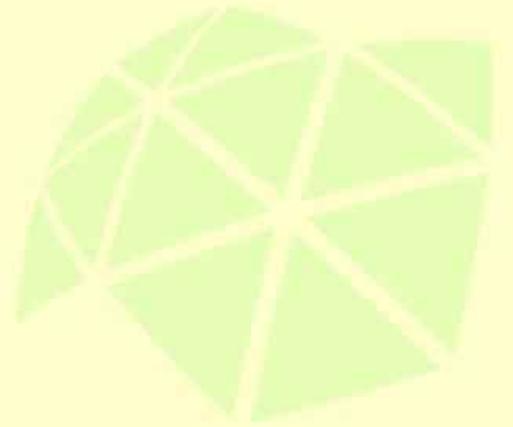


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*Ecologia e
Comportamento*



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Manuela Lombardi Brandão

*Sistemática e
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Manuela Lombardi Brandão

Social isolation in a group living fish impairs cognition

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2014

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, Área de Concentração – Ecologia e Comportamento, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Campus de São José do Rio Preto.

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Dedico este trabalho

Aos meus pais, Manoel e Marcia que, com muito amor, carinho e dedicação, me deram um exemplo a seguir, me apoiaram incondicionalmente nas minhas escolhas e me inspiraram a ser a pessoa que hoje me orgulho de ser.

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“Quando o homem aprender a respeitar até o menor ser da criação, seja ele animal ou vegetal, ninguém precisará ensiná-lo a amar seu semelhante”.

Albert Schweitzer

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RESUMO

Para se ajustar a mudanças ambientais, os animais podem aprender sobre tais variações e modular seu comportamento de acordo com suas necessidades. Em alguns casos, no entanto, mudanças no ambiente social ou físico podem gerar estados de medo que levam a respostas de estresse fisiológico e que podem modificar a capacidade de um indivíduo em aprender, impactando negativamente o bem-estar desse animal. O isolamento social, por exemplo, além de estressante para animais sociais, pode proporcionar menor estimulação para esses indivíduos. Neste estudo, nós investigamos se o isolamento social prejudica habilidades de aprendizagem no ciclídeo *Cichlasoma paranaense*. Dois tratamentos foram comparados, um com indivíduos isolados e outro com peixes dominantes de um grupo social. A habilidade de associar uma pista visual com a comida acessível (recompensa) foi medida em uma tarefa espacial. De fato, menos peixes isolados foram capazes de aprender a tarefa. O resultado observado não se deveu a uma diferença na motivação geral para nadar, se alimentar ou explorar as opções de forrageamento. Os resultados indicam que o isolamento social para uma espécie de peixes normalmente social pode prejudicar a aprendizagem.

Palavras-chave: cognição, aprendizagem, memória, interação social, bem-estar.

ABSTRACT

*To adjust to changes in the environment, animals can learn about the changes to help them modulate their behaviour as needed. Sometimes, however, changes in the social or the physical environment can generate fear states that trigger a physiological stress response which modifies an individual's capacity to learn and can have a negative impact on the welfare of the animal. Social isolation, for example, besides being stressful for social animals, may provide less stimulation for these individuals. Here, we investigated whether social isolation impairs learning skills in a cichlid fish, *Cichlasoma paranaense*. Two treatments were compared, one with isolated individuals and another with dominant fish from a social group. The ability to associate a visual landmark with an accessible food reward was measured in a spatial task. Overall, fewer isolated fish were able to learn the task. The result was not because of a difference in general motivation to swim, feed, or explore the foraging options. The results indicate that social isolation for a normally social species of fish can impair learning.*

Keywords: cognition, learning, memory, social interaction, welfare.

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Esta dissertação está em formato de artigo e foi editada de acordo com as normas da revista científica *Applied Animal Behaviour Science*. No entanto, as figuras e tabela se encontram no meio do texto nesta versão (e não após as referências, como solicitado pela revista) com o intuito de facilitar a visualização e leitura do trabalho por parte da banca.

1 Social isolation in a group living fish impairs cognition

2

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13

14 **ABSTRACT**

15 In variable environments, animals can monitor and learn about factors that change to help them
16 adjust their behaviour to cope with such changes. Sometimes, however, the ability to learn and
17 modify behaviour can be impaired. For example, challenges in the social or the physical
18 environment can generate fear states that trigger a physiological stress response that can
19 compromise an individual’s capacity to learn. In addition to the direct negative effect stressors may
20 have on an animal, an impaired learning ability may be further detrimental to an animal’s welfare
21 as it may prevent the animal from adjusting its behavior to cope with an altered environment.

22 Social isolation, for example, can trigger stress responses in social animals that are used to living
23 within a group and this may impair cognitive ability in the isolated animals. Yet social isolation and
24 individual housing are often used as a way to track individual identity in research experiments. To
25 investigate the effects of isolation on cognition, we tested whether social isolation impairs learning
26 skills in a cichlid fish, *Cichlasoma paranaense*. Two treatments were compared, one with isolated
27 individuals and another with dominant fish from a social group. The ability to associate a visual
28 landmark with an accessible food reward was measured in a spatial task. Overall, fewer isolated

29 fish were able to learn the task. The poor performance in the isolated fish could not be explained
30 by differences in their general motivation to swim, feed, or explore the foraging options. The results
31 therefore suggest that social isolation for a normally social species of fish can impair learning.

32

33 Keywords: cognition, learning, memory, social interaction, welfare.

34

35 **1. Introduction**

36 When animals are housed in captive environments, it is sometimes necessary to keep them
37 in conditions that are not natural for that particular species (Clubb & Mason, 2003). Taking a group
38 living animal and putting it into isolation, for example, might be expected to induce negative
39 affective states that could impair the animal's welfare (Olsson & Westlund, 2007; Galhardo et al.,
40 2011). For instance, if social, group living animals are housed in isolation for research purposes,
41 such negative affective states could influence the behaviour of the animal in ways that compromise
42 the research.

