

Females of the sailfin tetra prefer red illuminated environments

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Contributions

EAB was involved in conceptualization of the experiment, experimental work, formal analysis, writing (review and editing) and funding acquisition. THSP participated in conceptualization of the experiment and writing (review and editing). JZ participated in conceptualization of the experiment and writing (review and editing). HS participated in conceptualization of the experiment and writing (review and editing). SK participated in conceptualization of the experiment, writing (review and editing), funding acquisition, and project management.

Abstract

The preference for colours has profound impacts on the fitness of visually oriented species, influencing the search for food resources and mates of better quality while also modulating various physiological and behavioural features. Such processes may be more important to sexually dimorphic species, especially coloured ones since visual signals play an important role in intra- and intersexual communication. Amazon small streams vary widely regarding the amount of dissolved organic carbon resulting in black (red-biased) and clear (mostly transparent) water types. The sailfin tetra *Crenuchus spilurus* is a small sexually dimorphic species composed of two main genetic lineages that live in black and clear water types. Recently, we have shown that the female sexual preference for male ornaments colouration differs between individuals inhabiting black and clear water types. Females from black waters do not choose males primarily based on colouration under strongly red-biased lighting, but females from clear waters do, which suggests that the importance of red colours for sexual behaviour differs between lineages of the sailfin tetra. Here, we evaluated the sailfin tetra female environmental colour preference through the choice between a red and a full spectrum illuminated environments. Females from black waters and clear waters preferred red-lightened environments. Such results suggest that the females are attracted to the red colouration outside the context of mating, irrespective of the prevailing lighting condition of their native environment. This preference for red illuminated environments may be important for several processes, including physiological and behavioural features.

Key-words: environmental colouration choice; water colour bias; visually oriented fish; Amazon water types; habitat choice; Amazon fish

Introduction

Colours and their preferences have profound impacts on individuals' lives and fitness of visually-oriented species. Environmental lighting conditions can modulate physiological and behavioural responses, such as feeding (Duray et al. 1996), growth (Downing and Litvak 2000), reproduction (Volpato et al. 2004) and aggression (Höglund et al. 2002). Aquatic environments highly vary in their underwater light,

affecting individual colour sensitivity and possibly driving habitat/environment colour preferences.

Colour preference is often attributed to a pre-existing bias within the visual sensory system (Luchiari et al. 2007; Spence and Smith 2008), a complex network involving intricate processes of visual perception and interpretation. The visual sensory system relies on various components, including opsin genes that encode proteins crucial for photoreceptor function. The expression levels of these opsin genes and peak spectral sensitivity (λ_{max}) contribute to the individual's perception of colours. Individuals may prefer coloured objects that match the prevalent environmental lighting conditions, appearing brighter under such specific environments (Endler 1993). Conversely, the colour contrast between objects and their surroundings, rather than their expected brightness, might play a more pivotal role in shaping colour preference (Johnson et al. 2013). Consequently, multiple ecological factors and evolutionary mechanisms likely influence preferences for lighting environments and colours. Such preferences are usually closely tied to advantageous outcomes in terms of visual detection of food resources and potential mates (Rodd et al. 2002; Spence and Smith 2008).

The preference for a particular colour and a specific environmental lighting condition can be more important to sexually dimorphic and coloured species since colours play an important role in intra- and inter-sexual communication (Endler and Houde 1995; Boughman 2001). Amazon waters can be classified into three different types depending on their apparent colouration as seen from land: black, blue (= clear, *sensu* Sioli 1984), and white (Wallace 1853). These water types highly vary in their physical and chemical characteristics (Sioli 1984; reviewed in Borghezan et al. 2021). Such water classification was conceived based on observations of the main river channels in the Amazon. However, small forest streams located in non-flooded regions (locally termed *igarapés*) can only be classified into two of these categories: black and clear waters. Black waters are rich in dissolved organic carbon (DOC), which absorbs light, particularly blue colours (short wavelength spectrum). Also, DOC scatters the light, reducing its intensity under black waters. Therefore, due to the abundance of DOC, black waters are yellow/red biased environments, while clear waters are mostly transparent (Mendonça et al. 2005; Costa et al. 2013).

