

The interaction of innate and imposed colour perception: a behavioural approach

Ana C. Luchiari · Jessica J. Oliveira

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Abstract Nile tilapia were raised from eggs to 2 months of age under a coloured light regime (violet, blue, green, yellow, and red) and then tested for colour preference in a multiple chamber maze with different colour options. Fish were observed individually during three days at 8, 11, 14, and 17 h, every 2 min for 20 min and the visit frequency in each compartment was analyzed. Young Nile tilapia kept under yellow and red light showed preference for yellow and red, respectively. Fish held under violet, blue, and green light did not show any colour preference or avoidance. These results imply that environmental colour affects colour preference of Nile tilapia, possibly due to light-dependent shift of visual pigments in the retina, indicating that colour preference is not an innate response. This conclusion reinforces the idea that environmental colour modulates fish physiological and behavioural processes.

Keywords Fish · Vision · Colour · Preference · Development

Introduction

Colour perception is a helpful feature for animals to discriminate details in the environment. It is supported by cone cells distributed in the retina and behavioural responses suggesting colour discrimination (Spence and Smith 2008; Knott et al. 2013). Atypical light environments during the early post-natal phase can severely

change the formation and function of the visual system, impairing colour discrimination or even inducing biased colour vision (Kröger et al. 2003; Spence and Smith 2008).

Fish can see colours such as blue, green, or near infrared, and proper colour vision seems to play an important role during the transition to exogenous feeding since most fishes are sight feeders. Some studies suggest the developmental programming of spectral processing and colour vision is genetically determined (Roorda and Williams 1999; Hofer et al. 2005). However, fish spectral sensitivity seems to be influenced by the environmental light spectrum from where they develop (Bejarano-Escobar et al. 2012). There is evidence that rearing fish under different visual environments induces differential neural plasticity in the retina and also changes the optomotor response to chromatic stimuli (Kröger et al. 2003).

While there are many studies regarding the genetic and developmental basis of colour vision in animals, processing wavelength information is still ambiguous in many aspects. Thus, if behavioural patterns are modulated during development, colour preference (choice for a place that confers a better visual perception, acuity) would depend on the light wavelength animals are exposed to during the first days of life. It is known that Nile tilapia cone opsins code for distinct visual pigments, and juveniles express seven different genes while only four genes are expressed in adults (Spady et al. 2006). However, there are no studies describing behavioural changes in colour perception during this period. Additionally, as far as is known, there is no evaluation of behavioural colour preference during the early life stages of Nile tilapia.

Besides the ontogenetic plasticity of the spectral transmittance of visual pigments and cellular organization, some influence from the nature of the post-natal lighting environment might be expected. In a previous study, we

A. C. Luchiari (✉) · J. J. Oliveira
Departamento de Fisiologia, Centro de Biociências,
Universidade Federal do Rio Grande do Norte,
1510, Natal 59078-970, Rio Grande do Norte, Brazil
e-mail: analuchiari@yahoo.com.br

reported that naïve juvenile Nile tilapia prefer yellow, based on multiple-colour choice tests (Luchiari et al. 2007). Here we used a behavioral approach to investigate changes in innate colour preference in Nile tilapia by rearing fish from eggs to two months old under a specific coloured light and testing their preference in a multiple-colour choice test.

Materials and methods

Housing parents and young

Adult Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) housed for about one year in an indoor 1200 L tank (ca. 1 fish/6 L) were used as our stock population for reproduction. During the housing period, temperature averaged 26 ± 1 °C. Oxygen-saturated water with low levels of ammonia (<0.5 ppm) and nitrite (<0.05 ppm) was provided. The photoperiod was set from 0600 to 1800 hours (c.100 lx at water surface). Food was offered in excess (>5 % of fish biomass, Fri Aqua 46 PB, composition according to manufacturer: protein 46 %, fat 26 % and energy 25.8 MJ/kg, FriRibe®, Brazil) once a day.