43 Animals perceive and interpret environmental information through cognitive processes, and
44 use learning and memory to help them modify their behavior (Braithwaite, 2006). To gain a better
45 understanding of how such cognitive processes function, we can compare how different animals
46 learn and remember information (Bshary et al., 2002). In the last two decades, there has been a
47 considerable growth in knowledge about animal cognition, particularly in birds and mammals
48 (Dukas, 2004), however, fewer studies have focused on groups such as fish (Huntingford, 2003;
49 Braithwaite, 2006). Yet, it is becoming increasingly recognized that many species of fish are
50 capable of developing sophisticated behaviors that require the integration of different kinds of
51 information and that are presumably underpinned by complex cognitive processes (Braithwaite &
52 Salvanes, 2008).

53 From an evolutionary perspective, the brain structure of vertebrates and many of the
54 cognitive processes that these systems support appear to be relatively conserved (Broglio et al.,
55 2005; Salas et al., 2003; Portavella et al., 2004). Fish, for example, have been shown to have
56 several homologous brain regions found in other vertebrates (Nieuwenhuys & Meek, 1990; Salas

57 et al., 2003). Huntingford (2003) describes how several learning skills in fish are comparable to
58 those seen in mammals; for example, fish exhibit social learning, they develop migratory strategies
59 and they can learn to avoid places that predators frequent (Brown & Laland, 2003). In addition to
60 this, many fish species are capable of learning complex spatial relationships and can use
61 landmarks, geometry and form mental maps (Rodríguez et al., 1994; Odling-Smee & Braithwaite,
62 2003a). These specialized learning skills in fish depend upon a brain structure in the telencephalon
63 known as the dorsal lateral area (DL) that has been proposed to be homologous to the
64 hippocampus of birds and mammals (Broglia et al., 2003). Fish use their learning skills to provide a
65 degree of behavioral flexibility that aids survival and development. Their cognitive processes are
66 supported by changes in neural plasticity that promotes adaptive responses when they are
67 challenged by new situations (Kandel, 2001; Ebbesson & Braithwaite 2012).

68 Two factors that play a significant role in shaping an animal's nervous system are
69 environmental and social interactions (Shumway, 2010). An environment that is rich in stimuli is
70 more complex and promotes neural growth and learning (Salvanes et al. 2013). With respect to the
71 social environment, the social brain hypothesis suggests that, the more complex the social
72 interactions between individuals, the greater the development of the nervous system underpinning
73 the behavior of those animals (Byrne & Whiten, 1988; Dunbar, 1998). Moreover, social
74 observation, and learning that arise from these observations, provides an individual the ability to
75 adjust its behaviour rather than having to spend time and energy learning by trial and error
76 (Manassa et al., 2013). Thus, social interactions provide neural stimulation that increase the
77 number of synaptic connections and promotes neurogenesis (Coss & Globus, 1978; Young et al.,
78 1999; Dunlap et al., 2006) that help to support neural plasticity and learning.

79 Social individuals, deprived of interactions even for a short-term, period can exhibit a
80 reduction in brain cell proliferation, which can lead to impaired learning and memory processes
81 (Han et al., 2011). Furthermore, a lack of social interaction can lead to stress in social individuals,
82 which can impair learning skills and can be detrimental to memory retention (de Quervain et al.,
83 2000; Barreto et al., 2006) because neurons can be damaged by increased exposure to
84 glucocorticoids (McEwen & Sapolsky, 1995; Sørensen et al., 2013).

85 Fish are commonly used as model organisms in biomedical trials, or to test evolutionary
86 biology hypotheses (e.g. Panula et al. 2010; Howe et al., 2013; Bell & Aguirre, 2013). Teleost fish
87 exhibit the greatest neurogenesis seen in any adult vertebrate (Zupanc, 2008), making them an
88 excellent model animal to study the relationship between social organization, environmental
89 challenges and their effects on neural systems (Bshary et al., 2002). Many of the fish species used
90 in experiments would normally live in social groups, but certain kinds of experiment require that the
91 fish are kept in isolation (Gómez-Laplaza & Morgan, 2000). Social isolation in teleost fish has been
92 shown to alter aggressive behavior (Gonçalves-de-Freitas & Mariguela, 2006), locomotor and
93 feeding activity (Gómez-Laplaza & Morgan, 2003), and the neuroendocrine system (Hannes &
94 Franck, 1983). The aim of the current study was to extend these observations and to address
95 whether social isolation in a social species of fish also impairs cognitive performance.

96 Cichlids are a family of social animals that live in several kinds of habitat and show a range
97 of complex social behaviors (Barlow, 2000; Shumway, 2010). Many species of cichlids are
98 maintained as ornamental fish (Barlow, 2000) and they are often used in research (Bshary, 2002;
99 Shumway, 2010; Seehausen, 2014), but due to their aggressive behavior they are frequently
100 maintained in isolation (e.g. Gonçalves-de-Freitas & Mariguela, 2006). Thus, we use the social,
101 neotropical cichlid fish *Cichlasoma paranaense* to test whether social isolation affects learning
102 skills. The learning performance of fish kept in social isolation were compared to fish that were
103 group housed and allowed to develop dominance hierarchies within their social group.

104

105 **2. Methods**

106 2.1 Subjects and rearing conditions

107 Juvenile *Cichlasoma paranaense* of unidentified sex (adults of this species are sexually
108 monomorphic) were held in outdoor tanks at the Instituto de Biociências, Letras e Ciências Exatas,
109 UNESP, São José do Rio Preto, SP, Brazil. Fish were acclimated to laboratory conditions in 500L
110 indoor tanks (ca one fish per 5L; 27°C and 12h light/dark cycles) for at least 15 days. Biological
111 filters and constant aeration ensured water quality. Fish were fed to satiation with a commercial
112 ration for tropical fish, Pirá® 28% protein, twice a day.

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2.2 Experimental design

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We compared spatial learning ability in two groups of fish; one consisted of socially isolated individuals ($n = 15$) and the other was made up of the most dominant individual sampled from different social groups of four fish ($n = 14$). We chose to compare isolated and dominant fish because dominant individuals in this species can become territorial and will exclude other fish from their territory, but this behavior is a choice by dominant individuals to live in relative social isolation, unlike forced isolation that occurs when fish are placed into a tank of their own in captive situations (Figler & Einhorn, 1983).

