

High biodiversity may improve fish welfare

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ABSTRACT

In humans, it has been demonstrated a preference for environments with high biodiversity over those with low biodiversity. Moreover, exposure to environments with high biodiversity reduces stress and improves psychological well-being and health. It has been hypothesized that these effects might be the result of selection because individuals able to identify and settle in rich and productive environments with higher biodiversity likely obtain more resources. If this hypothesis is true, the effects of biodiversity on the welfare might be present also in non-human species. To test it, we investigated whether plant biodiversity levels affect welfare and preferences in a fish, the guppy *Poecilia reticulata*. In a first experiment, guppies' preference for environments with high biodiversity was assessed with a dichotomous choice test. No significant preference was detected, with fish spending equal time in areas with high (3 plant species) and low (1 plant species) biodiversity. In the second experiment of this study, the effect of biodiversity on welfare was examined by raising guppies in different environmental conditions: high biodiversity (3 plant species), low biodiversity (1 plant species), and control conditions (no plants). Behavioral indicators of welfare suggested a positive effect of both high and low biodiversity treatments, although a particular plant species used in the treatments had significant influence. Notably, physiological indicators suggested that the effect on welfare was enhanced in the biodiversity treatment, with fish from this group displaying lower basal cortisol levels. Overall, our results partially support the hypothesis that high biodiversity might positively influence the welfare of guppies, although this effect was primarily due to improved physiological conditions.

1. Introduction

In humans, it has been demonstrated that there is a clear preference for environments rich in biodiversity over those characterized by lower biodiversity (Hu et al., 2022; Hao et al., 2024; Lindemann-Matthies, 2017). For example, a study conducted in Baoji, a town in China (Gao et al., 2019) used an on-site questionnaire to analyze people's perceptions and preferences regarding biodiversity and the recreational value of urban green spaces. The results showed that the participants were able to accurately perceive the level of biodiversity and tended to prefer engaging in recreational activities in environments characterized by high biodiversity. This preference is likely driven by the consequences of exposure to high-biodiversity environments, which not only reduces stress but also enhances psychological and physical well-being in humans (Fuller et al., 2007; Carrus et al., 2015; Southon et al., 2018; Wood et al., 2018; Wolf et al., 2017; Young et al., 2020).

It has been hypothesized that such effects may stem from an evolutionary selective process: if biodiversity predicts the quality of an habitat, particularly in terms of resource availability and the opportunities it offers (Xu et al., 2020; Cardinale et al., 2006; Waide et al., 1999; Yang et al., 2017), individuals more efficient at identifying and settling in high-biodiversity habitats might be more likely to thrive (Håla and Flegel, 2021; Pekkonen et al., 2013). If this hypothesis proposed to explain the aforementioned human responses to biodiversity is true, its implications could extend beyond humans, potentially affecting other animals as well. In line with this reasoning, a growing body of research shows that non-human animals express robust preferences for certain environmental factors such as structural complexity, shelter availability, substrate characteristics, and that these factors significantly influence their welfare (Godýn et al., 2019; Ramankevich et al., 2022; Bashaw et al., 2016; Gatto et al., 2022), improving animals' physiology, behavior, and even cognitive abilities (Carbia, Brown, 2019;

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Montalbano et al., 2022; Kempermann, 2019). However, to the best of our knowledge, the specific role of biodiversity in influencing animal welfare remains underexplored.

To explore whether a non-human animal may respond to biodiversity in a way similar to that observed in humans, we performed two experiments with a teleost fish, the guppy (*Poecilia reticulata*). Fish have been increasingly used to investigate the evolutionary roots of behavior in vertebrates, both because of their interesting phylogenetic position at the base of the lineage and their potential for rapid and extensive phenotyping, which is particularly useful for exploring novel hypotheses. Most major behaviors and cognitive abilities observed in mammals have also been reported in fish, including in the guppy (e.g., Agrillo et al., 2012; Lucon-Xiccato et al., 2017; Burns, 2008). Although it is not always clear whether these traits are homologous, the similarity has made fish a valuable model group in this field (Miklósi and Andrew, 2006; Norton and Bally-Cuif, 2010). In several fish species, a number of studies have already described clear-cut spontaneous preferences for specific environmental elements, such as plants, gravel substrate, and artificial enrichments (Bender et al., 2025; DePasquale et al., 2019; Gatto et al., 2025; Krueger et al., 2020; Maia, Volpato, 2018; Schroeder et al., 2014).

