Do captive fish need cognitive enrichment? A test with a puzzle feeder in guppies

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Abstract

For many mammalian and avian species, it has been proposed that cognitive enrichment, such as puzzle feeders, can improve welfare in captive conditions. A well-established method to evaluate the need of cognitive enrichment is to observe the preference of the animal between freely available food and a puzzle feeder. We investigated this preference in a teleost fish, the guppy. In most of our experimental trials, guppies first chose the feeder with freely available food over the puzzle feeder, in contrast with what was observed in most other species. Nevertheless, guppies' number of choices for the puzzle feeder was significantly greater than zero. Moreover, after consuming the freely available food, most of the guppies tackled the puzzle feeder. This pattern of results suggests that guppies displayed a certain interest in the puzzle feeder that was overshadowed by their strong attraction towards the free food. Interestingly, several measures of performance indicated that female guppies responded more positively towards the puzzle feeder as compared to the males, suggesting sex differences in the preference for cognitive enrichment. In conclusion, our study highlights the potential significance of cognitive enrichment for captive fish. Considering that the number of individual fish maintained in captivity exceeds by far that of any other vertebrate group, it is paramount to investigate cognitive enrichment in other teleost species.

Keywords: animal welfare; enrichment; fish cognition; *Poecilia reticulata*; problem-solving; sex differences.

Introduction

In recent decades, there has been increasing interest in the welfare of captive animals because of social and normative pressures (e.g., Fraser, 2008; Koknaroglu et al., 2013). Environmental enrichment, which involves providing biotic and abiotic stimuli in the environment, is a well-established method for improving welfare (Ellis, 2009; Bayne, 2018; Makecha & Highfill, 2018). Research has shown that animals reared with environmental enrichment develop a more natural suite of behaviors (Ellis, 2009; Mason et al., 2007; Salvanes et al., 2013; Mkwanazi et al., 2019), experience reduced stress (Benaroya-Milshtein et al., 2004; Crone et al., 2021; Godyń et al., 2019), and, interestingly, exhibit increased productivity (Bolt & George, 2019). This likely happens because enrichment provides an environment that is more similar to the natural one, which has shaped most of the evolutionary history of the species.

Stimulation of the cognitive system likely plays a role in mediating some of the benefits of enrichment. Cognition controls individuals' behaviour and consequently, shapes their interactions with the environment (Shettleworth, 2001). Hence, the cognitive abilities displayed by a species are the results of selection due to the natural environment (Lee & Thornton, 2021; Holekamp & Benson-Amram, 2017) and are strictly tied to the tasks individuals are required to perform in the wild (Kotrschal & Taborsky, 2010). Consequently, animals maintained in captivity without the possibility to execute natural cognitive tasks might display reduced welfare. This emphasizes the need to study and develop cognitive enrichment strategies for captive animals (Clark, 2017; Meehan & Mench, 2007).

Studies on cognitive enrichment are currently available for several species of mammals (De Jonge et al., 2008; De Rosa et al., 2003; Gottlieb et al., 2011; Clark et al., 2013; Rosemberg et al., 2020; Schipper et al., 2008; Van Os et al., 2018) and birds (Coulton et al.,1997; Jensen et al., 2022; Smith et al., 2021; van Zeeland et al., 2013). A common

finding of these studies is that individuals presented with the choice between a freely available resource (e.g., food) and the same resource that can be accessed by solving a problem (e.g., a puzzle feeder) would prefer the latter option. Typically, this is considered evidence that cognitive enrichment increases animals' welfare.

The literature on cognitive enrichment exhibits a significant taxonomic bias. It's unclear whether teleost fish, the most numerous vertebrates held in captivity, share the same cognitive enrichment requirements as mammals and birds. Given the wide range of cognitive abilities displayed by fish (e.g., Brandão et al., 2019; Brown, 2012; Bshary et al., 2014; Gierszewski et al., 2017; Lucon-Xiccato et al., 2020), we hypothesise that also captive fish prefer to interact with cognitive enrichment. We tested this hypothesis in the guppies, *Poecilia reticulata*. Guppies are one of the most common aquarium species, and among the teleosts with ample evidence of problem-solving skills (Lucon-Xiccato et al., 2020; Mair et al., 2021; Montalbano et al., 2020). In our experiment, we observed guppies' choice between a freely available food reward and one accessible by solving a puzzle as a form of cognitive enrichment.

Materials and Methods

Subjects

The experimental subjects were 16 adult guppies (approximately 1 year old) of both sexes (8 males and 8 females). The subjects belonged to a domestic strain called 'snakeskin cobra green'. We selected this strain because domestic guppies are usually less fearful than their wild counterparts (Swaney et al., 2015). In pilot studies using this procedure, we encountered difficulties when training wild subjects; they often failed to habituate to interactions with the experimenter and exhibited a high level of stress and fear. In addition, our domestic guppy strain has reduced sex size difference, which could potentially introduce

another confound in our study. We selected only females with no sign of abdominal distension, and therefore, no signs of pregnancy.

