

1 **Captivity-induced behaviour and spatial learning abilities in an enigmatic,**
2 **aquifer-dwelling blind eel, *Rakthamichthys digressus***

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20
21

22 **Abstract**

23

24 We investigated the impact of captive life on behaviour and learning abilities in an enigmatic,
25 aquifer-dwelling blind eel, *Rakthamichthys digressus*. Of eight major behavioural traits
26 related to exploration and activity in a novel arena, four were significantly altered by life in
27 captivity. While the startle response upon introduction into the arena and overall swimming
28 away from the walls increased after captivity, inactivity exhibited immediately after the
29 startle and the reaction to an external disturbance decreased. We also observed behavioural
30 syndromes between ‘startle responses’ and ‘horizontal wall following’, and between ‘overall
31 activity’ and ‘vertical wall following’; however, these behavioural syndromes were not
32 altered by maintenance in captivity. Interestingly, this blind-eel failed to learn a simple
33 spatial task in a Y-maze apparatus. Captive-associated behavioural changes in *R. digressus*
34 may influence their survival after reintroduction into natural habitats, and such changes must
35 be taken into account while developing protocols for ex-situ conservation and subsequent
36 release.

37

38 **Keywords:** conservation, ethology, human-fish conflicts, reintroduction, subterranean

39 **1. Introduction**

40

41 Subterranean ecosystems, extreme environments characterized by darkness, truncated food
42 webs and food scarcity, yet harbouring exceptional biodiversity are highly vulnerable to
43 environmental changes [1]. Being highly restricted, and having small population sizes and
44 low resilience, anthropogenic threats could have serious consequences on the survival of
45 most subterranean fauna [2–3]. Though habitat loss, and contamination and overexploitation
46 of groundwater are widely regarded as major stressors to subterranean biodiversity [4], there
47 are also emerging threats and challenges in many regions of the world that have been poorly
48 addressed.

49

50 A unique example, from the Western Ghats of India is ‘human-fish conflict’, where
51 subterranean species are killed as their presence in wells is mistakenly linked to poor water
52 quality, and some species of eels mistaken for snakes and killed on purpose [5]. Home-stead
53 wells in this region are also cleaned annually and fish encountered during such times are also
54 killed. To prevent this, many fish are rescued from dug-out wells and maintained in captivity
55 until a suitable subterranean habitat is available for their release. Such captive maintenance
56 may last for several months depending on the season of capture – e.g., rescued fish during
57 summer may require a captive environment until their release during the subsequent
58 monsoon.

59

60 Unlike epigeal fishes in which translocation to artificial habitats are known to modify
61 behavioural traits and syndromes [6–8], no such information is currently available on
62 subterranean taxa. Understanding behavioural changes in captivity, and developing
63 appropriate management protocols are critical for the success of conservation strategies such
64 as reintroductions and translocations. This can be undertaken by altering physical properties
65 of the environment to suit the sociobiology of the species. In many cases, it may also be
66 required that individuals which have undergone behavioural modifications in captivity may
67 need to be provided ‘life skill training’ [9–11] to re-organize their behavioural characters and
68 ensure improved survival on reintroduction.

69

70 Focusing on an enigmatic, blind, synbranchid eel, *Rakthamichthys digressus*, we explore the
71 influence of captivity on their behavioural traits and syndromes. As this species is known to
72 inhabit a spatially complex ecosystem, i.e., narrow pores inside aquifer-bearing lateritic rocks

73 [12], they are expected to have an excellent capacity for spatial learning. Therefore, to test
74 this hypothesis, we assessed the spatial learning ability of *R. digressus* using a standard maze
75 apparatus. Our study is the first to explore these behavioural aspects on blind, aquifer-
76 dwelling eels, and provides important scientific evidence to facilitate the development of
77 conservation strategies for subterranean fish taxa.

78

79 **2. Materials and methods**

80

81 (a) Maintenance and husbandry

82

83 *Rakthamichthys digressus* (N=24) were collected from homestead dug-out wells as part of a
84 rescue effort and transferred to the laboratory where they were housed in pairs in well-aerated
85 glass aquaria (39×26×30 cm). Pieces of Poly Vinyl Chloride (PVC) pipe (diameter 2.5 cm;
86 length 15 cm) were provided as shelters and water level was maintained at a height of 16 cm.
87 We paired fishes slightly different in their body length (< 1 cm) to facilitate individual
88 identification. Fish were fed with tubifex worms ad libitum in the afternoon, and uneaten
89 food siphoned out after 30 minutes. Water temperature was maintained at 25°C and the room
90 was maintained under a 12h:12h LD cycle. All fish were healthy until the completion of
91 experiments, and no mortality was observed.