In the first experiment, we investigated whether guppies displayed a preference for environments with different levels of plant biodiversity through a dichotomous choice test. The subjects had the option to spend time in an area containing three plant species or in an area with only a single plant species. In the second experiment, we assessed the long-term impact of plant biodiversity on the welfare of guppies by raising them for 30 days in one of three distinct conditions: high biodiversity (three plant species), low biodiversity (one plant species), or a control condition (no plants). After this period, we measured both behavioral and physiological indicators of welfare, including anxiety-like behavior and basal cortisol levels, to evaluate the effects of biodiversity.

2. Materials and methods

2.1. Subjects

In the two experiments of this study, we used 591 guppies overall ($N = 51$ in experiment 1 and $N = 540$ in experiment 2). These individuals were descendants of wild guppies of the lower Tacarigua River (Trinidad), reared in our laboratory since 2002 in a population of approximately 1000 individuals at the time of this study. They were maintained in mixed-sex groups within 400-L plastic tanks, under a controlled light/dark cycle of 12 h provided by 30 W fluorescent lamps

(Sylvania GRO-LUX, Milano, IT). The tanks were kept with water conductivity at $606.7 \pm 60.18 \mu\text{S}/\text{cm}$, nitrite levels below $0.1 \text{ mg}/\text{L}$, and nitrate concentrations below $50 \text{ mg}/\text{L}$. The guppies were fed twice daily with a diet of commercial tropical flakes (Sera Vipan Nature) and *Artemia salina* nauplii (Ocean Nutrition, Essen, BE). All subjects were randomly selected from the stock population the day of birth, ensuring that they were naive to any experimental manipulations and had not been previously exposed to the experimental conditions.

2.2. Experiment 1 - Preference for high versus low biodiversity

The apparatus and procedures for the preference experiment closely followed those described in Varracchio et al. (2025). The observation of each subject was conducted in a rectangular aquarium ($42 \times 16 \times 15 \text{ cm}$) filled with 10 cm of water (Fig. 1 A). Illumination was provided by a LED strip (natural light, 6500 K, TMR, distributed by ELCART, IT), and fish behavior was recorded using an IMX179 (ELP, Shenzhen Ailipu Technology Co., Ltd., CN; resolution: 3264×2448) video camera and a software ('MultiViewer') developed at the University of Murcia's Computer Systems Department. Thirty identical test apparatuses were used simultaneously, and the 51 subjects were tested in two consecutive blocks under identical conditions. Each subject was tested only once and experienced a single plant species in the low-biodiversity sector.

Real plants of the species *Egeria densa*, *Bacopa caroliniana*, and *Hygrophila polysperma* were used as stimuli. These species were selected for logistic reasons: they are commonly used in tropical freshwater aquaria, easy to maintain under laboratory conditions, and exhibit comparable structural characteristics, allowing us to manipulate plant species richness while keeping overall structural complexity as constant as possible across treatments. To ensure consistency in the stimuli, plants were trimmed to individual stems of 6 cm in length. Each stem was anchored to a small ceramic biofilter ring to prevent floating (Fig. 1 A). During each trial, three stems from the three different plant species were placed at one end of the tank, while three stems of one species were positioned at the opposite end. To control for potential side biases, the arrangement of plant species was alternated across trials.

The testing procedure involved randomly selecting a newborn guppy from the maintenance tanks and placing it at the center of the experimental apparatus. The subject then remained in the apparatus undisturbed for 5 days, until we performed the behavioral observation. This interval was chosen based on a previous study showing that it allows investigation of guppies' habitat preferences without the confounding effects of responses to a novel environment. During the interval, the subjects were fed twice daily. The behavioral observations were

