

## Effects of environmental colour on growth of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), maintained individually or in groups

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### Summary

Examined was the influence of different colours of light on the growth of adult Nile tilapia (*Oreochromis niloticus*) held either individually (initial size  $12.8 \pm 1.1$  cm;  $62.9 \pm 15.6$  g) or in groups (initial size  $7.8 \pm 0.8$  cm;  $17.2 \pm 6.4$  g). Various colours (blue 434.5 nm, violet 430.0 nm, red 609.7 nm, green 525.2 nm and yellow 545.2 nm) did not affect weight gain of fish held individually (initial to final weights: blue  $62.5 \pm 18.4$  to  $72.9 \pm 16.0$  g, violet  $63.5 \pm 17.8$  to  $78.0 \pm 19.1$  g, green  $62.1 \pm 13.0$  to  $72.9 \pm 15.7$  g, and yellow  $60.4 \pm 20.4$  to  $71.0 \pm 21.1$  g); red seemed to restrict growth (initial to final weights:  $65.8 \pm 13.3$  to  $71.8 \pm 10.8$  g). Final weight differences were observed among individuals in groups maintained under blue, violet, red and green light (smaller and larger fish: blue  $13.2 \pm 5.0$  and  $18.9 \pm 7.0$  g, violet  $17.3 \pm 5.2$  and  $23.8 \pm 4.7$  g, red  $14.7 \pm 3.3$  and  $23.9 \pm 4.7$  g, and green  $19.4 \pm 7.8$  and  $28.6 \pm 8.1$  g); however, under the yellow light there were no differences in final weights (smaller fish  $19.1 \pm 4.8$  g; larger fish  $26.2 \pm 5.2$  g). Under the red light, heterogeneity in growth was observed earlier than with the other colours. It is therefore suggested that the red colour might have some harmful effects on Nile tilapia growth, limiting weight gain when fish are individually maintained, and with weight differences increasing when fish are held in groups. On the other hand, the yellow light seems to be positive for Nile tilapia, as it appears not to affect individually-held fish, but reduces variation in growth of group-maintained fish, promoting growth homogeneity.

### Introduction

Light is one of the environmental factors deeply impinging on fish life. Seen from a fish perspective, light can have several life-affecting characteristics: quality, quantity and periodicity. Among these, quality is the least studied (Boeuf and Le Bail, 1999). Experimental designs using lighting or tank colouration have shown significant differences in behavioural and physiological responses in fishes (Fanta, 1995; Papoutsoglou et al., 2000; Volpato et al., 2004). This probably occurs because an aquatic environment comprises many colours and fish have very different visual systems for detection and response (Levine and MacNichol, 1982).

Some fish species have demonstrated sensitivity to the colour of the light. For instance, silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) have best growth under green light (Radenko and Alimov, 1991; Ruchin et al., 2002); the guppy (*Poecilia reticulata*) improves under blue light

(Ruchin, 2004); pikeperch (*Sander lucioperca*) growth is better under red light (Luchiar et al., unpublished); and the goldfish (*Carassius auratus*) is able to discriminate both colour and brightness of light (Neumeyer et al., 1991). Modulation of physiological and behavioral responses can be expected since fish have the physiological capacity to distinguish colours (Wheeler, 1982; Pitcher, 1993) and the visual environments can be blue, green or near-infrared (Levine and MacNichol, 1982). Indeed, an important intrinsic factor associated with colour perception is the absorbance by eye pigment. Spady et al. (2006) determined the presence of seven opsin genes, code for seven photosensitive pigments in Nile tilapia. These authors observed that tilapia have photosensitive pigments ( $\lambda$  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.