The guppy population has been maintained in our laboratory since 2012, and at the time of the study was composed of approximately 1000 individuals. Before the experiments, the subjects were kept in mixed-sex groups in 400-L plastic tanks under standardized laboratory conditions: water temperature 27 ± 1 °C; conductivity 606.7 ± 60.18 µS/cm; nitrite levels < 0.1 mg/L and nitrate < 50 mg/L. The maintenance tanks were also provided with mechanical, chemical, and biological filters (Hailea Air Pump ACO-5505, Chaozhou, CN). Each aquarium also contained natural vegetation consisting of 4/5 plants (*Echinodorus bleheri* and *Taxiphyllum barbieri*). Illumination was provided by 30 W fluorescent lamps (Sylvania GRO-LUX, Milano, IT) set with 12 h of light (07:00 - 19:00). Fish were fed with commercial dry food (Sera Vipan Nature Tropical Flakes, DE) and *Artemia salina* nauplii (Ocean Nutrition, Essen, BE) twice per day.

All the fish used in the study were naïve. The experimenter randomly selected the subjects from the stock, ensuring that they did not show sign of distress or disease. No fish was excluded from the analyses (final sample size = 16 guppies).

STRANGE framework

Related to the potential experimental biases outlined by Webster and Rutz (2020) in the STRANGE framework, all the relevant information on the experimental subjects is disclosed in methods. Sampling bias can be excluded for the factors of age and sex. The sample might not be rapresentative of wild guppies' populations given the known behavioural differences (e.g., Swaney et al., 2015). Moreveor, as the subjects were collected by net from a large population, we cannot excluded a bias of selecting the bolder individuals (King et al.,

2013). This does not affect the conclusions of the study but should be considered when generalising its findings.

Experimental aquaria

We used eight identical experimental aquaria in which the subjects were housed individually. The aquaria, rectangular in shape (60 × 40 × 35 cm), were filled with 30 cm of water (Figure 1). To prevent the fish from seeing nearby subjects, we covered three of the external walls of each aquarium (the back and lateral walls) with green plastic. The front wall of the aquarium was covered with a mesh grid to allow the experimenter to observe the behaviour of the subject. Each aquarium was divided into two sectors by a green plastic panel (Figure 1). The larger, front sector was used for the testing. The smaller, rear sector was divided into three compartments (Figure 1). The two lateral compartments of the rear sector were separated from the central one by a mesh grid and contained water filters and plants (*Taxiphyllum barbieri*). In addition, each lateral sector housed two immature conspecifics as social companions to mitigate the potential stress of social isolation in the subjects. The central compartment of the rear sector served as a start box for the subject. A clear Plexiglas guillotine door (10 × 8 cm), controlled remotely by the experimenter, separated the front sector from the start box. The experimental aquaria were maintained in the same conditions (i.e., temperature and illumination) as the maintenance tanks. The lamp providing illumination was placed above the front sector of the aquaria.

Experimental procedure

The experimental procedure consisted of three sequential phases: 1) habituation to the apparatus and the procedure, 2) training to solve the problem, and 3) preference test for the puzzle feeder. The first two phases aimed to establish the conditions for the third phase, in

129 which we measured the preference for a puzzle feeder versus freely available food. To begin
130 the procedure, we transported the guppies to the experimental aquarium and immediately
131 started the habituation phase. The habituation phase lasted a fixed number of days (i.e.,
132 three). During this habituation phase, an experimenter provided food to the fish according to
133 a specific schedule that varied between days. The food used in this and the following
134 experimental phases was the same provided to the fish during the maintenance (Sera Vipan
135 Nature Tropical Flakes, DE). On Day 1, the experimenter fed each subject twice, following
136 the normal maintenance schedule. On the following day, the experimenter attracted the
137 subject into the start box using a Pasteur pipette filled with crumbled food dissolved in water.
138 To ensure consistent motivation, the amount of food provided in each trial was a small
139 portion of the fish's daily intake. Then, the experimenter lowered the guillotine door,
140 confining the subject in the start box. The experimenter moved the pipette in the front sector
141 and slowly released the food into the water, forming a cloud, trying to attract the attention of
142 the subject. When the subject appeared to notice the food (i.e., when it attempted to swim
143 through the transparent guillotine door), the experimenter raised the door, permitting the
144 subject to enter the front sector and consume the food. This feeding procedure was repeated
145 four times on Day 2 and eight times on Day 3.
146 After the habituation phase, we performed the training phase. The training phase
147 lasted a minimum of three days, although it could last longer if the subject did not achieve a
148 predetermined criterion. The scope of the training phase was to teach the guppies to feed
149 from grey plastic feeders (5 × 5 × 0.8 cm). The food was placed in a hole (Ø 10 mm and 4
150 mm deep) carved in the centre of the feeder. To maintain high feeding motivation throughout
151 the day, we delivered a small ration of food in each trial, approximately occupying 2 mm^2 of
152 the hole's surface. A control analysis showed that there was no variation in the choices made
153 by the fish across trials within a day (Generalized Linear Mixed-effects Model with logit link