91 The sailfin tetra *Crenuchus spilurus* Günther 1863 (Characiformes:
92 Crenuchidae) is a small-sized and sexually dimorphic Amazon fish species. In this
93 species, males possess dorsal and anal fins conspicuously ornamented in red and yellow
94 colours, and such colourful ornaments are used during an elaborated courtship
95 behaviour (Pires et al. 2016). A previous study has shown that the sailfin tetra is
96 composed of two phylogenetically distinct main lineages that inhabit black and clear
97 water types and are reproductively isolated (Pires et al. 2018). Female sexual preference
98 for the male colourful traits differs according to the underwater lighting environment
99 individuals live in. Females that live in black water conditions do not rely primarily on
100 the colouration of the males' ornaments, but instead, prefer males with larger ornaments
101 as mating partners. On the other hand, females living in clear water environments prefer
102 those males with redder ornament colouration as well as those males with larger
103 ornaments (Borghezan et al. 2023). Such divergence in female mating signal preference
104 is suggested to be driven by the perception of the males' ornament colouration. Black
105 water (which is red biased) increases the perceived intensity of the red colouration on
106 males' ornaments (shifting the ornament colour to a more saturated red colour), known
107 as spectrally masking effect; however, it also decreases the perceived variation among
108 males' ornaments colouration (Borghezan et al. 2023). Therefore, selecting males based
109 primarily on a trait with intense signals and low perceptual variation may be costly to
110 females living in black waters, since they could choose low-quality males that would
111 appear like high-quality ones due to the environmental condition (Hunt et al. 2004).

112 The sailfin tetra feeds on several red-coloured food resources, such as fruits of
113 the buriti palm tree *Mauritia flexuosa* and Chironomidae larvae (Pires et al. 2016),
114 suggesting that the red colours are still important for other processes. Also, the sailfin
115 tetra possesses several copies of the long wavelength sensitivity (LWS) gene: three
116 copies of LWS1, which are sensitive to red colours, and two copies of the LWS2 gene,
117 which are green sensitive; along with a copy of SWS2, which is blue sensitive
118 (Borghezan et al. 2024). The LWS genes encompass most of the sailfin tetra colour
119 vision (Escobar-Camacho et al. 2020; Borghezan et al. 2024). However, the sailfin tetra
120 spectral sensitivity is suggested to be dynamic, and visual adaptations for black and
121 clear waters may arise from the differential gene expression level, rather than the
122 differences in the LWS λ_{max} among lineages (Borghezan et al. 2024). Therefore, due to

the high relevance of the red colours for the sailfin tetra, we can expect both lineages to show a preference for red colours outside the mating context; however, this preference could be more intense in those individuals living in clear waters, since they are also attracted by the red colours in male ornaments (i.e. inside the mating context). Here, we investigated the female preference for red illuminated environment through environmental colour choice. This experiment was used to test female colour preference since environmental colours can affect fish physiology, behaviour and the details individuals can discriminate in the environment, thereby providing them with various advantages, including feeding and mating opportunities (Yokoyama 2000; Luchiari et al. 2007).

Material and Methods

Ethical statement

Fish collections in Brazil were carried out under IBAMA permanent licence SISBIO #10199-1 to JZ. Experiments were conducted following Brazilian federal law 11.794/2008. This study was approved by the Ethical Committee for Animal Use in Experiments of Kyoto University (WRC-2019-009A).

Fish sampling and housing

We collected the sailfin tetra individuals from populations that represent the two main lineages (Rio Negro - Black water, and Amazonas lineages - Clear water) and CJ1 population. CJ1 population phylogenetically belongs to the Clear water lineage (Pires et al. 2018). Also, the colour pattern (size and number of yellow egg spots in the anal fin) and intensity of the red coloured part on the dorsal fin in CJ1 population males ornaments are similar to those individuals from other populations from clear water lineage (Pires et al. 2019; Borghezan et al. 2023). However, CJ1 population individuals live nowadays under black water condition. CJ1 female mating preference for male red ornamentation is similar to those from black water lineage females (Borghezan et al. 2023). Therefore, this population provides a good opportunity to evaluate the convergent evolution of female red colour preference to black waters. Black water lineage individuals were sampled in an *igarapé* inside an urban forest fragment in Manaus (3°06'22.8"S; 59°58'40.5"W), Amazonas state, Brazil. Clear water lineage