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Before the learning tests, the fish were housed for 10 days in aquaria (30 x 30 x 40 cm) for habituation (isolated fish) and hierarchy establishment (group-housed fish). The group-housed fish were filmed for 10 min every two days to quantify their aggressive interactions using an ethogram developed for *C. paranaense* (see Table 1). A period of 10 days was used as this has previously been shown to be sufficient to reduce the influence of prior social experience in fish (e.g. Dunlap et al., 2006; Barreto et al., 2006).

129

Table 1: An ethogram of the main aggressive interactions seen in *C. paranaense*

Observed behavior	Description of behavior
Lateral threat	A fish approaches quickly towards its opponent that swims away
Chasing and fleeing	A fish swims in the direction of its opponent, causing the opponent to flee
Undulation	A fish intensively undulates its body antero-posteriorly against the opponent
Lateral attack	A fish approaches its opponent and bites its body
Pushing	A fish with its open mouth touches the lateral flank of its opponent, moving the opponent
Pulling fins	A fish bites its opponent's fin, moving the opponent away by pulling the

fins

Mouth fighting Two fish approach one another and simultaneously bite their jaws. They may swim in a circle while keeps their mouth attached

130

131 Social groups were formed by putting four individuals of heterogeneous size (between 6.0 -
 132 10.0 cm standard length) together. Each group's hierarchy was determined by calculating a
 133 Dominance Index (DI = individual attacks/ total group attacks), with the dominant fish (alpha)
 134 scoring the closest to 1.0 DI (e.g. Oliveira & Almada, 1996; Gonçalves-de-Freitas et al., 2008;
 135 Barreto et al., 2011). Before grouping, the fish were anesthetized with tricaine methanesulfonate,
 136 MS222, (Sigma-Aldrich/China) (20mg/L) and tagged below their dorsal and opercular scales using
 137 Visible Implant Elastomer tags, VIE tags, (Northwest Marine Technology, Inc./USA) to allow
 138 individual identification. Isolated individuals were also anaesthetized and tagged so that they
 139 experienced the same handling and manipulation as the grouped animals.

140

141 2.3 Social learning

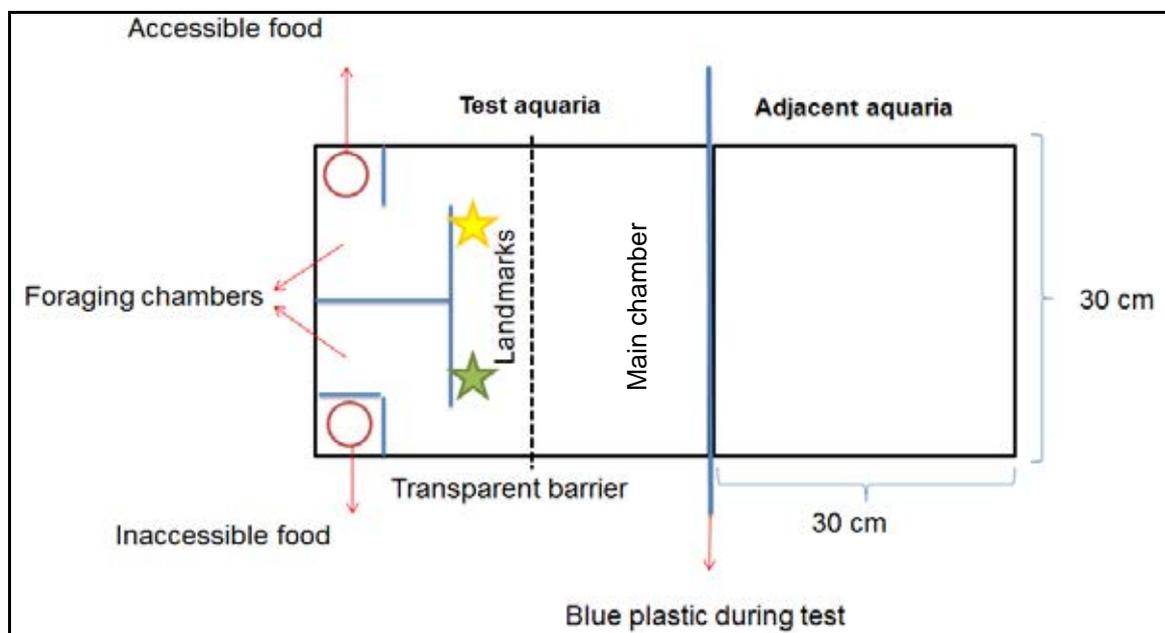
142 Dominant and isolated fish were transferred to individual test aquaria (40 x 30 x 40 cm; 48L),
 143 where they remained until the end of experiment. The side and back walls were covered with blue
 144 plastic to avoid visual contact between replicate tanks. Blue was used because it has been shown
 145 to reduce stress in another species of cichlid fish, the Nile-tilapia (*Oreochromis niloticus*) (Volpato
 146 & Barreto, 2001). The front wall was left uncovered and this was turned so that it faced a separate
 147 aquarium where the subordinate group members were housed in the case of the dominant fish, or
 148 an empty aquarium for the isolated individual. The test aquarium was divided into three chambers:
 149 two for foraging and one where the fish spent the rest of its time (see Fig. 1).

150 When the trials were not being run, the foraging chambers were separated from main
 151 chamber by an opaque plastic partition that prevented fish accessing that part of the tank. At the
 152 start of a trial, this opaque plastic partition was removed and another opaque barrier was placed
 153 between the test aquarium and the adjacent aquarium so all test fish were visually and physically
 154 isolated at the time of testing. A transparent barrier was then placed between the foraging

155 chambers and the main chamber to allow the foraging chambers to be baited while the test fish
 156 was confined in the main chamber (see Fig. 1). Two cups with food (fresh shrimp) were placed in
 157 each foraging chamber but in only one of these was the food accessible (inaccessible food was
 158 placed into a cup with a perforated plastic cover which allowed the fish to smell the food, but it
 159 could not be consumed). After two minutes, a video camera over the top of the tank was switched
 160 on and the transparent barrier was removed, allowing test fish access to the foraging chambers.
 161 The latency before fish began swimming after the transparent barrier was removed was noted.