154 function and binomial error distribution: $\chi^2_1 = 0.687$; P = 0.407), suggesting no marked
155 changes in motivation. In each trial of the training phase, following the placement of the
156 subject into the start box, the experimenter placed two feeders on the bottom, in the frontal
157 sector (Figure 1). Then, the experimenter placed the food inside the holes of the two feeders.
158 As the puzzle, the experimenter placed a light blue disc (1.2 × 0.2 cm) over the hole of one of
159 the two feeders, randomly alternating between the right and left sides. The experimenter then
160 lifted the guillotine door and left the subject to consume the food for 15 min to before
161 removing both feeders. We administered eight training trials, separated by a 30-min interval,
162 on each day of the training phase (total = 24 trials). The hole coverage gradually increased
163 over the three training days, from approximately 50% to almost complete occlusion. If the
164 subject fed from the feeder with the disc in at least six out of eight trials of each training day,
165 it was admitted to the next training day; otherwise, the subject had to repeat the day. On
166 average the subjects took 3.5 days (range 3-7) to complete the training phase.
167 In the preference test, the subjects underwent a series of 100 trials divided into 10
168 consecutive days (i.e., 10 trials per day), following the general procedure described in the
169 training phase. However, the hole in one of the two feeders was always completely occluded
170 by the disc (i.e., the puzzle feeder). Therefore, the subject could choose whether to feed on
171 the feeder with the free food or on the puzzle feeder. The experimenter recorded the first
172 choice of the subject in each trial. Additionally, the experimenter recorded whether the
173 subject made a second choice within the allowed time (15 min). Last, using a stopwatch, the
174 experimenter recorded the latency to feed in both the first and the eventual second choice of
175 each trial, starting from when the subject entered the front sector.
176
177 Statistical analysis

All analyses were conducted in RStudio (version 2022.02.3, http://www.rstudio.com). The descriptive statistics in the Result section are mean ± standard deviation. The threshold for the statistical significance was set at $p = 0.05$. In our analysis, we focussed on four different measures of performance: the first choice between the puzzle feeder and the feeder with the free food; the latency to feed in the first choice; the second choice; and the latency to feed in the second choice.

We analysed the subjects' first choice to evaluate the presence of a preference for the puzzle feeder over the feeder with the free food. As the dependent variable, we calculated the percentage of trials in which each subject selected the puzzle feeder as the first choice (hereafter 'overall first-choice preference'). The overall first-choice preference was calculated across the entire preference test (i.e., 100 choices per fish). First, we used a one-sample t-test to determine whether the overall first-choice preference was greater than random preference. The random preference for the binary choice test was set at 50%. Then, using a one-sample one-tailed t-test, we also evaluated whether the overall first-choice preference was higher than 0%. An overall first-choice preference that is not significantly greater than 0% would indicate that the subjects actively avoided the puzzle feeder. We also performed an additional analysis on the first-choice that considered the trend across the different trials. In this latter analysis, we used the first choice for the puzzle feeder per each trial separately, obtaining a repeated measures variable with 100 observations per subject. For each trial, the dependent variable was coded as: '0' = the subject chose the feeder with the free food; '1' the subject chose the puzzle feeder. We analysed this repeated measures variable with a Generalized Linear Mixed-effects Model with logit link function and binomial error distribution (GLMM, 'glmer' function from the 'lmerTest' R package). We fitted the GLMM with sex as a fixed effect with two levels, day (a numeric variable with 10 possible values: 1, 2, 3...10) as a

covariate, and subject ID as a random effect. We estimated effects' significance of the parameters using the 'Anova' function of the 'car' R package.

The second variable investigated, the latency to feed in the first choice, had a repeated measures structure, with one datapoint per each trial. The latency was always log-transformed before running the analysis due to a right-skewed distribution. We fitted a Linear Mixed-Effect Model (LMM, 'lmer' function from the 'lmerTest' R package) with the subject's sex (male or female) and chosen feeder (feeder with free food or puzzle feeder) as fixed effects, day as covariate, and subject ID as random effect. We assessed the effect of parameters via Satterhwaite's degrees of freedom method ('anova' function). Additional models divided by sex were performed to investigate significant interactions.

