



## Effects of photoperiod on somatic growth and gonadal development in male Nile tilapia

Rodrigo Diana Navarro<sup>1\*</sup>, Samantha Campos de Sousa<sup>1</sup>, Yvonaldo Wladimir Saldanha Bizarro<sup>1</sup>, Rodrigo Fortes da Silva<sup>2</sup> and Fernanda Keley Silva Pereira Navarro<sup>3</sup>

<sup>1</sup>Laboratório de Biotecnologia em Organismos Aquáticos, Faculdade de Agronomia e Veterinária, Universidade de Brasília, Campus Universitário Darcy Ribeiro, 70910-970, Brasília, Distrito Federal, Brazil. <sup>2</sup>Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil. <sup>3</sup>Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Águas Lindas de Goiás, Goiás, Brazil. \*Author for correspondence. E-mail: [navarrounb@gmail.com](mailto:navarrounb@gmail.com)

**ABSTRACT.** Rotation and translation of the Earth subject the living organisms to cyclic changes of environmental factors. This study evaluated the effects of photoperiod on growth and gonadal development in Nile tilapia (*Oreochromis niloticus*). In a completely randomized design experiment, juvenile males were distributed into nine tanks (10 fish per tank) and maintained for 60 days under three different light treatments with three repetitions each. The treatments were: T1 - 0 h photoperiod (tanks covered with lids and black plastic); T2 - 12 hours photoperiod (tanks uncovered during photoperiod to provide natural light); and T3 - 24 hours photoperiod (tanks covered with lids equipped with lamps). No significant differences were found between treatments for body length, gonad weight or gonadosomatic index, but body weight was higher in fish subjected to T3 compared with other treatments. Furthermore, significant increases in tubular lumen and germinal epithelium were observed in fish exposed to T1 and T3, respectively. Thus, the manipulation of photoperiod in Nile tilapia culture systems can improve production and consequently increase the economic return on investment.

**Keywords:** fish, testicular development, light, dark.

## Efeito do fotoperíodo sobre o crescimento e o desenvolvimento gonadal em macho de tilápia do Nilo

**RESUMO.** Rotação e translação da Terra submete os organismos vivos a alterações cíclicas de fatores ambientais. Objetivou-se avaliar o efeito do fotoperíodo no crescimento e desenvolvimento gonadal em tilápia do Nilo (*Oreochromis niloticus*). Em um delineamento experimental inteiramente casualizado, machos jovens foram distribuídos entre nove tanques (10 peixes por tanque) e mantido por 60 dias sob três tratamentos de luz diferentes, com três repetições de cada um. Os tratamentos foram: T1 - 0h de fotoperíodo (tanques cobertos com tampas e folhas de plástico preto); T2 - 12h fotoperíodo (tanques descobertos durante o fotoperíodo para fornecer luz natural); e T3 - 24h fotoperíodo (tanques cobertos com tampas equipados com lâmpadas). Não foram observadas diferenças significativas entre o tratamento em relação ao comprimento do corpo, peso das gônadas ou índice gonadosomático, mas o peso corporal foi maior nos peixes submetidos a T3 em comparação com outros tratamentos. Além disso, aumentos significativos na luz tubular e epitélio germinal foram observadas em peixes expostos a T1 e T3, respectivamente. Assim, a manipulação do fotoperíodo em sistemas de cultivo de tilápia do Nilo pode levar a uma melhora na produção e, conseqüentemente, a um aumento no retorno econômico sobre o investimento.

**Palavras chaves:** peixe, desenvolvimento testicular, luz, escuro.

### Introduction

The temporal organization of an organism is expressed in response to various environmental stimuli including photoperiod, temperature, salinity and availability of food and water. These external cues play important roles in adjusting circadian and/or annual rhythms, mediated by specific biological mechanisms, within limits that are well defined for each species (CARR et al., 2006;

BLANCO-VIVES; SÁNCHEZ-VÁZQUEZ, 2009; BLANCO-VIVES et al., 2010; FALCÓN et al., 2010; NAVARRO et al., 2014).

The photoperiod exerts the largest influence on animal biorhythms, giving rise to alterations in weight gain, food intake, energy consumption, locomotion and other physiological parameters (NAVARRO; NAVARRO, 2012; NAVARRO et al., 2013). The photoperiod, along with other synchronizing agents, can adjust the circadian and/or

annual rhythms, influencing the fish growth, survival, reproduction and stress (YANTHAN; GUPTA, 2007; MENDONÇA et al., 2009).