individuals were sampled in a small stream near Iquitos (4°0'46.20"S; 73°27'47.70"W), Peru, and CJ1 population individuals were collected in a small stream in the outskirts of the municipality of Coari (4°07'09.9"S; 63°04'29.9"W), Amazonas state, Brazil. At the laboratory, fishes were housed in 92 L aquariums separated by lineage and sex for at least 30 days prior to the experiment. Their housing tanks were kept close to the windows that covered over half of the wall of the laboratory providing indirect natural light and photoperiod (12:12 h light:dark cycle). We used dark cloth on the windows to control the amount of light that reached the laboratory, effectively simulating the natural canopy-shaded condition of an Amazon forest stream. An air conditioning system maintained the laboratory at a constant temperature of 24°C, simulating the natural temperature of Amazon *igarapés*. Natural and artificial plants were added to the tanks and over 10 pieces of PVC pipes (10 cm long, 25 mm in diameter) to provide shelter. Aquariums were filled using artesian well water from the Negro River basin at Manaus, and had similar limnological parameters to those found in the black water basin (pH at 5.5 and conductivity at 20 $\mu\text{S cm}^{-1}$) except for high DOC concentration. Therefore, the underwater lighting condition of the tanks was similar to those found in clear water *igarapés* (mostly under full-spectrum light). The same water source was used during the experiments. The absorbance of the water used in the housing and experimental tanks can be found in Supplementary Material 1. Because we used the same water source throughout our experiments, we evaluated only the effect of the underwater lighting condition on the female environmental colour preference.

The majority of sailfin tetra individuals are between 3 and 4.5 cm long (standard length). This species does not actively swim around the tanks, and its territory is only defended at a very close range near PVC pipes; thus, aggressive interactions were rarely observed. Water changes (25%) were weekly performed. Fishes were fed commercial pelleted food for aquarium fishes *ad libitum* once a day. All individuals used in the experiments were adults since the smallest individual measured 34.04 mm of standard length (minimum = 34.04 mm, maximum = 46.77 mm, average = 40.84 mm, $\pm$ 3.20 mm SD), 8.34 mm larger than the estimated size at first maturity for this species (25.7 mm, Pires et al. 2016).

Experimental setup

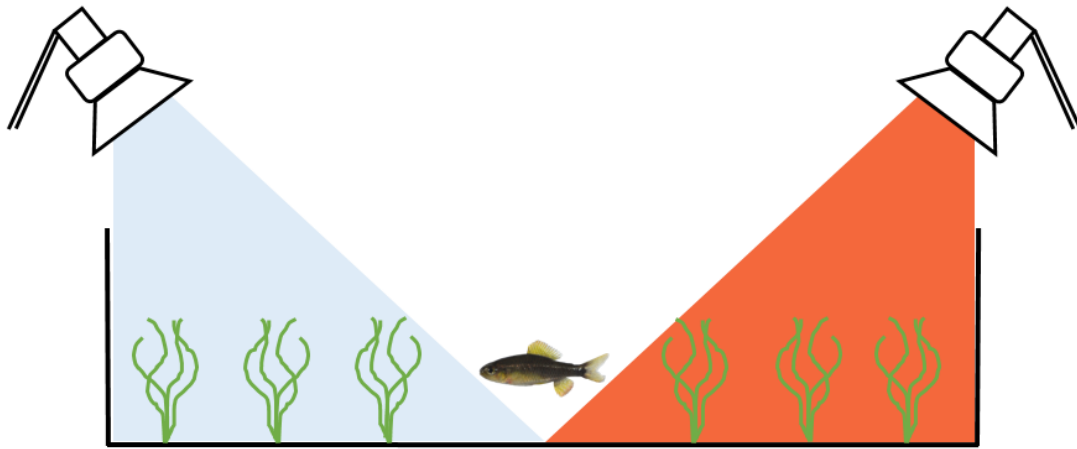
We used an aquarium (60x15x15 cm) to evaluate the environmental colour preference. The aquarium was half illuminated by a red light and half by a full spectrum light source (Figure 1). Such colours simulate the general tendency of clear and black water underwater lighting conditions in Amazon *igarapés*. On each side of the experimental aquarium were introduced six artificial plants (similar to those found in their natural environments - *Cabomba* sp.) that could potentially be used as shelters. We recorded the experimental tank with a camera positioned above the experimental tank. The experimental tank, light sources, and recording equipment were sheltered within a dark chamber to prevent any interference from external light during the trials. The experimental tank was rinsed and the water was completely renewed after each trial to avoid chemical cues from influencing subsequent trials. We used the same full spectrum light source (GrassyLeDio RX072 - GLRX072/AQ) for both, full spectrum and red light, treatments; however, for the red light treatment, the full spectrum light was filtered out by a red colour gelatin filter (Supplementary Material 2). We equalized the intensity of light sources to 250 lux for both lighting treatments using two dimmer switches, one for each light source. Importantly, the 250 lux of light intensity was controlled after the light had passed through the red light colour filter. Each light source was arranged within a lamp reflector designed to optimize the confinement of the light source to each lateral region of the experimental tank. The setup was implemented in a completely dark room to achieve optimal conditions. While there might be a slight elevation in light intensity at the peripheries of the experimental tank due to the placement of light sources at these locations, efforts were made to measure a consistent light intensity of 250 lux at a standardized distance of 5 cm from the edge of the experimental tank, aiming to maintain uniform illumination on both sides. The position of the light source, red biased and full spectrum light, was randomized among the trials to avoid side bias. Such procedures in our experimental setup were established to avoid any possible side bias in the study and this sense adheres to the STRANGE framework (Webster and Rutz 2020).