92

93 (b) Experiment 1: Exploratory behaviour and syndromes

94

95 Exploratory behaviour of *R. digressus* was studied using a rectangular open-field apparatus.
96 An aquarium (50 × 39 × 30 cm) with 2 × 2 cm grids marked on the bottom to quantify
97 locomotor activity was the open field used in this experiment. This apparatus was filled with
98 water up to a height of 15 cm and a Compact Fluorescent Lamp (CFL; 20W) lighted the
99 apparatus from above. After acclimation period of seven days, subject fish were introduced
100 individually into the middle region of the open field. After allowing six minutes for the fish
101 to explore the arena, a small aquarium net (10.16 × 7.62 cm, handle length 29.21 cm and
102 weight 38 grams) was dropped from a height of 15 cm at a point 10 cm away from the head
103 of the subject fish and retrieved [13]. Fish recuperated quickly and the behaviour was
104 recorded for two minutes after dropping the net, and the fish subsequently transferred back to
105 its home tank (Trial 1). Subject fish were tested again using the same apparatus and protocol
106 after 45 days to examine the impact of captive life on these behaviours (Trial 2). All

107 experiments were conducted during the day time and recorded using a Handycam (Sony
108 HDR-CX405) fixed above the open field apparatus.

109

110 We compared syndromes between behavioural traits in trials 1 and 2 to improve our
111 understanding of the effect of captivity on behavioural syndromes – the tight linkage between
112 various components of exploratory behaviours.

113

114 (c) Experiment 2: Spatial learning ability

115

116 Spatial learning ability was studied using a ‘Y-maze’ [14] made of plexiglass sheets fixed
117 inside an aquarium (50 × 39 × 30 cm) divided into two chambers - A (7 × 39 × 30 cm) and B
118 (43 × 39 × 30 cm). The starting arm of the maze (31 × 2 × 30 cm) was connected to the start
119 chamber (chamber A) by a guillotine door, and both choice arms (31 × 2 × 30 cm) were
120 placed in chamber B. One of the arms of the Y-maze was closed at the end (blocked arm),
121 while the other led to an open area (12 × 35 × 30 cm) which the fish could explore upon
122 entry. A 20 W CFL lit the apparatus from above. In this experiment, we used the same
123 individual fish that were part of trials 1 and 2, after providing an interval of 7 days. Fish was
124 introduced individually in the start chamber, and an acclimatization time of five minutes was
125 provided before opening the guillotine door. Fish behaviour was recorded for 15 minutes,
126 after which it was returned to its home tank. All 24 individuals were tested once daily for six
127 consecutive days following the same protocol.

128

129 (d) Analysis

130

131 Videos of experiments 1 and 2 were analysed using Behavioral Observation Research
132 Interactive Software (BORIS) [15]. Eight behavioural parameters were quantified from the
133 videos of the experiment 1 and three parameters from experiment 2 (Table S1). Analyses
134 were carried out using Linear Mixed Modelling (LMM) using R version 3.6.1 [16].
135 Behavioural data (after log transformation) followed normal distribution, and were used as
136 dependent variables in the analysis. Trial number (trial 1 refers to the assay performed in the
137 first week after transfer to laboratory conditions, and trial 2 to that performed after 45 days in
138 captivity) was considered an independent variable. Significant effects of trial number suggest
139 that dependent variable (behavioural traits) was altered as a consequence of captive life.