However, information is scarce as to the influence on fish of the colour of the light. In fact, to understand the physical parameters that act on fish performance it is essential to develop protocols that can maximize survival and growth under aquaculture conditions. Some influences of the colour of the light on individually-held fish have been studied (as cited above), but these effects on group-reared fish have not been addressed. In fish farms the fish are maintained in groups, where feed intake, growth and behaviour, in contrast to isolated conditions, can change. Hayward et al. (2000) have shown that group-holdings impede total expression of compensatory growth of hybrid sunfish (*Lepomis cyanellus* × *L. + macrochirus*) when compared to individually-held fish. However, social interactions can affect individual growth rates positively and negatively (Sogard and Olla, 2000). Benefits of grouping include increased encounter rates with patchy distribution of prey in natural habitats, increase time for foraging and decrease vigilance time (Pitcher et al., 1982); however, group membership can also reduce the growth rate through competition and social stress (Sogard and Olla, 2000).

In suitable colour conditions the fish may spend more energy on growth than they would under inappropriate colour environments. In fish farms, different colours may affect fish vision, e.g. have an influence on the food intake and the signals for hierarchical status. Hence, some specific colours may improve growth and productivity. Food intake and growth can be regarded as valuable parameters for estimating general performance and the well-being of fish under culture conditions.

Thus, the aim of this study was to test the colour of the light on growth of individually held Nile tilapia (*Oreochromis niloticus*) and to test the effects of the same colours on the

growth of this species held in groups. Three additional colours were chosen from the two poles (blue and red) of the visible light spectrum: violet, green and yellow representing the mid-wavelengths. It was hypothesized that the colour of the light would affect Nile tilapia growth performance as well as influence growth differences, depending on the hierarchy of the fish groups, by decreasing or intensifying individual differences. This information is important for improvements in Nile tilapia aquaculture.

### Materials and methods

Nile tilapia were held for 5 months in a 1500-L indoor tank (density  $6 \text{ kg m}^{-3}$ ) in the laboratory of the Departamento de Fisiologia, Universidade Estadual Paulista, as a stock population. Water was continuously recirculated and aerated, and temperature averaged  $24^\circ\text{C}$ . The photoperiod was 12L : 12D (c. 150 lx at water surface; all light intensity measurements made with a LD-240 digital luxmeter, Instrutherm, São Paulo, Brazil). Throughout the study, fish were fed commercial dry food (manufacturer's proximate composition: protein 36%, fat 8%; Purina® LTDA, Campinas, SP, Brazil) offered once a day in excess.

#### Trials with individually maintained fish

Effect of the colour of the light on growth was studied in Nile tilapia individually held in glass aquaria for 30 days. Thirty aquaria ( $40 \times 25 \times 20 \text{ cm}$ ; water volume 15 L; biomass  $4.2 \pm 1.0 \text{ g L}^{-1}$ ) were covered on the sides with white canson paper; the top was covered with a blue, violet, red, green or yellow gelatin filter, setting luminosity at around 120–150 lx (6 aquaria of each colour), illuminated with white fluorescent tubes using a photoperiod 12L : 12D. Before the experiment colour wavelengths were measured under fluorescent light; values were: blue 434.5 nm, violet 430.0 nm, red 609.7 nm, green 525.2 nm and yellow 545.2 nm.

The aquaria were filled with filtered and aerated  $24^\circ\text{C}$  water (pH 7.2, levels of ammonia  $<0.5 \text{ ppm}$  and nitrite  $<0.05 \text{ ppm}$ ); 20% of the water in each aquarium (3 L) was exchanged every 3 days. Air was also provided in each aquarium via air stones connected to air pumps. Oxygen concentration and temperature were measured with a YSI oxygen meter (Ysi 85 DO, Ysi Inc., Yellow Springs, OH, USA) each day before feeding time; oxygen was always above  $5.0 \text{ mg L}^{-1}$ ; temperature varied from 23 to  $25^\circ\text{C}$ .