162 Visual landmarks in the form of green or yellow star shaped symbols were placed at the
 163 entrance of each foraging chamber. Each fish experienced one color and side consistently
 164 signaling the accessible food chamber, and the other color and side indicating inaccessible food
 165 (Fig. 1). Several species of fish have been shown to use such visual and spatial cues to help them
 166 learn foraging tasks (Odling-Smee & Braithwaite, 2003a). The side on which food was made
 167 accessible and the color of the rewarded chamber landmark were counterbalanced across both the
 168 dominant and isolated treatment groups.

169



170

171 **Figure 1:** Schematic view of test aquarium seen from above. There were two possible routes that
 172 fish could take to find food (guided by a green or yellow star shaped symbol). The rest of the tank
 173 area, the main chamber, is where fish remained when the test was not being run. The adjacent

174 aquarium, on the right, was where subordinate fish were maintained for so that the dominant fish
175 had visual contact with the rest of their social group. This tank was empty for individuals in the
176 isolated treatment.

177

178 Individuals were tested for 15 minutes, twice a day between 8:00h-10:00h and 14:00h-
179 16:00h, for 15 days. A learning criterion of nine correct choices (defined as entering the correct,
180 accessible food chamber first and consuming the food) out of ten trials was used (e.g. Odling-
181 Smee & Braithwaite, 2003b). Fish were trained until they reach the criterion or until they had
182 completed 30 trials without achieving the learning criterion and so categorized as no-learners. On
183 completion of the experiment, the fish were euthanized with benzocaine and dissected to permit
184 sex identification. The isolated group consisted of revealing seven males and eight females, and
185 the dominant group consisted of two males and 12 females.

186

187 2.4 Statistical

188 The number of individuals that reached the learning criterion within a treatment was
189 compared using a binomial test. A comparison of fish that learned and did not learn the task
190 between the isolated and social groups was compared using a Chi Square Contingency Table two-
191 tailed test. Only data from fish that learned the task was analyzed, here the number of trials
192 needed for each fish to reach the criterion was compared after log transformation, using unpaired t
193 test.

194 The motivation of the two groups to perform the task was analyzed by looking at the latency
195 for the fish to consume the food reward, their latency to start swimming at the beginning of the trial
196 and the number of times an individual entered a foraging chamber. These data were compared in
197 unpaired t tests (after log transformation). The aggressiveness of dominant fish (measured during
198 the initial stage when the dominance hierarchies were becoming established) that learned or did
199 not learn the task was also compared using unpaired t tests (log transformed data). A Pearson
200 correlation was used to compare the aggressiveness of dominant fish that achieved the learning
201 criterion. Finally, as there were unequal numbers of males and females in the treatments groups,

202 we compared the possible effect of sex within and between treatments using a Fisher's Exact test.
 203 We used $p < 0.05$ for statistical significance.

204

205 2.5 Ethical approval

206 This study was conducted according to the ethical guidelines of the Brazilian National
 207 Council for the Control of Animal Experiments (CONCEA) and was approved by the Ethical
 208 Committee of Animal Experimentation of the Universidade Estadual Paulista (UNESP, SP, Brazil,
 209 permit 063/2012).

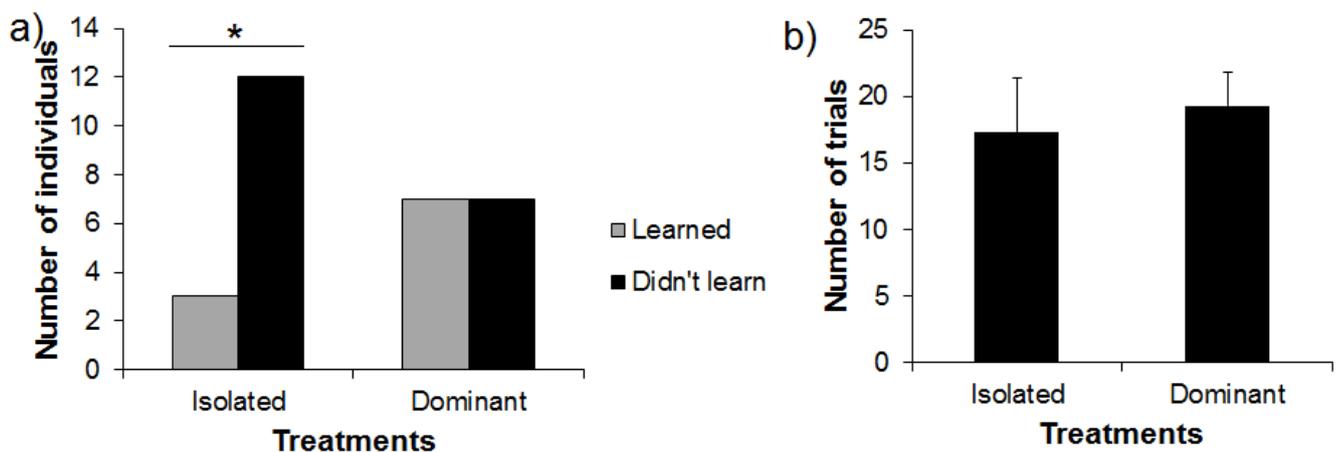
210

211 3. Results

212 3.1 Frequency and latency for learning

213 Only three out of 15 fish in the isolated treatment were able to achieve the learning criterion,
 214 (Binomial test, $p = 0.03$; Fig. 2a). Whereas, in the dominant group seven fish learned the task and
 215 seven did not (Binomial test $p > 0.05$). A comparison between the two treatments showed a non-
 216 significant trend that more fish from the dominant treatment group learned the task ($\chi^2 = 2.88$, $p =$
 217 0.09 ; Fig. 2a). Using only fish that learned the task, a comparison between the two treatments
 218 found no difference in the rate at which fish reached the learning criterion (Mann-Whitney test, $U =$
 219 8.5 ; $Z = -0.46$; $p = 0.65$ Fig. 2b), although it should be noted that the samples size in this
 220 comparison are low ($n = 7$ Dominant, $n = 3$ Isolated).

