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Coloration impacts sexual behavior through dominance without any color preferences in the short-living fish *Nothobranchius furzeri*

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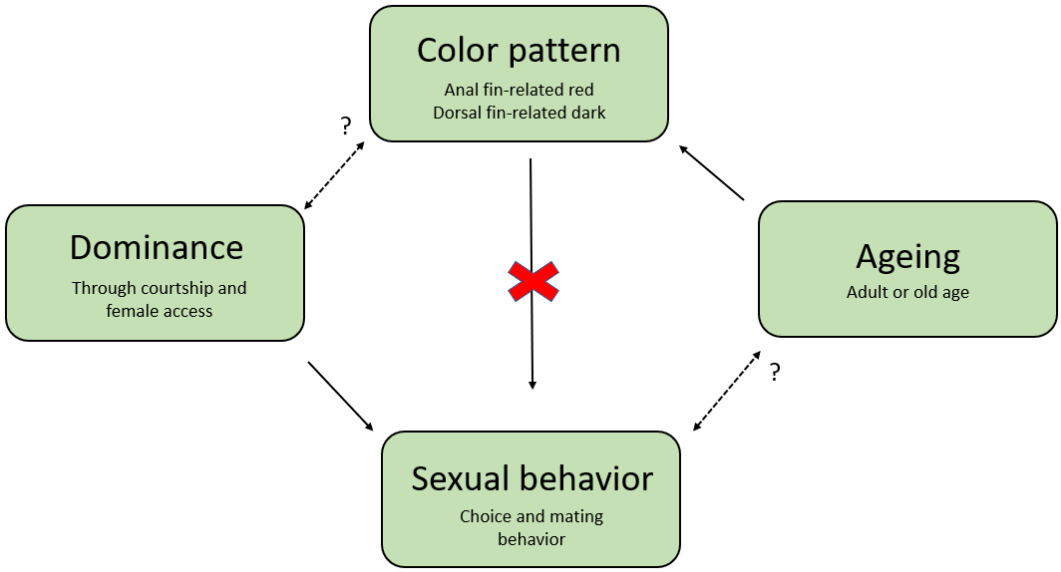
This thesis is dedicated to my beloved father. I hope you are proud of me wherever you are.

Abstract

Color is a particularly available and quantifiable phenotype to assess the different pressures exhibited through natural and sexual selection. Color is dynamic and deeply involved in a lot of processes including communication and adaptation. In that case, color is useful phenotype to study both sexual behaviors, environment-related changes and ageing. In this study, the shortest-lived vertebrate model *Nothobranchius furzeri* was first used to understand how color patterns vary under social and stressful condition following ageing process. The effects of color traits on sexual and non-sexual behaviors were assessed to highlight some features. New methods are described here providing an accessible and open-source way to uniformly assess color patterns in killifish. It has been reported that color and pattern area increase following adult stage but do not fade at older age. One failed to highlight the effect of chemical stress on morphological trait, but it is suspected to speed up color appearance rather than pattern morphology. More importantly, it is strongly suggested that some color patterns in *N. furzeri* are intrasexually selected as they rely on social context like dominance rather than mate choice. This is strengthened by the fact that some patterns fade after 12 weeks of development, following low dominance pressure. In this paper two patterns were identified that might be involved in dominance and sexual behavior in *N. furzeri*; anal fin-related red and dorsal fin-related dark patterns with multiple evidences. Further studies are now expected to explain how dominance expresses itself between males and whether dominance alone allows the maintenance of such bright color pattern in turbid environment.

Keywords: Color – mate choice – sexual behavior - social context – ageing – Turquoise killifish – *Nothobranchius furzeri*

Graphical abstract



**Plain lines represent proven effects where dotted lines represent hypothetical effects rose in the discussion. Interrogation points represent unknown effects which can also impact those traits*

Introduction

In living animals, color is one of the most relevant phenotypes to understand the balanced equilibrium drawn between natural and sexual selection. The fact that both environment and mate choice can influence the same trait is vital to understand phenotypical-environmental mismatch and deleterious gene flow caused by multiple selective pressures. Color is no exception to this and is a particularly available and quantifiable phenotype to assess (**Cellerino *et al.*, 2016; Cuthill *et al.*, 2017; Endler & Mappes, 2017**). In the case of sexual behavior, color can facilitate courtship and preference in some species but can also increase mortality through predation as described in the Red Queen hypothesis (**Van Valen, 1974**). This has been well detailed (**Candolin, 1998; Heinen-Kay *et al.*, 2015; Hurtado-Gonzales *et al.*, 2010; Nokelainen *et al.*, 2012**) even though it might be still debated in specific cases (**Roulin, 2016**).

Color is dynamic (**Mäthger *et al.*, 2003; John *et al.*, 2021; Stuart-Fox & Moussalli, 2009**) and one of the most important means of communication between species (**Cuthill *et al.*, 2017; Leclercq *et al.*, 2010**), playing a major role in adaptation, conspecific and heterospecific communications (**Supplementary information I & II**). For the past decades, the number of animal coloration studies has significantly increased, bringing this field of biology in a real new era (**Cuthill *et al.*, 2017**). Evidence on the effects of coloration on sexual behavior are ubiquitous and well documented in birds (**Dunn *et al.*, 2015; Nolan *et al.*, 2010**), insects (**Kemp & Rutowski, 2011; Tigreros *et al.*, 2014**), mammals (**Caro & Mallarino, 2020; Dubuc *et al.*, 2016**) or fish (**Kodric-Brown, 1985; Maan *et al.*, 2010)**. It is also known that color can affect both intrasexual (**Hiermes *et al.*, 2016; John *et al.*, 2021**) and intersexual communication (**Cuadrado, 2000; Schweitzer *et al.*, 2015**). Those are particularly present in highly sexually dimorphic species, which are suited model to study the effect of color on sexual selection. From all of the species of birds, insects, mammals and fish, teleost fish are the ones to focus on as it is one of the most enriched clades in animal realm (**Reece *et al.*, 2012)**. This will provide a great diversity in morphological size and shape to study deeply morphological skin color variations in vertebrates **(Leclercq *et al.*, 2010).** Furthermore, color patterns as well as color-producing mechanisms are more diverse and elaborated in fish than any other vertebrate (**Irion & Nüsslein-Volhard, 2019; Luo *et al.*, 2021; Maan & Sefc, 2013**). In water, visual cues are key components of communication as it is a visually more complex environment than land (**Lythgoe, 1988**).

Color is created by the way incident light is absorbed by pigments or scattered by cells or molecular structures (**Supplementary information III)**. Pigments are mainly contained inside vesicles of particular cells called chromatophores which are progenitors of more specific pigments-containing

cells like melanocytes (**Leclercq *et al.*, 2010**). Those cells are a huge component of fish' skin and their positioning are the basis of most color patterns (**Kelsh, 2004; Nüsslein-Volhard & Singh, 2017).** On the other side, light scattering allows a non-pigmentary based coloration called structural coloration (**Mouchet & Deparis, 2021**). This is known to occur in iridophores cells by the way guanine crystals face light sources (**Funt *et al.*, 2017; Levy-Lior *et al.*, 2010 ; Yoshioka *et al.*, 2011**). This scattering may also be done by extracellular crystalline surfaces composed of fibrous structures like collagen (**Giraud *et al.*, 1978; Mitov, 2012**). Pigments are known to be genetically encoded in vertebrates' genomes and their expression can be modified through molecular mechanisms such as epigenetics (**Faulk & Dolinoy, 2011; Shorter *et al.*, 2012).** A lot of genes have been highlighted to create colors notably in melanin or purines pathways (**Leclercq *et al.*, 2010; Luo *et al.*, 2021**). In the case of light scattering, it is still unclear how guanine-based crystals or extracellular fibrous structures can be influenced by specific gene sequence or expression. Color in fish is often a combination of different cell types and mechanisms which can lead to a huge diversity of patterns and change strategies (**Singh & Nüsslein-Volhard, 2015).**

Those color changes can arise from diverse parameters including ageing (**Hsu *et al.*, 2008; Nikiforov-Nikishin, 2022).** It is also known that social context can impact color patterns as ranking and dominance may often rely on color in fish (**Culumber & Monks, 2014; Hiermes *et al.*, 2016; John *et al.*, 2021**). Finally, environmental conditions including anthropogenic disturbances are being studied for their impacts on coloration whether it is water pollution (**Gomes-Silva *et al.*, 2020**) or climate change **(López-Idiáquez *et al.*, 2022; Roulin, 2014)**. Those parameters could then indirectly impact sexual behavior and mate choice in fish. Nevertheless, little is known regarding how those parameters interact and if their effects can be mitigated by the interactions they performed. Based on this knowledge gap, it is hypothesised that ageing can function as a backdrop on all these conditions, which will at last impact fitness (**Reichard, 2017**). It would then be insightful to study to what extent ageing may impact color and its interactions with social context or environmental disturbances to understand how it will influence mate choice and fitness.

The turquoise killifish *Nothobranchius furzeri* is a cyprinodont fish considered as an emerging model for ageing (**Platzer & Englert, 2016; Reichard & Polačik, 2019**), quantitative genetics (**Cellerino *et al.*, 2016**) and ecotoxicological studies (**Thoré *et al.*, 2020**). It is the shortest-living vertebrate bred in laboratory where all known ageing process are rapidly reached in a range of six months **(Supplementary information IV).** *N. furzeri* is naturally distributed in South-East African freshwater ponds in an area spanning between Zimbabwe, Mozambique, and South Africa. This fish reproduces continuously from sexual maturity until death, and top reproduction activity is

reached at the adult stage (**Cellerino *et al.*, 2016**). Social structure is relatively well settled as males dominance rely on size and coloration (**Cellerino *et al.*, 2016**; **Reichard & Polačik, 2019).** It is a highly sexually dimorphic species where males display two different color morphs expressed by a red or yellow caudal fin (**Cellerino *et al.*, 2016)**. In *N. furzeri*, it appears that color pattern is strongly sexually selected (**Haas, 1976**). As a result, it is known that color may impact mate choice in this species (**Cellerino *et al.*, 2016**). A previous study showed that this choice might also rely on vigorous courtship from males but also on sympatric interactions previously performed (**Reichard & Polačik, 2010**). Therefore, a recent study proposed *N. furzeri* as an emerging model for behavioral studies and mate choice (**Johnson *et al.*, 2020**), as little is known about coloration preferences in females (**Cellerino *et al.*, 2016**). Some studies have been carrying work on preferences between the two males morphs (**Johnson *et al.*, 2020; Reichard & Polačik, 2010**) but no preferences have been highlighted among a particular morph so far. Following the opportunity this species represents for ageing, colors are also known to fade following old age but it has not been described for precise pattern among a particular male morph though.

The origin of those colors is still unknown as the causal link between environmental conditions and coloration pattern has not been made so far (**Reichard & Polačik, 2019**). However, genetic implications might explain some parts of color as melanin pathway is suspected to play his role in yellow morph males albeit no single gene seems to stand out (**Ng'oma *et al.*, 2014; Valenzano *et al.*, 2009**). Color characterization itself has already been carried out in other species relatively close to our model (**Domínguez-Castanedo *et al.*, 2021**; **Nikiforov-Nikishin *et al.*, 2022**). Although colors have already been strongly linked to sexual behaviors, some of them need also to be recontextualized with male personality traits such as boldness. This trait is known to affect social interactions and particularly mate choice in fish network as the bolder the male, the more he will interact with a male or female partner **(Bierbach *et al.*, 2015; Godin & Dugatkin, 1996; Pike *et al.*, 2008)**.

The main objective of this study is to investigate the variation and effects of coloration patterns and male personality on mate choice in a social and environmentally disturbed context throughout the lifespan of the turquoise killifish *N. furzeri*. To that end, the main objective was split in two different ones:

Firstly, a pure description of coloration patterns following ageing, social context and environmental disturbances has been made. All colors were described on each part of the body, based on colored area and L*a*b* chromaticity coordinates at different timepoints. Secondly, fish behaviors were assessed through two different tests; a male-female interaction in a mate choice test adapted from **Reichard and Polačik (2010),** and a shelter test to evaluate non-sexual behavioral traits in males.

Material & methods

Breeding

300 *Nothobranchius furzeri* eggs have first been ordered from Microbiotest™ (Gent, Belgium). Yellow morph males from the GRZ lineage were used which originally comes from the Gonarezhou National Park in Zimbabwe (**Cellerino *et al.*, 2016**). Those fish (F0) have been raised together until their fifth week of development in a seven liter tank (35,5 x 23,5 x 12 cm, , L x W x H) filled with system water which consists in type II deionized water supplemented by Instant Ocean® sea salt (Spectrum Brands™, Blacksburg, VA, USA) until reached 600µS/cm conductivity, maintained at 27 ± 1°C, pH 7 ± 0,5. Fish were kept in this stable environment with 10:14 day/night cycle and 50% air humidity. Since their hatching, fish have been fed twice a day with *Artemia salina* nauplii (**Annex I**), guarantying *ad libitum* feeding. Water was changed following a precise schedule: partially changed (1/4) every two days and almost entirely (3/4) every seven days. After five weeks, fish were moved to smaller tank in harem (one male and three females per tank) in order to reproduce. From that moment, thawed *Chironomus sp.* larvae (Ocean Nutrition™, Essen, Belgium) have been added to fish diet. The same feeding diet has been kept for all *N. furzeri* individuals grown for this study. From the fifth developmental week, sandboxes were put in each tank and the eggs laid were collected at least every other day but preferably each day. Eggs have been conserved on coconut fiber at 17°C first. Once a sufficient amount of eggs was reached, all of them have been put in development at 28°C until hatching.

Hatching

After three weeks of development, all ready-to-hatched eggs have been selected and put in a 1.5L solution of humic acid 0.33g/L (Sigma-Aldrich™, Saint-Louis, MO, USA) during 24h. Afterwards, all non-hatched eggs have been removed and humic acid solution has been consciously diluted during the next 24h to reach 100% system water after 48h post-hatching. Following that, 116 individuals were hatched and composed our F1 generation on which our experiments were based on. To collect some metrics, all fish were moved to 6-well plates. All steps described above are derived from **Polačik *et al.*, 2016**.

Conditions

From that moment, fish have been separated in several groups to assess the different conditions, which were social context and environmental perturbations. Firstly, fish were divided in

isolated or community condition to assess the impact of social context on coloration and behavioral traits. To impose environmental perturbations, fish were exposed to pyrethroids pesticides like permethrin which are accessible components to mimic anthropogenic disturbances commonly used in behavior and reproduction (**Jaensson *et al.*, 2007; Moore & Waring, 2001**).

Isolated fish were exposed in 6-well plates where each well was supported by an opaque circle to prevent any visual contact between individuals. Each morning during seven consecutive days, wells have been filled with 10mL of 100µg/L permethrin solution (C1) in 100% DMSO or 0µg/L in 100% DMSO (Control). For the community group, fish were moved to seven-liter tank filled with C1 condition or control and renewed each morning. After the first-week exposure, all solutions were replaced by 100% system water. Consecutively at two and five weeks development, fish were removed from their tank to finally reach three-liter individual tanks for isolated fish while community group were moved to 50-liter tank (58,7 x 39 x 30 cm). In the community group, fish were divided into groups of ten depending on their size and their sex (two-three females per male) in enriched water tanks (**Annex II**).