140 Identity of individual fish was used as the random factor in these analyses. For examining
141 changes in behavioural syndromes after captivity, we examined effect of the interaction term
142 between behavioural traits and trial number in the linear mixed model. We performed this
143 analysis by comparing models with, and without such interaction terms. Models with lowest
144 AIC values were considered the best fits, and models with $\Delta AIC > 2$ significantly poorer fits.
145 If models with interaction between behavioural traits and trial number were significantly
146 better fits than those without, then we inferred that behavioural syndrome was altered by
147 captivity. In the case of experiments examining the spatial learning ability, data on selection
148 of the arm of Y maze, a binary choice situation, was modelled as a binomial distribution.
149 Hence, Generalized Linear Mixed Models (GLMM) was used. The packages used were
150 ‘ggplot2’, ‘lmerTest’, and ‘lme4’ [16]

151

152 **3. Results**

153

154 (a) Effect of captivity on behavioural traits and syndromes

155

156 Life in captivity affected certain behaviour traits such as sudden movements upon
157 introduction into the novel arena (startle response, $\beta = 10.05$, $p = 0.05$) and ‘swimming away
158 from the walls’ ($\beta = 8.60$, $p = 0.02$) which significantly increased, and ‘duration of rapid
159 swimming’ in response to dropping the net (reaction, $\beta = -7.89$, $p = 0.001$) and ‘total time
160 spent in rest during exploration’ (rest, $\beta = -7.73$, $p = 0.002$, Figure 1a; Table 1) which
161 decreased. These results are substantiated by the fact that models without trial as an
162 independent factor were poorer fits for these traits ($\Delta AIC > 2$; Table 2). However, duration of
163 inactivity bout immediately after startle (‘inactivity after startle’ $\beta = -4.54$, $p = 0.06$), ‘moving
164 along the wall of the apparatus towards the water surface’ (vertical wall following, $\beta = 0.91$,
165 $p = 0.83$, Figure 1a), and ‘swimming along the wall parallel to it’ (horizontal wall following
166 behaviour, $\beta = -1.55$, $p = 0.86$, Figure 1a) and ‘total activity’ ($\beta = 7.17$, $p = 0.40$, Figure 1a)
167 were not affected by captivity. We did not observe any significant difference in the fit of
168 models of these behavioural traits with, and without trial, as independent factor ($\Delta AIC < 2$;
169 Table 2).

170

171 In terms of behavioural syndromes, the ‘startle response’ was negatively correlated with
172 ‘horizontal wall following behaviour’ ($\beta = -0.24$, $p < 0.0001$; Figure 2a). Full Linear Mixed
173 Models of ‘startle response’ as response variable with interactions between ‘wall following

174 behaviour' and trial revealed that the model without interaction was the best fit for 'startle
175 response' ($\Delta AIC = 1.93$ for the model with interaction; Table 2). This suggests that the
176 behavioural syndrome between 'startle response' and 'swimming along walls' was not
177 influenced by 45 days of captivity. Similarly positive correlation noted between 'vertical wall
178 following' and 'activity' ($\beta = 1.33$, $p < 0.0001$; Figure 2b) was also found to be uninfluenced
179 by the captive life. No linkage was observed between any other behaviour parameters
180 studied.

181

182 (b) Spatial learning

183

184 No significant change was observed in the 'latency to leave the start chamber' (LMM: $\beta = -$
185 1.73 , $p = 0.34$; Table 1; Figure 1b), 'time spent at junction of the Y-maze' before choosing an
186 arm (LMM: $\beta = -0.19$, $p = 0.68$; Figure 1b), and 'selecting one arm over the other' (GLMM-
187 binomial: $\beta = -0.02$, $p = 0.83$), over repeated testing conducted for six days. This revealed
188 that *R. digressus* did not develop preference towards any choice arm, indicating that it failed
189 to learn this simple spatial task.

190

191 4. Discussion

192

193 Temporary translocation of threatened fish from their natural habitat to captivity, and
194 reintroduction during favourable times is a strategy for avoiding permanent loss of
195 individuals or fragmentation of populations during harsh seasons [17,18]. Translocation and
196 subsequent life in captivity for 45 days significantly increased the 'startle response', 'time
197 spent swimming away from the walls', and 'rest taken during the exploration' in *R. digressus*.
198 Meanwhile, the reaction towards a fear inducing stimulus became weaker. Startle response
199 exhibited by a fish in open field is attributed to fear, or handling by humans [19,20].
200 However, separating the effect of these two parameters on the enhanced startle exhibited by
201 captive *R. digressus* is difficult [20]. Another behavioural expression of fear, i.e., 'reaction,'
202 which diminished in captivity suggests that handling by humans may be potentially
203 promoting startle response.