221



222

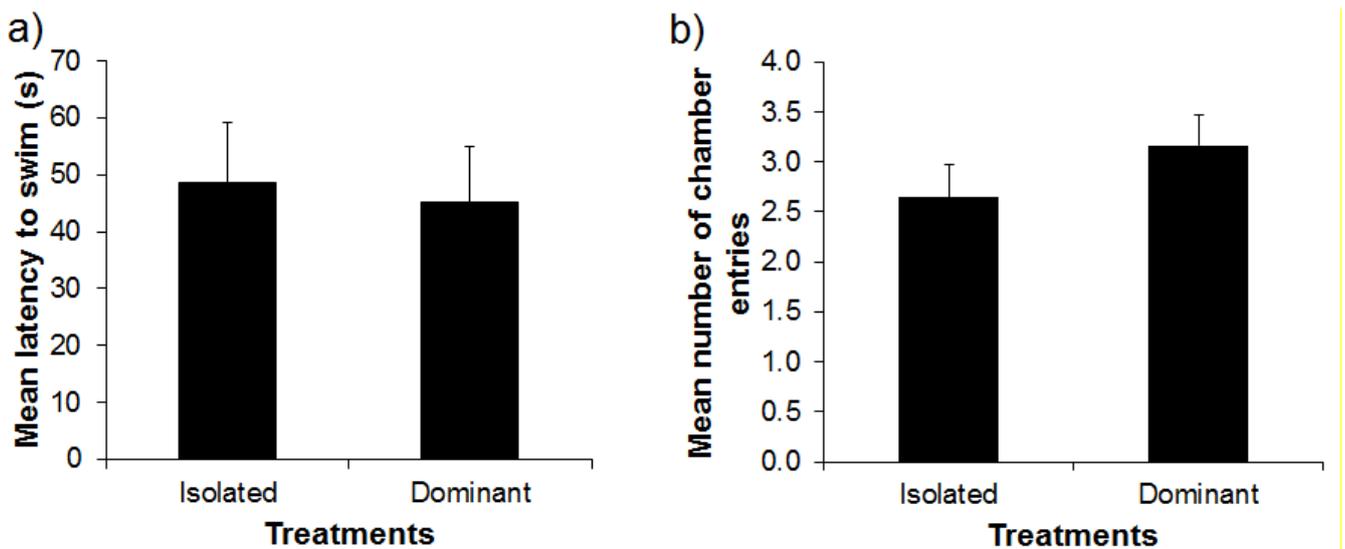
223 **Figure 2:** a) Number of individuals that achieved the 9/10 learning criterion in each treatment (
 224 Binomial test); b) Mean \pm SE of the number of trials taken for fish to learn the task (Mann-Whitney).
 225 * indicates significant difference ($p < 0.05$).

226

227 3.2 Activity and motivation profile

228 The latency of fish to start swimming once the test had started was similar between
 229 treatments (mean \pm SE for isolated individuals was 48.63 ± 10.69 seconds and for dominant ones,
 230 45.25 ± 9.61 seconds; unpaired t test, $t = 0.31$, $p = 0.75$; Fig. 3a). There was also no difference in
 231 the number of times that isolated or dominant treatment fish entered the foraging chambers (mean
 232 \pm SE for isolated fish was 2.65 ± 0.33 and for dominant fish was 3.16 ± 0.30 ; unpaired t test $t = -$
 233 1.43 , $p = 0.16$; Fig. 3b).

234



235

236 **Figure 3:** Mean \pm SE a) latency in seconds for fish to start swimming in each trial; b) number of
 237 times that the fish entered one of the foraging chambers in each trial.

238

239 3.3 Feeding activity

240 There was no difference between treatments in terms of the latency to start feeding in the
 241 trials (mean \pm SE for isolated individuals was 489.47 ± 58.81 seconds and for dominant fish,
 242 340.71 ± 38.23 seconds; unpaired t test, $t = 1.58$, $p = 0.12$).

243

244 3.4 Aggressiveness

245 There was no difference in the aggressiveness of the dominant individuals that learned or did
246 not learn the task (aggressiveness measures were scored from the videos taken during the
247 hierarchy establishment; mean \pm SE number of aggressive confrontations from fish that learned
248 the task, 97.86 ± 24.5 , and from individuals that did not learn the task, 174.33 ± 42.08 ; unpaired t
249 test, $t = -1.38$, $p = 0.19$), nor was there a correlation between the aggressiveness of those fish that
250 learned and the latency for these fish reach the learning criterion (Pearson correlation, $p = 0.47$).

251

252 3.5 Sex effects

253 In the isolated fish treatment, one male of six and two females of six fish learned the task. In
254 the dominant treatment group, seven females fish learned the task and five did not. Neither of the
255 two male fish learned the task. Thus there were no consistent sex differences in learning
256 performance. The unequal sex ratios in the two treatment groups were a result of the inability to sex
257 the fish prior to the behavior trials as the two sexes are monomorphic.

258

259 4. Discussion

260 As predicted, cichlid fish that experienced ten days of social isolation were impaired when
261 tested in a spatial learning task; only three out of 15 fish were able to reach the learning criterion.
262 In contrast, half of the fish (seven) that were scored as dominant individuals in a socially housed
263 treatment achieved the learning criterion. To predict the location of the accessible food reward, the
264 fish had to associate a visual landmark and, or, the position (left or right) of one of two food reward
265 chambers. The results suggest that social isolation has a negative effect on the fish and disrupts
266 cognitive ability. Yet, keeping fish in isolation is not uncommon in captive settings, particularly in
267 research laboratory situations where fish are used as experimental subjects (Gómez-Laplaza &
268 Morgan, 2000; Galhardo & Oliveira, 2013).