Initial fish lengths ( $12.8 \pm 1.1 \text{ cm}$ ) and weights ( $62.9 \pm 15.6 \text{ g}$ ) were measured before introduction into the colour regime. There were no statistical differences in fish sizes in colour treatments (ANOVA  $F = 1.87$ ,  $P = 0.12$ ;  $n = 6$  aquaria of each colour). Each fish was fed by hand with dry food once a day (3% of fish weight) at 11.00 hours until apparent satiation (fish were fed as long as the pellets in the water column were eaten, avoiding overfeeding); uneaten food was removed after 1 h. Throughout the experiment water temperature averaged  $24 \pm 1^\circ\text{C}$ , pH ranged from 6.2 to 6.5, water oxygen concentration was above  $5 \text{ mg L}^{-1}$ , and nitrite and ammonia were lower than 0.1 and  $0.05 \text{ mg L}^{-1}$ , respectively.

Fish length (to 0.1 cm) and weight (to 0.01 g) were measured every 10 days. Specific growth rate [ $\text{SGR} = (\text{Ln } w_2 - \text{Ln } w_1) * 100 / t$ ] was determined, where  $w_2$  and  $w_1$  are final and initial weights, and  $t$  is time in days. Weight gain and coefficient of variation of final weight [ $\text{CV} = (\text{SD} / \text{average weight}) * 100$ ] were also calculated.

#### Trials with small groups of fish

The effect of the colour on growth of group-held fish was determined by keeping groups of four Nile tilapia in glass aquaria for 30 days. Thirty aquaria ( $60 \times 40 \times 30 \text{ cm}$ ; water volume 50 L; biomass  $1.4 \pm 0.5 \text{ g L}^{-1}$ ) were covered on the sides with white canson paper; the top was covered with a blue, violet, red, green or yellow gelatin filter; luminosity and water quality were as described for the trials with isolated fish. Every 3 days, 20% of the water in each aquarium (ca. 10-L) was exchanged with filtered and aerated water. Air was supplied in each aquarium by air stones. Oxygen concentration and temperature were measured daily, as explained above.

Groups of four fish of similar size (length  $7.8 \pm 0.8 \text{ cm}$ ; weight  $17.2 \pm 6.4 \text{ g}$ ) were reared under different colours of light for 30 days. Initial fish lengths were the same, however initial weights varied somewhat. Even with some variation, initial weights were not statistically different among treatments (ANOVA  $F = 0.94$ ,  $P = 0.53$ ;  $n = 8$  aquaria for each colour; 32 fish per colour).

Fish were hand-fed with dry food once a day at c. 11.00 hours until apparent satiation. During the experiment water averaged  $24 \pm 1^\circ\text{C}$ , and pH, DO, ammonia, nitrite and photoperiod were the same as in the trial with individually raised fish.

Length (to 0.1 cm) and weight (to 0.01 g) were measured at the beginning of the experiment and every 10 days thereafter. Fish in each group were marked with a cut on the tail (up, down, both or middle), whereby the fish were first anesthetized and then cut using benzocaine ( $40 \text{ mg L}^{-1}$ ) to avoid stress. Because of fin regeneration, the fin of the fish was re-cut on each measurement day, and the specific growth rate (SGR), weight gain and coefficient of variation (CV) of the final weight were calculated.

Data were analyzed by ANOVA (one-way or repeated measures) and Tukey's test when normal distribution and equal variance was shown, otherwise either the Kruskal–Wallis or Friedman test was followed by the Dunn's test. The Student  $t$ -test was used to compare initial and final weights.