Studies evaluating colour preference can also control for the intensity of the light that is sensed by the focal individual, instead of controlling the light intensity that is used for the experiment. This can be achieved by multiplying the irradiance (intensity measured in spectral interval per surface area) of each light colour source by the known

spectral sensitivity of the individuals (Li et al. 2018). However, such methodology is hard to apply to the sailfin tetra. The sailfin tetra opsins among individuals living in clear and black waters are nearly identical and they are not expected to differ in their λ max. However, differences in visual sensitivities of the sailfin tetra exposed to clear and black waters are expected to arise from the differential gene expression level (Borghezan et al. 2024). Therefore, since the visual spectral sensitivity of the sailfin tetra is dynamic and changes according to the environmental underwater lighting condition (the underwater lighting condition of black water Amazon *igarapés* can change based on the daily rainfall), we could not estimate the light intensity necessary for the individuals to sense an equal light intensity in our treatments, as it has been done in other studies (e.g. Li et al. 2018). Consequently, it is possible that light intensity in our experiments could have been perceived differently by the individuals who were tested. However, the full-spectrum and red lighting treatments in our experiment are similar to the natural conditions of clear and black water types, respectively, in which the sailfin tetra lives; therefore, our experimental set up represents a good approach to evaluate environmental colour preference among lineages of the sailfin tetra.

Experimental procedure

The experiment was conducted as follows: a fish was gently caught in the housing tank, introduced into a small container (around 1L) and then transferred to the experimental aquarium. This was done to reduce possible stress to the testing individual due to the handling and transferring of testing individuals between tanks. The fish was kindly introduced in the middle of the experimental aquarium and we recorded its movement for 46 minutes. The first 6 minutes of each record were considered acclimation time and were removed from the analysis. We measured the time spent in each environmental colour (in seconds) for 40 minutes.



248

249 Figure 1. Schematic representation of the experimental aquarium in lateral view. The
 250 blue light on the left side represents the full spectrum light/clear water environment,
 251 while the orange light on the right side represents the red light/black water environment.
 252 The green lines represent artificial plants.

253

254 Each fish was tested only once. A total of 51 trials were performed. Individuals
 255 that have not contacted at least the edge of both illuminated environments (excluding
 256 the moment of fish insertion in the experimental tank) within 10 minutes of the
 257 experimental time were excluded from the analysis. This was done to prevent possible
 258 noises in the results due to the individuals that potentially failed to express their
 259 environment colour preference (cases of stress, possibly due to handling). Ten
 260 individuals failed to contact the edge of the two lightened environments within 10
 261 minutes and were excluded from the analysis. Therefore, 41 trials were used to fit the
 262 statistical model. We assessed the environment choice of 15 females from black water
 263 lineage, 9 from CJ1 population and 17 from clear water lineage. Individuals from black
 264 water lineage and CJ1 population live under black water type, while those from clear
 265 water lineage live under clear water type.

266

267 *Statistical analysis*

268 We first calculated the proportion of the time individuals spent in the red
 269 illuminated environment (i.e. total time spent in the red environment divided by the total

experimental time, in seconds). Values higher than 0.5 indicate a preference for the red colour, conversely, values lower than 0.5 indicate a preference for the full spectrum environment. We then fitted a one-sample t-test comparing the mean proportion against the null hypothesis value of 0.5 for each lineage. However, I fitted a Wilcoxon signed rank test, which is the equivalent non-parametric test, for the clear water lineage, in which data failed to show a normal distribution (Shapiro-Wilk normality test, $W = 0.6235$, $p\text{-value} < 0.001$). We also performed an additional test for each lineage to investigate if the environment colour preference was influenced by the first illuminated environment (red or full spectrum environments) females swam to. This test could evaluate if individuals' decision was biased by the first illuminated environment they had contact with when we inserted them into the experimental tank. For that, we fitted a Welch's t-test for the black water lineage, and a Wilcoxon test for the clear water lineage, using the proportion of the time individuals spent in the red illuminated environment as our dependent variable and the first lightened environment females swam to as our independent variable. Because in seven out of nine cases, individuals from CJ1 population swam to the red illuminated environment, a statistical model was not possible to fit.