269 A comparison between fish that did learn the task revealed that they appeared to do so at a
270 similar rate as the number of trials taken to achieve the learning criterion was not different for either
271 treatment group. Cichlids are considered relatively plastic fish in that changes in their social

272 environment are readily seen in both behavioral and physiological processes (Huffman et al.,
273 2012). A comparison of motivation across the two groups tested here revealed no differences in
274 the latency to begin swimming at the start of a trial, the number of foraging chamber entries, or
275 how quickly the fish started to feed on the food reward. Thus compromised motivation in the
276 socially isolated fish does not seem to explain their poor learning performance.

277 In situations where there are high levels of environmental variation, or when an immediate
278 action to some kind of challenge is required, an animal's nervous system must respond quickly and
279 learning processes can help refine how the animal behaves. Indeed, fish that live in enriched
280 environments, with stimuli richness exhibit a greater level of neural activity and a superior ability to
281 learn (von Krogh et al., 2010; Salvanes et al., 2013). Although stimulation provided by the physical
282 environment is important, social interactions can also exert a considerable influence over neural
283 plasticity (Dunbar & Shultz, 2007). Bshary (2011) proposes that social interactions have an
284 important role in individual natural selection concerning the evolution of cognitive processes. In our
285 study, a lack of social stimulation appears to have impaired the ability of the fish to associate food
286 with a particular landmark and position.

287 Territorial fish, like *Cichlasoma paranaense*, need to be able to recognize the boundaries of
288 their territory, and to readily track the position of shelter so that they can quickly seek refuge when
289 threatened (Braithwaite, 2006). Thus, we expected that the *C. paranaense* tested in the experiment
290 would use the spatial cues and would learn how to reliably locate the food reward. Yet most of
291 isolated individuals were unable to learn the association between the spatial cues and the
292 accessible food reward.

293 It seems likely that the stress induced by social isolation could have generated the learning
294 deficit seen in isolated fish. A study by Øverli and colleagues (2002) found that only six days of
295 social isolation were sufficient to provide chronic stress in rainbow trout, *Oncorhynchus mykiss*. It
296 has also been shown that social isolation in the cichlid fish, *Archocentrus nigrofasciatus*, resulted in
297 an increase in cortisol levels (Earley et al., 2006). Social stressors have been found to directly
298 reduce cell proliferation in the telencephalon of rainbow trout (Sørensen et al., 2011) and they are
299 associated with the loss of neurons that support learning and memory processes (Zhang et al.,

300 2006). Although we did not measure stress indicators, it seems likely that the ten days of social
301 isolation challenged the *C. paranaense* in such a way that it impaired their spatial learning.

302 Alternative behaviors that could be compromised in the isolated fish, such as swimming
303 (Gómez-Laplaza & Morgan, 2003) and feeding (Øverli et al., 2002) were also quantified. Isolated
304 fish have previously been found to exhibit reduced swimming activity after a 10 day period in
305 isolation (Gómez-Laplaza & Morgan, 1991). A decrease in activity could be adaptive for individuals
306 that find themselves in social isolation because being solitary may make an individual more prone
307 to danger such as predation, but decreased activity would lower an individuals' detectability
308 (Gómez-Laplaza & Morgan, 1991; 2003). For feeding activity, previous work has described how
309 introducing a fish into a new environment can lead to a cessation of feeding activity (Øverli et al.,
310 2002). Thus, to confirm that impaired performance in the learning task was altered because of an
311 effect on learning, and not some alternative change in motivation induced by the reduction in social
312 stimulation, we compared the exploratory and feeding profiles of dominant and isolated fish. We
313 found no differences in latency to explore, the number of entries into the foraging chambers or in
314 the latency to start feeding. This suggests that the isolated and dominant fish had similar levels of
315 motivation to find the food (reward), so the differences between the treatment groups do seem to
316 represent differences in learning.

317 Finally, for the dominant fish we related their aggressiveness, measured during the
318 dominance hierarchy establishment phase, with their ability to learn. We wanted to explore this
319 relationship because studies have previously shown positive correlations between aggression and
320 boldness, with more aggressive fish being more willing to explore new environments (Sih et al.,
321 2004, Conrad et al., 2011; Ruiz-Gomez & Huntingford, 2012) and, consequently, they may be in a
322 position to update and change their behavior compared to their more timid, less exploratory
323 conspecifics. Thus, we evaluated whether learning ability in the dominant fish correlated with their
324 aggressiveness. However, we did not find significant differences between animals with higher or
325 lower aggressiveness indices and their learning skills, suggesting that there is no association
326 among these factors at least with regards to spatial learning.

327 The results from this study indicate that a lack of social stimulation can impair learning skills
328 and, consequently, social isolation may influence the behavior of fish used in experiments.
329 Combining these observations with previous research that shows how isolation of naturally group
330 living fishes triggers a stress response, it seems likely that social isolation has a negative effect on
331 the welfare of the isolated individuals (Øverli et al., 2002). The act of isolating normally group living
332 fish may interfere with how the fish judge situations and adjust their behavior (von Krogh et al.,
333 2010). This could also have implications for ornamental fish as individuals are often kept in
334 isolation to avoid fights (Gonçalves-de-Freitas & Mariguela, 2006).