## Results

#### Trials with individually raised fish

The colour of the light had no effect on weight gain of Nile tilapia (ANOVA,  $F = 0.16$   $P = 0.96$ ). However, fish reared under blue, violet, green and yellow increased their weight significantly from the beginning to the end of the experimental period (Student  $t$ -test, blue:  $t = -3.64$   $P = 0.02$ , violet:  $t = -9.80$   $P < 0.01$ , green:  $t = -6.40$   $P < 0.01$ , yellow:  $t = -4.40$   $P = 0.01$ ) whereas tilapia under the red light showed no statistical differences in weight gain (Student  $t$ -test,  $t = -0.92$   $P = 0.41$ ), and initial and final weight of fish under the red light was statistically similar (Fig. 1a). SGR did not differ among the tested colours (Kruskal–Wallis,  $H = 1.46$   $P = 0.83$ ; Fig. 1b). According to the CV of fish final weight in each colour, the yellow light had the highest variance (CV = 29.82), while fish under the red colour showed the lowest variance (CV = 15.99) (Fig. 1c). There were no mortalities during the experimental phase.

#### Trials with small fish groups

Fish were classified based on final size, from a (largest) to d (smallest) in each aquarium. Under blue, violet, red and green

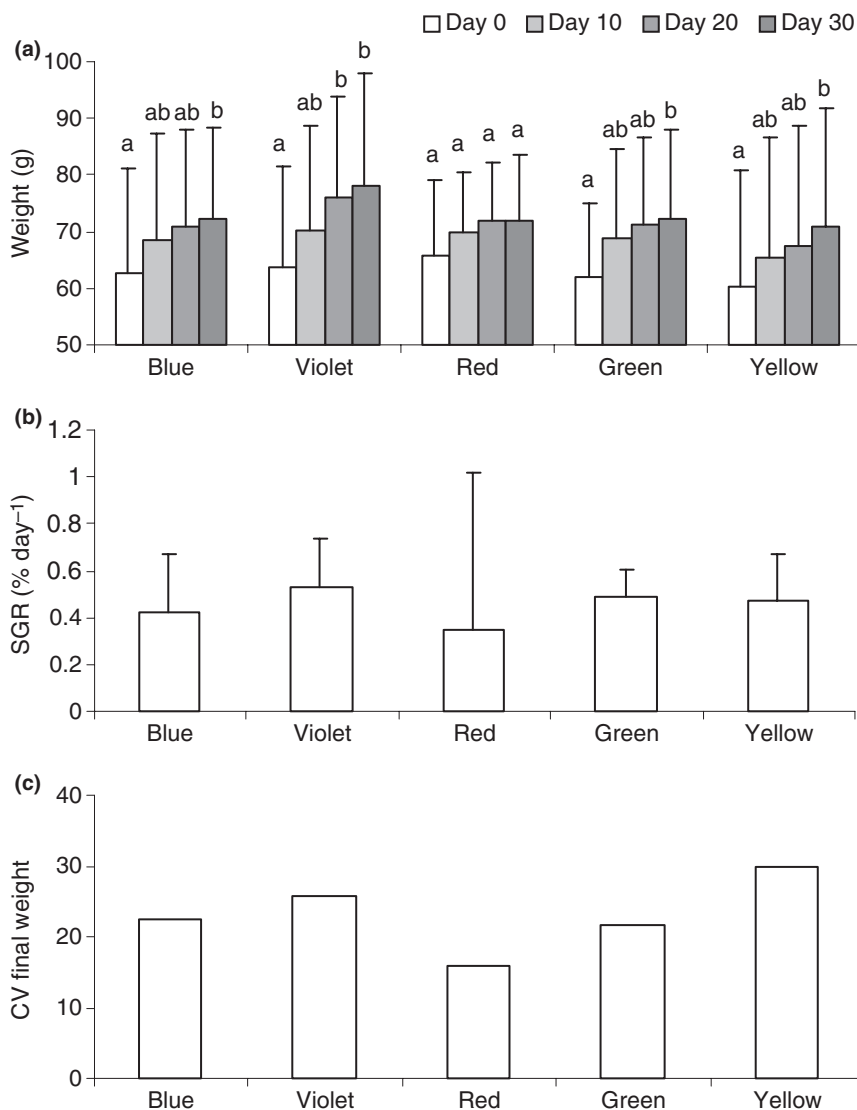


Fig. 1. Effects of light colour on fish growth. (a) Weight, (b) specific growth rate (SGR) and (c) coefficient of variation (CV) of final weight of individually raised Nile tilapia (*Oreochromis niloticus*) held under different environmental colours ( $n = 6$ ). (a) Bars = mean weight (g)  $\pm$  SD at days 0, 10, 20 and 30; different letters = statistical differences among days of sampling (RM ANOVA,  $P < 0.05$ ). (b) Bars = mean SGR  $\pm$  SD at end of the experiment; no differences among fish raised under different colours. (c) Bars = CV [(mean weight / SD) \* 100] of fish final weight under each colour

light there were significant differences in final weights among fish within each group (ANOVA, blue:  $F = 7.62$   $P < 0.01$ , violet:  $F = 14.70$   $P < 0.01$ , red:  $F = 6.67$   $P < 0.01$ , green:  $F = 7.06$   $P < 0.01$ ); however, under the yellow light there were no differences in final weights (ANOVA,  $F = 2.27$   $P = 0.10$ ; Fig. 2a). Weight differences among individuals in the groups could already be observed at