The analysis of the second choice of subjects was performed following the modelling approach described for the first-choice repeated measures data. Indeed, the second choice data consisted of repeated measurements (100 observations per subject) and was coded as a binary variable: '0' = the subject ate from a single feeder; '1' = the subject ate from both feeders. We fitted a GLMM with sex (male or female) and the first choice (feeder with free food or puzzle feeder) as a fixed effect, day as a covariate, and subject as a random effect. The latency to feed in the second choice (log-transformed) was analysed via a LMM as previously described for the latency to feed in the first choice.

Results

Spontaneous preference for the puzzle feeder in the first choice

Overall, the guppies selected the puzzle feeder as the first choice in 17.38 ± 9.41% of trials. The resulting overall first-choice preference for the puzzle feeder was significantly lower than expected by chance (one-sample t test versus 50% preference: $t_{15} = 13.871$; P <

0.001; Figure 2a). However, the overall first-choice preference for the puzzle feeder was significantly greater than 0 (one-sample t test: $t_{15} = 7.387$; $P < 0.001$; Figure 2a).

The analysis across the 10 days of testing showed that the first choice of the subjects was affected by their sex ($\chi^2_1 = 16.311$; $P < 0.001$). Females were more likely to select the puzzle feeder compared to the males (female preference: 23.88 ± 7.10%; male preference: 10.88 ± 6.53%; Figure 2a). The analysis showed no significant change over time in the first choice ($\chi^2_1 = 1.739$; $P = 0.187$) and no significant sex × day interaction ($\chi^2_1 = 0.484$; $P = 0.487$; Figure 2b).

The analysis of the latency to feed in the first choice revealed a significant effect of the sex (females: 17.91 ± 10.25 s, males: 21.20 ± 9.10 s; $F_{1,36} = 24.180$, $P < 0.001$), a significant effect of the feeder chosen ($F_{1,1574} = 7.388$, $P = 0.007$), a significant interaction sex × feeder chosen ($F_{1,1574} = 9.094$, $P = 0.003$), and a significant interaction sex × day ($F_{1,1571} = 17.149$, $P < 0.001$). No other interactions were significant (Ps > 0.08). We performed two analyses separating the subjects by their sex to investigate the two significant interactions. Results indicated that males took longer to feed when they chose the puzzle feeder compared to when they chose the feeder with the free food ($F_{1,790} = 12.538$, $P < 0.001$). Conversely, females did not show differences in the latency to feed between the two types of choice ($F_{1,784} = 0.068$, $P = 0.794$; Figure 2c). Moreover, females showed an increase in the time to consume the food over testing days ($F_{1,783} = 22.632$, $P < 0.001$), but males did not ($F_{1,789} = 3.247$, $P = 0.072$; Figure 2d). The interactions in the models separated by subjects' sex were not significant (Ps > 0.2).

Second choice

Overall, the guppies consumed the food from both feeders in 92.81 ± 6.51% of trials. The model across the 10 days of the preference test revealed a significant effect of the first

choice on the percentage of trials completed ($\chi^2_1 = 3.996$; P = 0.046; Figure 3a). Fish were more likely to make a second choice after selecting the puzzle feeder (97.25 ± 4.86%) than after selecting the feeder with the free food (92.34 ± 6.60%). The analysis found no significant effect of sex (females: 91.87 ± 8.51%; males: 93.75 ± 4.06%; $\chi^2_1 = 0.041$; P = 0.840) and no significant effect of the experimental day ($\chi^2_1 = 0.907$; P = 0.341). The interaction day × sex was significant ($\chi^2_1 = 5.652$; P = 0.017; Figure 3b). Post-hoc analyses separated by subjects' sexes indicated that males showed a small increase in the percentage of trials completed across testing days ($\chi^2_1 = 5.078$; P = 0.024), while females did not alter their choice across days ($\chi^2_1 = 0.868$; P = 0.352).

The analysis of the second-choice latency found that males took significantly longer than females to start feeding (females: 76.55 ± 44.85 s, males: 113.74 ± 35.75s; $F_{1,43}$ = 13.161; P < 0.001; Figure 3c). This effect was mostly evident at the beginning of the experiment (sex × day interaction: $F_{1,1464} = 6.311$; P = 0.012; Figure 3d). There was no significant effect of the first choice ($F_{1,1466} = 1.933$; P = 0.165) and no other significant interactions (Ps > 0.4). Post-hoc analyses separated by subjects' sexes indicated that males decreased the latency to complete the task over days ($F_{1,741} = 5.329$, P = 0.021), while females increased their latency ($F_{1,726} = 4.339$, P = 0.038; Figure 3d).

