

Environmental blue light prevents stress in the fish Nile tilapia

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Abstract

The present study aimed to test the effects of blue, green or white light on the stress response of the Nile tilapia, *Oreochromis niloticus* (L.). Each color was tested on two groups of isolated adult Nile tilapia (8 replicates each): one being subjected to confinement stress, and the other not (control). A different environmental color was imposed on each compartment by covering the light source with cellophane of the respective color (green or blue; no cellophane was used for white light). The intensity of green, white and blue lights was 250, 590 and 250 lux, respectively. Basal plasma cortisol levels were determined for each fish prior to the experimental procedures. The fish were confined by being displaced toward one side of the aquarium using an opaque partition for 1 h both in the morning and the afternoon of the two consecutive days of the test. At the end of this 48-h period, plasma cortisol levels were measured again. Basal cortisol levels (ng/ml) were similar for each group (ANOVA, $F_{(2,42)} = 0.77$, $P = 0.47$). Thus, plasma cortisol levels were analyzed in terms of variation from their respective basal level. After confinement, plasma cortisol levels were not increased in fish submitted to a blue light environment. Thus, blue light prevents the confinement-induced cortisol response, an effect not necessarily related to light intensity.

Key words

- Stress
- Animal welfare
- Light color
- Cortisol
- Nile tilapia
- *Oreochromis niloticus* (L.)

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The visual environments of fish are blue, green or near infrared (1) and fish have cone cells which enable them to discriminate colors (2). Despite this, very few studies have been devoted to understanding the effects of background or light color on fish biology. Even so, some interesting effects have been reported. In salmonids, background color decreases egg development and increases egg mortality (3). In other fish families, effects of environmental color have also been described, such as changes in schooling, fright reaction, and color attractiveness in *Sardinops caerulea* (4), *Oplegnathus fasciatus*, *Mono-*

canthus cirrhifer, *Cybium nipponium*, *Sphaeroides niphobles*, and *Sphyaena japonica* (5), and increased survival and growth in the Eurasian perch larvae, *Perca fluviatilis* (6).

The above mentioned studies show both improvement and disruption of fish welfare caused by environmental color, supporting the interest in better understanding such effects which may also be related to rearing conditions. In fisheries, the environmental conditions should undoubtedly be monitored to guarantee fish welfare. In these attempts, however, compatibility with tank color has been largely neglected. Actually, the inter-

nal-wall color of the artificial fish environment has been chosen almost by chance.

The present study aims to understand the relation between welfare and environmental light color in the Nile tilapia, a fish where stress has been investigated (7-9) and which is considered to be the most extensively cultivated cichlid species in the world. Blue and green lights were tested because they are widespread in fish environments (1). Also, Loukashkin and Grant (4) and Kawamoto and Takeda (5) showed that fish are attracted to green and blue lights and avoid red light. Additionally, a previous study of the characid fish, *Brycon cephalus*, has suggested that these colors may affect reaction to stressors (10).

One hundred adults of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1759), held for about one year in a 1200-l tank, were the stock population. During this time, the water temperature averaged 24°C, with continuous aeration through a biological filter and photoperiod from 6:00 to 18:00 h. Food was offered *ad libitum* once a day. Forty-eight fish were matched for size (mean \pm SD: standard length: 14.02 \pm 0.77 cm; body weight: 110.16 \pm 19.01 g) and randomly distributed in each experimental group. Sex was determined but no association with the results was found.

The effect of light color on stress was studied in Nile tilapia completely isolated in glass aquaria (40 x 25 x 20 cm) for 15 days before experimentation. Stress levels were determined basally and immediately after each color condition (blue, green and white) was imposed on stressed and nonstressed groups (six treatment; 8 fish each). After this experimentation period, the blood of all 48 fish was sampled for stress reevaluation.

The study was carried out in three completely isolated compartments. Each compartment was supplied with white fluorescent illumination. A different environmental color was imposed on each compartment by covering the light source with cellophane of

the respective color (green or blue; no cellophane was used for white light). The intensity of light reaching each aquarium was: green = 250, blue = 250, and white = 590 lux.