204

205 Blind subterranean fishes which possess minimal sensory range are highly dependent on
206 tactile stimuli and lateral line sense organs to perceive their environment [21,22]. This may
207 explain why they follow the walls of the novel environment so that their snouts bestowed

208 with lateral line sensory organs could receive more sensory stimulus. Previous research has
209 demonstrated that impairment of the lateral line system is associated with reduced ‘wall
210 following behaviour’ [23]. Though the exact reason for the reduction in ‘time spent near wall’
211 could not be understood, diameter of snout of the eel (where lateral line pore system is
212 pronounced [24]) was observed to have reduced in captivity (results not shown).

213

214 Moving individuals from their natural habitat to captivity has influenced behavioural
215 syndromes in many epigeal fishes [7,8]. In *R. digressus*, a negative correlation between
216 ‘startle response’ and ‘horizontal wall following’, and a positive linkage between ‘activity’
217 and the ‘vertical wall following’ was observed. Individuals that exhibit higher levels of startle
218 response due to higher neophobia spend more time near the sides of the open field in species
219 ranging from rodents to fishes [25–27]. Active *R. digressus*, spending more time in ‘vertical
220 wall following’ could either be due to the inability to recognize the presence of wall [23], or
221 searching for biologically significant resources similar to its function in natural habitats.
222 Although boldness - propensity to take a risky decision is positively correlated with activity
223 in many epigeal fishes [28,29], ‘startle response’, duration of the ‘inactivity after the startle’,
224 and ‘reaction’, which are potential indirect measures of boldness [30,31] failed to show any
225 association with activity in *R. digressus*. Furthermore, life in captivity for 45 days neither
226 changed any existing behavioural syndromes, nor generated new associations between
227 behaviour traits in this species.

228

229 In spite of a short sensory range, blind fishes have been known to learn spatial properties of
230 their environment [32,33]. However, in *R. digressus*, latency to leave the start chamber did
231 not change and no preference was developed towards any of the choice arms of Y maze
232 indicating the inability to learn this simple spatial task. This lack of learning may be due to
233 the negative effects of captive conditions, short duration of exposure (15 minutes for 5
234 consecutive days) to the maze or neither of the choice arms being a strong reward [34].
235 Hence, analysing the spatial learning ability in *R. digressus* immediately after collecting from
236 the natural habitat and providing more time to familiarise with the spatial properties of the
237 apparatus is essential to distinguish between whether this inability is a species-specific
238 characteristic or the consequence of captive life.

239

240 Captivity altered certain behavioural traits such as ‘increased startle response’ and
241 ‘swimming away from the wall’, and ‘reduced fear response’ in *R. digressus*, which may

242 increase its predation risk when reintroduced into its natural habitat. Hence, to mitigate such
243 adverse effects of captive life, increasing complexity of artificial habitat [9], soft release [35],
244 and life skill training protocols [11] should be considered for improving the success of their
245 reintroduction.

246

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248

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252

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370 areas of high-and low-predation pressure. *J. Comp. Physiol. B* **175**, 305–312.

371

372 **Table 1.** Influence of 45-day captivity on various elements of exploratory behaviour
373 exhibited by *Rakthamichthys digressus* (Experiment 1). Experiment 2 represents the effect of
374 repeated exposure to Y maze for five consecutive days on the spatial learning ability in this
375 species. Statistics used were LMM in all cases except arm choice (GLMM), which was a
376 binary data. *= P<0.05, **= P<0.01, ***= P<0.0001.

377

| 378 | S. No. | Behavioural parameters | β | p |
|-----|---|-------------------------------|----------|----------|
| 380 | Experiment 1 (Exploratory behaviour) | | | |
| 381 | 1. | Startle | 10.05 | 0.05* |
| 382 | 2. | Inactivity after startle | -4.54 | 0.06 |
| 383 | 3. | Rest | -7.73 | 0.002** |
| 384 | 4. | Swimming away from walls | 8.60 | 0.02* |
| 385 | 5. | Horizontal wall following | -1.55 | 0.86 |
| 386 | 6. | Vertical wall following | 0.91 | 0.83 |
| 387 | 7. | Activity | 7.17 | 0.40 |
| 388 | 8. | Reaction | -7.89 | 0.001** |
| 389 | Experiment 2 (Spatial learning) | | | |
| 390 | 9. | Maze entry | -1.73 | 0.38 |
| 391 | 10. | Time spent at the junction | -0.19 | 0.70 |
| 392 | 11. | Arm choice | -0.02 | 0.83 |

393

394

395 **Table 2.** Model comparisons of linear mixed models (LMM) with various behavioural traits
 396 of *Rakthamichthys digressus* studied during the ‘Trials 1 and 2’ of experiment 1. Each row
 397 shows the results of different models with degrees of freedom and difference in AIC (Akaike
 398 Information Criterion) values from the best-fitted model. The models are ordered with
 399 increasing AIC values.