Behavioral test

Furthermore, some behavioral tests were performed to assess the effects of color on behavioral traits. All tests have been realized following three accurate timepoints: 5 (W5), 12 (W12) and 17 (W17) weeks development, each representing an important stage of life which can be summarized as young, adult and old (**Cellerino *et al.*, 2016 ; Reichard & Polačik, 2019**). Those tests only implied male individuals as they are the interest to study the link between coloration and reproductive behavior. During experimental period, tested males remained in individual tank to promote accurate identification.

Firstly, a shelter test has been carried out to assess the boldness of male individuals (N = 36) (**Annex III**). As this test does not induce social interactions, each male individual was used (isolated and community group combined). Each test has been filmed by a Sony HDR-CX625 (Sony group™, Tokyo, Japan) camera linked to Ethovision XT 15® software (Noldus™, Wageningen, The Netherlands) to collect data. For this test, the total time spent in the arena as well as the travelled distance for each male (reported to size and time) were calculated. Afterwards, a mate choice test (N = 20) was performed to assess the potential role of coloration in sexual behavior (**Annex IV**). Fish are gathered in groups of nine, six males randomly selected from the pool of tested male, and three females, randomly selected from community tanks. The

test has been done in triplicate as each pair of males has been tested by all the three females. Here, the mean time spent by female in front of each male as well as the time spent courting by males were calculated.

Photography

To describe the evolution of coloration patterns, males (N = 36) were photographed at each timepoint using a Pentax K10D® camera with Pentax-DA 18-55mm macro lens (Pentax Ricoh Imaging™, Tokyo, Japan). A standardized portable setup (so-called "Blackbox") was built in order to provide standardized light conditions for each fish photography (**Figure 1** & **Annex V**).

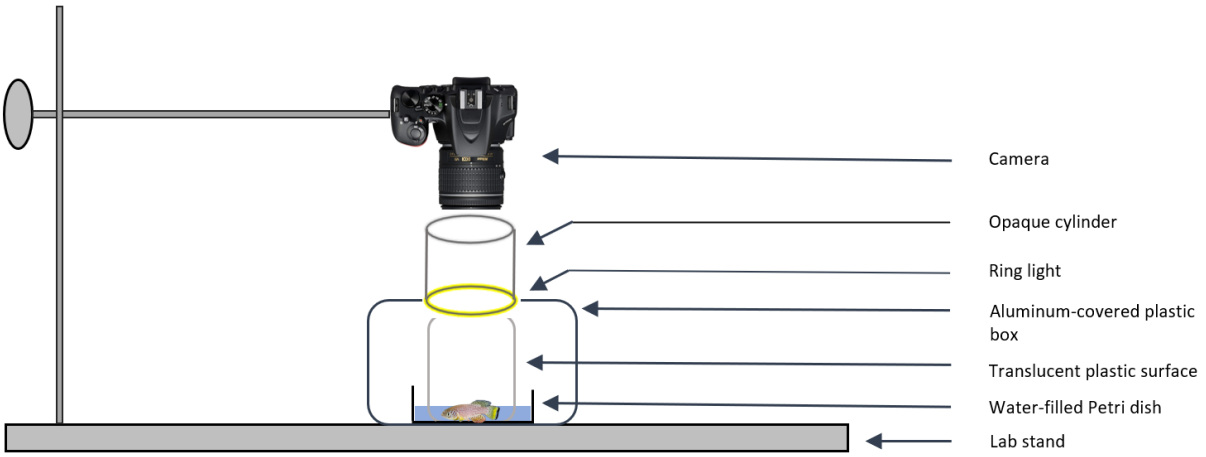


Figure 1. Design of the portable Blackbox composed of an opaque cylinder (7-cm diameter, 8-cm high), a plastic ring (26-cm diameter, 10-cm high) on which was fixed a ring light (5000 K) extended by a translucent plastic cylinder (diameter of 6 cm). The latter enable a diffused light environment, which is used around all fish for photography. A Pentax camera was held on a lab stand, to prevent any camera movement during photography, at 18,5 cm high from the sample.

This prototype makes it possible to take quality pictures in the field for small fish and is a low-cost complement to other devices already on the market like the *Photafish system* (**García-Melo *et al.*, 2019**). To reinforce standardized conditions, camera settings remained the same for all our fish samples (ISO-100, F22, 1/6 s, f=50 mm). Color calibration remained necessary here and was provided by standard 24-patch ColorChecker classic target (X-Rite Inc., Grand Rapids, MI, USA), which has been proven to be a stable calibrator used in various studies (**Potash *et al.*, 2020; Sunoj *et al.*, 2018**).

In each picture, white (#f3f3f2) and dark (#343434) spot calibrators were placed alongside our specimen for following image processing (**Annex VI**). Images have been saved in .PEF (Pentax Electronic Format) to constitute a stock of RAW format pictures. This kind of format is preferred for scientific coloration measurements as it provides the best image quality without

compressing any color information namely Red-Green-Blue (RGB) values (**Stevens *et al.*, 2007**).

Image processing

Three pictures were taken for each male individual to prevent any disturbance caused by blurring or dusting to jeopardize our images quality. The best out of the three was chosen and imported in Rawtherapee v5.8 software to undergo preliminaries image treatment. Pictures were adjusted by adding a neutral profile and exported in Tagged Image File Format, .TIFF. To standardize all our sRGB values, the micatoolbox plugin (**Troscianko & Stevens, 2015**) was used in the free open-source software ImageJ v1.53s (**Schneider *et al.*, 2012**) to transform our pictures in multispectral images. Color was selected in terms of hue, saturation and brightness using the "Color threshold" tool in ImageJ as used in **Maan *et al.*, 2004 & Selz *et al.*, 2016** (**Annex VII**). The same three parameters were kept for each selected pattern at each timepoint. In terms of patterns, one focused on the body-related red; body-related blue; yellow and dark on the caudal fin; red and dark on the dorsal fin; as well as the red on the anal fin (**Figure 2**).

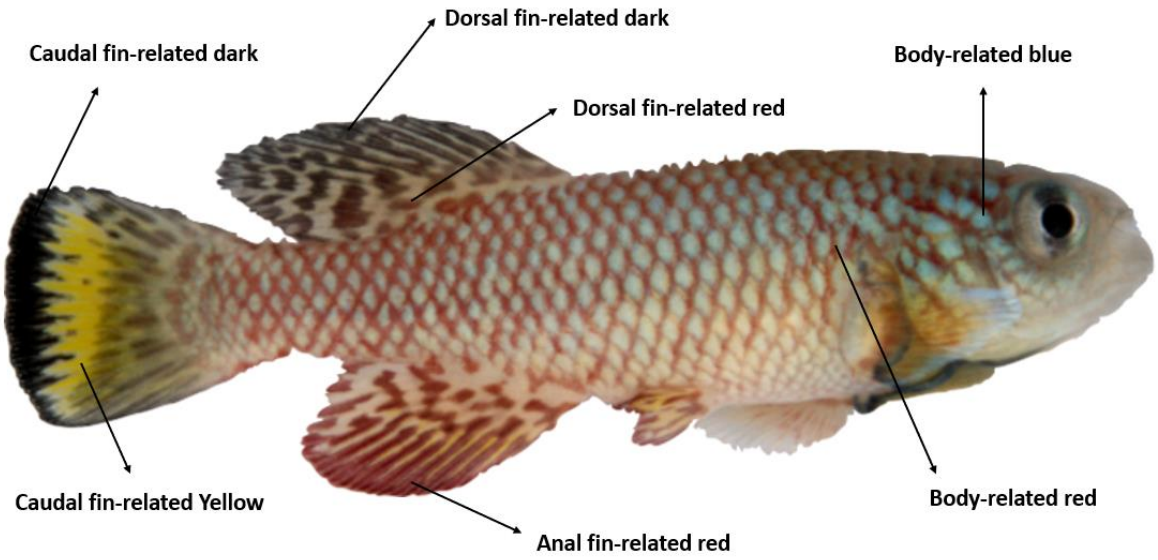


Figure 2. Chosen patterns for the coloration study of *N.furzeri* represented on a 12-week-old male

Those were chosen as they represent the main colors displayed on the more socially and sexually implicated body parts of *N. furzeri* **(Cellerino *et al.*, 2016).** For each pattern the area was calculated by the number of selected pixels reported in millimeters. The area has been normalized by the area of the interest zone (i.e., fin or body area) and expressed as a percentage of fish surface (body or fin) covered by each color. For each color pattern, the mean sRGB values were extracted. Those sRGB values have been converted into CIE 1976 L*a*b* color

space using MATLAB v8.2.0.29 (The MathWorks®, Natick, MA, USA). Those are known to be standardized and pretty well fitted to the human vision (**Renoult *et al.*, 2015; Schanda, 2007**) and their coordinates were used afterwards to investigate chromatic differences (**Annex VIII**). Furthermore, related pattern data were completed by Hausdorff fractal dimension (FD), which gave information about the complexity of each pattern (**Mandelbrot, 1983**). For that, only the fin-related patterns and the body-related blue were kept as they are size-limited and concrete patterns. We used in this study the box counting method (**Halley *et al.*, 2004**) with the "Fractal box count" tool in ImageJ (**Ristanovic *et al.*, 2014)**. This widely used method has proved to be useful in various topics in biology so far (**Jovani & Tella, 2007; Lothaire *et al.*, 2022; Pérez-Rodríguez *et al.*, 2013; Smith *et al.*, 1996**) (**Annex IX**). All those data have been gathered in Excel software (Microsoft Corporation™, Redmond, WA, USA) prior to statistical analyses.

Anesthesia and euthanasia

Before taking pictures, all fish were anesthetized to prevent any movements to jeopardize photography quality as well as sharpness. To do so, each male fish was put in a 1L solution of Ethyl 3-aminobenzoate methane sulfonate 0,4g/L (MS-222; Sigma-Aldrich™, Saint-Louis, MO, USA) buffered with 20 mL $NaHCO_3$ (30g/L) for 60 s to reach sedation. Afterwards, the fish was placed in a 6-mL water-filled Petri dish, which was positioned under the previously described photography setup. The fish was consequently placed in a recovery tank filled with system water until all movements were recovered. Finally, the fish was returned to the original water tank.

Statistics

All data were analysed using R.studio v2022.07.2 (**R Core Team, 2020**). Due to the large number of variables, PCA analysis was first performed to sort out the variables and use the ones that explain the most significantly the distribution of individuals. Spearman's correlation matrix was performed between colors and behaviors and significativity checked with rho (ρ) and p-values. A p-value of $<0,05$ was accepted as statistically significant. Main patterns were described with areas and color values with means ± standard deviations (sd) and variation coefficients (*V*). To assess the effects of the different conditions on color, nested linear models were performed on each sorted variables. Best models were chosen with type II ANOVA and AIC value and normality was checked using Shapiro-Wilk test. If normality was verified, ANOVA was completed with Tukey post-hoc analysis with Estimated Marginal Means

function (emmeans; r package "*emmeans*"). If not, significativity was verified by non-parametric Kruskal-Wallis test. Once the color variables were sorted and assessed, they were used in other linear models to assess behavioral effects. Redundancy analysis (RDA) was also performed to see any correlation between color and behaviors *a priori*.

Results

After PCA reduction, 20 color variables were kept for following analyses. Those variables draw the analysis in the same direction according to new dimension projection in PCA (**Annex X**).

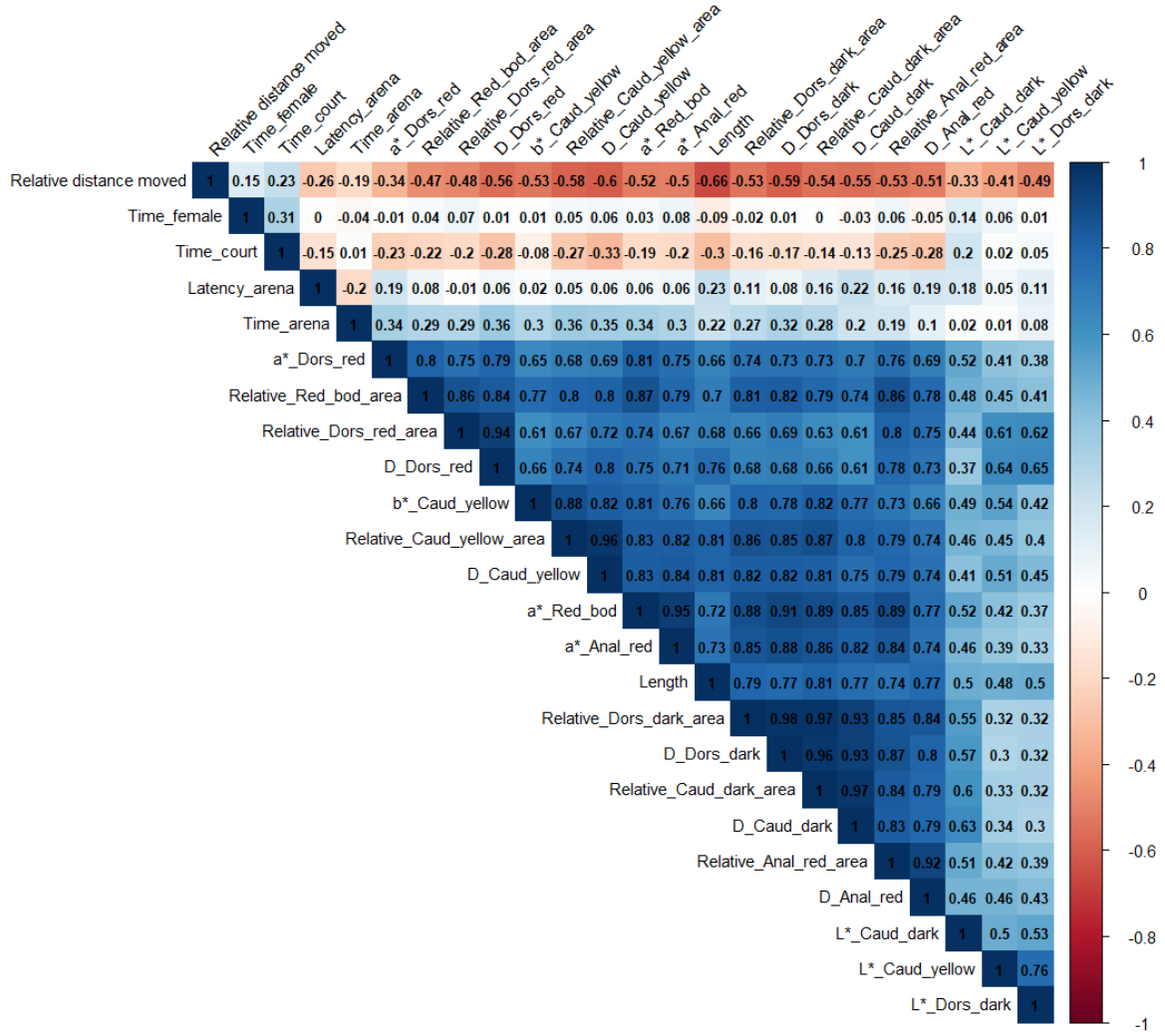


Figure 3. Spearman's correlation matrix between color and behavioral variables. Values are hanging between strongly correlated (dark blue) to strongly anti-correlated (dark orange) with no correlation at all represented by white square. All morphological variables are gathered below the sixth line of the matrix. Anal fin area was had based on interest rose by previous work done in the lab.

Following that, Spearman's correlation matrix (**Figure 3**) was performed and showed that all color variables were correlated between them, Spearman's correlation coefficient ρ ranging between 0.3 for light correlation to 0.98 with significant p-values for strong correlation such

as FD and relative area (**Annex XI & XII**). Following that and for the sake of simplification, only areas and color values were used for patterns description.