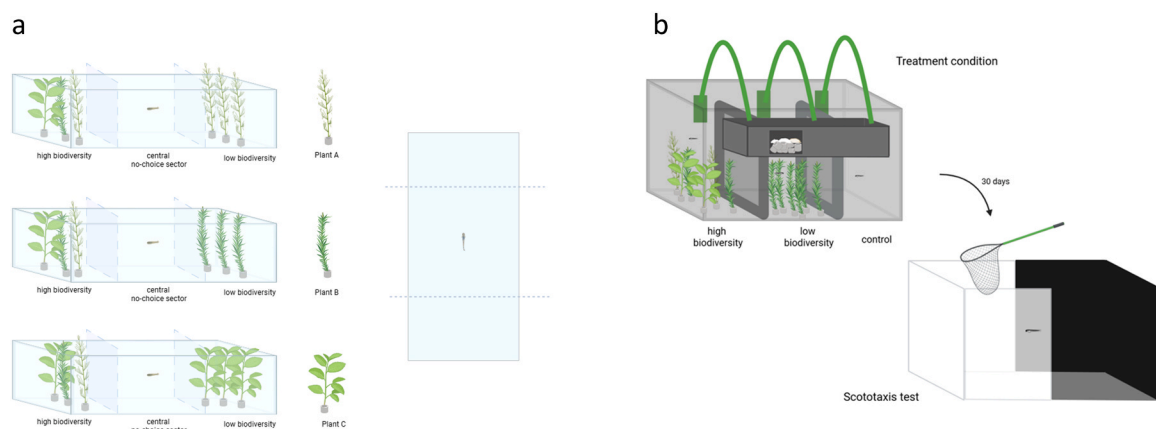


Fig. 1. Experimental apparatuses used in the two experiments. (A) Preference test (experiment 1): guppies were tested in a rectangular aquarium divided into three virtual sectors, with three plant stems of different species (high-biodiversity) at one end and three stems of a single species (low-biodiversity) at the opposite end. (B) Experimental treatments setup (experiment 2): large aquaria divided into three compartments corresponding to the three treatments (control, low-biodiversity, high-biodiversity). Water flow was allowed through perforated dividers to ensure homogeneous conditions. The scototaxis test apparatus used to assess anxiety-like behavior is also shown.

performed during a 20-minute session (starting at 10:00 AM) and subsequently analyzed using software developed by our research group ('Ciclic Timer') to track the time spent in three sectors, each corresponding to one third of the apparatus (Fig. 1 A): 1) the sector with high biodiversity, 2) the central no-choice sector, and 3) the sector with low biodiversity, as described by Varracchio et al. (2025). The preference for the high-biodiversity sector was calculated excluding the time spent in the central sector.

2.3. Experiment 2 - Effect of biodiversity on welfare

2.3.1. Experimental treatments

In this experiment, we employed two separate cohorts of 270 newborn guppies for logistic reasons. The first cohort was used to study behavioral indicators of welfare, and the second cohort for the physiological indicators. The individuals of each cohort were randomly split into three experimental groups ($N = 90$ per group), each assigned to one of three experimental conditions: high biodiversity (3 plant species), low biodiversity (1 plant species), and a control condition (no plants). These three experimental groups within a cohort were then further divided into three replicates each ($N = 30$ per replicate per group), because the treatment would be administered in three large, rectangular aquaria ($40 \times 60 \times 35$ cm), each divided into three distinct compartments (each compartment $40 \times 20 \times 35$ cm; 28 L of water). Within each aquarium, one compartment was assigned to each treatment condition (high biodiversity, low biodiversity, and control), resulting in three independent replicates per treatment. Each compartment housed 30 newborn guppies (overall $N = 90$ fish per aquarium, belonging to the three different experimental groups; fish density = approximately 1 fish per liter of water). Six aquaria were used in the study (three per cohort), with three independent replicates per treatment within each cohort. Each aquarium was a closed, non-recirculating water system; however, water was allowed to circulate between compartments to maintain consistent conditions across treatments. This was achieved using dividers made of two layers of perforated black plastic panels with a fine mesh in between, allowing water flow while preventing physical and visual contact between individuals (Fig. 1B). Each compartment was also equipped with a biological filter. The external walls of the aquaria were covered with black plastic panels to minimize external disturbance.

In the high biodiversity condition, the compartment contained nine stems of live plants, representing three different species chosen for logistic reasons: three stems of *Limnophila heterophylla* 'sessiliflora' (Plant A), three of *E. densa* (Plant B), and three of *B. caroliniana* (Plant C). The low biodiversity condition featured nine stems of a single plant species per compartment. Each low-biodiversity compartment contained one of the three plant species. For each compartment, and thus for each group of fish of the low-biodiversity treatment, the plant species remained the same throughout the entire treatment. The compartments assigned to the control condition had no plants, serving as a baseline to assess the effects of plant presence on guppies' welfare.