Discussion

Cognitive enrichment is increasingly recognized as a tool to improve the welfare of mammals and birds in captivity, but its value for teleost fish has yet to be experimentally investigated. In this study, we examined the interest of guppies towards a cognitive enrichment consisting of a puzzle feeder. When given the choice between the puzzle feeder and freely available food, the subjects chose the former option in approximately 20% of the trials. This first analysis might indicate that guppies were not attracted by the cognitive

276 enrichment. Given this interpretation, the behaviour of guppies represents an exception to
277 what has been observed so far. Previous studies have shown that numerous animal species,
278 including humans, chimpanzees, macaques, pigs, domestic dogs, maned wolves, rats,
279 giraffes, chickens, jungle fowl, and pigeons, display a clear preference for various forms of
280 cognitive enrichment (de Jonge et al. 2008; Godyń et al., 2019; Inglis et al. 1997; Jensen et al.
281 2002; Sasson-Yenor and Powell 2019; Schipper et al., 2008; Vasconcellos et al. 2012), a
282 behaviour that is usually referred to as contrafreeloading (Osborne, 1977). However, this
283 preference has not been observed in domestic cats (Delgado et al., 2022) and in grizzly bears
284 (McGowan et al. 2010). While it is not possible to exclude that some species are not attracted
285 by foraging problems, it should be considered that the preference may be difficult to observe
286 if the puzzle is not appropriate to the species' natural foraging behaviour. Hence, it still is
287 important to evaluate other types of enrichment in guppies and teleosts in general. The study
288 of problem-solving in fish is limited, which makes it challenging to design puzzles tailored to
289 their foraging behavior.
290 When we looked more in detail into the behaviour of guppies during the experiment,
291 we found evidence of a more complex situation compatible with a certain interest in the
292 puzzle feeder. In a significant number of trials, guppies initially opted to obtain the food from
293 the puzzle feeder and even after eating the freely available food, the guppies almost always
294 consumed the food in the puzzle feeder. Therefore, a careful interpretation of our data does
295 not allow us to reject the hypothesis that guppies did show some form of interest towards
296 cognitive enrichment. Guppies' preference for the puzzle feeder may not have been absent but
297 rather overshadowed by a strong attraction to the freely available food. It is relevant to
298 remember that the guppy is a social fish that lives in group and displays strong competition
299 over food resources (Ward et al., 2006). This could make guppies particularly impulsive,
300 leading them to choose the easiest and most immediate food source first, and then shift their

interest toward food that requires problem-solving. In line with this hypothesis, it has been previously demonstrated that guppies choose the most conspicuous food source in sight even when another option has more food (Lucon-Xiccato et al., 2015). For the main goal of this study, which was investigating a potential form of cognitive enrichment, this more comprehensive interpretation of guppies' behaviour has an important consequence. The interest in the puzzle feeder, even if moderate, suggests that cognitive enrichment may have some relevance for fish welfare. As mentioned earlier, it might be necessary to design puzzles that are better suited to guppies to observe a clear preference.

An unexpected finding of our study was the presence of notable sex differences. Female guppies showed a stronger first-choice preference for the cognitive enrichment compared to males. However, it should be noted that males did not entirely avoid the cognitive enrichment: after consuming the freely available food, the males showed an interest in consuming the food in the puzzle feeder that was not significantly different from females. Therefore, it can be deduced that the sex difference is restricted to the initial choice between the two options. Sexual differences have been increasingly documented in cognitive research in fish (reviewed in Lucon-Xiccato, 2022). A previous study has shown that female guppies outperformed males in a similar problem-solving task involving dislodging a disc (Lucon-Xiccato et al., 2020). Thus, a straightforward explanation for our results would be that female guppies were more likely to choose the puzzle due to their higher ratio of solution. This explanation warrants further testing because it implies a cognitive process that grants guppies a form of awareness. For example, males might possess an understanding of their limited problem-solving skills and prioritize the easier foraging option in their choices.

A second sex difference regarded temporal aspects of the performance. In most trials, females were faster than males to obtain food in their first choice (even if they chose the puzzle feeder more often). Similarly, the latency to perform the second choice was greater for

males, at least in the initial phases of the experiment. Males, but not females, also showed higher latency when choosing the puzzle feeder compared to the free food. Interestingly, in an earlier study on the same population of guppies, it was found that males took consistently less time than females to solve a visual discrimination task, a finding that was interpreted in terms of males' greater impulsivity (Lucon-Xiccato & Bisazza, 2016). The reason behind the difference in the direction of the sex difference in speed between the two studies is currently unclear. Perhaps, the aforementioned greater performance of females in problem solving is involved (Lucon-Xiccato et al., 2020) or differences in feeding motivation.

