish. *PLoS ONE* **7**(5), e36606.
204   (doi:10.1371/journal.pone.0036606).

205 10. Nakayama S., Johnstone R.A., Manica A. 2012 Temperament and Hunger Interact to Determine
206   the Emergence of Leaders in Pairs of Foraging Fish. *PLoS ONE* **7**(8), e43747.
207   (doi:10.1371/journal.pone.0043747).

208 11. Leblond C., Reebs S.G. 2006 Individual leadership and boldness in shoals of golden shiners.
209   *Behaviour* **143**(10), 1263-1280. (doi:doi:https://doi.org/10.1163/156853906778691603).

210 12. Krause J., Reeves P., Hoare D. 1998 Positioning Behaviour in Roach Shoals: The Role of Body
211   Length and Nutritional State. *Behaviour* **135**(8), 1031-1039.
212   (doi:doi:https://doi.org/10.1163/156853998792913519).

213 13. Romenskyy M., Herbert-Read J.E., Ward A.J.W., Sumpter D.J.T. 2017 Body size affects the
214   strength of social interactions and spatial organization of a schooling fish (*Pseudomugil*
215   *signifer*). *Royal Society Open Science* **4**(4). (doi:10.1098/rsos.161056).

216 14. Reebs S.G. 2001 Influence of body size on leadership in shoals of golden shiners, *Notemigonus*
217   *crysoleucas*. *Behaviour* **138**(7), 797-809.

218 15. Polverino G., Bierbach D., Killen S.S., Uusi-Heikkilä S., Arlinghaus R. 2016 Body length rather
219   than routine metabolic rate and body condition correlates with activity and risk-taking in
220   juvenile zebrafish *Danio rerio*. *Journal of Fish Biology* **89**(5), 2251-2267.
221   (doi:10.1111/jfb.13100).

222 16. Landgraf T., Bierbach D., Nguyen H., Muggelberg N., Romanczuk P., Krause J. 2016
223   RoboFish: increased acceptance of interactive robotic fish with realistic eyes and natural motion
224   patterns by live Trinidadian guppies. *Bioinspir Biomim* **11**(1), 015001.

225 17. Landgraf T., Nguyen H., Schröer J., Szengel A., Clément R.G., Bierbach D., Krause J. 2014
226   Blending in with the Shoal: Robotic Fish Swarms for Investigating Strategies of Group
227   Formation in Guppies. In *Biomimetic and Biohybrid Systems* (eds. Duff A., Lepora N., Mura A.,
228   Prescott T., Verschure P.M.J.), pp. 178-189, Springer International Publishing.

229 18. Mönck H.J., Jörg A., Falkenhausen T.v., Tanke J., Wild B., Dormagen D., Piotrowski J.,
230   Winklmayr C., Bierbach D., Landgraf T. 2018 BioTracker: An Open-Source Computer Vision
231   Framework for Visual Animal Tracking. *arXiv:180307985*.

232 19. Jolles J.W., Fleetwood-Wilson A., Nakayama S., Stumpe M.C., Johnstone R.A., Manica A.
233   2015 The role of social attraction and its link with boldness in the collective movements of

Bierbach et al.