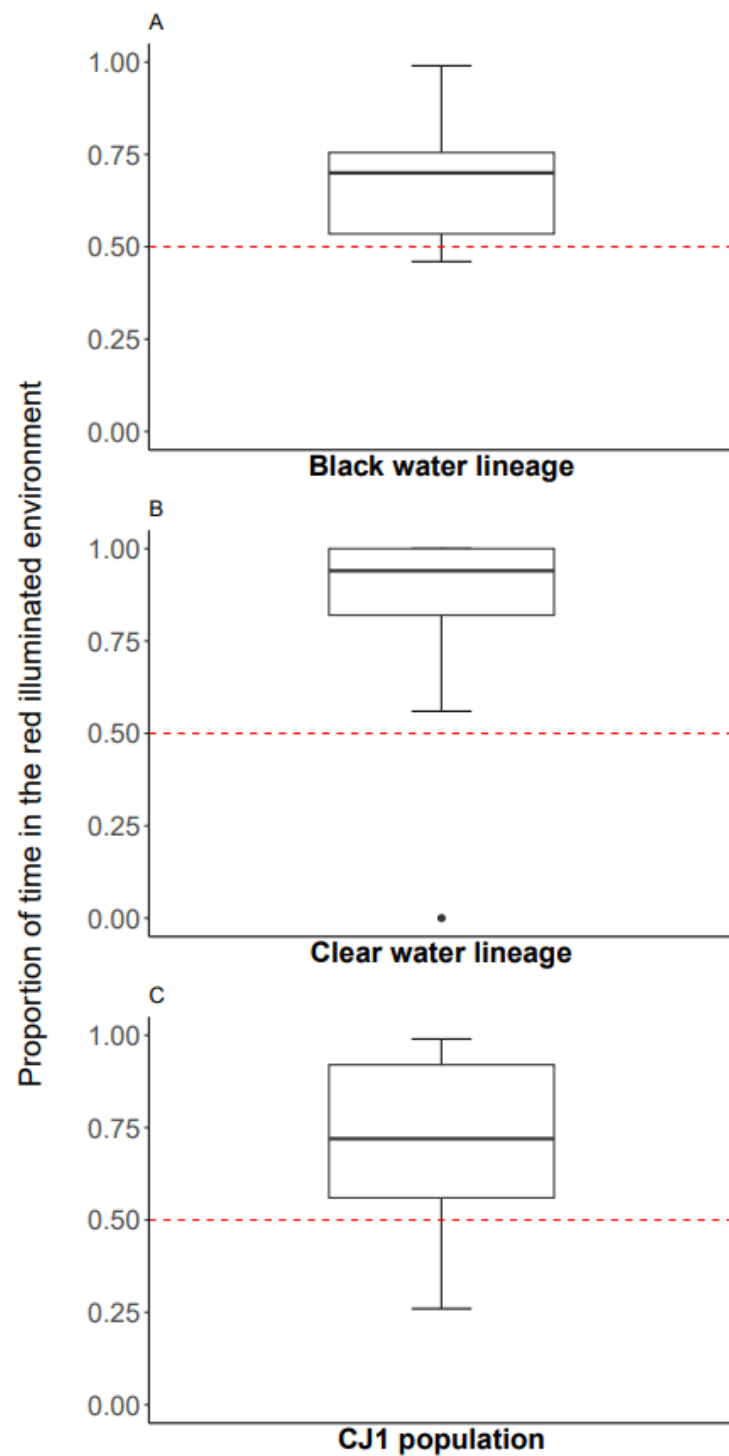
We also compared the strength of the red colour preference among lineages. The proportion of time spent in the red environment was used as the dependent variable and lineages (black water, clear water and CJ1 population) as the independent variable in a Kruskal-Wallis test. The Kruskal-Wallis test was used since the proportion of time spent in the red environment was not normal (Shapiro-Wilk normality test, $W = 0.896$, $p\text{-value} = 0.001$). We checked for pairwise comparisons using Dunn's test with the Bonferroni correction. All analysis was performed in R version 4.2.2.

Results

Females from black water lineage spent longer in the red illuminated environment than in the full spectrum illuminated environment (One-sample t-test: $p\text{-value} = 0.0008$) (1446.73 ± 548.43 seconds in the red light against 710.6 ± 388.23 seconds in the full-spectrum light, mean $\pm$ standard deviation) (Figure 2A). Similarly, females from the clear water lineage (Wilcoxon signed rank exact test: $V = 144$, $p\text{-value}$

= 0.0005) (2051.82 ± 599.69 seconds in the red light against 347.58 ± 600.05 seconds in the full-spectrum light, mean $\pm$ standard deviation) (Figure 2B) and CJ1 population (1606.44 ± 605.06 seconds in the red light against 693.55 ± 570.52 seconds in the full-spectrum light, mean $\pm$ standard deviation) (One-sample t-test: p-value = 0.0414) (Figure 2C) spent longer in the red illuminated environment than in the full spectrum illuminated environment.