day 20 under the red colour (ANOVA,  $F = 4.25$   $P = 0.01$ ), but not evident in the other colours (ANOVA,  $P > 0.05$ ). However, no weight gain differences were found among fish of the same size-category among different colours (ANOVA, fish a:  $F = 1.17$   $P = 0.18$ , fish b:  $F = 1.37$   $P = 0.27$ , fish c:  $F = 2.32$   $P = 0.08$ , fish d:  $F = 1.34$   $P = 0.28$ ).

SGR of each fish within a group differed under blue, violet, red and green lights, but not under yellow light (ANOVA, blue:  $F = 3.29$   $P = 0.05$ , violet:  $F = 3.57$   $P = 0.03$ , red:  $F = 6.68$   $P = 0.02$ , green:  $F = 3.69$   $P = 0.03$ , yellow:  $F = 0.49$   $P = 0.70$ ; Fig. 2b). Fish under the red light showed a higher CV in final weight than fish under the yellow light (Kruskal–Wallis,  $H = 16.3$   $P < 0.01$ ; Fig. 2c).

In general, fish showed better growth performances when maintained under the yellow colour than under the blue or red environment (ANOVA,  $F = 5.38$   $P < 0.01$ ). Fish mortality was observed on occasion right after weighing; the replicate where

this occurred was excluded from the experiment. All colour trials were initiated with eight replicates; total number of deaths in the entire experiment varied from one fish in the yellow, two in the violet and red, and three in the green and blue treatments.

## Discussion

This study showed that the colour of the light did not affect weight gain in Nile tilapia when fish were held individually (Fig. 1a); however, the yellow colour seemed to support more homogeneous growth (Fig. 2a,b) of fish in groups, whereas the red colour increased growth variability, as expressed by CV of final weight (Fig. 2c). Also, in individually-held fish the red colour seemed to inhibit growth.

The effects of yellow light on Nile tilapia could be expected, as yellow was shown to be the species' colour preference (Luchiari et al., 2007); it presents many opsins that are maximally absorbed at mid-wavelength light in the retina (Spady et al., 2006). Effects of the colour of the light on fish growth were observed in rainbow trout, as well as being linked to colour preference (Luchiari and Pirhonen, 2008) and to the proportion of visual pigments in the retina of this species (Tsin and Beatty, 1977). The presence of colour pigments has been

