

Bierbach et al.

1 **Guppies prefer to follow large (robot) leaders irrespective** 2 **of own size**

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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.

27 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*), Reeb
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

57 **Methods**

58 ***Study organism and maintenance***

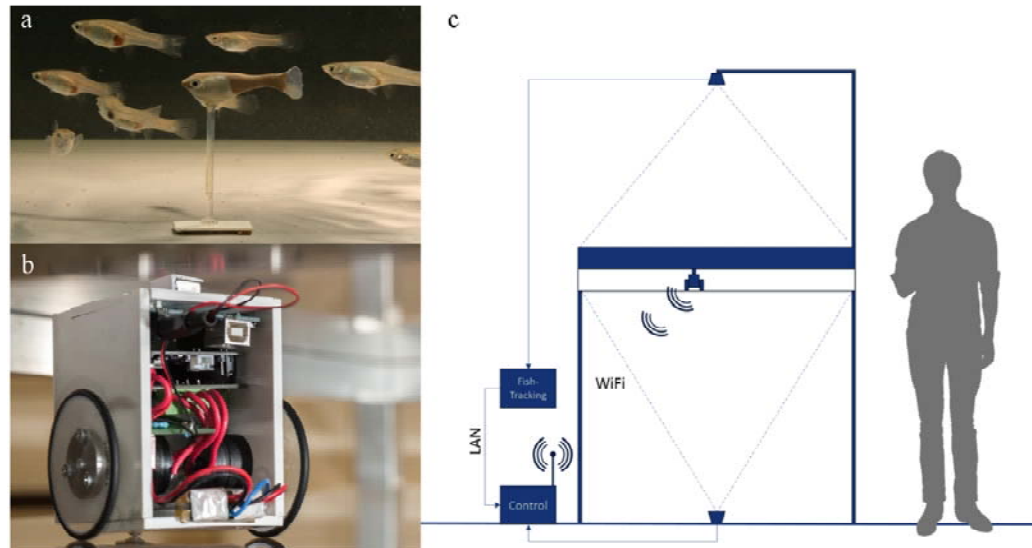
59 We used Trinidadian guppies (*Poecilia reticulata*) that were descendants of wild-caught fish from the
60 Arima River in North Trinidad. Test fish came from large, randomly outbred single-species stocks
61 maintained at the animal care facilities at the Faculty of Life Sciences, Humboldt University 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

66 ***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.



77

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

81

82 **Experimental setup**

83 To provide live guppies with differently-sized Robofish leaders, we used three replicas that differed
84 only in body size ($r_1=20$ mm standard length (SL); $r_2=25$ mm SL, $r_3=30$ mm SL, see Figure S1). As
85 we used transparent screws to attach the replica to its magnet foot, all replicas regardless of size kept
86 the same distance to the water surface (1 cm, at 10 cm water level). Similarly, we used live test fish
87 (only females to avoid sex effects) that were preselected into three body size classes: 20 mm (ranging
88 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,
89 mean= 30.0 $n=33$]. Thus, we had a balanced two-way design with the factors leader size (three levels)
90 and live fish size (three levels).

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

Bierbach et al.

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.

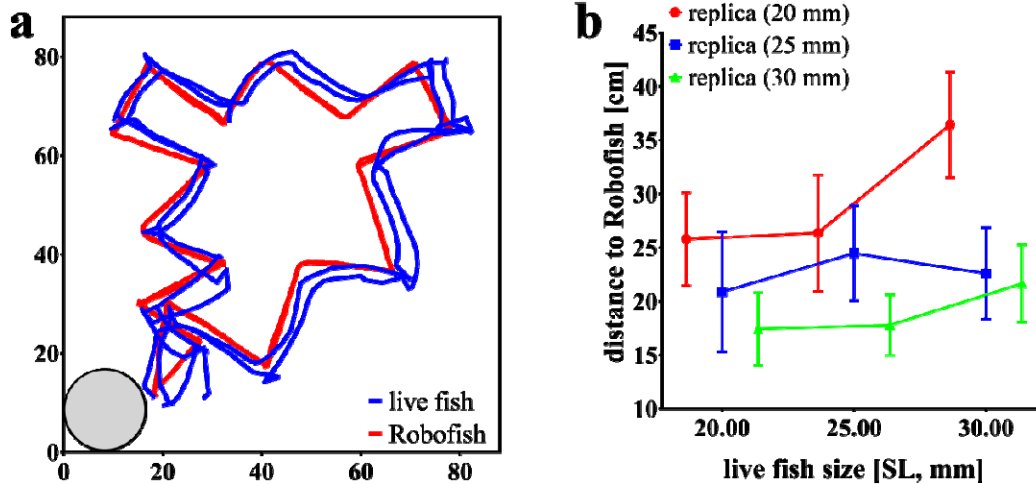
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112 **Results**

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 \times 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 (\pm S.E.M) for each size combination.

128

129 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

Bierbach et al.

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 Reeb's [14] we found no evidence that larger individuals follow less
159 than smaller ones. This can be due to species-specific difference as Reeb's [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 shy 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

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178 authors declare no competing interests.

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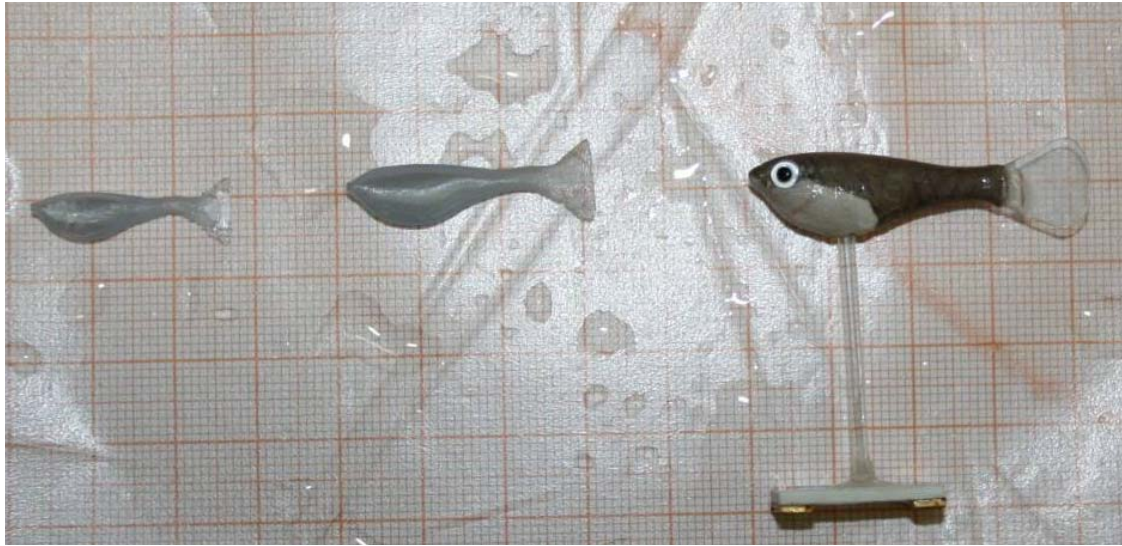
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278

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 later 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.

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287