There was no significant difference between the proportion of the time individuals spent in the red illuminated environment when they first swam to red or full spectrum illuminated environments for black water lineage (Welch Two Sample t-test: $t = 0.49259$, $df = 12.433$, p-value = 0.6309), and for the clear water lineage (Wilcoxon rank sum test: $W = 28$, p-value = 0.6462). However, females from the CJ1 population spent a higher proportion of the time in the red illuminated environment when they first swam into the red illuminated environment (7 out of 9 cases) (Figure 3).



314

315 Figure 2. Proportion of the time females spent in the red illuminated environment for
 316 the three lineages of the sailfin tetra. a) Black water lineage, b) Clear water lineage, and
 317 c) CJ1 population. The red dashed line at 0.50 marks the point where no colour
 318 preference is expected (i.e. when the time individuals spent in red illuminated

environment is equal to the time spent in the full-spectrum one). The sample size for black water lineage, clear water lineage and CJ1 population is 15, 17 and 9, respectively.

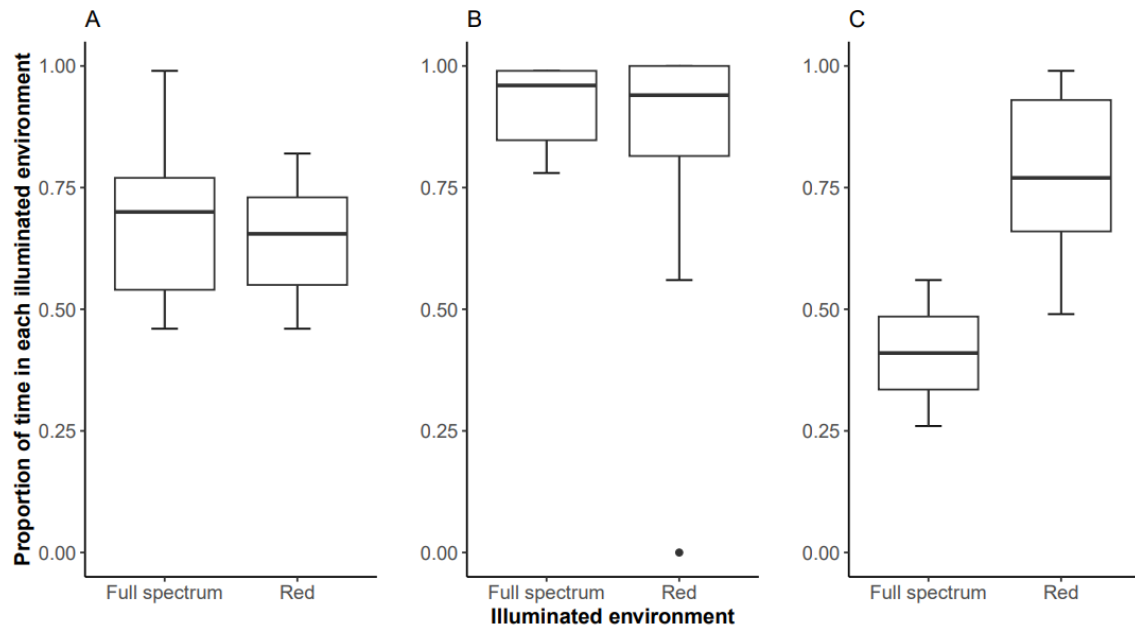


Figure 3. Proportion of the time females of the sailfin tetra spent in the red illuminated environments when they swam firstly to full spectrum and red illuminated environments. a) Black water lineage, b) Clear water lineage, and c) CJ1 population. The sample size under the full-spectrum and red light conditions, respectively, are shown inside the parentheses for black water lineage (9, 6), clear water lineage (6, 11) and CJ1 population (2, 7).

The proportion of the time spent in the red environment differed among lineages (Kruskal-Wallis chi-squared = 12.177, df = 2, p-value = 0.002). The comparison of the red colour preference among lineages suggested that the red colour preference is stronger in females of the clear water lineage than for females from black water lineage (Pairwise comparisons using Dunn's test, p-value = 0.001) and CJ1 population (Pairwise comparisons using Dunn's test, p-value = 0.031) (Figure 4). There was no significant difference between the strength of the red colour preference between females of the

black water lineage and CJ1 population (Pairwise comparisons using Dunn's test, p-value = 0.885).