234 three-spined sticklebacks. *Animal behaviour* **99**(0), 147-153.
235 (doi:http://dx.doi.org/10.1016/j.anbehav.2014.11.004).
236 20. Bierbach D., Landgraf T., Romanczuk P., Lukas J., Nguyen H., Wolf M., Krause J. 2018 Using
237 a robotic fish to investigate individual differences in social responsiveness in the guppy.
238 *bioRxiv*. (doi:10.1101/304501).
239 21. Fisher H.S., Mascuch S.J., Rosenthal G.G. 2009 Multivariate male traits misalign with
240 multivariate female preferences in the swordtail fish, *Xiphophorus birchmanni*. *Animal*
241 *behaviour* **78**(2), 265-269. (doi:https://doi.org/10.1016/j.anbehav.2009.02.029).
242 22. Fisher H.S., Rosenthal G.G. 2007 Male swordtails court with an audience in mind. *Biology*
243 *Letters* **3**(1), 5-7. (doi:10.1098/rsbl.2006.0556).
244 23. Sommer-Trembo C., Plath M., Gismann J., Helfrich C., Bierbach D. 2017 Context-dependent
245 female mate choice maintains variation in male sexual activity. *Royal Society Open Science*
246 **4**(7). (doi:10.1098/rsos.170303).
247 24. Gierszewski S., Müller K., Smielik I., Hütwohl J.-M., Kuhnert K.-D., Witte K. 2017 The virtual
248 lover: variable and easily guided 3D fish animations as an innovative tool in mate-choice
249 experiments with sailfin mollies-II. Validation. *Current Zoology* **63**(1), 65-74.
250 (doi:10.1093/cz/zow108).
251 25. Stowers J.R., Hofbauer M., Bastien R., Griessner J., Higgins P., Farooqui S., Fischer R.M.,
252 Nowikovsky K., Haubensak W., Couzin I.D., et al. 2017 Virtual reality for freely moving
253 animals. *Nature Methods* **14**, 995. (doi:10.1038/nmeth.4399
254 https://www.nature.com/articles/nmeth.4399#supplementary-information).
255 26. Jones K.A., Croft D.P., Ramnarine I.W., Godin J.-G.J. 2010 Size-Assortative Shoaling in the
256 Guppy (*Poecilia reticulata*): The Role of Active Choice. *Ethology* **116**(2), 147-154.
257 (doi:10.1111/j.1439-0310.2009.01727.x).
258 27. Agrillo C., Dadda M., Bisazza A. 2006 Sexual Harassment Influences Group Choice in Female
259 Mosquitofish. *Ethology* **112**(6), 592-598. (doi:10.1111/j.1439-0310.2006.01188.x).
260 28. Herdman E.J.E., Kelly C.D., Godin J.-G.J. 2004 Male mate choice in the guppy (*Poecilia*
261 *reticulata*): Do males prefer larger females as mates? *Ethology* **110**(2), 97-111.
262 (doi:10.1111/j.1439-0310.2003.00960.x).
263 29. Pocklington R., Dill L. 1995 Predation on females or males: who pays for bright male traits.
264 *Animal behaviour* **49**, 1122-1124.
265 30. Krause J., Ruxton G.D. 2002 *Living in groups*. Oxford, Oxford University Press.
266 31. Bierbach D., Makowicz A.M., Schlupp I., Geupel H., Streit B., Plath M. 2013 Casanovas are
267 liars: behavioral syndromes, sperm competition risk, and the evolution of deceptive male mating
268 behavior in live-bearing fishes. *F1000Research* **2**, 75.
269 32. Strandburg-Peshkin A., Twomey C.R., Bode N.W.F., Kao A.B., Katz Y., Ioannou C.C.,
270 Rosenthal S.B., Torney C.J., Wu H.S., Levin S.A., et al. 2013 Visual sensory networks and
271 effective information transfer in animal groups. *Current Biology* **23**(17), R709-R711.
272 (doi:http://dx.doi.org/10.1016/j.cub.2013.07.059).
273 33. Hoare D.J., Krause J., Peuhkuri N., Godin J.G.J. 2000 Body size and shoaling in fish. *Journal of*
274 *Fish Biology* **57**(6), 1351-1366. (doi:10.1111/j.1095-8649.2000.tb02217.x).
275 34. Harcourt J.L., Ang T.Z., Sweetman G., Johnstone R.A., Manica A. 2009 Social Feedback and
276 the Emergence of Leaders and Followers. *Current Biology* **19**(3), 248-252.
277 (doi:http://dx.doi.org/10.1016/j.cub.2008.12.051).

278

Bierbach et al.

279 **Supplemental Information**
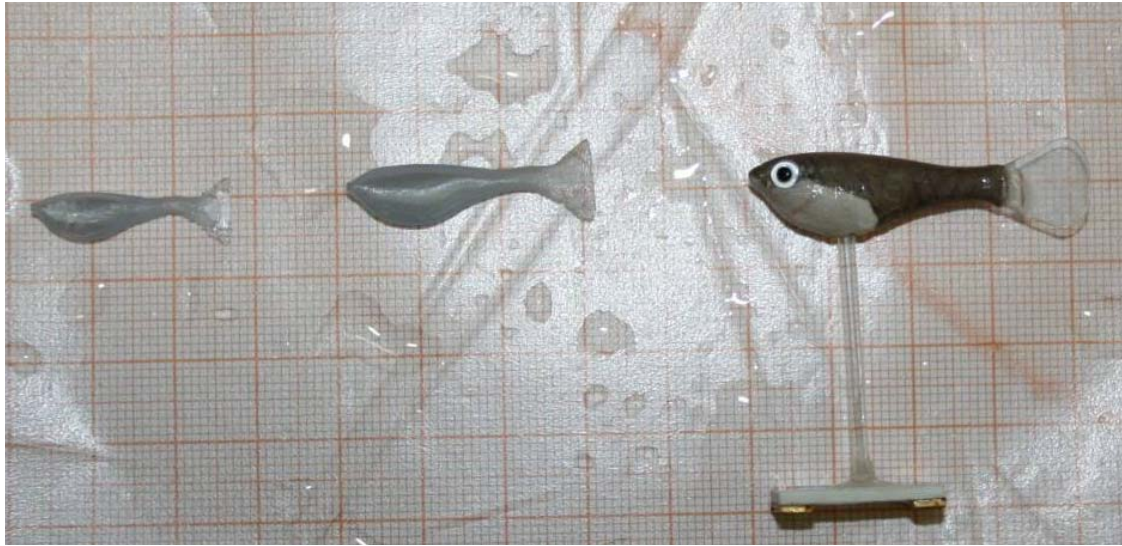
280

281 **Figure SI:** Photograph of differently-sized replicas. Left (20 mm SL) and middle (25 mm SL) replicas

282 are unprocessed 3D printed blanks that were latter on equipped with glass eyes and color-painted as

283 shown for the 30 mm replica on the right.

284 **Video SI:** Example of a 30 mm live guppy that follows a 25 mm Robofish replica. The live fish leaves

285 the cylinder at 01:04 min.

286

287