335

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341

342 **References**

- 343 Barlow, G.W., 2000. The cichlid fishes: nature's grand experiment in evolution. Basic Books, USA,
344 335p.
- 345 Barreto, R.E., Arantes Carvalho, G.G., Volpato, G.L., 2011. The aggressive behavior of Nile tilapia
346 introduced into novel environments with variation in enrichment. *Zoology* 114, 53-57.
- 347 Barreto, R.E., Volpato, G.L., Pottinger, T.G., 2006. The effect of elevated blood cortisol levels on
348 the extinction of a conditioned stress response in rainbow trout. *Hormones and Behavior* 50,
349 484-488.
- 350 Bell, M.A., Aguirre, W.E., 2013. Contemporary evolution, allelic recycling, and adaptive radiation of
351 the threespine stickleback. *Evolutionary Ecology Research* 15, 377-411.
- 352 Braithwaite, V.A., 2006. Cognitive ability in fish. *Fish Physiology* 24, 1-37.
- 353 Braithwaite, V.A., Salvanes, A.G.V., 2008. Cognition: Learning and memory. In: Magnhagen, C.,
354 Braithwaite, V.A., Forsgren, E., Kapoor, B.G. (Eds.), *Fish Behaviour*. Science Publisher, Inc,

- 355 USA p. 33- 60.
- 356 Broglio, C., Gomez, A., Duran, E., Ocana, F.M., Jimenez-Moya, F., Rodríguez, F., Salas, C., 2005.
357 Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial,
358 temporal and emotional memory in actinopterygian fish. *Brain Research Bulletin* 66, 277-281.
- 359 Broglio, C., Rodríguez, F., Salas, C., 2003. Spatial cognition and its neural basis in teleost fishes.
360 *Fish and Fisheries* 4, 245-255.
- 361 Brown, C., Laland, K.N., 2003. Social learning in fishes: a review. *Fish and Fisheries* 4, 280-288.
- 362 Bshary, R., Wickler, W., Fricke, H., 2002. Fish cognition: a primate's eye view. *Animal Cognition* 5,
363 1-13.
- 364 Bshary, R. 2011. Machiavellian Intelligence in fishes. In: Brown, C., Laland, K., Krause, J. (Eds.),
365 *Fish Cognition and Behavior*, Second edition. Blackwell Publishing, Ltd, UK, pp. 277-297.
- 366 Byrne, R.W., Whiten, A., 1988. Machiavellian Intelligence: social expertise and the evolution of
367 intellect in monkeys, apes, and humans. Oxford Science Publications, USA, 413p.
- 368 Clubb, R., Mason, G., 2003. Captivity effects on wide-ranging carnivores. *Nature* 425, 473-474.
- 369 Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B., Sih, A., 2011. Behavioural syndromes in
370 fishes: a review with implications for ecology and fisheries management. *Journal of Fish*
371 *Biology* 78, 395-435.
- 372 Coss, R.G., Globus, A., 1978. Spine stems on tectal interneurons in jewel fish are shortened by
373 social stimulation. *Science* 200, 787-790.
- 374 de Quervain, D.J.F., Roozendaal, B., Nitsch, R.M., McGaugh, J.L., Hock, C., 2000. Acute cortisone
375 administration impairs retrieval of long-term declarative memory in humans. *Nature*
376 *Neuroscience* 3, 313-314.
- 377 Dukas, R., 2004. Evolutionary biology of animal cognition. *Annual Review of Ecology, Evolution*
378 *and Systematics*, 35, 347-74.
- 379 Dunbar, R.I.M., 1998. The social brain hypothesis. *Evolutionary Anthropology* 6, 178-190.
- 380 Dunbar, R.I.M., Shultz, S., 2007. Evolution in the social brain. *Science* 317, 1344-1347.
- 381 Dunlap, K.D., Castellano, J.F., Prendaj, E., 2006. Social interaction and cortisol treatment increase
382 cell addition and radial glia fiber density in the diencephalic periventricular zone of adult

- 383 electric fish, *Apteronotus leptorhynchus*. *Hormones and Behavior* 50, 10-17.
- 384 Ebbesson, L.O.E., Braithwaite, V.A. 2012. Environmental effects on fish neural plasticity and
385 cognition. *Journal of Fish Biology* 81, 2151-2174.
- 386 Earley, R.L., Edwards, J.T., Aseem, O., Felton, K., Blumer, L.S., Karom, M., Grober, M.S., 2006.
387 Social interactions tune aggression and stress responsiveness in a territorial cichlid fish
388 (*Archocentrus nigrofasciatus*). *Physiology & Behavior* 88, 353-363.
- 389 Figler, M.H., Einhorn, D.M., 1983. The territorial prior residence effect in convict cichlids
390 (*Cichlasoma nigrofasciatum* Gunther) - temporal aspects of establishment and retention, and
391 proximate mechanisms, pp. 157-183.
- 392 Galhardo, L., Vital, J., Oliveira, R.F., 2011. The role of predictability in the stress response of a
393 cichlid fish. *Physiology & Behavior* 102, 367-372.
- 394 Galhardo, L., Oliveira, R.F., 2013. The effects of social isolation on steroid hormone levels are
395 modulated by previous social status and context in a cichlid fish, *Hormones and Behavior*,
396 doi: 10.1016/j.yhbeh.2013.10.010
- 397 Gonçalves-de-Freitas, E., Mariguela, T. C., 2006. Social isolation and aggressiveness in the
398 Amazonian juvenile fish *Astronotus ocellatus*. *Brazilian Journal of Biology* 66, 233-238.
- 399 Gonçalves-de-Freitas, E., Teresa, F.B., Gomes, F.S., Giaquinto, P.C., 2008. Effect of water
400 renewal on dominance hierarchy of juvenile Nile tilapia. *Applied Animal Behaviour Science*
401 112, 187-195.
- 402 Gómez-Laplaza, L.M., Morgan, E., 1991. Effects of short-term isolation on the locomotor-activity of
403 the angelfish (*Pterophyllum scalare*). *Journal of Comparative Psychology* 105, 366-375.
- 404 Gómez-Laplaza, L.M., Morgan, E., 2000. Laboratory studies of the effects of short-term isolation
405 on aggressive behaviour in fish. *Marine and Freshwater Behaviour and Physiology* 33, 63-
406 102.
- 407 Gómez-Laplaza, L.M., Morgan, E., 2003. The influence of social rank in the angelfish,
408 *Pterophyllum scalare*, on locomotor and feeding activities in a novel environment. *Laboratory*
409 *Animals* 37, 108-120.
- 410 Han, X., Wang, W., Xue, X., Shao, F., Li, N., 2011. Brief social isolation in early adolescence