Regardless of the underlying mechanism, which remains unclear, the findings on sex differences have implications for the welfare of captive fish. Males and females may have different requirements in terms of cognitive enrichment. If this is true, housing conditions for fish should be shaped also according to the sex of the fish maintained. Many fish farms contain stocks of only one sex (e.g., Budd et al., 2015; Bye & Lincoln, 1986). Furthermore, research on fish welfare should always include subjects of both sexes when developing new enrichments. Our results align with previous reports in highlighting the presence of intraspecific variation in the response to cognitive enrichment (Smith et al., 2021). Intraspecific variability in enrichment needs might be common across species. Besides the sex of the individuals, other factors not addressed in the present work, such as age and personality, deserves attention in future studies.

In conclusion, our study shows that guppies show limited, but not absent, attraction in a puzzle feeder provided as cognitive enrichment, although with a potential sex difference. It is necessary to further evaluate the effects of cognitive enrichment on fish welfare and consider the inclusion of these enrichments in captive settings.

Declarations

Ethical Approval

The experiments of this study were conducted in accordance with the current legislation of our country (Italy, D.L. 4 Marzo 2014, n. 26). The Ethical Committee of the University of Ferrara reviewed and approved the experimental procedures (protocol no. TLX 1-2020-PR).

Competing interests

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: C.V., E.G., C.B., and T.L.-X.; Methodology: C.V., E.G., and T.L.-X.; Formal analysis and investigation: C.V. and E.G.; Writing - original draft preparation: C.V. and T.L.-X.; Writing - review and editing: C.V., E.G., C.B., and T.L.-X.; Funding acquisition: E.G., C.B., and T.L.-X.

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Availability of data and materials

The data of this study are available as Supplementary Data.

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585

586 **Figure captions**

587 Figure 1. Aerial view of the apparatus used in the experiment. Letters in the figure indicate
588 the following: a = rear sector with guillotine door; b = lateral sectors with social companions
589 and natural plants; c = puzzle feeder; d = feeder with free food.

590

591 Figure 2. (**a**) Overall percentage of puzzle feeder as a first choice (grey) and divided for
592 males (blue) and females (green). (**b**) a percentage of males (blue) and females (green) in
593 preference for the puzzle feeder across days. (**c**) first-choice latency in choosing the puzzle
594 feeder or the feeder with free food divided for males (blue) and females (green). (**d**) first-
595 choice latency (log-transformed data) per experimental days of males (blue) and females
596 (green) divided for the type of choice (puzzle feeder: solid line; feeder with the free food:
597 dotted line). In panels (a) and (c) data points represent each subject; in panels (b) and (d) data
598 points represent mean ± standard error. Dotted light grey-line represent chance performance
599 (50% correct responses).

600

601 Figure 3. (**a**) Qverall percentage of completed task divided for the first-choice (puzzle feeder:
602 light grey; feeder with free food: grey). (**b**) percentage of completed task across days divided
603 for males (blue) and females (green). (**c**) second-choice latency divided for males (blue) and
604 females (green). (**d**) second-choice latency (log-transformed data) across days divided for
605 males (blue) and females (green). In panels (a) and (c) data points represent each subject; in
606 panels (b) and (d) data points represent mean ± standard error. Dotted light grey-line
607 represent chance performance (50% correct responses).