Each fish was displaced by an opaque partition which confined it to a small area (less than 10% of the total aquarium volume) for 1 h, a method adapted from Auperin et al. (8). This confinement was imposed in the morning and afternoon of two consecutive days.

Throughout experimentation, water temperature averaged 26.47 \pm 0.78°C, pH ranged from 6.2 to 6.5, water was oxygen-saturated (>5 mg/l), and nitrite and ammonia were lower than 0.1 mg/l and 0.05 mg/l, respectively. The photoperiod was set up from 6:00 to 18:00 h.

Stress was evaluated on the basis of plasma cortisol levels determined by radioimmunoassay. Each fish was anesthetized with benzocaine (80 mg/l) and blood was sampled (~0.6 ml) by cardiac puncture using heparinized (liquemine®, Roche, Rio de Janeiro, RJ, Brazil) syringes. Blood was transferred to glass test tubes and centrifuged at 3000 rpm for 10 min. Plasma (0.3 ml) was collected with a semi-automatic digital micropipette, transferred to 1-ml Eppendorf tubes and stored frozen (-22°C) until required for assay.

Mean cortisol levels are shown in Table 1. Basal cortisol levels obtained for the six conditions (green, blue and white for stressed or nonstressed fish) were compared by ANOVA (Table 1). Since no significant difference was found, the difference between pre- and post-plasma cortisol was independently compared by the unpaired Student *t*-test between stressed and nonstressed groups for each color (Figure 1). Confinement increased cortisol levels in fish held in green and white light, but no effect occurred when these fish were maintained under blue light.

The present study showed that blue light prevents an increase of stress-induced cortisol in the Nile tilapia.

A prior analysis was conducted to determine the reliability of the physiological responses expressed by the fish in this experiment. The Nile tilapia showed mean basal plasma cortisol levels ranging from 16.43 to 39.22 ng/ml (Table 1), which is in accordance with basal levels described in other studies for this and other fish species. Basal cortisol levels for the Nile tilapia ranging from 5 to 50 ng/ml and from 5 to 15 ng/ml were reported by Auperin et al. (8) and by Barcellos et al. (9), respectively. In a related cichlid, *Oreochromis mossambicus*, basal cortisol ranged from 20 to 60 ng/ml (11). Other studies have also shown similar basal results ranging from 10 to 50 ng/ml for other fish families, such as the cyprinid *Cyprinus carpio* (12), and the salmonids *Oncorhynchus clarkii* (13) and *O. mykiss* (14). As stated by Haddy and Pankhurst (15), it is difficult to establish basal levels of cortisol in fish mainly because of several factors which may be affecting their life, such as season, time of the day, etc. Despite this, confinement in the present study clearly increased cortisol, thus supporting the assertion that the fish were stressed compared with their previous condition.

The response of the fish held in the blue environment is interesting, showing inhibition of cortisol increase. Few studies have been devoted to the effects of color on fish and most of them are not directly concerned with stress. As far as we know, this is the first study showing such a cortisol-suppressing effect of environmental light color in fish. Other reports have focused on the effects of environmental color on behavior (4,5,16), growth and survival (6). Effects of color background on chromatophore-regulating hormones have also been reported and Groneveld et al. (17) found that the response of the melanin-concentrating hormone to background color is not strictly related to the pituitary-interrenal axis in tilapia. However, the present study supports the idea that color influences this axis, thus showing that color

may affect different biological systems.

The effects of environmental color have been investigated using mainly background color instead of light color. Although background color is probably more useful for recommendations of color of interior aquarium walls, light color - like those used in the present study - may also have practical consequences for indoor fish production or even fish transportation. Moreover, if the light color has an effect on fish physiology, the color of the inner walls of containers or aquaria should also be chosen accordingly.