The treatment lasted for 30 days, after which we conducted behavioral measurement in the first cohort of fish and physiological measurements in the second cohort. The treatment setup was carefully maintained under consistent environmental conditions for the whole treatment. The water temperature was kept at a constant 28°C, and the illumination was provided by LED strips (natural light, 6500 K, TMR, distributed by EL CART, IT) and fluorescent lamps (Sylvania GRO-LUX, 15 W) positioned above the tanks. A 12-hour light/dark cycle was implemented to mimic natural conditions for both the plants and the fish.

2.3.2. Behavioral indicators

Behavioral testing was conducted using the scototaxis test (Maximino et al., 2010, 2010) at the end of the treatment. The test was performed in two identical rectangular tanks ($20 \times 28 \times 20$ cm), each

equally divided into a white and a black compartment (Fig. 1B), allowing two fish to be tested simultaneously. This test assesses anxiety-like behavior based on the natural tendency of fish to reduce their exploration of white areas under stressful conditions (Maximino et al., 2010, 2010). At the beginning of each trial, a single fish was gently netted from the treatment tanks and placed at the center of the apparatus, at the junction between the two compartments. Each trial lasted 20 min, during which behavior was recorded from above using a digital video camera (HDR-CX405, Sony Europe B.V., Weybridge, UK) mounted on a tripod. The apparatus was illuminated by warm-white, fluorescent lamps (320 lux). Testing was conducted in the morning between 8:00 AM and 1:00 PM and was completed over six consecutive days under identical environmental conditions. Fish from different treatment groups were tested each day to minimize potential day-related bias. Recorded videos were analyzed with custom software (Ciclic Timer) to quantify the total time spent in each compartment.

A white preference index was calculated for each fish as the ratio of time spent in the white compartment to the total time spent in both compartments. Higher values of this index indicate reduced anxiety-like behavior (i.e., greater willingness to explore the white area), while lower values reflect heightened anxiety-like behavior (Maximino et al., 2011; Gebauer et al., 2011).

A total of $N = 173$ fish were tested: 47 from the control group, 63 from the low biodiversity group and 63 from the high biodiversity group. No individuals were excluded from the testing. This final sample size reflects mortality during the treatment period, which, to the best of our knowledge, was not attributable to any specific fault or issue in the experimental setup. Between trials, the apparatus was cleaned and refilled with fresh system water to eliminate olfactory cues.

2.3.3. Physiological indicators

To ensure that behavioral testing did not influence the cortisol readings (Onarheim et al., 2022), a new group of guppies, naïve to behavioral procedures, was used. At the end of the 30-day treatment, 180 guppies ($N = 60$ per group, divided into three aquaria within specific treatment compartments) were assigned to the physiological measurements and were euthanized in accordance with national regulations and institutional ethical guidelines by an anesthetic overdose (MS-222; Merck, Germany). Under a dissection microscope, after removing brains for future analysis, the whole-body tissue samples were collected for cortisol quantification. For each treatment, we obtained 3 pools of 20 fish each, resulting in three biological replicates per treatment (9 pools overall). Pooling was necessary to obtain sufficient tissue for cortisol quantification. The pools of fish from the low-biodiversity (single plant species) experimental group were formed by mixing an equal number of individuals treated with each of the different plant species. The pools of fish body were homogenized in phosphate-buffered saline solution. After overnight storage at -20°C , tissue homogenates were thawed and refrozen twice, then centrifuged to collect the supernatant into a new centrifuge tube for biochemical analysis. Basal body cortisol levels were measured using a Fish Cortisol Competitive ELISA kit (CusaBio, USA), following the manufacturer's protocol. This method is based on the competitive inhibition enzyme immunoassay principle, involving the interaction between pre-coated cortisol and cortisol within the samples, and has a cortisol detection range of 3.12–200 ng/mL and is well-established for use in teleost fish (Zheng et al., 2024; Aly et al., 2025; Tang et al., 2021).

Briefly, all reagents and samples were brought to room temperature prior to use. In each well, 50 μL of sample or standard was mixed with 50 μL antibody and incubated at 37°C for 40 min. Each well was then washed three times with the wash buffer and 100 μL of HRP conjugate was added immediately. The plate was covered and incubated in the dark at 37°C for 30 min and then subjected to five washes. Color development was initiated by adding 90 μL of TMB substrate to each well and incubating the plate at 37°C for 20 min. The reaction was stopped by adding 50 μL of stop solution. The optical density of each

well was determined within 5 min using a microplate reader, the Multiscan FC Spectrophotometer (Thermoscientific, USA) at a wavelength of 450 nm. The cortisol concentration in the sample was calculated based on the standard curve, prepared by plotting the optical density at 450 nm of cortisol standards (0–200 ng/mL) derived from serially diluted cortisol stock (200 ng/mL).