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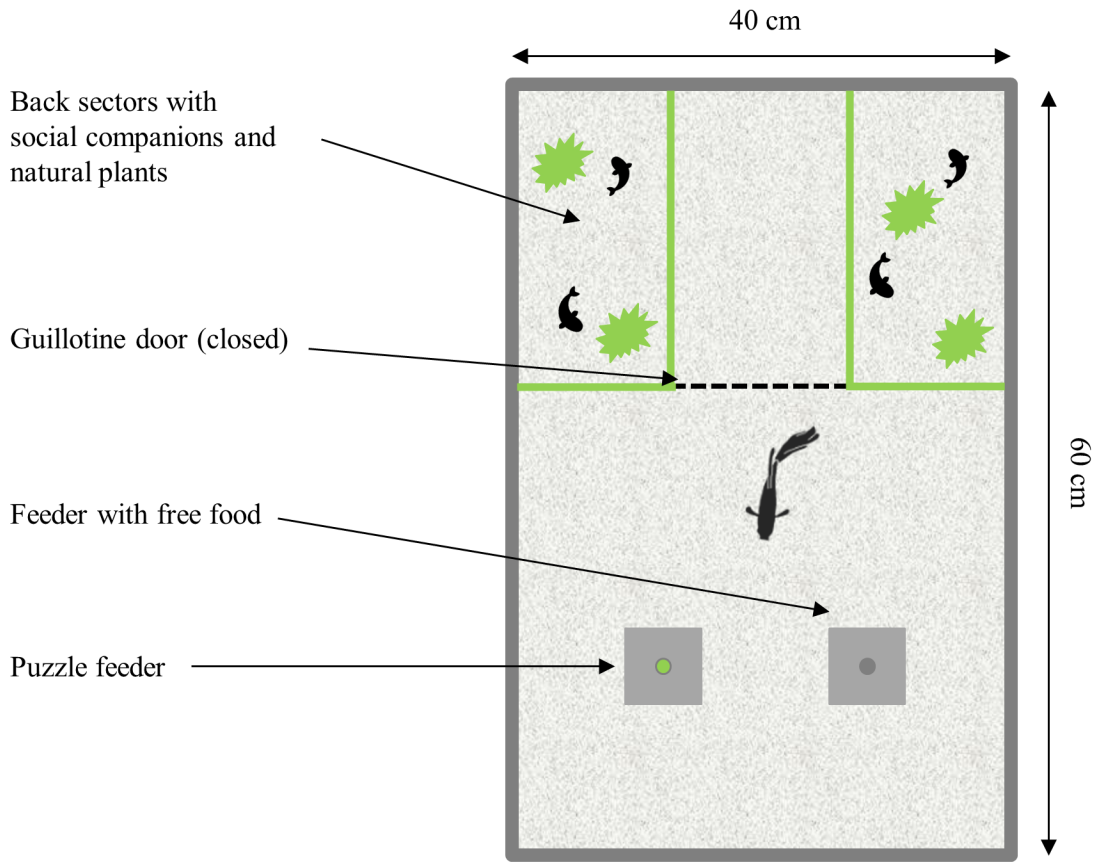

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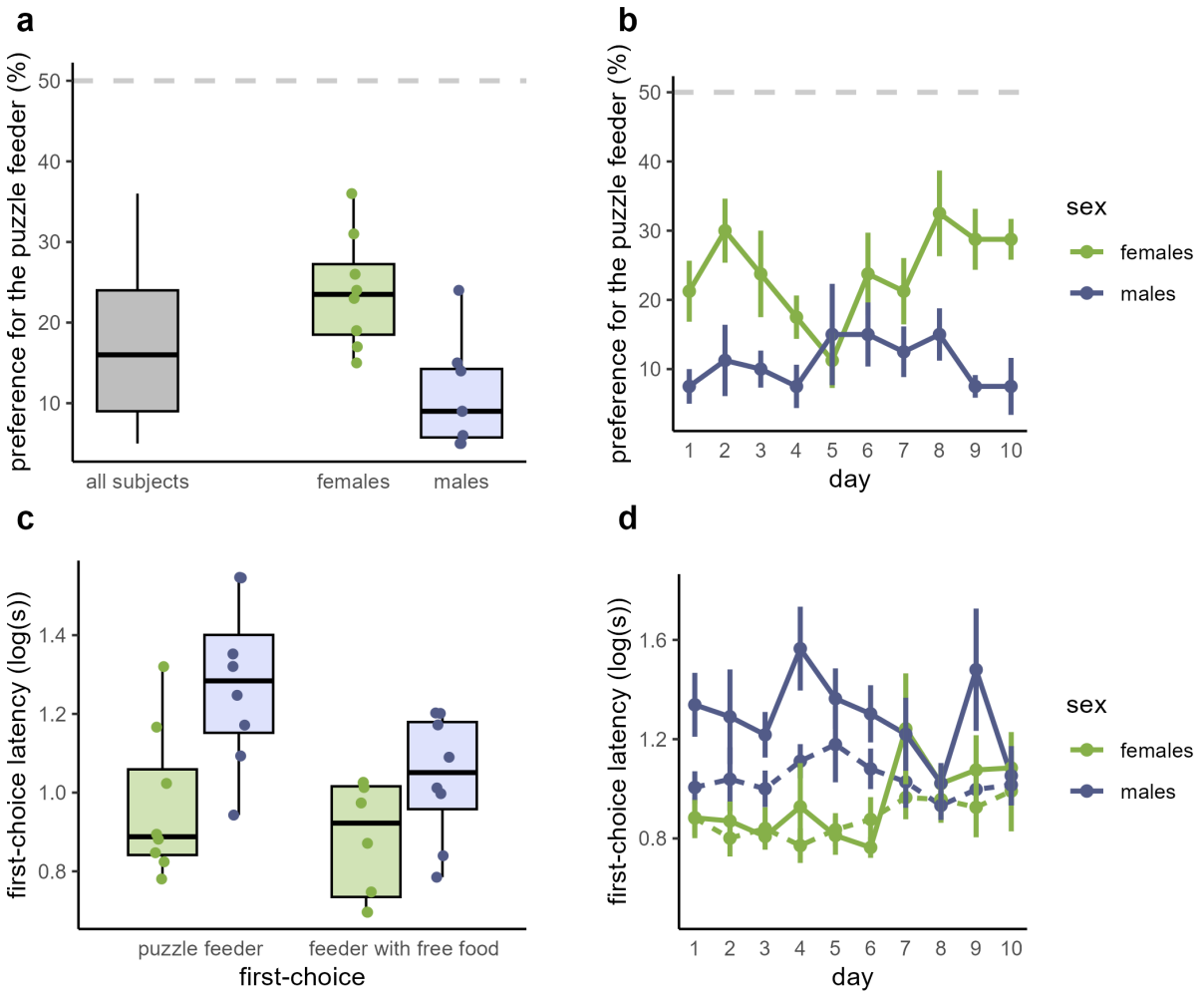
612 Figure 1