Pattern description

Male individuals are characterized by several fin- and body-related patterns. Body-related red and blue are distributed in a checkerboard pattern all along the body with an extensive blue spot on the gills. Caudal fin pattern is recognizable by a long yellow band supplemented by dark circular arc on the external side. Yellow is often tainted with dark spots, which can be quite variable between individuals. Dorsal fin is mainly dominated by dark and white stripes with an inclusion of some red stripes. Anal fin is dominated by red stripes on white background, which can be sometimes completed by small yellow stripes as visible in **Figure 2**. Main values of color and *V* value for fin-related patterns are described in **Table 1.**

A

	W5	W12	W17
Caudal fin-related pattern			
Yellow b* value	10,45 ± 14,53	37,36 ± 4,04	33,92 ± 4,38
Relative yellow area	2,00 ± 4,55	37,97 ± 9,57	32,61 ± 11,93
Dark L* value	8,57 ± 11,44	14,98 ± 2,78	16,34 ± 2,68
Relative dark area	4,26 ± 6,86	29,32 ± 11,75	30,85 ± 12,52
Dorsal fin-related pattern			
Red a* value	10,02 ± 6,57	17,16 ± 4,65	16,57 ± 4,35
Relative red area	7,07 ± 10,57	30,73 ± 11,31	26,92 ± 11,08
Dark L* value	11,87 ± 14,51	24,18 ± 3,63	24,36 ± 3,07
Relative dark area	3,77 ± 7,56	44,87 ± 20,04	42,44 ± 17,60
Anal fin-related pattern			
Red a* value	19,67 ± 6,92	25,80 ± 3,00	25,75 ± 3,06
Relative red area	25,67 ± 27,30	75,06 ± 10,67	72,09 ± 9,15

B

	W5	W12	W17
Caudal fin-related pattern			
Yellow b* value	139,12	10,82	12,90
Yellow area	227,61	25,21	36,58
Dark L* value	133,48	18,57	16,41
Dark area	160,96	40,07	40,58
Dorsal fin-related pattern			
Red a* value	65,57	27,09	26,25
Red area	149,51	36,81	41,18
Dark L* value	122,29	15,02	12,58
Dark area	200,39	44,66	41,46
Anal fin-related pattern			
Red a* value	35,17	11,61	11,86
Red area	106,32	14,22	12,69

Table 1. Numerical data of color (L*a*b* values) and area (%) for each selected timepoint (i.e., W5, W7 and W12). (A) mean ± sd values; (B) *V* value for each variable calculated by (sd/mean)*100%.

For each pattern variables, both color parameters and areas seem to increase with ageing which will be furtherly test through modelling. The same way, sd seems to decrease for color but increase for area. There is a lot of variation at the first timepoint for all variables, which drastically decreases following ageing. It can also be highlighted that anal fin-related red pattern is bigger at each timepoint and present one of the lowest values of *V* among all patterns.

Color x Conditions

Among morphological data, length has a strong correlation with all variables. As length is clearly explained by the age (ANOVA, $R^2 = 0.858$, AIC = 80.864, $p < 0.0001$), color variables could be expected to be significatively affected by ageing which fits with previous patterns description. The number of variables was reduced to color value (a*, b* or L*) and relative area for one pattern per fin only (yellow on caudal, dark on dorsal and red on anal fin) for the purpose of analysis simplification. For each variable, week, social context and permethrin concentration were tested through classic linear models (lm). Each variable proved to be significantly different following ageing at W12. However, no significant difference has been observed in any color variables following W17 as showed in **Table 2**.

			W12	*W17*
	R^2	*df*	*p*	*p*
Caudal fin-related pattern				
Yellow b* value	0,694	75	**<0,0001**	0,08
Yellow area	0,728	74	**<0,0001**	0,19
Dorsal fin-related pattern				
Dark L* value	0,21	78	**0,03**	0,97
Dark area	0,705	74	**<0,0001**	0,69
Anal fin-related pattern				
Red a* value	0,505	74	**<0,0001**	0,92
Red area	0,744	72	**<0,0001**	0,63

Table 2. Linear models results for each selected pattern. *df: degrees of freedom*.

What regards social condition, some significant differences have been highlighted for dorsal fin-related dark value and anal fin-related red area in **Figure 4**. All linear models' results are displayed in **Annex XIII**.

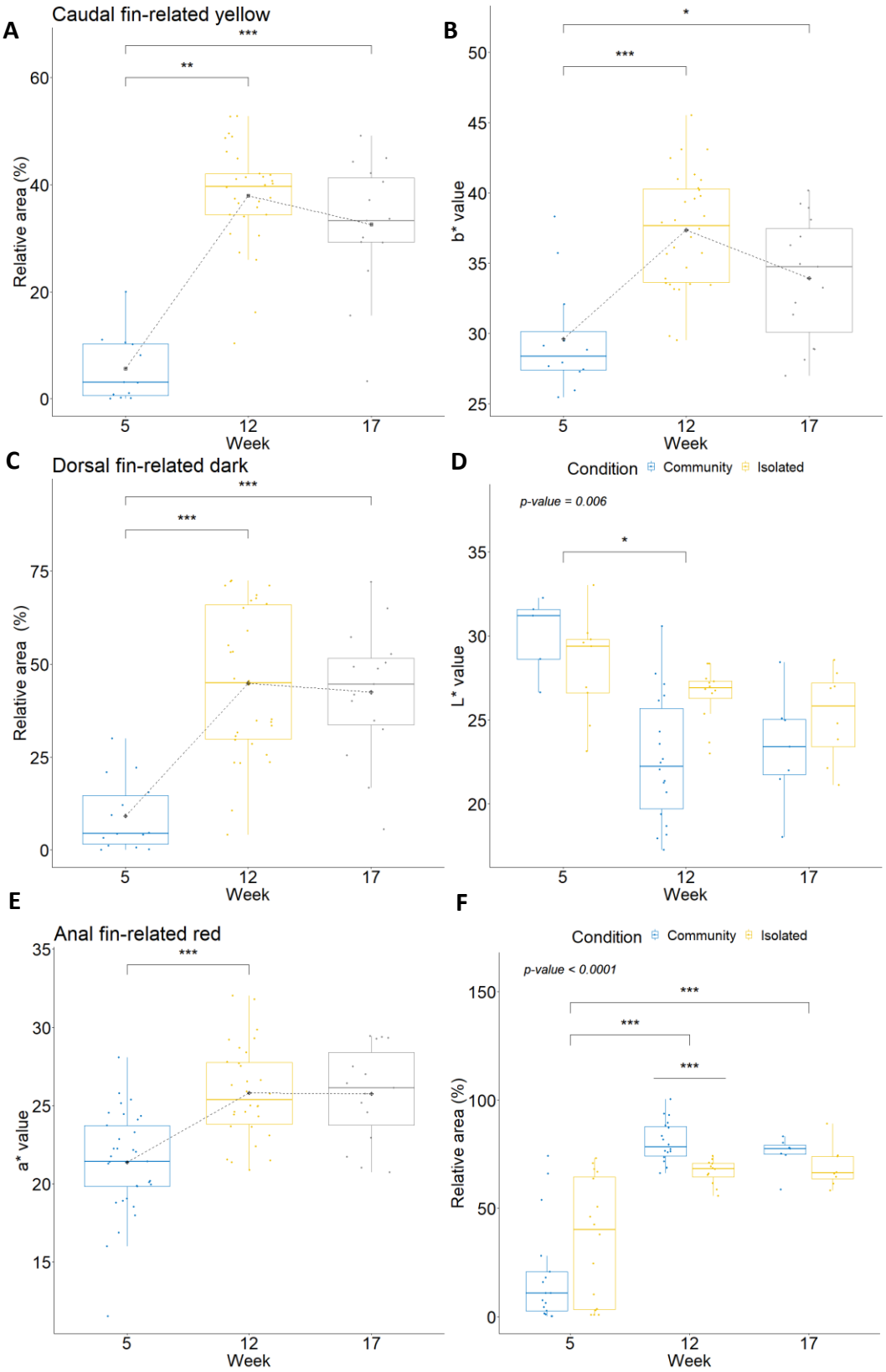


Figure 4. Boxplot graphs of the effects of ageing on (**A**) Caudal fin-related yellow area; (**B**) Caudal fin-related yellow value; (**C**) Dorsal fin-related dark area; (**D**) Dorsal fin-related dark value; (**E**) Anal fin-related red value; (**F**) Anal fin-related red area. Dark point represents the mean value which have been linked to one another with a dotted line. Social context has been tested significative for D and F with the value written on each graph through non-parametric Kruskal-Wallis test. **p*<0.05 ***p*<0.01 ****p*<0.001

Behavior x Color

As shown in **Figure 3**, behaviors are not all correlated between them unlike color variables, which is also visible on PCA analysis (**Annex XIV**). Only the time spent by female and the time of courtship performed by males undergo a light correlation (ρ = 0.31, p = 0.035). Morphological parameters also showed some correlations with behavioral data. In that case, courtship time values are lightly correlated with length (ρ = -0.3, p = 0.049) and FD of caudal fin-related yellow (ρ = -0.33, p = 0.032). Relative distance moved during shelter test is correlated with several morphological data including length (ρ = -0.66, p = 0.0001) and all other color components (ρ > 0.3, p <0.05). Redundancy analysis (RDA) was performed and confirmed that only the relative distance moved is negatively correlated with color variables (**Annex XV**).

Following linear model analyses, it is shown in **Figure 5** that female choice is significantly influenced by courtship behavior from male (p = 0.017) but also by the relative anal fin area (p = 0.01). No color variables are shown significant following type II ANOVA (p >0.05) (**Annex XVI**). However, courtship behavior is shown to be affected by length (p = 0.001) and dorsal fin-related dark lightness (p = 0.03).

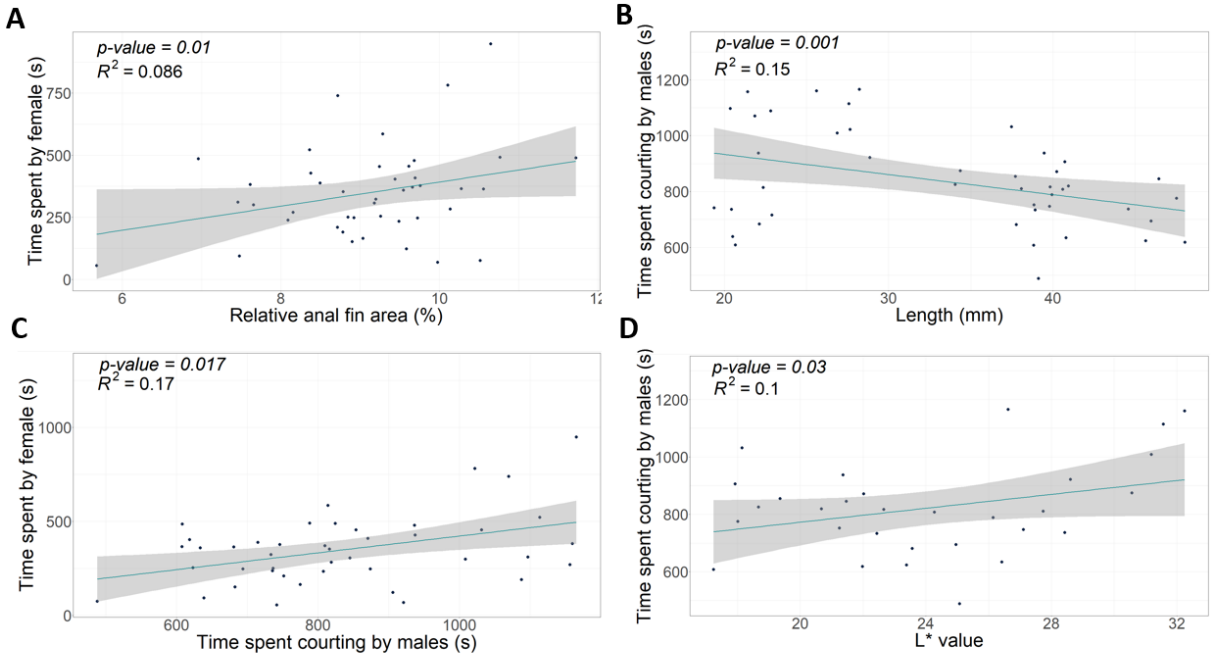


Figure 5. Linear regression of sexual behavior data based on linear model results. The above describes the significative effects of relative anal fin area (**A**) and courtship time (**C**) on the mean time spent by female in front of males. It is also described the effects of length (**B**) and dorsal fin-related dark lightness (**D**) on courtship time with significative p-value obtained by type II ANOVA.

Following that, other behavioral traits were characterized in shelter test and shown in **Figure 6**. Time spent in arena differed significantly depending on social condition (p = 0.03) but also with relative caudal fin-related yellow area (p = 0.002) (**Annex XVII**). The relative distance moved in arena also differed significantly with the length of the fish (p <0.0001). However, no significant result emerged for the latency before the fish arrived in arena (Kruskal-Wallis; p >0.05).

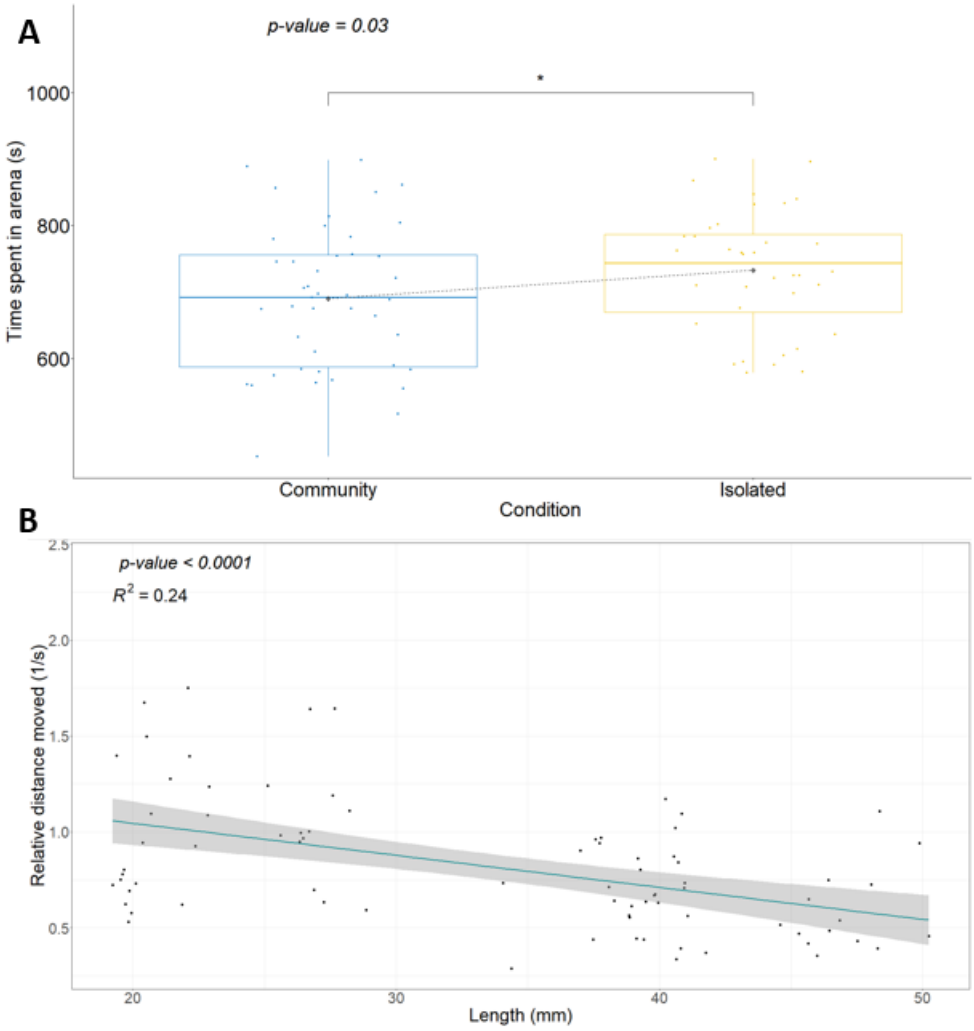


Figure 6. Linear model results for non-sexual behavioral traits. (**A**) Boxplot of the significative difference in time spent in arena between isolated and grouped males. Mean values are represented in dark spots linked with a dotted line. (**B**) Linear regression between length and relative distance moved.