2.4. Statistical analysis

All analyses were conducted in RStudio (version 2022.02.3, <http://www.rstudio.com>). The descriptive statistics in the Results section are presented as mean $\pm$ SE. The threshold for statistical significance was set at $p = 0.05$. In Experiment 1, we first compared the time spent in the three sectors (low biodiversity, central, and high biodiversity) with a repeated measures ANOVA. We then calculated a preference for the sector with high biodiversity as the proportion of time the subjects spent in that sector relative to the total time spent in both choice sectors (excluding the central no-choice sector). We compared this preference across the three plant species used in the low-biodiversity sector with one-way ANOVA. We also compared the preference against chance level (50%) using a one-sample t -test.

In Experiment 2, we evaluated the effect of the three different treatments on behavior using the time spent in the white compartment of the scototaxis tank as the dependent variable. A one-way ANOVA was conducted to test the effect of treatment (control, low biodiversity, high biodiversity). Thereafter, pairwise comparisons were conducted using Tukey's post-hoc test to explore differences between experimental groups. We also ran a one-way ANOVA to compare the behavior of the subjects from the low-biodiversity treatment that were exposed to different plant species (Plant A, Plant B, and Plant C). Survival was evaluated among experimental treatments using Chi-squared tests. Cortisol levels were compared among treatment groups using one-way ANOVA, followed by Tukey's post-hoc pairwise comparisons.

3. Results

3.1. Experiment 1

There was no significant difference between the time spent in the three sectors of the preference apparatus (repeated measures ANOVA: $F_{2,100} = 2.648$, $p = 0.076$). The plant species used as stimulus in the low-biodiversity sector did not significantly affect the preference for the sector with high biodiversity (one-way ANOVA: $F_{2,48} = 0.227$, $p = 0.798$). The preference for the sector of the apparatus with high biodiversity was not significantly different from chance level ($50.10 \pm 3.44\%$; one sample t -test vs. 50% preference: $t_{50} = 0.025$, $p = 0.980$; Fig. 2).

3.2. Experiment 2 – Behavioral indicators

Guppies spent an average of 324.71 ± 11.33 s in the white compartment of the scototaxis tank. A one-way ANOVA revealed a significant effect of treatment on the proportion of time spent in the white area ($F_{2,161} = 415.00$, $p < 0.001$; Fig. 3a). A Tukey's post-hoc test indicated significant differences between both plant-enriched groups and the control ($p < 0.001$ for both comparisons; Fig. 3a). Moreover, guppies exposed to the low biodiversity treatment spent significantly higher proportion of time in the white sector than those from the high biodiversity group ($p < 0.001$; Fig. 3a). These results indicate that the presence of plants reduced anxiety-like behavior in guppies, but that the effect was present also in the single plant treatment.

Further analysis showed that guppies of the low biodiversity treatment exposed to either Plant A, Plant B, or Plant C showed different behavior (one-way ANOVA: $F_{2,56} = 11.68$, $p < 0.001$; Fig. 3b). Post-hoc analysis indicated that Plant C determined a decrease in time spent in the white sector as compared to Plant A ($p < 0.001$) and Plant B

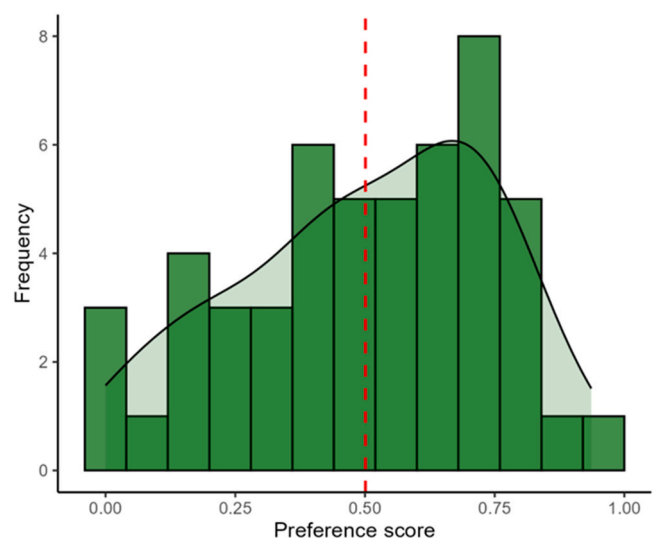


Fig. 2. Guppy preference for high-biodiversity sectors (experiment 1). Frequency distribution and probability density curve of time spent in the sector with high plant biodiversity. The dashed line indicates the mean.