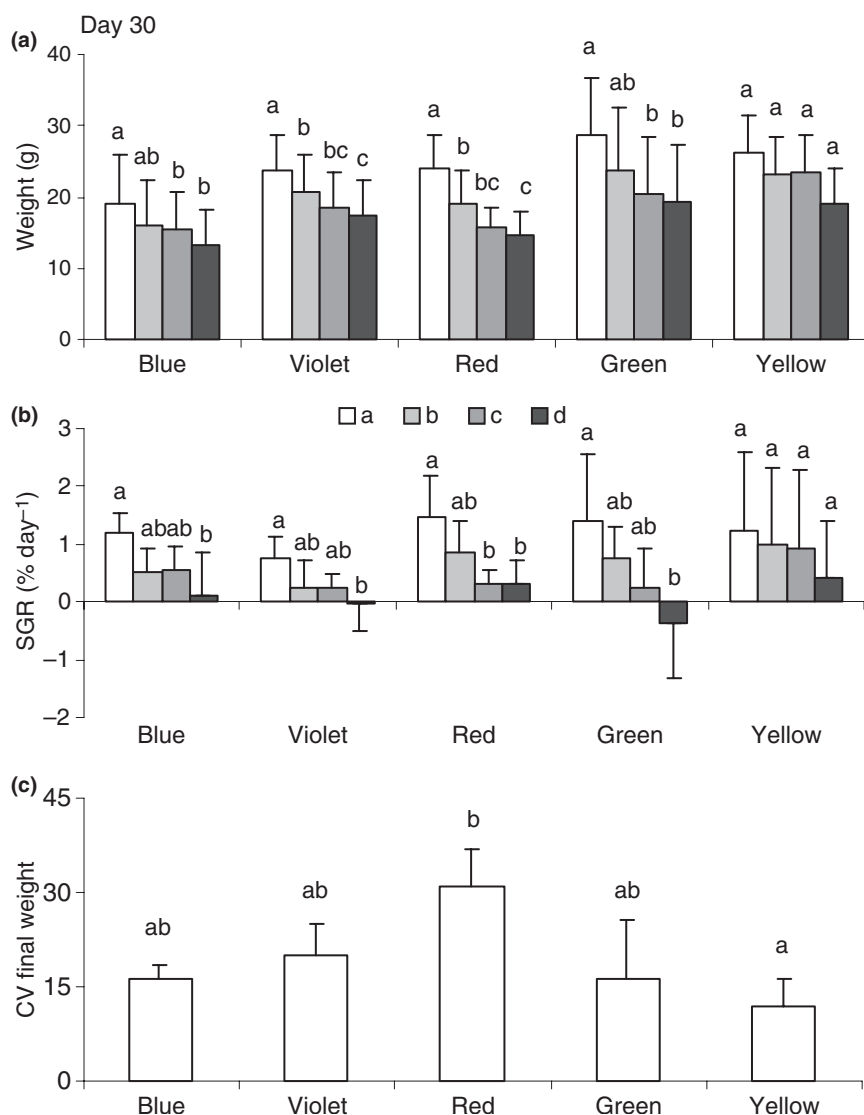


Fig. 2. Effects of light colour on fish growth. (a) Final weight, (b) specific growth rate (SGR) and (c) coefficient of variation of final weight from Nile tilapia (*Oreochromis niloticus*) groups of four fish held under different colours. Data presented as means values (blue and green n = 5, violet and red n = 6, yellow n = 7), error bars = SD. Each group of four fish classified from a (largest) to d (smallest) as their final size. Different letters = differences within each group under a colour: (a) and (b) one-way ANOVA, and (c) Kruskal–Wallis; P < 0.05. There was no difference in weight gain among fishes of the same size class (a, b, c or d) under different colours.

suggested as permitting the fish to have spectral sensitivity of their visual system to match the light environment (Bowmaker, 1990), although the present study could not explain the functional significance of yellow light effectiveness on Nile tilapia fitness. Nile tilapia is a tropical fish that in its natural environment experiences broad ranges of colour, comprising wavelengths between 500 and 570 nm (Kageyama, 1999), encompassing the green and yellow spectrum. According to Munz (1958) and Loew and Lythgoe (1978), vision sensitivity is adjusted to the spectral quality of the ambient light, which enables fish to catch the greatest number of photons available and enhance vision to permit better detection of predators or prey. Thus Nile tilapia visual pigments possibly better match wavelengths present in yellow light, which could account for the fish performance in such an environment.