In order to get adjusted to variations in brightness, fish possess a clock mechanism through which light-sensitive photoreceptors and humoral and neural systems inform the organism of the status of environmental light (FALCÓN et al., 2010).

Managing environmental conditions, particularly the photoperiod, can be an excellent tool by which to induce reproduction in fish since this synchronizer exerts a direct effect on the maturation of ovaries and testes (NAVARRO; NAVARRO, 2012). In teleostean fish, photoperiod influences the hypothalamic-pituitary-gonadal axis stimulating or inhibiting the production of gonadotropin-releasing hormone, pituitary hormones (follicle stimulating hormone and luteinizing hormone) and other hormones that modulate reproduction and gametocyte maturation (DAVIE et al., 2007).

The aim of the present study was to establish environmental lighting conditions that maximize gonadal development and growth of Nile tilapia (*Oreochromis niloticus*). This study aimed to evaluate the effect of photoperiod on growth and gonadal development in Nile tilapia (*Oreochromis niloticus*).

## Material and methods

The experiment was carried out in a climate-controlled room at the 'Núcleo de Tecnologia em Piscicultura e Pecuária, Secretaria de Agricultura e Desenvolvimento Rural', Brasília, Distrito Federal State, Brazil. The experiment was a completely randomized design in which 90 male Nile tilapia juveniles were assigned to three treatments with three repetitions each. Fish (mean weight  $83.65 \pm 2.5$  g) were distributed into six 500 L asbestos tanks (10 fish per tank) filled with 400 L water, and maintained under controlled conditions for 60 days.

The treatments were as follows: T1 - 0 hours photoperiod; boxes were covered with plastic lids underneath black plastic sheets to ensure total darkness; T2 - 12 hours photoperiod; boxes were completely uncovered during the photoperiod to allow illumination by natural light; T3 - 24 hours photoperiod; boxes were covered with plastic lids equipped with lamp holders and 20 W fluorescent lamps with a constant intensity of 1173 lx to provide 24 hours of constant illumination. The quality of water in each tank was monitored on a daily basis by measurement of pH, temperature and conductivity, and the levels of dissolved oxygen (determined with an oximeter) were maintained with the aid of an

aeration pump. Fish fed 5% body weight of a commercial fish diet containing 28% of protein and 3,100 kcal of digestible energy  $\text{kg}^{-1}$ , twice a day at 09:00 and 16:00 hour. After 15 min. of each meal, the leftovers were taken by siphoning the aquaria, so that all animals fed the same amount of feed and did not impair water quality. At the time of feeding, the lid has been opened and subsequently closed.

After the experimental period, specimens of Tilapia males fasted for 24 hours were taken and anaesthetized with a solution containing 65 ppm Eugenol for desensitization and then we determined biometric data collected gonads, which were weighed and fixed in Bouin solution and stored in 70% ethanol. The gonadosomatic index (GSI) was calculated with the expression  $[(\text{gonad weight}/\text{total body weight}) \times 100]$ . Sections of testis tissue were stained with hematoxylin-eosin and examined under the light microscope. Volumetric ratios of the components of the testicular parenchyma were established by the point counting method at 400x magnification using an optical microscope fitted with a 121-point eyepiece graticule. For each animal, points corresponding to the seminiferous tubules (germinal epithelium, tubular lumen and spermatozooids) and the intertubular compartments (connective tissue, blood and lymph vessels, Leydig cells) were counted in twenty randomly distributed fields. Each fish was considered an experimental unit. Histological preparations and morphological and morphometric analyses were performed in the pathology laboratory, University of Brasilia (UnB).