($p = 0.0128$), while no difference was observed between Plant A vs. Plant B ($p = 0.155$). The high-biodiversity group, consisting of an assemblage of three plant species, differed significantly from Plant A ($p < 0.001$) and Plant B ($p < 0.001$), but not from Plant C ($p = 0.999$; Fig. 3b) treatments in the low-biodiversity group. This suggested that the treatment with plant C (either alone or in combination with other plants) determined a decrease in the time spent in the white sector.

In addition, survival rates differed significantly among treatments (Chi-squared test: $\chi^2_2 = 8.238$, $p = 0.016$; Fig. 3c), with low- and high-biodiversity treatments showing higher survival compared to the control. Within the low-biodiversity condition (single plant), survival was lower for Plant A (50.00% survived subjects) compared to Plant B (73.33% survived subjects) and Plant C (86.67% survived subjects; Chi-squared test: $\chi^2_2 = 9.841$, $p = 0.007$).

3.3. Experiment 2 – Physiological indicators

Cortisol concentrations differed significantly among treatment groups (low biodiversity: 12.07 ± 1.03 ng/mL; high biodiversity: 9.58 ± 1.05 ng/mL; control: 16.58 ± 1.84 ng/mL; one-way ANOVA: $F_{2,6} = 6.81$, $p = 0.029$; Fig. 4). Post-hoc Tukey tests showed that the cortisol levels in the control group were significantly higher than in the three species treatment ($p = 0.025$). No significant differences were found between the three species and one species treatment ($p = 0.447$), nor between the control and one species treatment ($p = 0.125$). These results suggest that exposure to higher biodiversity (three species condition) reduced physiological stress in guppies compared to the control condition.

4. Discussion

Humans display an attraction to environments with high levels of biodiversity (Hu et al., 2022; Hao et al., 2024; Lindemann-Matthies, 2017), and, in such environments, they typically show increased well-being (Fuller et al., 2007; Carrus et al., 2015; Southon et al., 2018). The present study investigated whether a similar response to plant biodiversity could be observed in a fish species. Our findings provide limited, but significant, support for the idea that at least part of the effects observed in humans may also occur in other species. Specifically, we found that guppies kept in environments containing plants of different species had lower cortisol levels compared to guppies kept in barren environments. In contrast, the same reduction in cortisol was not

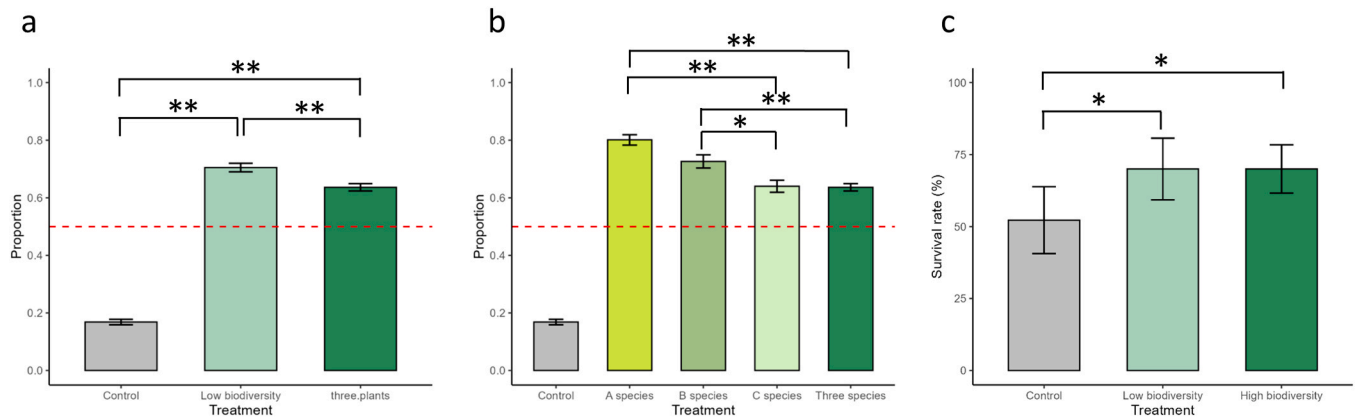


Fig. 3. Effect of biodiversity treatments on anxiety-like behavior in guppies (experiment 2). Bar plots show mean ($\pm$ SE) proportion of time spent in the white compartment of the scototaxis test. Asterisks indicate statistical significance: * = $p < 0.02$, ** = $p < 0.001$. (A) Cumulative data per treatment (control, low-biodiversity, high-biodiversity). (B) Low-biodiversity group divided by single plant species (Plant A: *L. heterophylla*, Plant B: *E. densa*, Plant C: *B. caroliniana*). The dashed line represents the reference value of 50%, a threshold for interpreting the preference variable. (C) Survival rate (%) after treatments.