Groups of two adult males and three adult females *Oreochromis niloticus* (16 ± 2.7 cm) were held in $60 \times 60 \times 40$ cm tanks with a 2 cm gravel layer to be used for nest building for reproduction. Water quality in the reproduction tank was maintained by a biofilter; an air stone supplied oxygen, and a thermostat maintained water temperature at 28 °C. The photoperiod was set as 12L:12D. Food was offered once a day ad libitum. Observation of the fish groups was done four times per day and fish were kept in these conditions up to mouthbrooding.

Five different couples (male + female Nile tilapia) from the groups described above supplied eggs for the tests. Immediately after one of the females had incubated eggs, we took her out of the water and removed all eggs from her mouth. For each brood, the amount of eggs was distributed among the coloured incubators (violet, blue, yellow, green, and red) and observed until all yolk had been absorbed. After this period, the larvae were transferred to coloured housing tanks (same colour as the incubator) and fed brine shrimp and larvae flakes until the age of two months. At least 30 larvae grew under each environmental colour condition. During this period water quality was maintained as in the breeding tanks.

Different environmental light colours were randomly chosen for each incubator and housing tank; colour was achieved by covering the tank with layers of gelatin filter (LeeFilters, Hampshire, England). Each coloured gelatin sheet was chosen by the light wavelength it offered when illuminated by fluorescent tubes (Osram 1900, T8W30,

1900 lumen, Osram, Osasco/SP Brazil): violet (Violet #344, $Y = 19.85$ %, $\lambda_{\max} = 424.8$ nm), blue (Lagoon blue #172, $Y = 25.36$ %, $\lambda_{\max} = 435$ nm), green (Jade #323, $Y = 32.00$ %, $\lambda_{\max} = 534$ nm), yellow (Yellow #101, $Y = 80.00$ %, $\lambda_{\max} = 546$ nm), and red (Sunset red #025, $Y = 26.37$ %, $\lambda_{\max} = 610$ nm). Information about the filters are name and number according to LeeFilters (www.leefilters.com). Y indicates transmission, and λ_{\max} is the maximum absorbance. Light intensity was set at c. 60 lx by adding layers of the same gelatin filter; the number of gelatin layers had no effect on the wavelengths mentioned above.

Behavioural preference test

For the colour preference tests, five transparent plastic tanks of 30 cm diameter were divided into five lateral compartments of similar size with a hole in the central region to allow the fish to move between compartments. Each compartment (central or lateral) had an identical area and was equally illuminated by white fluorescent light (Osram 1900, T8W30, 1900 lumen, Osram, Osasco/SP Brazil). Water depth was 10 cm and one air stone supplied oxygen in the middle of each tank. To provide different colours, a layer of the same gelatin paper used for the housing phase was chosen randomly for each compartment of the testing tanks; no paper was used on the central area. The gelatin filters were placed on the top and around the tank and light intensity was measured at the water surface using a luxmeter (Luxmeter Instrutherm LD-240; São Paulo-SP/Brazil). Light intensity was set at 60 lx for the compartments and 200 lx for the central area (not covered), preventing fish from staying there.

Colour preference of individual fish was observed for a period of 3 days. Eight 2-month-old Nile tilapia from each colour-raised group were tested for colour preference. Each fish was initially placed in a transparent tube in the middle of the tank for 10 min (1 fish per tank). After this period, the tube was removed and the “visit frequency” at each different compartment was observed. Visit frequency was directly observed throughout 3 days by checking the fish location every 2 min for 20-minute periods at 0800, 1100, 1400 and 1700 hours, totaling 40 observations per day. Food was not offered during the experimental days in order to prevent the fish from choosing one specific compartment due to a driving force/stimulus other than light colour. A starvation period of three days is not long enough to affect fish survival or well-being (Luchiari and Pirhonen 2008).