Discussion

New photoprocessing methods have been recently described to assess color patterns in killifish (**Dominguez-Castanedo *et al.*, 2021; Nikiforov-Nikishin *et al.*, 2022**). Nevertheless, sometimes those methods can be unsuitable, inaccessible or can jeopardize fish wellness. The new method

described above can provide an affordable and open-source way to uniformly assess color patterns. All the obtained color values respect the theoretical range of L*a*b* values (**Durmus, 2020**) and match with other color studies using CIELAB color space (**Culumber & Monks, 2014; Potash *et al.*, 2020**). According to the available literature, this is the first time CIELAB color space is successfully used to describe color pattern in killifish. That is an additional piece to reinforce the use of the standardized L*a*b* coordinates for animal coloration studies.

Regarding pattern description, a lot of variation appears at W5. At that precise time, fish are becoming sexually mature and, as a strongly sexually selected trait, color appears at that time too (**Reichard & Polačik, 2019**). Variation in color at W5 can then be explained by the individual variability of reached maturity as the analyses began the same day for each fish. In that way, some may have reached maturity before pictures were taken and others have not. Variation coefficient *V* also shows that both color and area of anal fin stay in the same range for all fish at each timepoint. This shows that anal fin-related red pattern may display some features as it looks well conserved between individuals.

Beside anal fin-related red, no pattern seems to differ from one another which fits Spearman's correlation matrix. All pattern parameters are correlated between them which implies that all patterns on *N. furzeri* skin vary together following conditions; either you are colored or you are not. This might be explained by the global selection of pattern as a whole. It is indeed quite rare that predation impacts only a precise phenotype rather than color in general as known in guppies (**Fuller, 2022**). This might also be explained by pleiotropic effects as pigmentary colors are often created by the same conserved mechanisms even though they are under the control of different signals (**Nusslein-Volhard & Singh, 2017**). The lack of independence between patterns rise the interest to study entire pattern at a large scale instead of individual patches (**Endler & Mielke, 2005; Kemp *et al.*, 2008**).

In this study, models were based on fin-related pattern only as one was dealing with a too small number of individuals (N = 36). Nevertheless, pure description of fin-related color can firstly give insights on the important roles played by fins particularly in social context (**Culumber & Monks, 2014**). Here, the lack of social interactions seems to impact dorsal fin-related dark color and anal fin-related red area as it might reduce dark color and red area. This is a conceivable effect as it is known that males threespine sticklebacks (*Gasterosteus aculeatus*) can increase body coloration when facing a male opponent (**Hiermes *et al.*, 2016**). However, this would have to be put in line with dominance as sometimes this confrontation could lead to color decreasing in some fish (**John *et al.*, 2021**). In *N. furzeri*, it is known that the brightest and colored males are dominant (**Cellerino *et al.*, 2016**). However, one do not know if male-male interactions affect coloration up or down.

Dominance might be expected to pull up patterns as some of them decay following isolation, but this still needs to be proven. Dominance pressure is the highest at top reproduction activity which means around W12. This is precisely the time at which social context impacts anal fin-related red area and dorsal fin-related dark value. This reinforces the potential impact of social context on a specific pattern. Moreover, anal fin-related red pattern here strengthens his potential as it is relatively conserved pattern among individuals but is relatively impacted by isolation.

Concentration of permethrin was also assessed for its possible impact on color. However, no significant effect emerges for color value in any pattern. In *N. furzeri*, no effect of permethrin on morphological traits has been observed so far (**Van den Broucke, 2022**). However, one does believe that permethrin may somehow impact the appearance of color. It was noticed that all males exposed to permethrin displayed their color at W5 while around 3/5 of the control males were colored at the same timepoint. The rest of them appaired colored at W12. However, no highlighting of that effect could be made as a daily examination of color was required. Color in fish is known to also respond to external stress (**Freitas *et al.*, 2014**) which can sometimes be chemicals (**Gomes-Silva *et al.*, 2020**). Effects of chemical pollutions on color and their mechanisms is still largely unknown though.

In fish, color pattern can vary following ageing which is also the case for our study. Based on that, colors were expected to be less conspicuous with younger and older males. Colors increased at the adult stage but no sign of decreasing at old age was observed despite visual observations in *N. furzeri* or *N. korthausae* (**Lucas-Sánchez *et al.*, 2011**). Since no effect was get, one could say that maybe scientists should consider W19 or W20 as an accurate timepoint for "old". However, this would require a huge amount of fish due to high mortality rate.

Nevertheless, the absence of color fading might also be a direct consequence of *N. furzeri* ecology. As this fish reaches sexual maturity after around five weeks, color could resist ageing process as males do not stop to reproduce until death. This would then imply that color plays its role in reproductive behavior. However, no color seems to impact mate choice in our study as previously described by **Reichard & Polačik (2010)**. The only morphological trait involved is the relative anal fin area which is the third clue that strengthen the potential of the anal fin-related red pattern. This completes some work previously done in our lab where anal fin area significantly covary with aggressiveness probably through dominance process (**Van den Broucke, 2022**). Anal fin then appeared as a strong candidate to unmask the implication of dominance in sexual behavior and color retention in *N. furzeri*.

Courtship behavior performed by males also seemed to impact female choice, as the more males court, the more females spend time in front of them. This relation might not be unidirectional as

males seem to court as a response to female approach which is not representative of male behavior alone. Indeed, female choice behavior is well established as when a male is chosen the female will go straight to him (**Reichard & Polačik, 2019**) but will go the other way around when he is not chosen (**Cellerino *et al.*, 2016**). Even though female choice might rely on herself, interaction male-female might be more important for mating than individual behavior.

Courtship time performed by males is also impacted by length and then ageing as the older the males the less they spend time courting female. This can also be put together with relative distance moved as it also decreases following ageing. The decrease in activity and courtship behavior might rely on energy trade off induced by reduced available energy at older age (**Genade *et al.*, 2005; Reichard, 2017**). It might also be expected that ageing may indirectly impact mate choice through behavior even though it has not been proved in this study. If some behaviors decreased following ageing but color did not, it would suggest that a stronger selective pressure is keeping color on top even at older age.

Courtship seems also correlated with dorsal fin-related dark lightness as the darker the dorsal fin the less the courtship time is spent. However, one must stay critical as the significative effect is slight and could also simply follow ageing as the pattern is getting darker in both conditions. Nevertheless, dorsal fin, such as anal fin, is also involved in mating dance (**Cellerino *et al.*, 2016**) and social context as it is impacted by it. Therefore, dorsal fin-related dark also appears as a candidate pattern to study the effect of phenotype on sexual behavior.

Finally, curiosity (latency before going in arena) and exploration (time spent in arena) were not impacted by ageing. However, exploration was slightly higher for isolated males than for grouped males. Those results were a bit unexpected and no matches with literature on *N.furzeri* were found. Some work showed significant impact of ageing on open-field exploration but those data could not be compared to exploration data from a shelter test (**Genade *et al.*, 2005**). At the start, we wanted to use shelter test to evaluate boldness in males. However, this test is more likely to assess explorative behavior rather than boldness in *N. furzeri* based on the absence of fright or predation stimuli (**Conrad *et al.*, 2011**). The only color variable, which significantly impacted non-sexual behavior in this study, was caudal fin-related yellow area. The bigger the yellow surface, the more males' individuals spent time in the arena. This result is questionable as no other results from any other test emerge for this pattern. Caudal fin-related yellow likely plays no role in sexual behavior but may rather be related to explorative behavior. However, as a unique result and as the number of individuals was small, this shall be confirmed by further studies. In fish, particular color patterns have already been linked to specific behavioral trait (**Kim & Velando, 2015**). In this study, one failed to link behavior trait with particular color. Color seems here to demonstrate a specific social

status rather than a behavioral trait. As shown in **Figure 3**, no significative relationship between behavioral variables has been made making impossible any link between curiosity, activity, or exploration at that stage. At this point, no evidence leads to assess the effect of a specific phenotype; color or behavior; on non-sexual behavior in *N. furzeri*.

Conclusion

This research provides a new method for accurate picture processing for small fish studies but can be dimensionally adaptable for bigger ones. Contrary to the expectations, colors in *N. furzeri* are not directly involved in mate choice as they are more intrasexually selected through social interaction like dominance. Nevertheless, colors indirectly impact mate choice as dominance relies on them and would impact access to females. In environmental conditions, dominance shapes interactions and courtship time, which will then at last impact fitness. By acting on activity and courtship through reduced energy, ageing may also impact access to females and then sexual behavior. Some ongoing results in the lab also showed that sexual behavior can impact ageing and survival in this species (**Minocci, unpublished data**). This would confirm the backdrop role of age on fitness and gives us insights on the evolution of particular phenotypes at older age. It might not be forgotten that chemical stress is also suspected to speed up color appearance in male individuals, but this is solely based on visual cues.

This study also provides two phenotype candidates for dominance and sexual behavior. First, the anal fin-related red pattern as it is really conserved between males, impacted by social context, involved in mating dance and influences female choice. The second one could be the dorsal fin-related dark pattern as it is also impacted by social context and involved in mating dance. More studies should now focus on how dominance expresses itself between males and which pattern can be highlighted as dominant one, confirming our candidates or not. More work should also investigate whether dominance alone allows the maintenance of color following ageing as well as how so strongly visual patterns are conserved in such trouble water. A multistep and long-term study is now needed to assess the implication of dominant phenotypes and whether ageing may impact color if scientists extend color studies further than 20 weeks of development. This study also confirms the potential of using *N. furzeri* as a relevant model for the study of mate choice and sexual behavior with ageing implications.

Supplementary information

I. Physical origins of coloration

In animals, color is made by the way incident light from the sun is absorbed or reflected by different structures or physiochemical mechanisms. In that perspective, three diverse types of color production exist (**Mouchet & Deparis, 2021**).

The first type is the selective absorption of light through chemical components called pigments. Those pigments can take different names depending on the color perceived by animal's eye, such as melanin for brown and black colors, and can be synthetized through complex biochemical processes (**Leclercq *et al.*, 2010**). Those pigments are mainly contained in particular cells called chromatophores, which are a huge component of animal integuments, in particular fish. Those cells can also have specific names according to the pigments that they host and derive from neural crest cells during embryogenesis (**Leclercq *et al.*, 2010**). Pigmentary color is the main type of color involved in integument coloration in animals (**Mouchet & Deparis, 2021**) and is also the mainly one involved in color changing organisms (**Stuart-fox & Moussalli, 2009**). In those organisms, the color change can arise from the density of chromatophores (morphological) or the movement of pigment molecules within the chromatophore itself (physiological). Such pigment-based coloration dynamics can really impact biological processes including crypsis and all processes relying on disguises in nature (**Stuart-fox & Moussalli, 2009**). Following that, color changes can largely improve strategy efficiency as they allow species to adapt to changing environments. Those changes can be relatively slow and take several days or weeks through differential gene expression under hormonal control (**Cortesi *et al.*, 2016; Leclercq *et al.*, 2010**). However, it can also be quite quick following intra- or extracellular elements migration (**Stuart-Fox & Moussalli, 2009**). The fact that pigmentation is the main color production type in animal is crucial to be understood from both the ecosystemic and ecotoxicological points of view. Indeed, as a major constituent of dye and clothing industry, pigments are also a huge part of organic pollution of water in the world (**Ashraf *et al.*, 2021**). Studying how those pigments work and how to replace them can really bring a new dynamics of innovation and help scientists to find environment-friendly method of dyeing in the clothing industry (**Ashraf *et al.*, 2021; Liyanapathiranage *et al.*, 2020**).

The second type relies on the interference occurring between light waves with specific wavelengths and nanostructures, which will result in specific color perceived by animals. This is what can be called "structural color" (**Mouchet & Deparis, 2021; Sun *et al.*, 2013**). In the context of animal coloration, those nanostructures can either be biopolymers like collagen or keratin and are called photonic structures. Although they are less abundant than pigmentary colors in living organisms, structural colors are ubiquitously present in animal kingdom and have huge advantage on pigments, which is resilience. This will imply that color resists to age and last across time. Structural colors can also give rise to iridescence, namely the variation in the perception of a color as a function of the viewing angle.

The third type, and the least common one, is the light emission coming from fluorescence, or bioluminescence,. Those mechanisms differ between them by the way light is emitted: when fluorescence comes from the absorption of UV light by fluorophore cells, bioluminescence arises from the biochemical transformation of luciferin and dioxygen through luciferase enzyme (**Mouchet & Deparis, 2021**).

In many animals, colors are often a combination of two or three of these ways which can lead to a huge diversity of pattern and change strategies (**Mouchet & Deparis, 2021**). In those contexts, it is vital to understand the setting up of those ways to understand which will impact the most coloration and then the ecosystem. The establishment of coloration has already been studied in some model fish, including the zebrafish *Danio rerio* (**Singh & Nüsslein-Volhard, 2015; Volkening & Sandstede, 2018**) and the neon tetra *Paracheirodon innesi* (**Yoshioka *et al.*, 2011**). In those models, insights in genetics, histology and morphology have been made to assess the setting up of structural and pigmentary coloration. The choice of models in laboratory are mainly driven by the easy maintenance, as for *Danio rerio* (**Rahman & Sulaiman, 2019**), or the particularly accurate overlap between the model and the subject as for coloration in neon tetra (**Yoshioka *et al.*, 2011**). Nevertheless, new insights are always needed, and scientists are increasingly looking for strong multidisciplinary model species to cope with new biological issues.

II. Coloration in animal realm

Animal coloration patterns are constituent parameters of all species and ecosystems around the world. However, those colors are somewhat often underestimated in the research area and particularly in ecosystem ecology and evolution. Coloration patterns are often well detailed in different strategies such as defense, attack and both conspecific and heterospecific communication though (**Leclercq *et al.*, 2010**). In fact, defense mechanisms are crucial for

prey's survival to keep predators away. These defense mechanisms can take the form of aposematism, mimicry or even crypsis.