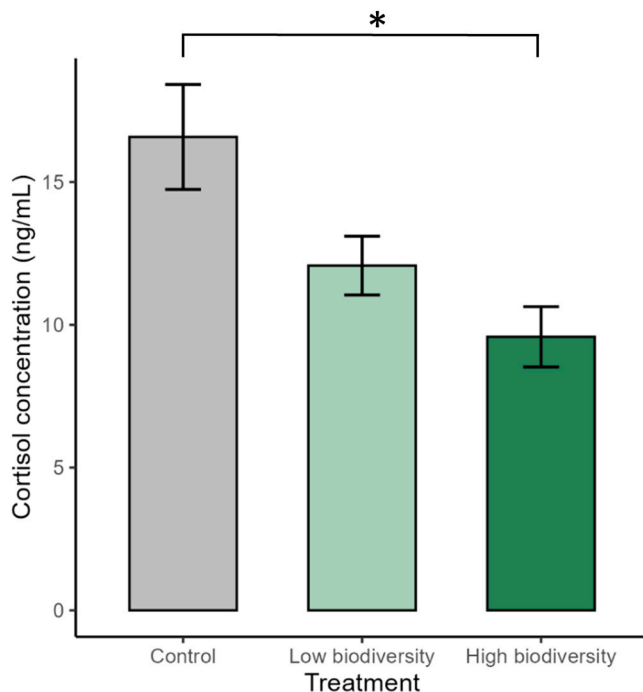


Fig. 4. Cortisol concentrations (ng/mL) of guppies after 30 days of treatment (experiment 2). Bars represent mean ($\pm$ SE) values per treatment. Asterisk indicates statistical significance: * = $p < 0.02$.

observed when plants belonged to a single species. This suggests that stress levels in guppies may be reduced by exposure to environments with higher plant biodiversity.

Interestingly, we did not detect positive effects of the high biodiversity treatment using behavioral indicators. In this case, guppies appeared to benefit simply from the presence of plants, regardless of the number of plant species. This is consistent with several studies showing that environmental enrichment, including natural elements, can improve welfare in fish and other animals (Ellis, 2009; Bayne, 2018; Makecha and Highfill, 2018; Gatto et al., 2022; Ramankevich et al., 2022; Bashaw et al., 2016). The presence of plants, regardless of species richness, also increased fish survival. This result should be interpreted with caution, as the mortality observed in our treatments was relatively high, albeit comparable to that reported in natural conditions (e.g., Reznick et al., 1996) and in some laboratory studies (e.g., survival

40–80%; Anka et al., 2016; Thasreefa et al., 2025). Nonetheless, it may provide additional evidence that environmental enrichment enhances animal welfare, in line with previous studies (Coulibaly et al., 2007; Fuss and Johnson, 1988; Gwak, 2003). Moreover, in the first experiment of this study, in which guppies were observed in an experimental setting where they could choose between a zone with a single plant species (simulating low biodiversity) and a zone with three plant species (simulating high biodiversity), the subjects did not exhibit a behavioral preference for the sector with higher biodiversity. This suggests that, although guppies may respond physiologically to biodiversity, they may not necessarily display a behavioral preference for it, or at least, not under the laboratory conditions we used, where there was no apparent cost or risk associated with choosing one environment over another.

The reasons for the difference between the physiological and the behavioral effects are currently unclear. They may reflect a lower sensitivity of behavioral indicators compared to physiological measures (Cavallino et al., 2023; Serra, Wolkers and Urbinati, 2018), although this interpretation seems unlikely given that the behavioral test detected differences even within the low biodiversity treatment for fish exposed to different species of plant. We can also speculate that for many guppies' activities the presence of plants itself, for instance as a shelter, is sufficient, while only for other more intrinsic aspects the presence of high biodiversity is relevant. Another aspect to consider is that although the fish from the present study originated from a wild population, they may have undergone some domestication process over several generations in captivity, potentially influencing their behavior towards the plants. Lastly, it is important to consider that we have analyzed only a single, and relatively small, aspect that contributes to environmental biodiversity, namely the presence of plants. Examining other factors, such as the diversity of prey or other organisms, as well as combinations of factors (DePasquale et al., 2019), could provide different results compared to the present study and even a behavioral preference for high-biodiversity environments.