400

| 401 Response Variable | Model | df | ΔAIC |
|------------------------------|--|-----------|-------------|
| 402 | | | |
| 403 | | | |
| 404 Startle | HorizontalWallFollowing * Trial + (1 FishID) | 6 | 1.93 |
| 405 | | | |
| 406 | HorizontalWallFollowing + Trial + (1 FishID) | 5 | 0 |
| 407 | | | |
| 408 | HorizontalWallFollowing + (1 FishID) | 4 | 2.58 |
| 409 | | | |
| 410 | Trial + (1 FishID) | 4 | 10.11 |
| 411 | | | |
| 412 | 1 + (1 FishID) | 3 | 21.26 |
| 413 | | | |
| 414 Activity | VerticalWallFollowing * Trial + (1 FishID) | 6 | 1.53 |
| 415 | | | |
| 416 | VerticalWallFollowing + Trial + (1 FishID) | 5 | 0.98 |
| 417 | | | |
| 418 | VerticalWallFollowing + (1 FishID) | 4 | 0 |
| 419 | | | |
| 420 | Trial + (1 FishID) | 4 | 24.99 |
| 421 | | | |
| 422 | 1 + (1 FishID) | 3 | 18.88 |
| 423 | | | |

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429

430 **Figures:**

431 **Figure 1.** (a) Influence of captivity on various elements of exploratory behaviour of
432 *Rakthamichthys digressus*; open and shaded boxplots represent Trial 1 (5th day) and Trial 2
433 (45th day) respectively. (b) Latency to enter the maze and time spent in the junction of the
434 choice arms by the test fish during spatial learning experiment conducted using Y maze.

435

436 **Figure 2.** Influence of captivity on the correlations between behaviour traits (a) startle and
437 horizontal wall following and (b) vertical wall following and activity in *R. digressus*. Each
438 dot represents individual fish (n = 24) and the grey shading represents 95% confidence
439 intervals.

Figure 1.