Aposematism is the strategy of coating a distinctive color judged as loomed to repel predators. The benefits of this strategy will go hand in hand with the density of the so-called signal and particularly memorable pattern like bright colors (**Mappes *et al.*, 2005**). However, those patterns are not everything such as contrasts: black background are also essential for learning and conspicuousness (**Aronsson & Gamberale-Stille, 2012**). As an example, scientists are aware of the well-known "black & yellow stripes" pattern which has been studied and experimentally assessed before (**Kauppinen & Mappes, 2003**). Aposematic patterns are often copied by other species to perform mimicry but not every time. It also happens that species copy coloration patterns that do not really have purpose, which still remains visual mimicry. Mimicry can be of two types: Batesian mimicry stands for the imitation of the color pattern of a harmful species by a harmless species (**Marchini *et al.*, 2017**) whereas Mullerian mimicry stands for the same imitation but between two harmless species **(Reece *et al.*, 2012**). The benefits of these strategies are only reaped in the case of under abundance of it compared to non-signaling organisms (**Reece *et al.*, 2012**). These benefits will also go hand in hand with particularly memorable pattern such as aposematic colors, discussed above. Crypsis is a kind of mimicry in which species can imitate the colors, the structures of its environment in order to hide. This strategy must be extremely specific to local habitat and is then highly subject to polymorphism in diverse species (**Stuart-Fox & Moussalli, 2009**). Moreover, crypsis is a particular strategy because it can take place for defense mechanisms but also for attack, like in ambushing species (**Reece *et al.*, 2012**).

Those subjects provide some detailed studies about coloration research. Those studies are essential to know the effect of color in a particular context. A real diversity of aposematic, mimicry and cryptic pattern has evolved among all taxonomic groups. Within this diversity, those patterns can go under polymorphism whilst monomorphism is expected. Scientists should then reach a paradox situation for color evolution as an efficient strategy is not over-selected by all species. This deadlock comes from the fact that scientists too often think about color alone, on the effects of color on one parameter but do not place in the environmental context itself. For example, aposematic and mimicry patterns, although they interact with predation, can also be driven by different selective pressures depending on fitness or environmental conditions (**Briolat *et al.*, 2019; Stevens & Ruxton, 2012**). That is also what can explain the

relatively high abundance of red, yellow, and black colors in varied habitats as they provide recognizable pattern for color discrimination in species (**Stevens & Ruxton, 2012**).

III. Conservation biology

As color clearly affects survival and fitness of species, it can then hugely impact the evolution of those species as much as how those ecosystems work. In a world under the influence of climate change, understanding ecosystems functioning and responses to disturbances must not be understudied. Moreover, coloration patterns are also expected to vary through climate change and anthropogenic disturbances (**López-Idiáquez *et al.*, 2022**), as an evolutionary response or not, which can make coloration shifts good indicators of environmental disturbances in some species (**Koneru & Caro, 2022**). These shifts in coloration are not always induced so that sometimes anthropogenic disturbances can lead to coloration mismatch as in the case of seasonal camouflage. Those mismatches can be a real concern for the retention of local populations, especially in specifically adapted species like the Snowshoe hares *Lepus americanus* (**Zimova *et al.*, 2016**) or the Alpine Rock Ptarmigan *Lagopus muta* (**Imperio *et al.*, 2013**). New insights in coloration studies would then improve our understandings in how those colors are made and perceived to prevent this phenomenon from harming conservation biology (**Koneru & Caro, 2022**).

Those conservation issues can also settle in new urban area as direct consequence of human disturbance. Indeed, since urbanization, new conservation problems emerge such as birds-windows collision (**Klem & Saenger, 2013**) or polarized light pollution (PLP) (**Horváth *et al.*, 2009**). Those problems pave the way to technical innovations as more studies are focusing on them. In this perspective, the way how colors and ultraviolet (UV) patterns are made and perceived by birds can help company design UV-reflecting glass with UV signals pattern which can prevent bird strikes in urban area (**Klem & Saenger, 2013; Madliger *et al.*, 2016).** On the other side, polarized light pollution is known to create ecological trap mostly for aquatic insects. These situations are created by smooths darks surfaces like asphalts, cars, buildings which reflect and polarize light in the visible range (390-700 nm) as well as in the UV (< 380 nm) (**Fraleigh *et al.*, 2021**). Since water is the most important natural source of polarized light; foraging behavior, orientation and reproduction of aquatic insects can then be impacted by this new urban pollution (**Robertson & Horváth, 2018**). As in the previous problem, studies on perception and, in this case, how PLP are perceived by insects can clearly pave the way to design less-polarizing urban surfaces to manage those newly created evolutionary traps (**Caro *et al.*, 2017; Robertson & Horváth, 2018**). Thus, coloration studies can clearly advance

research in conservation, urban ecology and technical innovations related to them. Nevertheless, these are not the only issues that color studies can help solving. Indeed, a non-exhaustive list contains several examples like urban heat islands (**Qi *et al.*, 2019**), dynamic iridescent structure (**Luo *et al.*, 2019**), or a lot of pure innovations related to biomimetics (**Caro *et al.*, 2017**).

IV. Nothobranchius furzeri as a new biological model?

Nothobranchius furzeri is a cyprinodont fish considered as an emerging model for ageing (**Platzer & Englert, 2016**), quantitative genetics (**Cellerino *et al.*, 2016**) or ecotoxicological studies (**Thoré *et al.*, 2020**). Indeed, this fish has some particular characteristics, which are directly related to his living place. *N. furzeri* is naturally distributed in African freshwater ponds in an area spanning between Zimbabwe, Mozambique, and South Africa. The different populations of *N. furzeri* are distributed on a gradient of aridity and rainfall which are the two mains environmental factors that confront the species. As those factors are very unpredictable in this kind of climate, the habitat of *N. furzeri* then consist of small water ponds, isolated from one another, where the turbidity will vary depending on vegetation's abundance (**Cellerino *et al.*, 2016; Reichard & Polačik, 2019**). The unpredictability of habitat quality has then forced this species to develop strategies as it can be seen in its life history traits.

In fact, *N. furzeri* is now reported as the vertebrate with the shortest life span, ranging from three to six months for some lines, with a sexual maturity reached after three to five weeks. Moreover, the individuals do not stop reproducing from their sexual maturity until they die. During this period, the females lay about 50 eggs per day which might then be impregnated, or not, by males (**Cellerino *et al.*, 2016**). *N. furzeri* also has an opportunistic and generalist diet, which is also related to his natural habitat (**Reichard & Polačik, 2019**). This species, as well as the genus *Nothobranchius* in general, produces eggs capable of entering diapause (developmental dormancy) consecutively at three distinct stages of their embryogenesis (**Figure 7**). Diapause stage two can persist for several years while diapause stage three, which corresponds to the stage when the embryo is ready to hatch, can persist for several weeks. This strategy allows eggs to hatch in good conditions depending on environmental unpredictability.

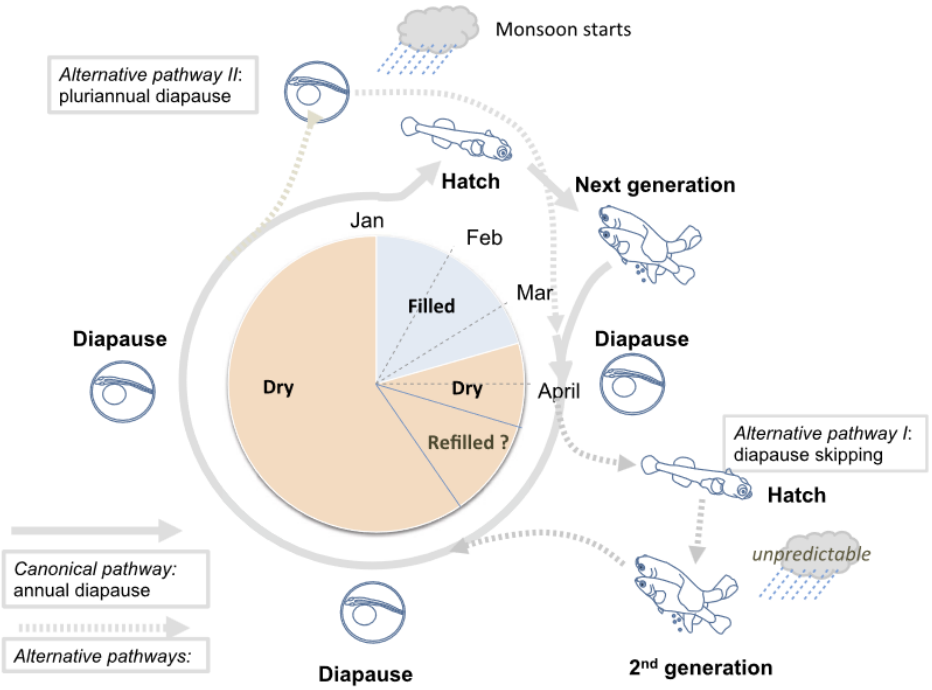


Figure 7. Life cycle of *N.furzeri* adapting to environmental conditions. As the habitat is only flood for three months, from January to March, this is the period when eggs hatch and juveniles rapidly reach sexual maturity to reproduce before extreme dry conditions come back. During that time, eggs laid by females can enter diapause to wait next preferable conditions. If the conditions do not come back soon, eggs can either die or choose to follow pluriannual diapause if timing is respected. In another case, if conditions are particularly good, eggs can skip diapause and hatch ahead of schedule (**Cellerino *et al.*, 2016**).

All these traits make *N. furzeri* an attractive and cost-effective biological model for multidisciplinary studies **(Reichwald *et al.*, 2015; Thoré *et al.*, 2020).** The parameters described above can easily be controlled in any laboratory to be replicated in any study. The best example of that is clearly the opportunity of diapause. Indeed, diapause stage two allows scientists to have a backup stock to be built up to ensure the continuity of the farm in the event of technical problems. Diapause stage three allows scientists to synchronize the hatching of eggs, even when they have not been collected at the same time (**Cellerino *et al.*, 2016**). Technical control of diapause is a crucial element to ensure efficient and reproducible rearing at different experimental designs.

Some of these specific parameters are straightforwardly related to emerging area in biology. It is obvious that the short lifespan of *N. furzeri* goes hand in hand with his importance in ageing research. Indeed, it will allow scientists to assess well-known criteria of ageing in vertebrates such as neoplasia or systemic failure (**Di Ciccio *et al.*, 2011**). Various mechanisms are also expected to decrease following the evolutionary theory of ageing like reproductive success

(**Reichard, 2017**). As age process will influence lot of different phenotypes, it would be interesting to study to what extent it may impact a particular phenotype. Following those interest, the genome of *N. furzeri* has been sequenced and annotated (**Reichwald *et al.*, 2015**), and then provide a good basis for genetic studies including those involving genome-engineering techniques as *Clustered Regularly Interspaced Short Palindromic Repeats* (CRISPR) (**Harel *et al.*, 2015**). Those advances reinforce the reasons why scientists should use this new model fish as well as insights in quantitative genetics (**Cellerino *et al.*, 2016; Platzer & Englert, 2016**). Perspectives on genome-wide studies might also be considered as they can represent a huge interest to complete our knowledge on coloration though (**San-Jose & Roulin, 2017**)

Coloration is not left out here as those patterns are universally available phenotype to be assessed for evolutionary studies (**Endler & Mappes, 2017; Cellerino *et al.*, 2016**). *N. furzeri* is indeed a highly sexually dimorphic species where pattern is strongly sexually selected (**Haas, 1976**). However, the origin of those colors is still blurry as the causal link between environmental conditions and coloration pattern has not been made so far (**Reichard & Polačik, 2019**). Nevertheless, depending on those environmental conditions, coloration pattern can impact sexual selection through mate choice (**Cellerino *et al.*, 2016**). In *N. furzeri*, as males are continuously ready to mate and fertilize eggs laid by females, those last can choose their males before laying eggs (**Cellerino *et al.*, 2016**). This is notably expressed by the female's approach to a displaying male (**Reichard & Polačik, 2019**). This choice might rely on vigorous courtship from males but also on sympatric interactions previously performed (**Reichard & Polačik, 2010**). As a response to that, novel study proposed *N. furzeri* as an emerging model for mate choice (**Johnson *et al.*, 2020**) as too little is known about coloration preferences in females (**Cellerino *et al.*, 2016**). That last point is the main part of this thesis as it will reinforce the use of *N. furzeri* in mate choice and sexual selection studies.

As seen earlier, coloration and behavior are strongly related phenotype in animals. In teleost, some coloration patterns have already been strongly linked to behaviors. Indeed, this type of analysis has already been conducted in the Honduran red point cichlid *Amatitlania siquia* (**Schweitzer *et al.*, 2015**), the blunthead cichlid *Tropheus sp*. (**Ziegelbecker *et al.*, 2021**) or even the guppy *Poecilia reticulata* in the context of mate choice (**Kodric-Brown, 1985**). The characterization of color alone has already been carried out in other species relatively close to our model, such as the American killifish *Millerichthys robustus* (**Domínguez-Castanedo *et al.*, 2021**) or the redtail notho *Nothobranchius guentheri* (**Nikiforov-Nikishin *et al.*, 2022**). However, based on available literature, no paper has yet described the link between coloration

patterns and real sexual behavior in *Nothobranchius furzeri*. Some of this work has already been done with 3D animations but still need to be confirmed with real fishes (**Johnson *et al.*, 2020**).

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Annexes

Annex I

The breeding of *Artemia salina* nauplii has been mastered in our lab for years. Nauplii are grown in a 5-liter aquarium filled with type II deionized water supplemented by Instant Ocean® sea salt (Spectrum Brands™, Blacksburg, VA, USA) until reached 25 ppt salinity.

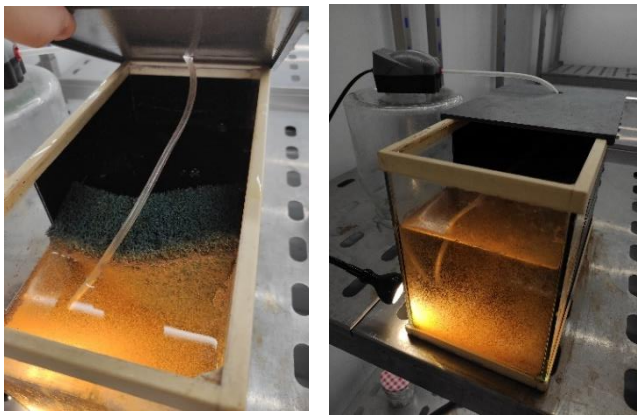


Figure I. Artemia's breeding tank. This tank is split in half by a foam allowing separation between unhatched cysts and nauplii. Cysts are put in the back of the foam with a bubbler 24 h before harvest. Nauplii are then vacuumed at the other side of the foam and flushed with distilled water before being used as food.

Annex II

Figure II. Community condition tank. Sandboxes were put to allow females to lay eggs and for males to assert dominance. Enrichment was provided by fake plants and a pipe.

Annex III

Figure III. Shelter test design. The arena consists of two elements: the shelter (A) and the circular open field (B), separated by a sliding door (C). This design is filled with system water which is changed between each test.

The test fish is introduced into the closed shelter for five minutes of acclimatization. After acclimatization, the sliding door is removed, and the fish is allowed access to the arena for 15 minutes test. Water was changed between each test.

Annex IV

Figure IV. Mate choice test design. The individuals are placed in a tank (20x30x15) filled with system water and divided into three compartments. The middle compartment is bigger to allow to make a choice between males. The two smaller compartments are for males and did not require too much space as we wanted female to correctly see males. Light was used under a cardboard to make sure that the light comes from below to facilitate video processing.

Between each replicate, the design has been turned around (180°) so that each male is not on the same side for the different females. Unlike Shelter test plan, water was not changed here between replicates in order to keep the same water conditions. Nevertheless, water has been changed when other males have been tested. After the test, fishes have been replaced in their original tank

Males and females have first been placed in an isolated compartment, without visual contact, in the tank for an acclimatization period of 10 minutes. After 10 minutes, eye contact has been re-established, and the test ran for 30 minutes.