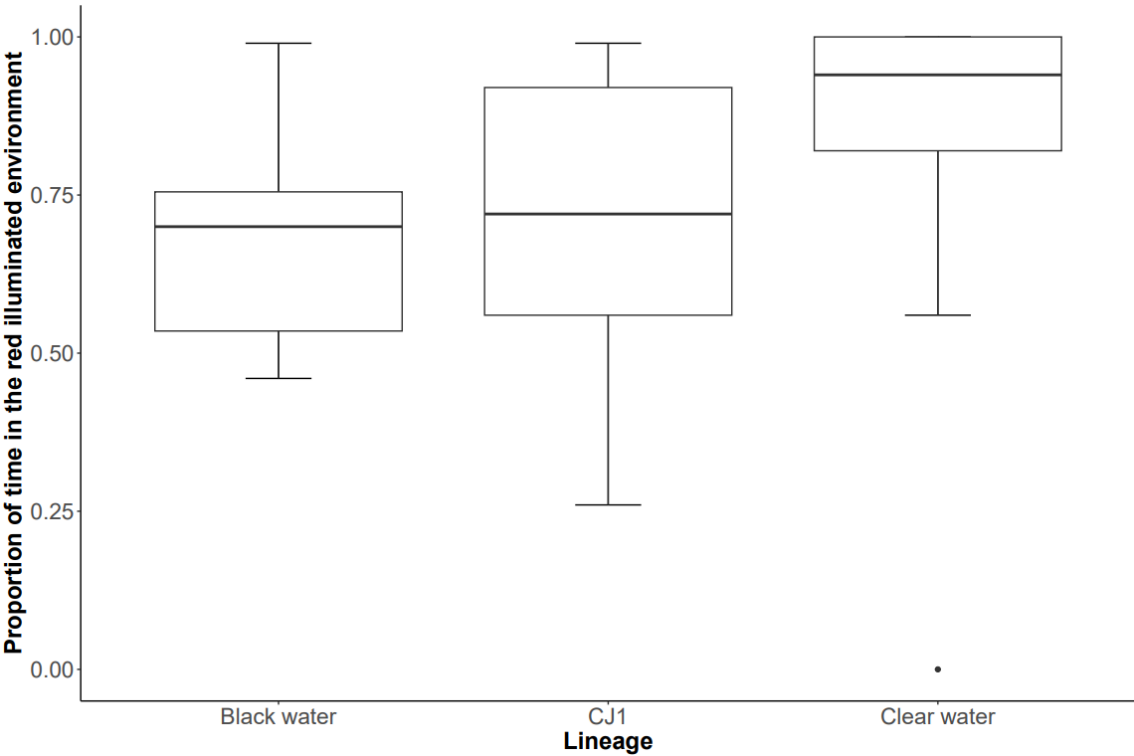


Figure 4. Proportion of the time spent in the red environment by females of the Black water, Clear water lineages and CJ1 population. The sample size for black water lineage, clear water lineage and CJ1 population is 15, 17 and 9, respectively.

Discussion

Our results show that females from all three groups strongly preferred red-biased over full-spectrum illuminated environments. Such preference seems not to be affected by the first contact individuals had with both red or full-spectrum illuminated environments for the black and clear water lineage. However, females from the CJ1 population spent a higher proportion of the time in the red illuminated environment when they had the first contact with this environment. However, it is important to highlight our small sample size for the CJ1 population (N=9). Also, the strength of the red environment colour preference was higher for clear water lineage females than for those females living in black waters.

The environmental colour preference may be related to the colour peak sensitivity (Luchiari et al. 2007; Little et al. 2019). In our previous analysis of the colour peak sensitivity, we did not find any difference between the LWS genes that are responsible for sensing red colours among lineages. However, differences in the red colour sensitivity may be achieved by the differential expression level of each LWS gene. Indeed, we suggested that the expression of different LWS genes and the colour peak sensitivity may be environmentally driven (Borghezan et al. 2024). However, the LWS genes account for more than 90% of the gene expression profile in the eyes of the sailfin tetra individuals caught in the wild (Escobar-Camacho et al. 2020), highlighting the potential importance of the red colours for the sailfin tetra. Therefore, even though all individuals tested in our experiment were kept under similar laboratory lighting conditions, it is likely that the environmental colour preference in both lineages of the sailfin tetra is driven by their high red colour sensitivity. However, it is important to highlight that our experimental set-up did not account for possible differences in the perceived light intensity by the focal individuals, and its effects on environmental colour preference. Future studies controlling for the opsin gene expression level, individuals' sensitivity under captivity conditions and light intensity can accurately evaluate the effects of light intensity and spectral composition on the sailfin tetra environmental colour preference.