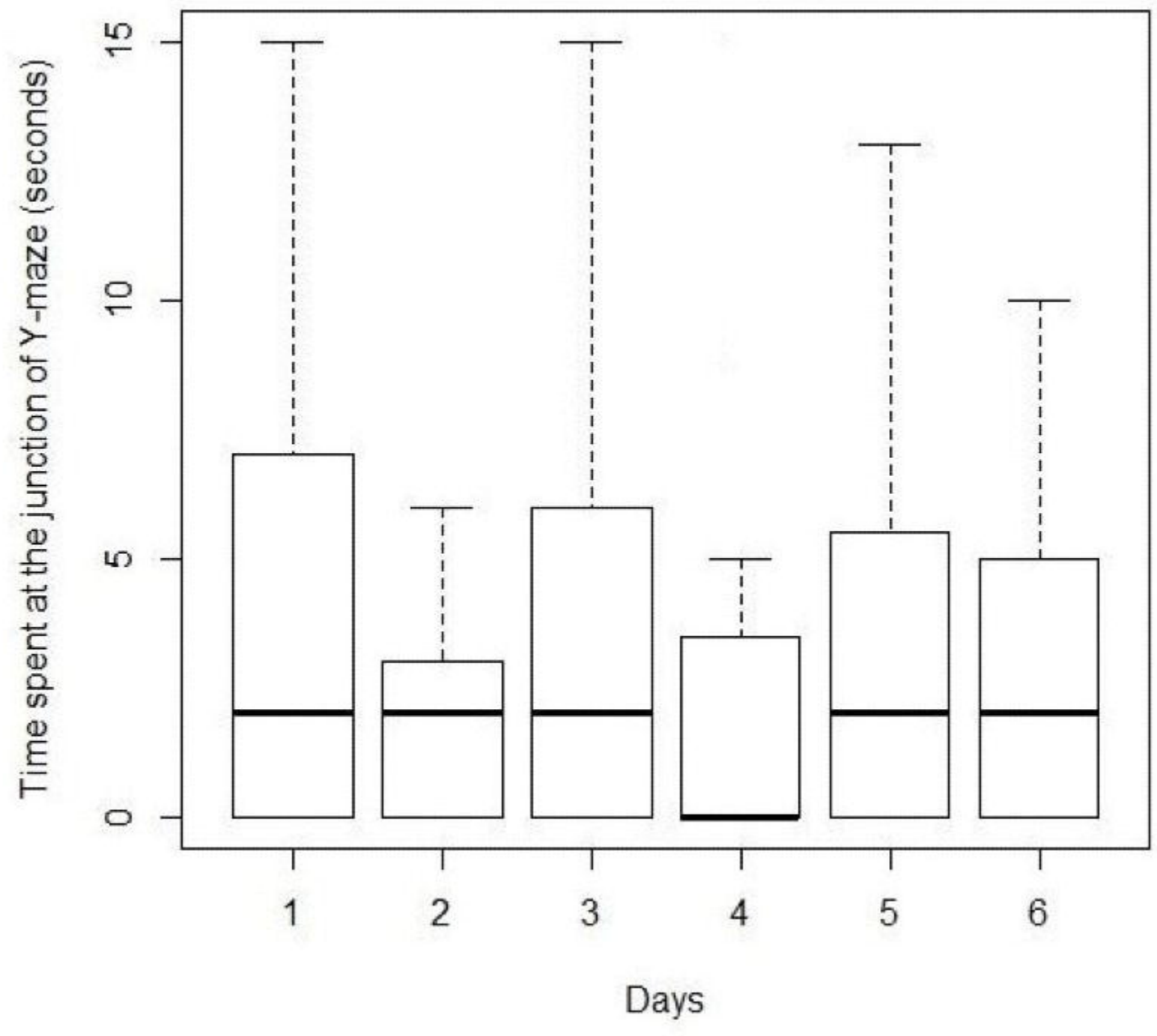
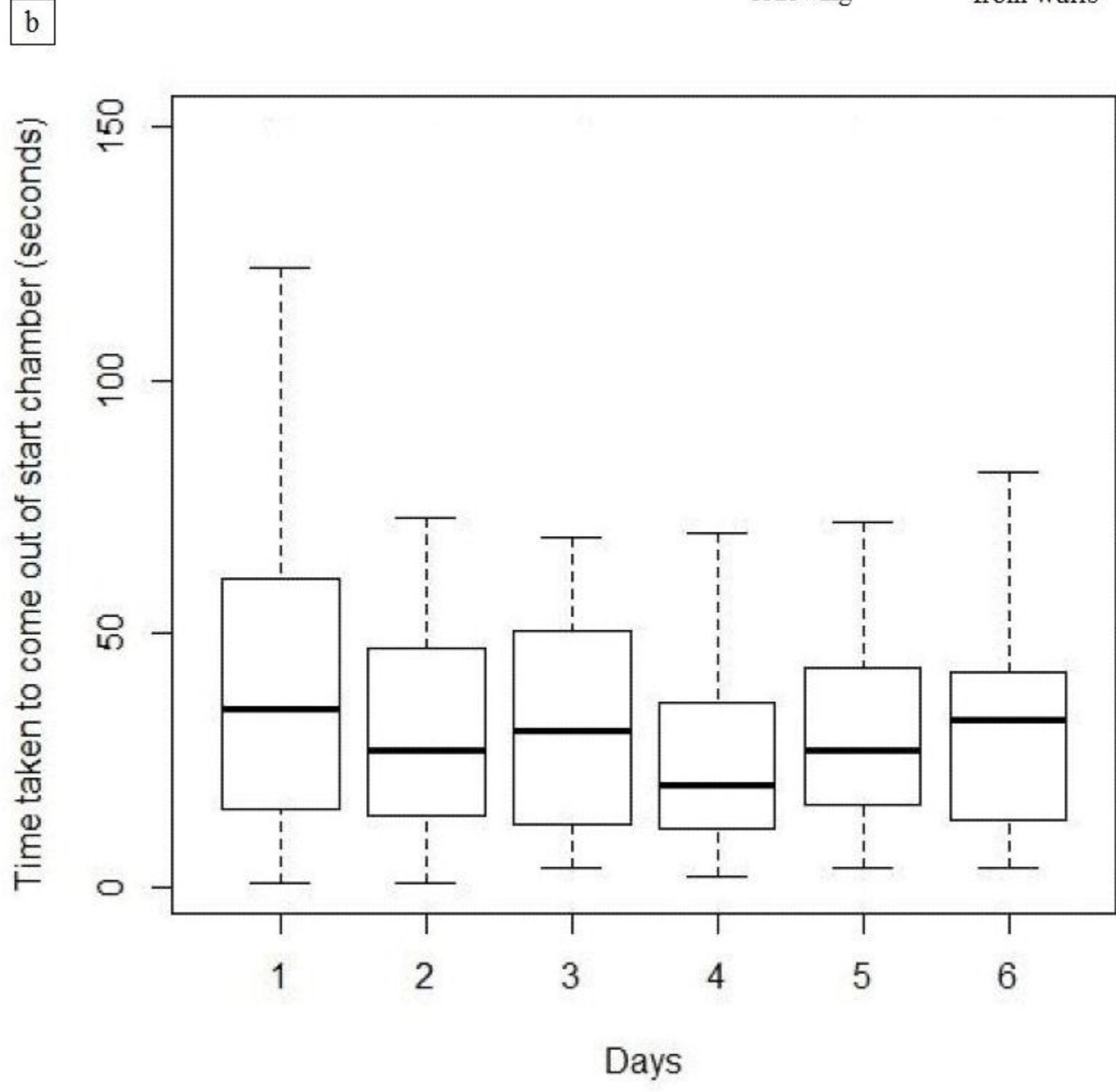