Annex V

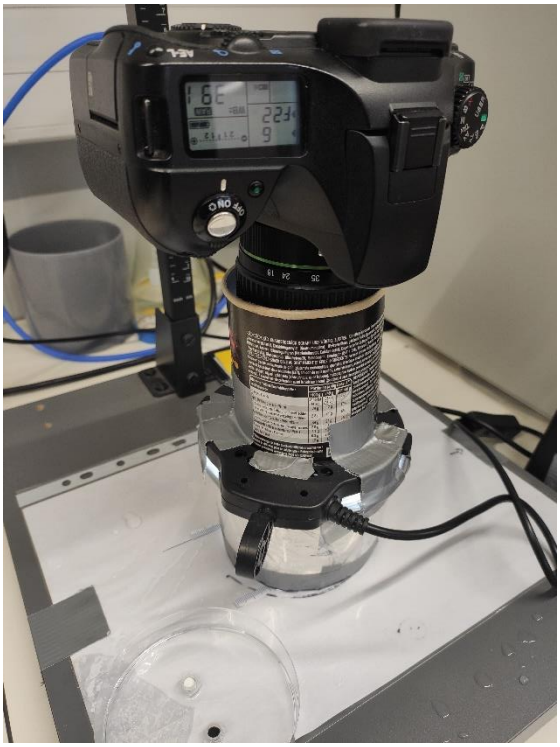


Figure V. First prototype of the Blackbox designed for this research.

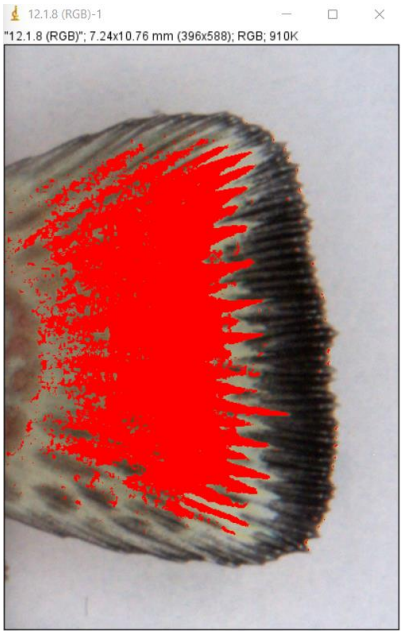
Annex VI

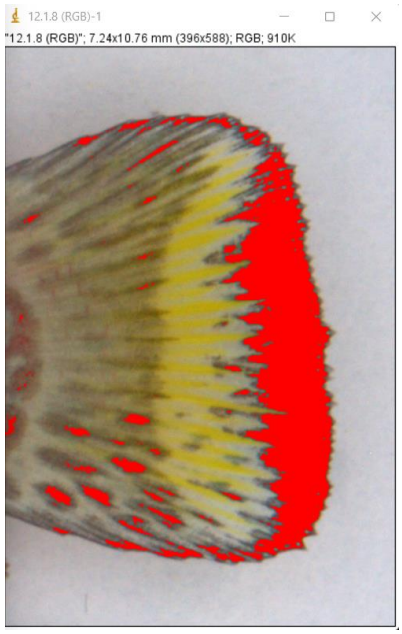
Figure VI. General overview of fish picture (above). The different selected patterns are described below: Body-related red (A), body-related blue (B), Yellow on the caudal fin (C), dark on the caudal fin (D), red on the dorsal fin (E), dark on the dorsal fin (F) and red on the anal fin (G).

A

B

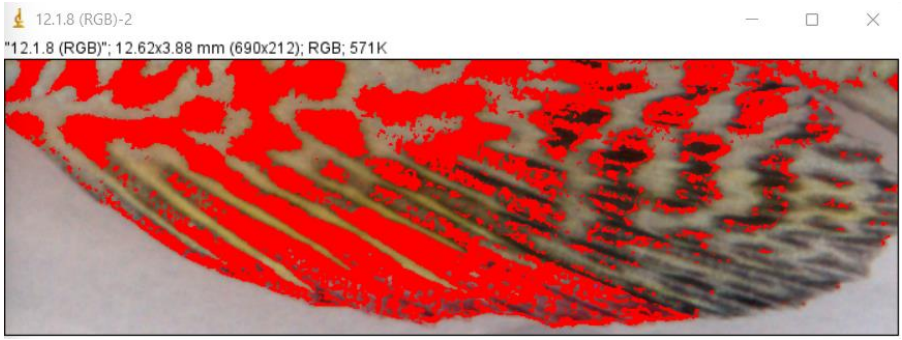
C


12.1.8 (RGB)-1
"12.1.8 (RGB)"; 7.24x10.76 mm (396x588); RGB; 910K

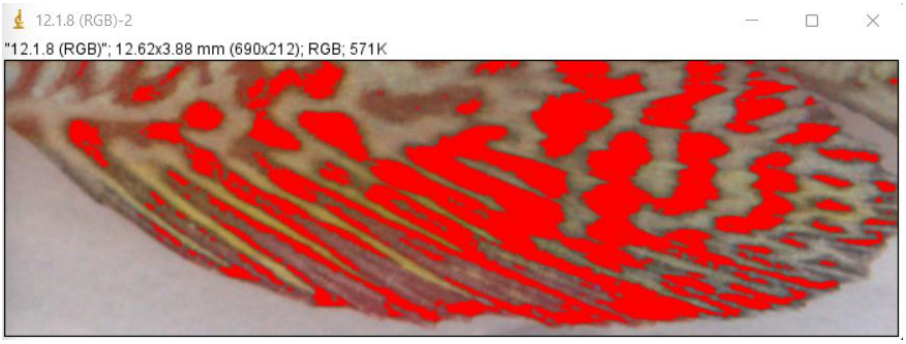

12.1.8 (RGB)-1
"12.1.8 (RGB)"; 7.24x10.76 mm (396x588); RGB; 910K

D

E


12.1.8 (RGB)-2
"12.1.8 (RGB)"; 12.62x3.88 mm (690x212); RGB; 571K

F


12.1.8 (RGB)-2
"12.1.8 (RGB)"; 12.62x3.88 mm (690x212); RGB; 571K

G


12.1.8 (RGB)-2
"12.1.8 (RGB)"; 10.12x4.24 mm (553x232); RGB; 501K

Annex VII

RGB color space

Color	Hue	Saturation	Brightness
Red	0-18	77-255 (30-100%)	77-255 (30-100%)
Blue	27-45	50-255 (20-100%)	77-255 (30-100%)
Yellow	115-170	77-255 (30-100%)	77-255 (30-100%)

YUV color space

	Luminosity=Y	Chroma component U	Chroma component Y
Black	0-80	0-255 (0-100%)	0-255 (0-100%)

Figure VII. Selected values for Hue, Saturation and brightness for each color. YUV color space was used for accurately extract black color.

Annex VIII

sRGB values are sometimes insufficient to compare chromaticity between samples. As sRGB coordinates provide point for each sample, the Euclidian distance (ΔE) between them could be calculated to assess the variability between individuals regarding chromaticity. However, RGB color space is not uniform so that distance between points cannot be compared even though they are at the same "visual" distance (**Mouchet & Deparis, 2021**). To avoid this, sRGB values are transformed into L*a*b* coordinates where L represents lightness and a/b are chromaticity coordinates (for red/green, and blue/yellow) in a uniform space. For more information on the mathematic transformation, it was explained by Mouchet & Deparis in their book *Natural Photonics & Bioinspiration* (**Mouchet & Deparis, 2021**).

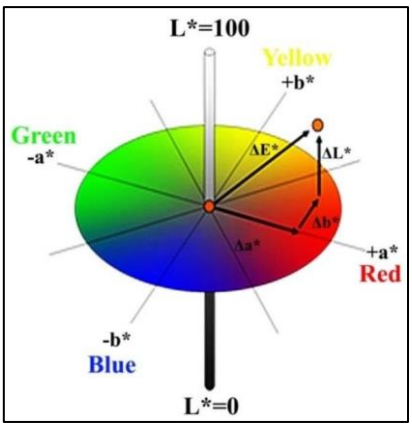


Figure VIII. L*a*b* color space representation. As a uniform color space, points can here be compared in terms of Lightness (ΔL) or chromaticity (Δa or Δb). Δ*E* here represents the distance between 2 points (**Nguyen *et al.*, 2018**).

Annex IX

In mathematics, a fractal is an object defined with a similar structure at any scale or resolution (**Halley *et al.*, 2004; Mandelbrot, 1983**). It is something naturally present in ecosystems, which has been the subject of much study recently. Fractals are used in biology to describe shapes and patterns where classic Euclidian geometry appears insufficient. Scientists can use fractals to calculate FD, which, following Mandelbrot's theory, "measure the object's ability to fill and Euclidian space *E* in which it is embedded" (**Halley *et al.*, 2004; Mandelbrot, 1983**).

It will then provide a numeric value to assess the shape and complexity of each selected object. FD is mainly included between 0, which is the dimension of a pixel alone, and 2, which represents a fully filled grid. The box counting method is a well-known technique to calculate FD. It calculates the number of boxes, from different sizes representing different scales, needed to cover the selected pixels (pattern). Once these values obtained, they are projected on a graph and a linear regression is calculated. The FD value is obtained by the slope of the line. FD were only calculated for small patterns here (Fins-related ones and body-related blue) as the box counting is not really adapted to wide-range pattern (**Halley *et al.*, 2004**).

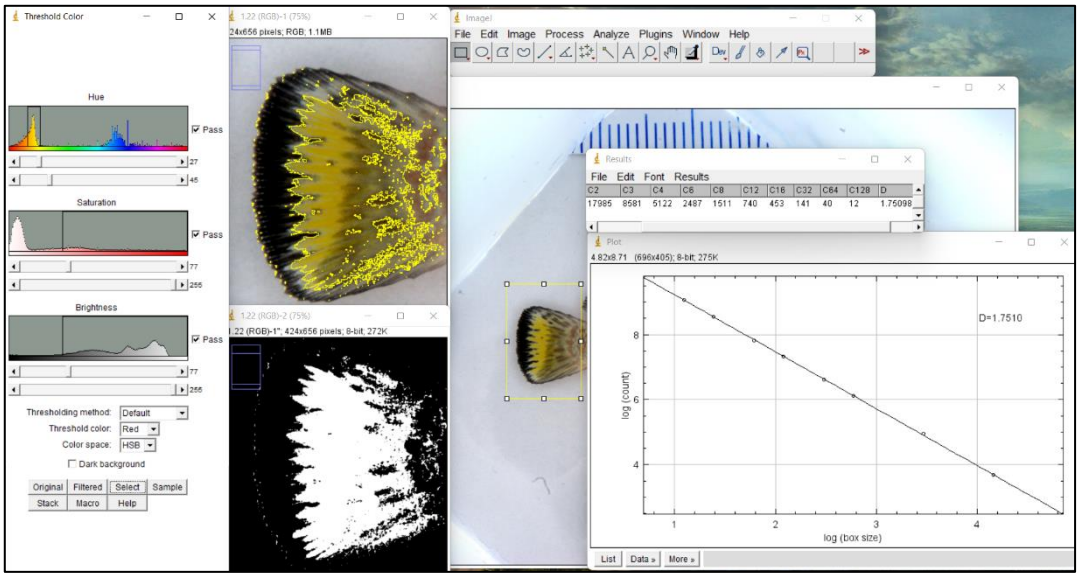


Figure IX. Here is an example on how that technique is applied to one of the selected patterns, the yellow on the caudal fin. Once the pattern selected (using "ColorThreshold" tool), the threshold color is changed for black & white, and the image is transformed to 8-bit. Box counting method is then applied for 2,3,4,6,8,12,16,32,64,128 size boxes and FD is automatically calculated. For more information on the mathematics background, it was fully described by Castrejón-Pita *et al.* in the framework of the investigation of butterfly wings (**Castrejón-Pita *et al.*, 2004**).

Annex X

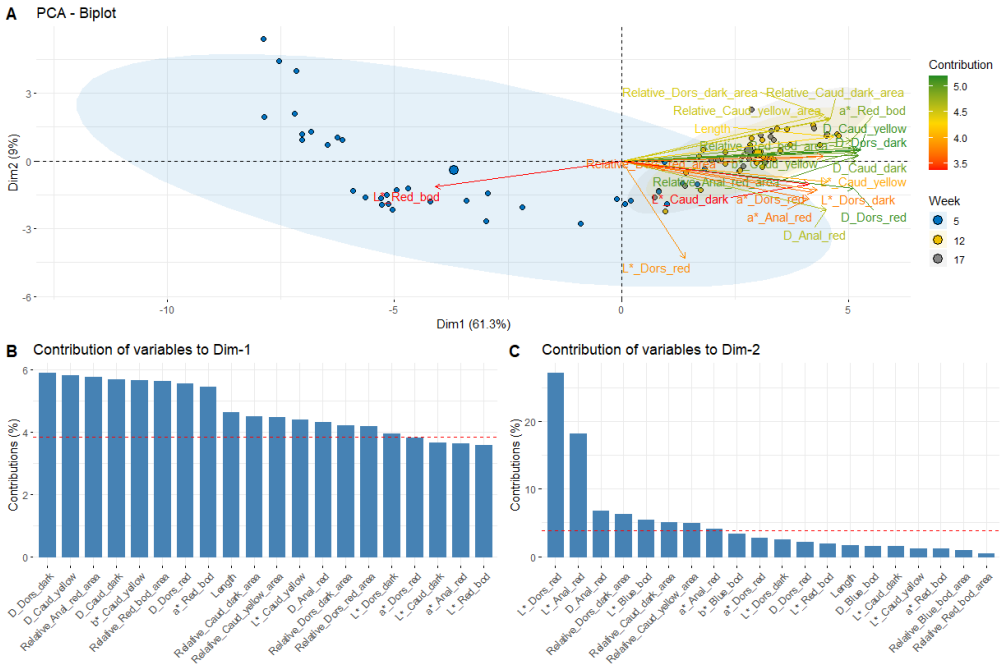


Figure X. Principal component analysis on all tested morphological variables.

Brut data might sometimes be complicated to understand. Here each of them are called by the variable followed or embedded by the pattern implicated. Our different patterns are: *Caud_yellow* and *Caud_dark* for caudal fin-related yellow and dark, *Dors_red* and *Dors_dark* for Dorsal fin-related red and dark, *anal_red* for Anal fin-related red. Thus *Relative_pattern_area*, *D_pattern*, *L*- a*- or b*_pattern* respectively represent the relative area, FD or L*a*b* value of the selected pattern.