For statistical analysis, the non-parametric procedure of Friedman ANOVA was used for multiple group analyses, since all attempts to obtain a normal distribution had failed. We also used the Friedman test because fish preference for one compartment over others provides dependent data. In

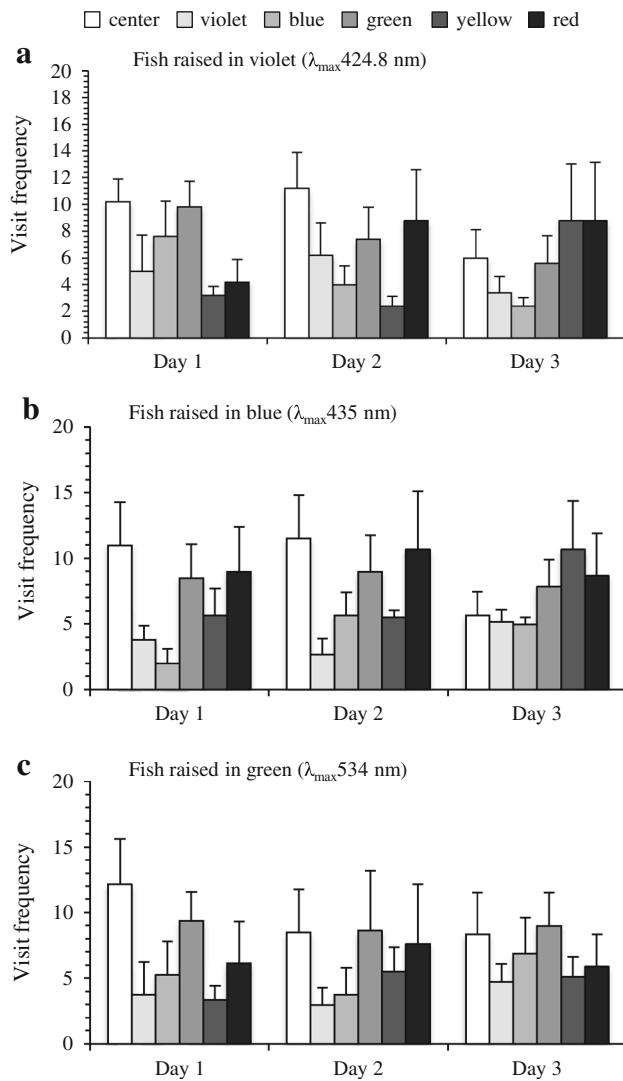


Fig. 1 Colour preference of juvenile Nile tilapia reared from eggs until two months old under **a** violet colour light, **b** blue colour light, and **c** green colour light. Bars represent the mean \pm SE visit frequency of 20-minute observation periods at 0800, 1100, 1400 and 1700 hours over the 3-day period (in total 120 observations per individual; $n = 8$). Gelatin Lee filters provided colour: violet (Violet #344, $Y = 19.85\%$), blue (Lagoon blue #172, $Y = 25.36\%$), green (Jade #323, $Y = 32.0\%$), yellow (Yellow #101, $Y = 80.0\%$), and red (Sunset red #025, 26.37%). There were no statistical differences between compartment visit frequency (Friedman $p > 0.05$). For statistical analysis values see “Results”

cases where the Friedman test was significant ($\alpha < 0.05$), the appropriate non-parametric multiple range test of Dunn was used to determine significant differences among compartments.

Results

On the first day, the 2-month-old Nile tilapia exposed previously to violet, blue, and green light did not show any