- 411 affects reversal learning and forebrain BDNF expression in adult rats. *Brain Research*
412 *Bulletin* 86, 173-178.
- 413 Hannes, R.P., Franck, D., 1983. The effect of social-isolation on androgen and corticosteroid levels
414 in a cichlid fish (*Haplochromis burtoni*) and in swordtails (*Xiphophorus helleri*). *Hormones*
415 *and Behavior* 17, 292-301.
- 416 Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., et al., 2013. The zebrafish reference genome
417 sequence and its relationship to the human genome. *Nature* 496, 498-503.
- 418 Huffman, L.S., Mitchell, M.M., O'Connell, L.A., Hofmann, H.A., 2012. Rising StARs: Behavioral,
419 hormonal, and molecular responses to social challenge and opportunity. *Hormones and*
420 *Behavior* 61, 631-641.
- 421 Huntingford, F.A., 2003. Foreword. *Fish and Fisheries* 4, 197-198.
- 422 Kandel, E.R., 2001. Neuroscience - The molecular biology of memory storage: A dialogue between
423 genes and synapses. *Science* 294, 1030-1038.
- 424 Manassa, R.P., McCormick, M.I., Chivers, D.P., 2013. Socially acquired predator recognition in
425 complex ecosystems. *Behavioral Ecology and Sociobiology* 67, 1033-1040.
- 426 McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. *Current Opinion in*
427 *Neurobiology* 5, 205-216.
- 428 Nieuwenhuys, R., Meek, J., 1990. The telencephalon of actinopterygian fishes. *Cerebral Cortex*
429 8A, 31-73.
- 430 Odling-Smee, L., Braithwaite, V.A., 2003a. The role of learning in fish orientation. *Fish and*
431 *Fisheries* 4, 235-246.
- 432 Odling-Smee, L., Braithwaite, V.A., 2003b. The influence of habitat stability on landmark use during
433 spatial learning in the threespined stickleback. *Animal Behaviour*. 65, 701-707.
- 434 Oliveira, R.F., Almada, V.C., 1996. On the (in)stability of dominance hierarchies in the cichlid fish
435 *Oreochromis mossambicus*. *Aggressive Behavior* 22, 37-45.
- 436 Olsson, I.A.S., Westlund, K., 2007. More than numbers matter: The effect of social factors on
437 behaviour and welfare of laboratory rodents and non-human primates. *Applied Animal*
438 *Behaviour Science* 103, 229-254.

- 439 Øverli, Ø., Pottinger, T.G., Carrick, T.R., Øverli, E., Winberg, S., 2002. Differences in behaviour
440 between rainbow trout selected for high- and low-stress responsiveness. *Journal of*
441 *Experimental Biology* 205, 391-395.
- 442 Panula, P., Chen, Y.-C., Priyadarshini, M., Kudo, H., Semenova, S., Sundvik, M., Sallinen, V.,
443 2010. The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of
444 relevance to human neuropsychiatric diseases. *Neurobiology of Disease* 40, 46–57.
- 445 Portavella, M., Torres, B., Salas, C., 2004. Avoidance response in goldfish: Emotional and
446 temporal involvement of medial and lateral telencephalic pallium. *Journal of Neuroscience*
447 24, 2335-2342.
- 448 Ruiz-Gomez, M.L., Huntingford, F.A., 2012. Boldness and aggressiveness in early and late
449 hatched three-spined sticklebacks *Gasterosteus aculeatus*. *Journal of Fish Biology* 81, 966-
450 976.
- 451 Salas, C., Broglio, C., Rodriguez, F., 2003. Evolution of forebrain and spatial cognition in
452 vertebrates: Conservation across diversity. *Brain Behavior and Evolution* 62, 72-82.
- 453 Salvanes, A.G.V., Moberg, O., Ebbesson, L.O.E., Nilsen, T.O., Jensen, K.H., Braithwaite, V.A.,
454 2013. Environmental enrichment promotes neural plasticity and cognitive ability in fish.
455 *Proceedings of the Royal Society B – Biological Sciences* 280, 20131331.
- 456 Seehausen, O., 2006. African cichlid fish: a model system in adaptive radiation research.
457 *Proceedings of the Royal Society B – Biological Sciences* 273, 1987–1998.
- 458 Shumway, C.A., 2010. The evolution of complex brains and behaviors in African cichlid fishes.
459 *Current Zoology* 56, 144-156.
- 460 Sih, A., Bell, A., Johnson, J.C., 2004. Behavioral syndromes: an ecological and evolutionary
461 overview. *Trends in Ecology & Evolution* 19, 372-378.
- 462 Sørensen, C., Bohlin, L.C., Øverli, Ø., Nilsson, G.E., 2011. Cortisol reduces cell proliferation in the
463 telencephalon of rainbow trout (*Oncorhynchus mykiss*). *Physiology & Behavior* 102, 518-523.
- 464 Sørensen, C., Johansen, I.B., Øverli, Ø., 2013. Neural plasticity and stress coping in teleost fishes.
465 *General and Comparative Endocrinology* 181, 25-34.
- 466 Volpato, G.L., Barreto, R.E., 2001. Environmental blue light prevents stress in the fish Nile tilapia.

- 467 Brazilian Journal of Medical and Biological Research 34, 1041-1045.
- 468 von Krogh, K., Sørensen, C., Nilsson, G.E., Øverli, Ø., 2010. Forebrain cell proliferation, behavior,
469 and physiology of zebrafish, *Danio rerio*, kept in enriched or barren environments. *Physiology*
470 & Behavior 101, 32-39.
- 471 Young, D., Lawlor, P.A., Leone, P., Dragunow, M., During, M.J., 1999. Environmental enrichment
472 inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Medicine* 5,
473 448-453.
- 474 Zhang, L., Zhou, R.L., Li, X.X., Ursano, R.J., Li, H., 2006. Stress-induced change of mitochondria
475 membrane potential regulated by genomic and non-genomic GR signaling: A possible
476 mechanism for hippocampus atrophy in PTSD. *Medical Hypotheses* 66, 1205-1208.
- 477 Zupanc, G.K.H., 2008. Adult neurogenesis and neuronal regeneration in the brain of teleost fish.
478 *Journal of Physiology-Paris* 102, 357-373.

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Assinatura