The attraction for red colour and red colour sensitivity are important components of the sailfin tetra diet, sexual and social behaviours. Such colour preference may be associated with the acquisition of food resources. The sailfin tetra feeds on several red-coloured items such as buriti palm tree *Mauritia flexuosa* fruits and Chironomidae larvae (Pires et al. 2016). Therefore, individuals might be more attracted to red environments, associating it with a potential food source. Most of the ripe fruits are orange/red coloured, and such colouration may indicate higher levels of carbohydrates and less unpalatable or potentially toxic secondary compounds. Therefore, the red colour preference for food items may be observed in several Amazon fish species. However, it is still unclear how the environmental red colour preference found in the present study can affect the preference for different coloured food items, and such interesting issue can be addressed in future investigations.

Environmental light also affects individuals' physiological states. Red light might provide a stimulating and enriched environment for fish, influencing their preference (Owen et al. 2010). It could impact their activity levels, stress response, or reproductive behaviour, leading them to choose red environments over full-spectrum ones. The ornaments of the sailfin tetra males are red coloured. Such ornaments are used during sexual behaviour (i.e. courtship behaviour) (Pires et al. 2016; Borghezan et al. 2023). The red colour preference found in the present study can also be associated to the sexual behaviour; and again, future investigations can address such interesting points and shed light on how colours affect their behaviours.

The sailfin tetra lineages live under clear and black water types. These water types differ in physical (light transmission) and chemical composition (reviewed in Borghezan et al. 2021). Interestingly, individuals of the sailfin tetra can assess and actively choose environments that chemically resemble *igarapés* over the main drainage basin water (Stefanelli-Silva et al. 2019). Similarly, couples under *igarapés* waters showed higher spawning success than those under the main black- or white- basin river water types (Stefanelli-Silva et al. 2019), suggesting that they are sensitive to the chemical and physical limnologic characteristics of the water. The red biased light in black water systems can decrease individuals' stress levels (measured as plasma cortisol concentrations), resulting in individuals being bolder (i.e. the exploratory behaviour) and more active when compared to those exposed to the white light (Owen et al. 2010). Such increased fish activity can also increase their feeding behaviour under red light conditions (Volpato et al. 2013). Such effects of the red light on individuals' physiological status may also make them more comfortable and less prone to diseases. Therefore, the red environmental colour preference in the sailfin tetra females may also represent a choice for environments that favour their physiological condition. Finally, the reproductive isolation of the sailfin tetra lineages is hypothesized to be shaped by differences in osmoregulation (Pires et al. 2018). Therefore, the chemical composition of the aquatic environment must play an important role in the fitness of the sailfin tetra individuals. Here, we acknowledge the importance of the chemical composition of the different water types; however, we tested the isolated influence of a physical composition (lighting bias) of the different water types on the female environment preference.

The red environmental colour preference was stronger for the individuals living under clear waters than for those living in black waters. Such stronger preference for red colour in clear waters individuals may relate to the colour preference in mating behaviour (Borghezan et al. 2023). Therefore, stronger colour preference in females may indicate a higher relevance of such trait for mating decisions. As the importance of colours for mating choice varies according to predation risk (Godin and Briggs 1996), the importance of colours for mating choice may vary according to the strength of the colour preference. Indeed, the sexual preference for a colourful trait has been hypothesized to be driven by a preliminary preference for such colouration (Taylor and Hunter 2016), and future studies can investigate how the strength of the colour preference may directly correlate with female mating preference. Interestingly, the strength of the red illuminated environment preference in the CJ1 population is similar to the black water lineage females, a result also found for their mate choice under different underwater lighting conditions (Borghezan et al. 2023), supporting the convergent evolution for black waters in the CJ1 population.

Even though we did not directly investigate the mechanisms of the environmental colour preference, we suggest that the females of the sailfin tetra are attracted to red colours even outside of the sexual context. Interestingly, the colour preference often matches the male sexual colourful traits in several species (Rodd et al. 2002; Madden and Tanner 2003). However, female mating preference for male ornament colouration differs in relation to the underwater lighting conditions individuals live in, with females from black water preferring red environments but with no effects of different lighting conditions on female mate acceptance (Borghezan et al. 2023). Therefore, because of the observed differences in the female preference for red colours under a mating context (Borghezan et al. 2023) and during non-reproductive activities for the sailfin tetra populations living in black waters, we suggest that the relevance of colours and their preference can vary according to the context and are shaped by environmental conditions.

Conflicts of interests/Competing interests

The authors declare that they have no competing interests.

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554