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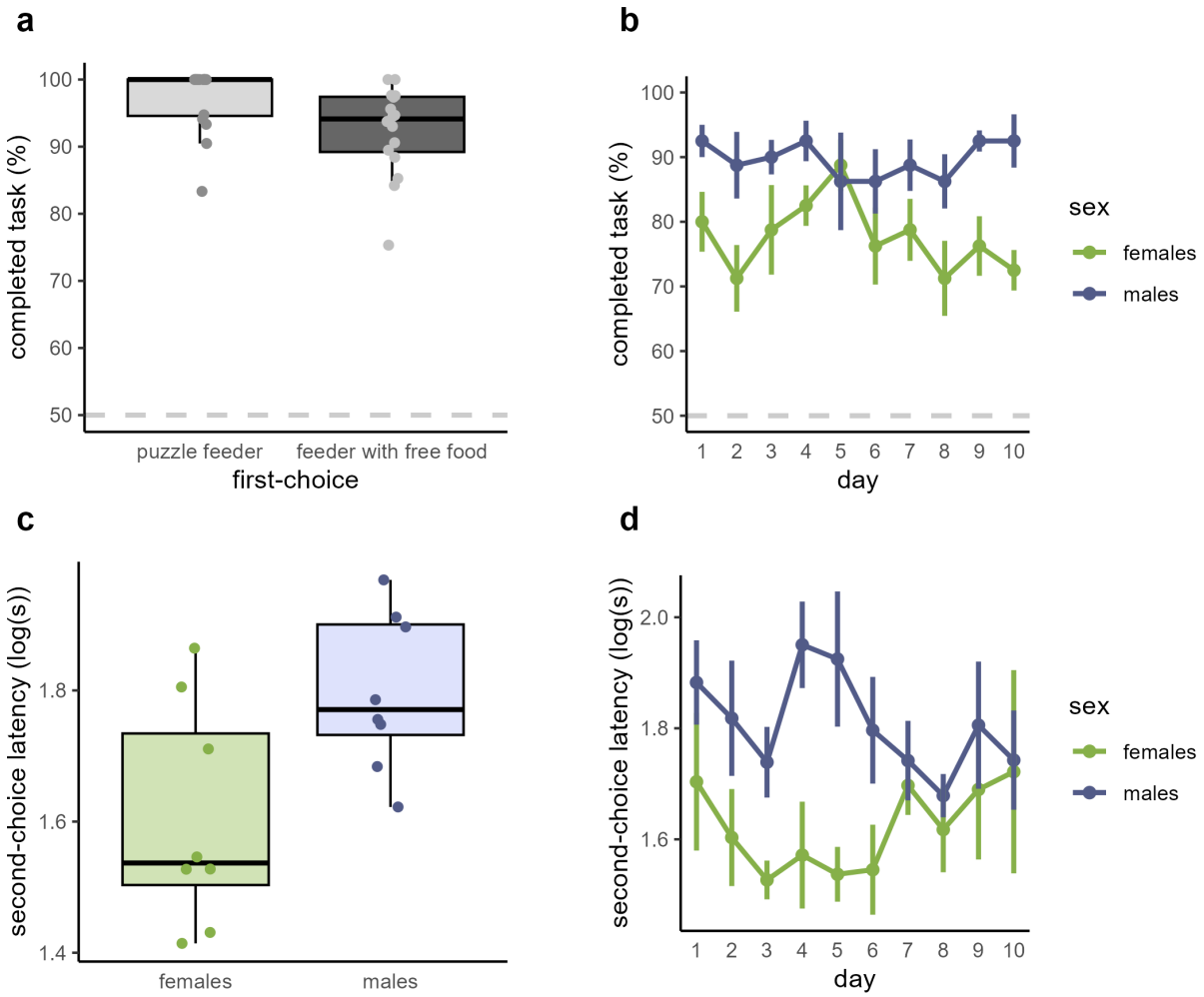
614 Figure 2



615

616 Figure 3