In this experiment fish were not held under white light, a natural state in Nile tilapia aquaculture, because the occurrence of heterogeneous growth for fish under these conditions is well documented. Many experiments address variability in growth of fish groups held under daylight conditions, for instance in Arctic charr (Jobling and Reinsnes, 1986), Midas cichlid (Valerio and Barlow, 1986), Nile tilapia (Fernandes and Volpato, 1993), and gilthead sea bream (Goldan et al., 1997). Thus, the use of specific light wavelengths in the present

experiment did not exclude the effects of white light, which is a mixture of several wavelengths, but did permit the possible contribution of each colour of the visible spectrum on growth. Although photoperiod and light intensity may greatly affect fish growth (Boeuf and Le Bail, 1999) through modulation of feed conversion (Taylor et al., 2006) or intake (Trippel and Neil, 2003), the colour of the light possibly influences more of the social interactions – and thereby growth as a secondary effect.

Fish held under red light, when isolated or grouped, showed decreased weight gain and increased growth heterogeneity within the group, respectively. The low mass gain under red light suggests that there is some negative effect of this colour on Nile tilapia growth. For this species the longest pigment wavelength absorbance is 561 nm (Spady et al., 2006), thus the red environment where the light wavelength is around 610 nm may restrict vision, as photons are not captured well by the cones. Indeed, several studies addressed the harmful effects of red light on growth rates of various fish species (Ruchin et al., 2002; Ruchin, 2004; Luchiaro et al., 2007; Luchiaro and Pirhonen, 2008), which concur with the present results with Nile tilapia. Ruchin (2004) suggested that the effects of red light might be explained by changes in energy metabolism, endocrinological changes, or by other biochemical or physio-

logical changes. On the other hand, unpublished results of Luchiari, Freire, Koskela and Pirhonen suggest red light as being the most favourable for rearing pikeperch, *Sander lucioperca* (L.). Although some colours can be found as having similar effects on the physiology and behaviour of various fishes, the present data do not permit any general comprehension of these effects; however, looking for similarities and differences in this phenomenon may be relevant and thus deserve further study.

Although yellow light appears to promote growth when compared to colours at either end of the visible spectrum, these results suggest that intake is not necessarily improved when using yellow light (food was offered in excess and remained in all aquaria after the feeding period). This result is arduous to elucidate and must be considered cautiously. Principally it must be remembered that the feed intake was not clearly measured, thus more data is needed to verify this result. Also, the mechanisms underlying the differences in intake and consequent growth between different colours remain unclear. These differences may have been associated in part with an increase in stress and aggression under an unsuitable colour (Goldan et al., 1997; Papoutsoglou et al., 2000).

Under an unsuitable colour in this study there were wide variations in fish sizes as well as high mortality. Survival of the entire group was better achieved under yellow light (87%), while blue and green light had the highest mortalities (37%). The presence of larger fish had negative effects on growth and survival of smaller fish; this variability can also affect growth and lead to mortality in fish groups (Uchmanski, 2000; Kendall and Fox, 2002; Smith and Fuimana, 2003). Indeed, it would be interesting to be able to distinguish the effect of the colour of the light on other parameters of fish life as well as determine the factors involved. To the best of our knowledge this issue has not been addressed elsewhere, and would certainly warrant further study.

In conclusion, these experiments indicated the value of yellow light in an optimal rearing environment. The yellow light appeared to promote growth in Nile tilapia groups; conversely, the red light seemed to suppress growth. Consequently, the original research hypothesis (see Introduction) proved to be correct. Based on these observations made under laboratory conditions it is suggested that the yellow light environment is the most favourable for rearing groups of Nile tilapia, even though the comparative effects of white light colour were not addressed.

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