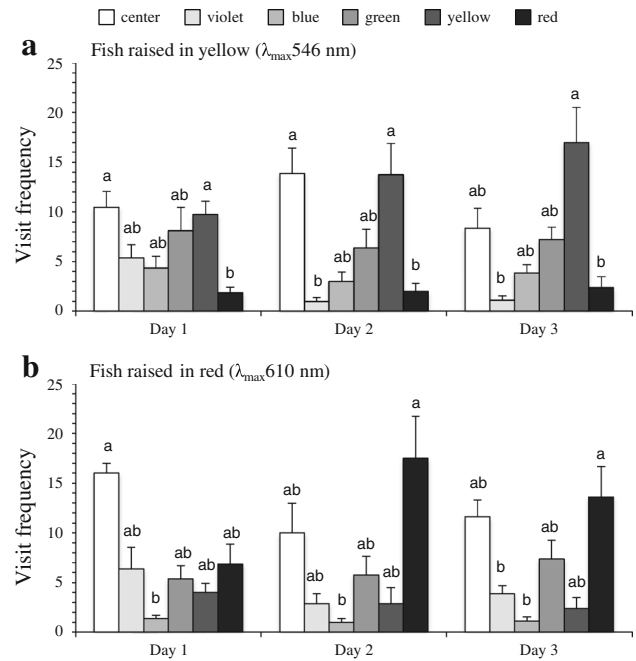


Fig. 2 Colour preference of juvenile Nile tilapia reared from eggs until two months old under **a** yellow colour light and **b** red colour light. Bars represent the mean \pm SE visit frequency of 20-minute observation periods at 0800, 1100, 1400 and 1700 hours over the 3-day period (in total 120 observations per individual; $n = 8$). Gelatin Lee filters provided colour: violet (Violet #344, $Y = 19.85\%$), blue (Lagoon blue #172, $Y = 25.36\%$), green (Jade #323, $Y = 32.0\%$), yellow (Yellow #101, $Y = 80.0\%$), and red (Sunset red #025, 26.37%). Statistical difference of fish visit frequency in each compartment in the same experimental day is indicated by different lower case letters, while at least one letter in common means no statistical value (Friedman $p < 0.05$)

preference for compartment colours (Friedman ANOVA; violet: $\chi^2 = 7.40$, $p = 0.37$; blue: $\chi^2 = 6.56$, $p = 0.25$; green: $\chi^2 = 11.05$, $p = 0.051$). On the second and third days, the fish showed again no preference for any of the compartments (Friedman ANOVA: 2nd day, violet: $\chi^2 = 5.60$, $p = 0.34$; blue: $\chi^2 = 8.12$, $p = 0.15$; green: $\chi^2 = 5.92$, $p = 0.31$; 3rd day, violet: $\chi^2 = 4.46$, $p = 0.48$; blue: $\chi^2 = 6.99$, $p = 0.22$; green: $\chi^2 = 3.16$, $p = 0.66$) (Fig. 1).

Fish raised under yellow light showed higher visit frequency to the center and yellow compartments on the first and second testing days (Friedman ANOVA: 1st day: $\chi^2 = 14.45$, $p = 0.013$; 2nd day: $\chi^2 = 25.04$, $p < 0.001$). On the third day, fish presented a preference for the yellow compartment (Friedman ANOVA: $\chi^2 = 22.43$, $p < 0.001$) (Fig. 2a). Fish held for two months under red light visited the central area (without colour) more often than all the other compartments on the first testing day (Friedman ANOVA, $\chi^2 = 16.27$, $p = 0.006$), but presented preference for the red compartment on the second (Friedman ANOVA: $\chi^2 = 18.77$, $p = 0.002$) and third days (Friedman ANOVA: $\chi^2 = 22.72$, $p < 0.001$) (Fig. 2b).

Discussion

Juvenile Nile tilapia showed varied colour preference depending upon the environment colour in which the fish were raised. While shorter wavelengths (violet, blue, and green) caused an absence of behavioral colour preference, the longer wavelengths (yellow and red) impelled fish to prefer the colour in which they were previously held. It seems that the wavelength of light during ontogenetic development modulates colour preference. This is the first study to show changes in fish colour preference depending on the environmental light wavelength fish were held in from embryo to late juvenile stages.

Colour vision depends on morphological substrates for colour perception: cone cells in the retina expressing pigments excited by different light wavelengths, and brain areas related to colour vision transduction and interpretation. Retinal development starts as early as 50–60 h post fertilization (Morris and Fadool 2005) and, in natural conditions, it is guided by gene expression that determines the number, location, and pigments λ_{\max} of each cone and rod cell. The Nile tilapia have seven opsin genes coding photosensitive pigments (λ_{\max}) at 360 nm (SWS1), 425 nm (SWS2B), 456 nm (SWS2A), 472 nm (Rh2B), 518 nm (Rh2Ab), 528 nm (Rh2Aa), and 561 nm (LWS) along the visible spectrum (Spady et al. 2006). However, information about the influence of the colour of the light on fish is scarce.

The retina, as any other tissue, develops in the way stated above when no external or internal stress is imposed on the fish. Changing the animals' natural light wavelength may directly interfere with normal retinal development, since the light that reaches the retina may stimulate or inhibit cell formation and growth (Autrusseau et al. 2011). In our study, fish development under all colour conditions induced a shift in the natural colour preference as seen either by a lack of colour preference or by the choice of the colour in which the fish were raised.