Annex XI

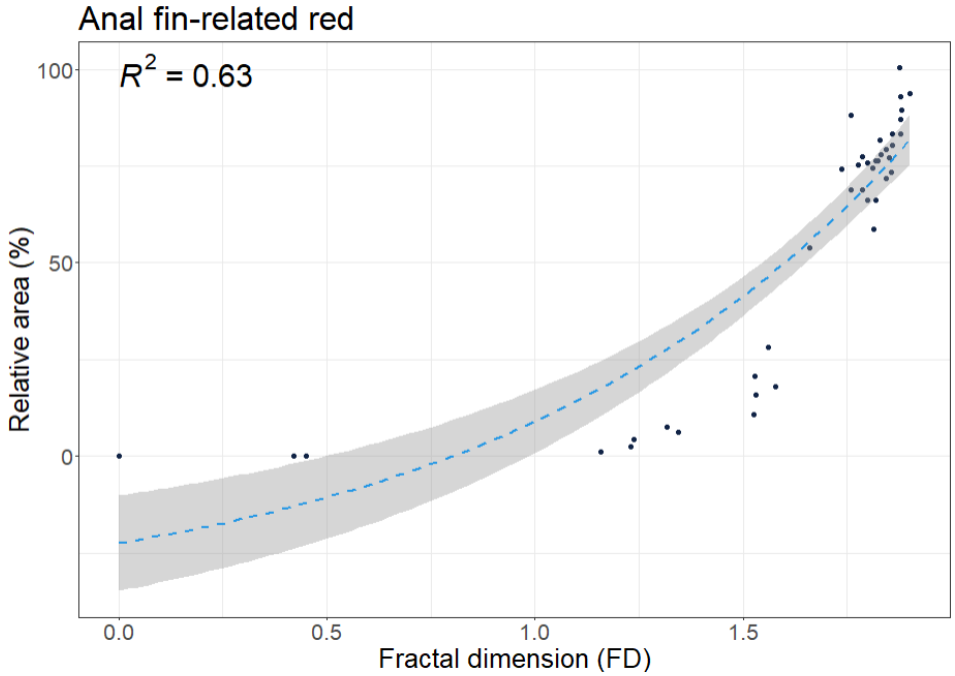


Figure XI. Exponential relation between FD and relative area which is conform to the calculus of FD as it relies on pixels count (Annex IX)

	Length	a*_Red_bod	L*_Caud_yellow	b*_Caud_yellow	L*_Caud_dark	a*_Dors_red	L*_Dors_dark	a*_Anal_red	Relative_Red_bod_area	Relative_Caud_yellow_area	Relative_Caud_dark_area	Relative_Dors_red_area	Relative_Dors_dark_area
Length	NA	6,97E-08	0,001049774	1,69E-06	0,00068118	1,33E-06	0,00071555	2,43E-08	1,98E-07	7,67E-11	5,63E-11	4,38E-07	2,09E-10
a*_Red_bod	6,97E-08	NA	0,005296578	4,93E-11	0,00040856	6,77E-11	0,01593817	0	3,64E-14	6,49E-12	1,33E-15	1,34E-08	7,99E-15
L*_Caud_yellow	0,0010498	0,0052966	NA	0,000203321	0,00065098	0,0063873	3,19E-09	0,0103189	0,002470586	0,002270571	0,029032012	1,21E-05	0,037691926
b*_Caud_yellow	1,69E-06	4,93E-11	0,000203321	NA	0,00095856	3,00E-06	0,0056272	4,15E-09	1,77E-09	8,88E-15	2,61E-11	1,17E-05	1,37E-10
L*_Caud_dark	0,0006812	0,0004086	0,000650979	0,000958564	NA	0,0003904	0,00026275	0,0020327	0,001085749	0,001839953	2,16E-05	0,003069976	0,000112825
a*_Dors_red	1,33E-06	6,77E-11	0,006387281	3,00E-06	0,00039043	NA	0,01093737	5,69E-09	1,20E-10	6,20E-07	2,19E-08	8,98E-09	1,60E-08
L*_Dors_dark	0,0007156	0,0159382	3,19E-09	0,005627203	0,00026275	0,0109374	NA	0,0300093	0,006205244	0,007895058	0,035317054	9,25E-06	0,035292136
a*_Anal_red	2,43E-08	0	0,010318921	4,15E-09	0,00203268	5,69E-09	0,03000929	NA	3,46E-10	1,16E-11	2,32E-13	7,34E-07	3,55E-13
Relative_Red_bod_area	1,98E-07	3,64E-14	0,002470586	1,77E-09	0,00108575	1,20E-10	0,00620524	3,46E-10	NA	1,85E-10	3,53E-10	1,01E-13	3,82E-11
Relative_Caud_yellow_area	7,67E-11	6,49E-12	0,002270571	8,88E-15	0,00183995	6,20E-07	0,00789506	1,16E-11	1,85E-10	NA	3,20E-14	1,14E-06	8,08E-14
Relative_Caud_dark_area	5,63E-11	1,33E-15	0,029032012	2,61E-11	2,16E-05	2,19E-08	0,03531705	2,32E-13	3,53E-10	3,20E-14	NA	6,92E-06	0
Relative_Dors_red_area	4,38E-07	1,34E-08	1,21E-05	1,17E-05	0,00306998	8,98E-09	9,25E-06	7,34E-07	1,01E-13	1,14E-06	6,92E-06	NA	1,19E-06
Relative_Dors_dark_area	2,09E-10	7,99E-15	0,037691926	1,37E-10	0,00011283	1,60E-08	0,03529214	3,55E-13	3,82E-11	8,08E-14	0	1,19E-06	NA
Relative_Anal_red_area	1,81E-08	2,22E-15	0,005475816	3,58E-08	0,00050356	4,51E-09	0,00919555	1,73E-12	1,07E-13	4,43E-10	3,36E-12	1,10E-10	3,21E-13
D_Caud_yellow	5,01E-11	6,17E-12	0,000507179	1,70E-11	0,00622391	3,69E-07	0,00263166	2,56E-12	1,04E-10	0	3,05E-11	4,92E-08	1,35E-11
D_Caud_dark	1,79E-09	5,98E-13	0,024518538	2,16E-09	5,43E-06	1,40E-07	0,04733351	2,20E-11	1,24E-08	1,07E-10	0	1,52E-05	0
D_Dors_red	2,70E-09	5,17E-09	4,52E-06	1,36E-06	0,01441905	2,10E-10	2,50E-06	8,87E-08	3,29E-12	1,11E-08	1,41E-06	0	5,40E-07
D_Dors_dark	1,55E-09	0	0,052933942	4,78E-10	6,21E-05	2,67E-08	0,03482758	1,09E-14	1,68E-11	3,67E-13	0	2,63E-07	0
D_Anal_red	1,31E-09	1,85E-09	0,001901045	1,59E-06	0,00168526	3,28E-07	0,00378666	1,30E-08	8,63E-10	1,63E-08	4,09E-10	5,30E-09	1,82E-12
Relative distance moved	1,75E-06	0,0003813	0,006845056	0,000258339	0,02896928	0,0263226	0,00097271	0,0005927	0,001590506	3,86E-05	0,000176858	0,000984066	0,000236544
Time_arena	0,1595014	0,025801	0,94173126	0,051396653	0,91565083	0,0260732	0,59739916	0,048398	0,055521554	0,018250972	0,073350555	0,055234091	0,080691281
Latency_arena	0,1418864	0,7088246	0,727941524	0,8879929	0,25971106	0,2139208	0,46395909	0,6877299	0,629894504	0,730929548	0,31982926	0,951297943	0,467634187
Time_female	0,5594654	0,8362562	0,699289195	0,961912387	0,37427576	0,9619352	0,97045836	0,6309922	0,814534098	0,773621576	0,986046385	0,656662663	0,918490819
Time_court	0,0489059	0,2276834	0,909985077	0,613812449	0,19954394	0,1348955	0,76839604	0,2020362	0,164890048	0,08322803	0,358573736	0,210059912	0,300680534

Relative_Anal_red_area	D_Caud_yellow	D_Caud_dark	D_Dors_red	D_Dors_dark	D_Anal_red	Relative distance moved	Time_arena	Latency_arena	Time_female	Time_court
1,81E-08	5,01E-11	1,79E-09	2,70E-09	1,55E-09	1,31E-09	1,75E-06	0,1595014	0,141886355	0,55946545	0,0489059
2,22E-15	6,17E-12	5,98E-13	5,17E-09	0	1,85E-09	0,000381313	0,025801	0,708824601	0,8362562	0,2276834
0,005475816	0,000507179	0,02451854	4,52E-06	0,0529339	0,001901	0,006845056	0,9417313	0,727941524	0,6992892	0,9099851
3,58E-08	1,70E-11	2,16E-09	1,36E-06	4,78E-10	1,59E-06	0,000258339	0,0513967	0,8879929	0,96191239	0,6138124
0,000503559	0,006223915	5,43E-06	0,014419	6,21E-05	0,0016853	0,028969277	0,9156508	0,259711057	0,37427576	0,1995439
4,51E-09	3,69E-07	1,40E-07	2,10E-10	2,67E-08	3,28E-07	0,026322563	0,0260732	0,213920756	0,96193524	0,1348955
0,009195548	0,002631664	0,04733351	2,50E-06	0,0348276	0,0037867	0,000972708	0,5973992	0,46395909	0,97045836	0,768396
1,73E-12	2,56E-12	2,20E-11	8,87E-08	1,09E-14	1,30E-08	0,000592654	0,048398	0,687729882	0,63099217	0,2020362
1,07E-13	1,04E-10	1,24E-08	3,29E-12	1,68E-11	8,63E-10	0,001590506	0,0555216	0,629894504	0,8145341	0,16489
4,43E-10	0	1,07E-10	1,11E-08	3,67E-13	1,63E-08	3,86E-05	0,018251	0,730929548	0,77362158	0,083228
3,36E-12	3,05E-11	0	1,41E-06	0	4,09E-10	0,000176858	0,0733506	0,31982926	0,98604639	0,3585737
1,10E-10	4,92E-08	1,52E-05	0	2,63E-07	5,30E-09	0,000984066	0,0552341	0,951297943	0,65666266	0,2100599
3,21E-13	1,35E-11	0	5,40E-07	0	1,82E-12	0,000236544	0,0806913	0,467634187	0,91849082	0,3006805
NA	2,98E-10	7,00E-12	9,49E-10	5,02E-14	0	0,000222339	0,2102066	0,318050184	0,69750272	0,1035897
2,98E-10	NA	6,60E-09	1,39E-10	1,38E-11	1,24E-08	2,03E-05	0,0234017	0,707878378	0,71819216	0,0315167
7,00E-12	6,60E-09	NA	1,63E-05	0	3,73E-10	0,000132155	0,1875224	0,147958637	0,87184569	0,3947421
9,49E-10	1,39E-10	1,63E-05	NA	4,19E-07	2,55E-08	0,000100531	0,0164523	0,707754877	0,9550209	0,0655695
5,02E-14	1,38E-11	0	4,19E-07	NA	1,07E-10	3,07E-05	0,037087	0,616644269	0,95414695	0,2723431
0	1,24E-08	3,73E-10	2,55E-08	1,07E-10	NA	0,000444673	0,50354	0,229142893	0,75094119	0,0723009
0,000222339	2,03E-05	0,00013215	0,0001005	3,07E-05	0,0004447	NA	0,2280642	0,085991466	0,33716358	0,1365694
0,210206624	0,023401671	0,18752245	0,0164523	0,037087	0,50354	0,228064228	NA	0,204695476	0,81752249	0,9448312
0,318050184	0,707878378	0,14795864	0,7077549	0,6166443	0,2291429	0,085991466	0,2046955	NA	0,98634462	0,3363096
0,697502719	0,718192158	0,87184569	0,9550209	0,954147	0,7509412	0,337163583	0,8175225	0,986344618	NA	0,041782
0,103589668	0,031516741	0,39474214	0,0655695	0,2723431	0,0723009	0,136569431	0,9448312	0,336309645	0,04178205	NA

Table I. Table of p-values extracted from Spearman's correlation matrix. Significative results are presented here as green where the red ones represent non significative values.

Annex XIII

b*_Caud_yellow				
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.21	-0.49 – 0.07	1.418e-01	75.00
Cohort2	-0.84 ***	-1.19 – -0.48	**9.949e-06**	75.00
Week12	1.21 ***	0.78 – 1.63	**2.709e-07**	75.00
Week12:Cohort2	0.58 *	0.05 – 1.10	**3.164e-02**	75.00
Week17	0.44	-0.06 – 0.94	8.513e-02	75.00
Week17:Cohort2	0.98 **	0.35 – 1.62	**2.991e-03**	75.00
Observations	81			
R^2 / R^2 adjusted	0.713 / 0.694			
AICc	129.417			

** p<0.05 ** p<0.01 *** p<0.001*

Kruskal-Wallis; p-value <0.0001

L*_Dors_dark				
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	0.36	-0.01 – 0.73	5.377e-02	78.00
Cohort2	-0.77 ***	-1.16 – -0.39	**1.419e-04**	78.00
Community	*Reference*			
Isolated	0.38 *	0.01 – 0.75	**4.663e-02**	78.00
Observations	81			
R^2 / R^2 adjusted	0.230 / 0.210			
AICc	203.680			

** p<0.05 ** p<0.01 *** p<0.001*

Kruskal-Wallis; p-value = 0.03

a*_Anal_red				
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	0.04	-0.35 – 0.44	8.212e-01	75.00
Cohort2	-1.11 ***	-1.61 – -0.62	**2.466e-05**	75.00
Week12	0.71 *	0.11 – 1.30	**2.117e-02**	75.00
Week12:Cohort2	0.79 *	0.05 – 1.53	**3.595e-02**	75.00
Week17	0.27	-0.44 – 0.97	4.518e-01	75.00
Week17:Cohort2	1.49 **	0.59 – 2.39	**1.429e-03**	75.00
Observations	81			
R^2 / R^2 adjusted	0.515 / 0.482			
AICc	184.488			

** p<0.05 ** p<0.01 *** p<0.001*

Relative_Caud_yellow_area				
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.29 *	-0.58 – -0.00	**4.984e-02**	74.00
Cohort2	-0.67 ***	-1.00 – -0.34	**1.334e-04**	74.00
Concentration100	-0.14	-0.35 – 0.08	2.063e-01	74.00
Week12	1.04 ***	0.64 – 1.44	**1.998e-06**	74.00
Week12:Cohort2	0.86 **	0.36 – 1.35	**1.005e-03**	74.00
Week17	0.81 **	0.34 – 1.28	**1.061e-03**	74.00
Week17:Cohort2	0.77 *	0.16 – 1.37	**1.375e-02**	74.00
Observations	81			
R^2 / R^2 adjusted	0.748 / 0.728			
AICc	121.416			

** p<0.05 ** p<0.01 *** p<0.001*

Kruskal-Wallis; p-value <0.0001

Relative_Dors_dark_area				
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.18	-0.48 – 0.12	2.401e-01	74.00
Cohort2	-0.69 ***	-1.04 – -0.34	**1.765e-04**	74.00
Concentration100	-0.29 *	-0.51 – -0.06	**1.335e-02**	74.00
Week12	1.04 ***	0.62 – 1.46	**5.094e-06**	74.00
Week12:Cohort2	0.73 **	0.20 – 1.25	**7.072e-03**	74.00
Week17	0.87 ***	0.37 – 1.36	**8.503e-04**	74.00
Week17:Cohort2	0.81 *	0.17 – 1.44	**1.362e-02**	74.00
Observations	81			
R^2 / R^2 adjusted	0.727 / 0.705			
AICc	129.265			

** p<0.05 ** p<0.01 *** p<0.001*

Kruskal-Wallis; p-value <0.0001

	Relative_Anal_red_area			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.30	-0.63 – 0.03	7.559e-02	72.00
Cohort2	-0.98 ***	-1.33 – -0.63	**3.810e-07**	72.00
Community	*Reference*			
Isolated	0.31	-0.03 – 0.65	7.102e-02	72.00
Week12	1.80 ***	1.31 – 2.30	**3.743e-10**	72.00
Week12:Cohort2	0.40	-0.12 – 0.93	1.326e-01	72.00
Week12:ConditionIsolated	-1.36 ***	-1.86 – -0.85	**1.041e-06**	72.00
Week17	1.13 ***	0.50 – 1.77	**6.482e-04**	72.00
Week17:Cohort2	0.93 **	0.29 – 1.57	**5.277e-03**	72.00
Week17:ConditionIsolated	-0.85 **	-1.48 – -0.22	**8.891e-03**	72.00
Observations	81			
R^2 / R^2 adjusted	0.770 / 0.744			
AICc	131.655			