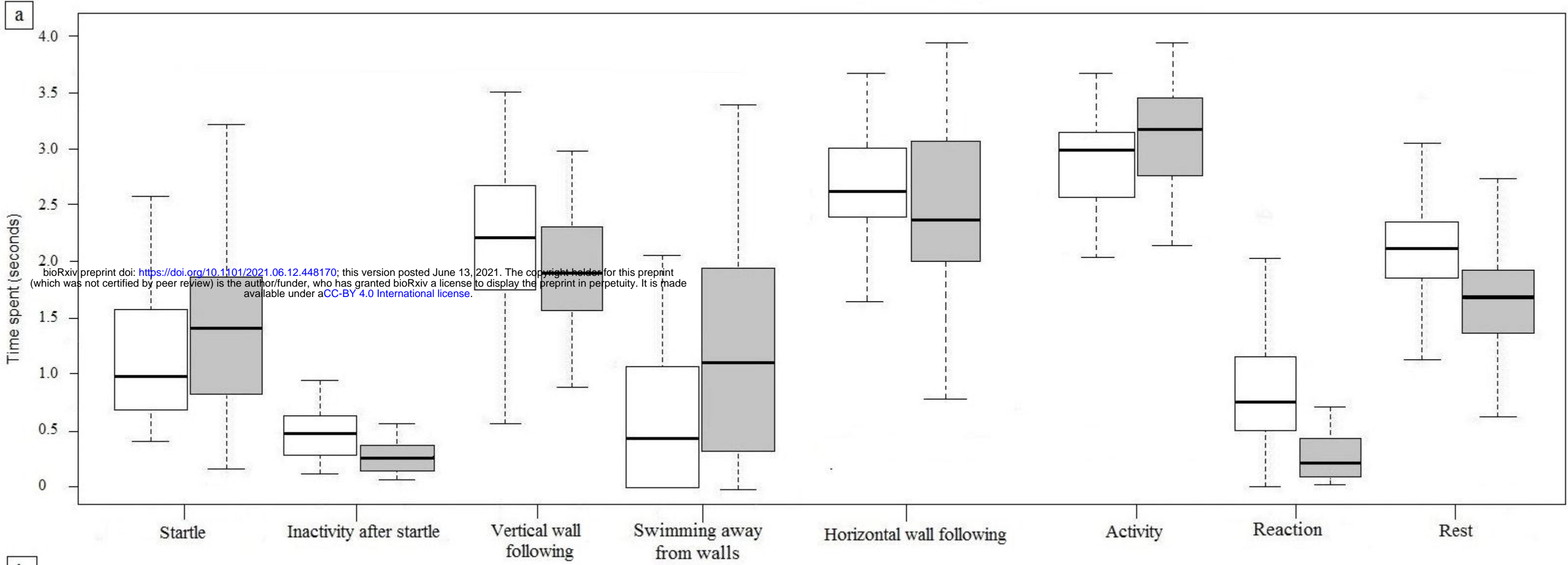


Figure 2.