Although Nile tilapia's natural preference is yellow (Luchiaro et al. 2007) and fish held under yellow also show the yellow preference, one could not reject the yellow interference on retinal development. The natural environment where Nile tilapia is found, the bottom and deep-water column of the Nile River, is reached by light comprising the wavelengths between 450 and 570 nm (Kageyama 1999), which encompasses the green and yellow parts of the visible spectrum. According to Lythgoe (1979), the colours of the light available to an animal are the main selective pressure for the cone pigments that allow colour vision. In this sense, the evolution of colour vision allowed the eyes to maximize photon capture in order to improve visual acuity (Cohen and Forward 2002). The natural yellowish water that Nile tilapia inhabit may have driven the

selection of genes expressing photosensitive pigments mainly in the yellow range of the visible spectrum (from 456 to 561 nm) throughout million of years of evolution. The natural gene expression may occur regularly when only natural light variation is present, but it may undergo significant changes when uncommon environmental regimes are imposed. While it is known that absence of light delays the development of normal colour vision, darkness does not impose any competitive advantage to a specific population of visual neurons as monochromatic light may do. Depending on the environmental condition, the range of wavelength of light that reaches the retina varies, and it seems that unequal stimulation of cone types may disrupt the cells' development, leading to an increase of some cell populations and a decrease of others (Autrusseau et al. 2011).

Our study shows that only medium to long wavelengths caused a shift in behaviour response to the specific colour previously experienced (inducing some conditioned preference), while the other monochromatic light affected colour preference by preventing a clear choice. Few studies have focused on the effect of a monochromatic environment on animal colour vision. The most complete study regarding the effects of wavelength on fish tested the retina morphology and optomotor responses of blue acara reared under five different wavelengths of light (Wagner and Kröger 2005). These authors noticed not only number, size, and function changes in cone and horizontal cells of the retina, but also some change in the optomotor response of the fish. However, the behavioural response was still inconclusive due to the fact that spectral sensitivity is more complex and cannot be addressed only by processes occurring in the outer retina.

Our results leave no doubt that the light spectrum affects Nile tilapia colour preference/perception, which is in accordance with other authors' findings (Carleton et al. 2008; Hornsby et al. 2013). We suggest that short wavelengths may prevent the normal development of some cone cell populations, which is in agreement with the results from Wagner and Kröger (2005) for the blue acara. On the other hand, the medium and long wavelengths may have stimulated some cones in excess, driving the animal to prefer that colour afterwards. According to Lythgoe (1979), the highest cone population that composes the retina allows fine discrimination and acuity for items against the background colour that matches these cones' λ_{\max} .

The natural Nile tilapia environment, mainly composed of yellowish light, may contribute to the ideal fine-tuning of the cones and rods, providing better visual perception and acuity for the discrimination of objects by several orders of magnitude in a stable background (Jacobs 2004). In this sense, the yellow ambient light provided in the experiment may have been very similar to the natural

lighting condition and thus, it allowed a functional balance between various chromatic channels, since the natural preference of Nile tilapia is also yellow.

The problem of setting a unique stimulation for colour vision development is that the system does not receive the varied quantity of different information on a time scale (in nature there is variation of day/night, seasons, daily climate, etc.). Normal development seems to be regulated by several mechanisms that adjust the system to the normal colour vision and discrimination. We know that many fish populations are under environmental pressure due to human activities that interfere with the light spectrum and many other natural aspects. Illumination is only a small piece of what anthropomorphic action can modify, but it is important to notice that it can also contribute to a chain of factors that may interfere with the fishes' (and other animals') environmental perception, conspecifics and mate discrimination, foraging, predator avoidance, etc., which is likely to have adverse consequences.

In conclusion, our results for Nile tilapia show that this species is deeply affected by wavelength features, which corroborates other studies that show light interference on ontogeny and behavioural processes and suggests that new behavioural protocols need to be developed to improve our understanding of the phylogenetic and phenotypic characteristics of vision.

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