Which color should this be? The present study showed that blue light was an effective inhibitor of the stress-induced cortisol response in the Nile tilapia. However, other colors have been shown to improve fish welfare in other species, such as green and blue in *Sardinops caerulea* (4), *Oplegnathus fasciatus*, *Monocanthus cirrhifer*, *Cybbium nipponium*, *Spheroides niphobles* and *Sphyraena japonica* (5), green in *Brycon cephalus* (10), and light grey in perch larvae, *Perca*

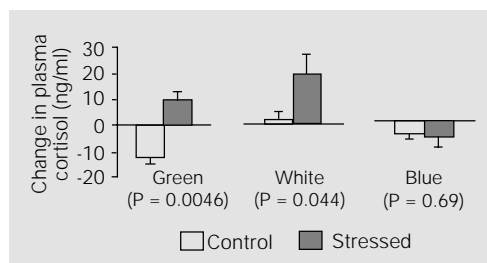


Figure 1. Effect of light colors on the stress response of adult Nile tilapia, *Oreochromis niloticus*. Data express the difference in plasma cortisol levels between the basal and post-handling conditions (see Table 1). P values were obtained by the unpaired Student t-test for each color condition.

Table 1. Mean (\pm SD) plasma cortisol levels (ng/ml, N = 8) of adult Nile tilapia, *Oreochromis niloticus*, before (basal) and after a color regime.

Group		Light color		
		Green	White	Blue
Control	Basal	28.10 \pm 19.92	16.43 \pm 6.54	39.22 \pm 21.27
	Post-color regime	15.64 \pm 7.88	17.66 \pm 7.49	34.61 \pm 15.58
Stressed	Basal	37.95 \pm 12.94	18.09 \pm 9.36	34.01 \pm 25.12
	Post-color regime	47.23 \pm 19.64	35.83 \pm 21.87	27.58 \pm 15.36

The fish were isolated under a white-light regime (12-h light/12-h dark) for 15 days and then submitted to the color regime. Basal mean values were not statistically different from each other (ANOVA, $F_{(2,42)} = 0.77$, $P = 0.47$).

fluviatilis (6). In diurnal fish, vision is the main sensory modality (4) and cone cells have been reported to be present (1,2), thus supporting an evolutionary significance for color discrimination in fish. Since in natural habitats environmental color is a characteristic which varies according to place, and may even change over time in the same habitat, the effect of color on the fish's life is expected to be associated with the predominant habitat color. According to Levine and MacNichol Jr. (1), the visual environments of fish are blue, green or near infrared, although the other studies cited above have shown mainly the two former colors as significant to the fish. Therefore, color effects on a fish's life seem to be a species-dependent phenomenon which may be related to specific habitat characteristics.

Fanta (16) argues that a blue background increases aggression in the Nile tilapia, and suggests that this color is not adequate for holding containers for this species. Conversely, the present data show that blue light has a protective role against stress for this same species. In fact, Schreck (18) suggests that conditions inducing behaviors differing from the standard pattern of the species may be supposed to be stressors, thus indicating that behaviors are reliable indicators of stress in fish. However, behavior interpretation must consider the environmental and evolutionary history of each species. Thus, aggression in the Nile tilapia should be interpreted considering its territorial habits (10). In fact, intraspecific aggression in territorial species is a natural behavior expected to be expressed when the fish are adjusted to their environment (stressors might decrease intraspecific aggression). Thus, the increased aggression caused by the blue background in the Nile tilapia reported by Fanta (16) may

indicate that the fish were more comfortable. Volpato (10) reached a similar conclusion studying the effects of light color on matrinxã, *Brycon cephalus*. This fish was more aggressive under green light and grew almost three times as much in this color, a finding supporting that this increased intraspecific aggression might imply that these fish were unstressed. Therefore, we reinforce that blue light improves the welfare of the Nile tilapia.

A last comment concerns the effect of light intensity or light color. Light intensity has been shown to affect several processes in fish (19,20) and light color or color background may affect light intensity. In the present study, the light intensities (measured in lux) of blue and green colors were the same, but the impact of these colors on the fish was strongly different. Thus, light intensity was probably not involved. The control condition showed a much higher light intensity; even so, these fish reacted in a manner similar to that of fish under green color (with a low lux value). Of course, these comments do not consider other light characteristics, such as wavelength. However, the present study emphatically shows that light color affects the life of Nile tilapia, opening new possibilities for further studies.

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