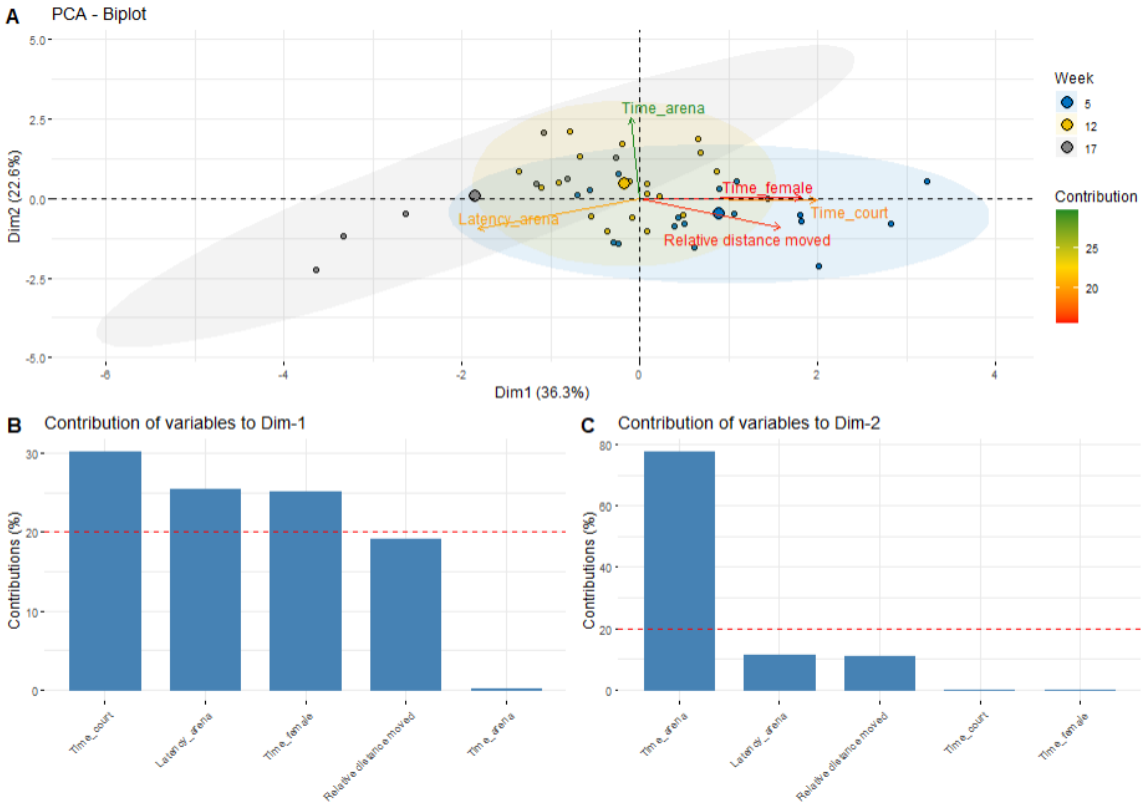
*p<0.05 **p<0.01 ***p<0.001

	Length			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.23	-0.50 – 0.03	8.080e-02	71.00
Cohort2	-0.69 ***	-1.00 – -0.37	**4.305e-05**	71.00
Concentration100	-0.21	-0.54 – 0.11	1.920e-01	71.00
Concentration100:Cohort2	-0.22	-0.56 – 0.11	1.915e-01	71.00
Week12	0.69 ***	0.33 – 1.06	**3.259e-04**	71.00
Week12:Cohort2	0.58 **	0.21 – 0.95	**2.855e-03**	71.00
Week12:Concentration100	0.32	-0.04 – 0.68	8.160e-02	71.00
Week17	2.04 ***	1.58 – 2.49	**3.425e-13**	71.00
Week17:Cohort2	0.12	-0.34 – 0.58	6.056e-01	71.00
Week17:Concentration100	0.19	-0.26 – 0.65	3.952e-01	71.00
Observations	81			
R^2 / R^2 adjusted	0.885 / 0.870			
AICc	79.473			

*p<0.05 **p<0.01 ***p<0.001

Table II. Linear models results for pattern variables. Cohort effect has been add for all our models as it represents significant and non-explainable variability. Kruskal-Wallis p-value are relative to the table above them.

Annex XIV



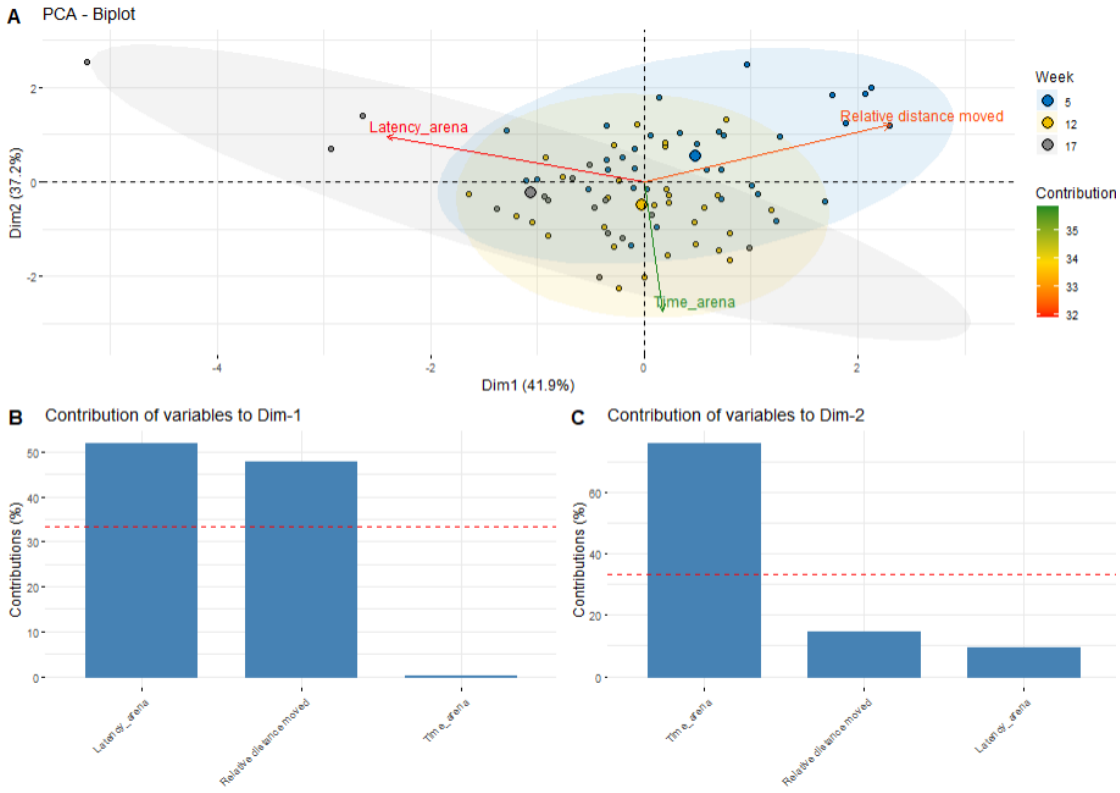


Figure XII. Principal component analysis of behavioral data. The first one is for all the data (only for community males) and the second one is for shelter test data (on all males).

Brut data:

- Time_female represents the mean time spent by females in front of each male during mate choice test
- Time_court represent the time spent by each male courting female during mate choice test
- Time_arena is the total spent in the arena during shelter test
- Relative distance moved is the distance moved in the arena reported to time spent in arena and the size of the fish.
- Latency_arena is the time before the first appearance of fish in the arena

Annex XV

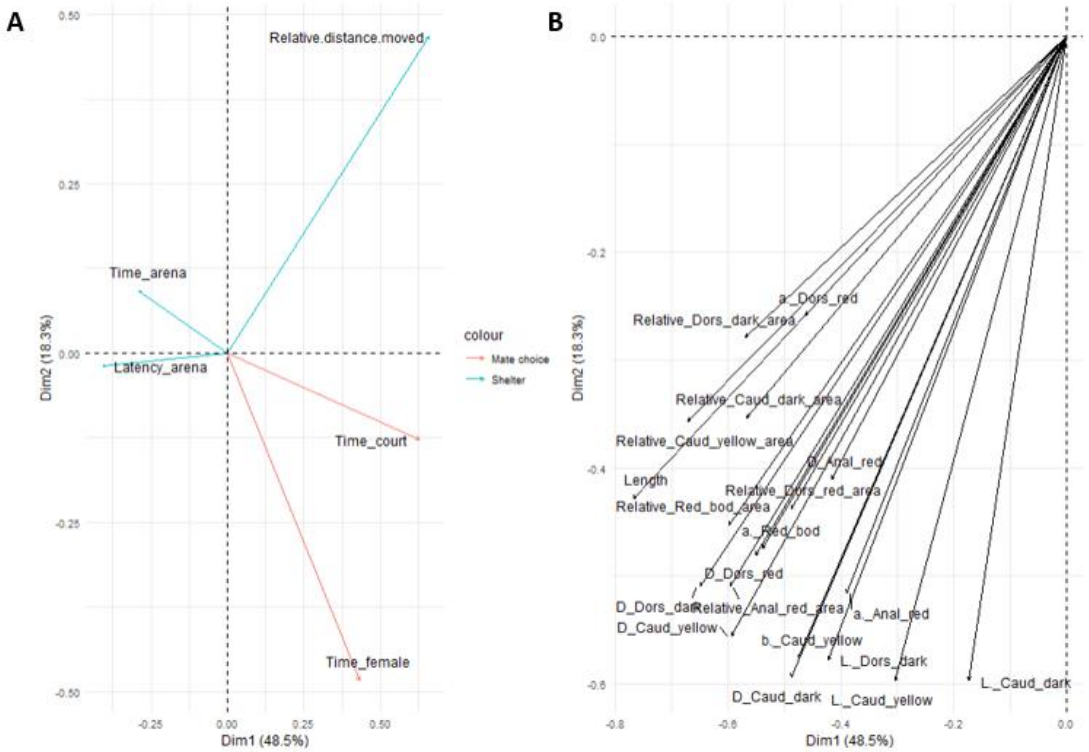


Figure XIII. Redundant analysis (RDA) on behavioral and color data. (A) Projection of the principal component analysis results for behaviors on new axis depending on color data; (B) Color data projected on RDA axis. Anti-correlation between color variables and relative distance moved is highlighted here.

Annex XVI

	Time_female			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	-4.95 ***	-7.70 – -2.21	**7.527e-04**	40.00
Relative_anal_area	0.33 *	0.08 – 0.57	**1.032e-02**	40.00
Time_court	0.00 **	0.00 – 0.00	**4.278e-03**	40.00
Observations	43			
R^2 / R^2 adjusted	0.268 / 0.231			
AICc	116.421			

** p<0.05 ** p<0.01 *** p<0.001*

	Time_court			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	1140.09 ***	956.34 – 1323.84	**2.846e-15**	39.00
L*_Dors_dark	5.89 *	0.49 – 11.29	**3.342e-02**	39.00
Length	-12.05 **	-18.91 – -5.19	**1.019e-03**	39.00
Time_female	56.03 *	10.34 – 101.71	**1.753e-02**	39.00
Observations	43			
R^2 / R^2 adjusted	0.348 / 0.298			
AICc	557.571			

** p<0.05 ** p<0.01 *** p<0.001*

	Time_arena			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	648.63 ***	610.15 – 687.12	**2.487e-47**	76.00
Community	*Reference*			
Isolated	46.83 *	3.92 – 89.73	**3.283e-02**	76.00
Relative_Caud_yellow_area	1.84 **	0.71 – 2.97	**1.797e-03**	76.00
Observations	79			
R^2 / R^2 adjusted	0.160 / 0.137			
AICc	949.503			

** p<0.05 ** p<0.01 *** p<0.001*

	Relative distance movedbis			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	1.65 ***	0.96 – 2.34	**9.720e-06**	77.00
Length	-0.05 ***	-0.07 – -0.03	**4.469e-06**	77.00
Observations	79			
R^2 / R^2 adjusted	0.241 / 0.231			
AICc	207.501			

** p<0.05 ** p<0.01 *** p<0.001*

Figure XIV. Results of linear models concerning behavioral data. Latency time is not displayed here as no significant results emerge from Kruskal-Wallis test.

Annex XVII

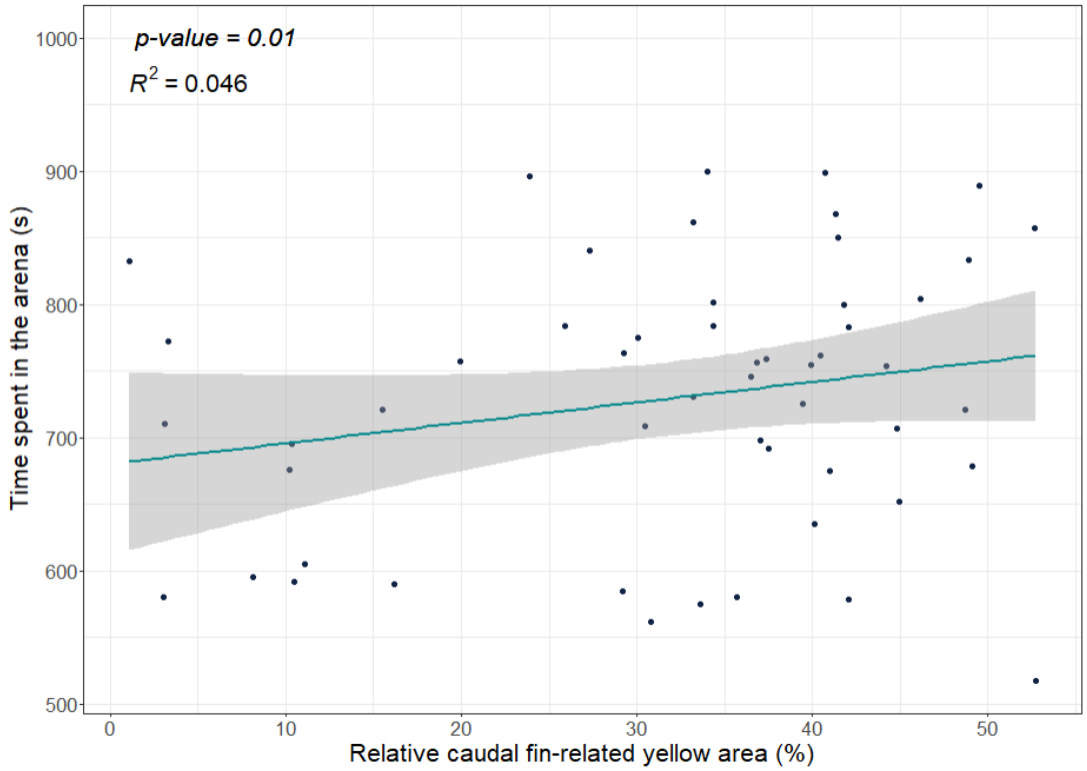


Figure XV. Linear regression of explorative behavior by the relative area of caudal fin-related yellow pattern.