Overall, our results suggest that the effects of biodiversity on well-being may not be exclusive to humans. Other species may also experience some of the same benefits when in environments with high biodiversity. This challenges the idea that the human response is purely cultural, for example, due to positive societal attitudes toward natural environments, or a by-product of humans having evolved in natural habitats while individuals now mostly live in artificial environments, thus experiencing a mismatch between their evolved and current habitats. Instead, our data indicate that the effects of biodiversity can be present in other species and appear from birth, suggesting they are innate. This raises the possibility that natural selection has shaped animals to be more sensitive to highly biodiverse environments. Note that

the presence of a selective effect does not exclude that experience also plays a role in animals' preference for high-biodiversity environments. Besides an innate preference, there could also be modulations that are due to experience with a certain environment. Thus, also for humans, we cannot exclude that the observed effects are actually a mixture of these two factors, innate preference and experientially driven choices.

In addition to replicating our findings in guppies, it is important to examine whether similar responses occur in other species. Moreover, our study did not address the ultimate mechanisms underlying the observed effects. Specifically, we do not know why a guppy would benefit from an environment with a greater variety of plant species. A leading hypothesis is that environments with higher biodiversity tend to provide more resources (Warfe, Barmuta, 2004; Cardinale et al., 2006; Messmer et al., 2011), but this remains to be experimentally verified. Another important question is whether different types of biodiversity elicit different effects, which seems entirely possible given that even different plant species determined within a certain degree, different effects. For example, would guppies respond positively to higher animal biodiversity? High diversity of small animals, such as invertebrates, could be beneficial as a food source, but other fish species could signal increased competition for resources. In some cases, other species could also be predators, further complicating the expected response. To this, it should be added that studies in other fish species have also highlighted specific preferences for non-biological elements that contribute to the complexity of the habitat, such as specific types of substrates (Maia and Volpato, 2018). Therefore, these elements could also affect the preference of fish, and performing more comprehensive studies examining multiple aspects of diversity is paramount to fully understand the environmental preference of the fish.

From an applied perspective, our results have implications for both conservation biology and aquaculture. A well-documented consequence of anthropogenic environmental change is the reduction of biodiversity at multiple levels within ecological communities (Keck et al., 2025; Klinke and Brown, 2026; Prakash and Verma, 2022). Our study suggests that such reductions may negatively affect fish welfare in those habitats, strengthening the argument for biodiversity conservation. Furthermore, millions of animals are kept in captivity for human purposes (Mason, 2010), with fish representing the largest proportion of individuals (Braithwaite & Huntingford, 2004). Concerns about the welfare of captive animals are growing due to both societal and regulatory pressures (Koknaroglu and Akunal, 2013). A common strategy for improving welfare in captivity is environmental enrichment, such as providing natural plants whenever possible (Bender et al., 2025; Näslund and Johnsson, 2016). Our findings suggest that enrichments may be more effective when they are more diverse, at least in the case of plant-based enrichment. It is also possible that this principle could apply to artificial enrichment materials, though this remains to be tested. Furthermore, it is worth noting that our methodology for experiment 2 aimed to focus on the effects mediated by the perception of high plant biodiversity and therefore, attempted to homogenize the water conditions across treatments. If higher biodiversity of plants also produces different physico-chemical conditions in the water, this could further enhance fish welfare (Tsang and Gerlai, 2022). While not directly relevant to the question of the present study, this is still a factor worth exploring within the broader scope of developing better enrichment to improve fish welfare.

Ethical approval

The experiments of this study were conducted following 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 2022_1).

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CRediT authorship contribution statement

Chiara Varracchio: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Tyrone Lucon-Xiccato:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Giorgio Bertorelle:** Writing – review & editing, Funding acquisition, Conceptualization. **Cristiano Bertolucci:** Writing – review & editing, Conceptualization. **Elena Frigato:** Writing – review & editing, Methodology, Conceptualization. **Francesco Pio Paci:** Writing – review & editing, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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