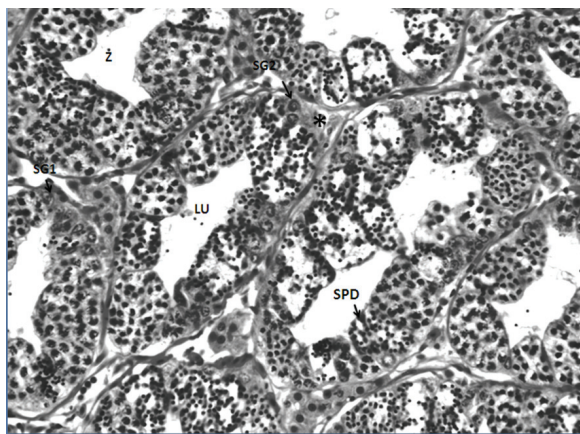
Data were tested for normality using the Kolmogorov-Smirnov test and, where appropriate, subjected to Napierian logarithm transformation. Differences between treatments (categorical variables) with respect to the dependent variables (fish length and weight, testis weight, gonadosomatic index, percentages of tubular and intertubular compartments) were evaluated using one-way analysis of variance (ANOVA) and the Tukey pairwise comparison or the Duncan multiple comparison tests at 5% significance level (SAS, 2007). Statistical analyses were run using the software PAST 1.92.

## Results

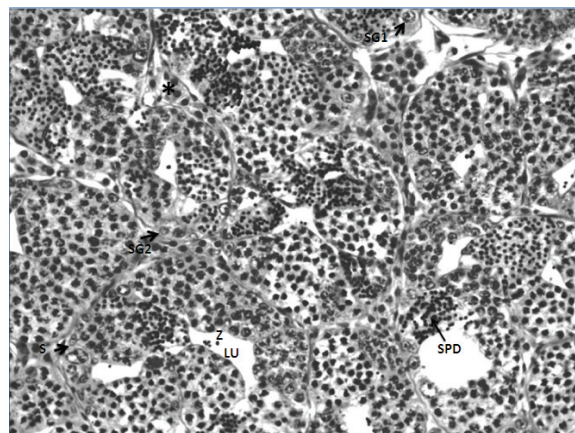
The water quality parameters presented the following mean values:  $23.74 \pm 0.55^\circ\text{C}$  for water temperature, 5.5 for pH,  $6.1 \pm 0.36 \text{ mg L}^{-1}$  for dissolved oxygen, 0.35 for ammonia and 0.10 for  $\text{NO}_2$  according to Navarro et al. (2012).

Body weight of male Nile tilapia that had been exposed to a 12 hours photoperiod (T2) were significantly lower than those maintained under 24 hours dark (T1) or 24 hours light (T3) conditions (Table 1). The highest values of body weight were observed in fish of the treatment T3. In contrast, body length, weight of testes and GSI values were not significantly influenced by the light regimes. Microscopic analysis of testis revealed that the tubular lumen was significantly increased when fish were exposed to T1 compared with the other treatments, whereas the germinal epithelium was significantly increased after exposure to T3 (Table 2). On the other hand, no significant differences between treatments were detected concerning the intertubular compartment variables.

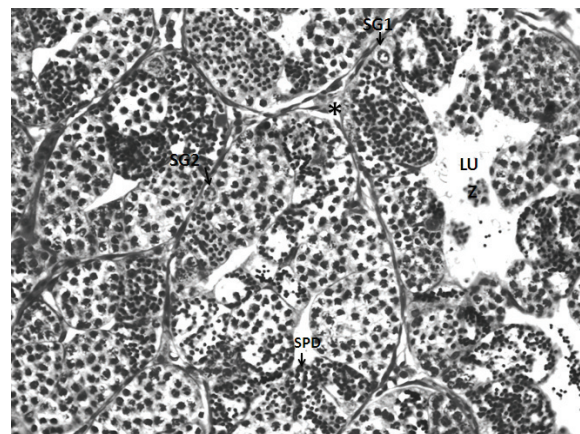
The microscopic characteristics of Nile tilapia testis maintained under different photoperiods are shown in Figures 1-3.



**Figure 1.** Light micrograph (hematoxylin-eosin staining) of testis of Nile tilapia males that had been maintained under a 0 hour photoperiod (treatment T1) for 60 days. Legend: SG1, primary spermatogonia; SG2, secondary spermatogonia; SPD, spermatids; LU, lumen of seminiferous tubules; Z, spermatozooids; \*testicular interstitial space.



**Figure 2.** Light micrograph (hematoxylin-eosin staining) of testis of Nile tilapia males that had been maintained under a 12 hours photoperiod (treatment T2) for 60 days. Legend: SG1, primary spermatogonia; SG2, secondary spermatogonia; SPD, spermatids; LU, lumen of seminiferous tubules; Z, spermatozooids; \*testicular interstitial space.



**Figure 3.** Light micrograph (hematoxylin-eosin staining) of testis of Nile tilapia males that had been maintained under a 24 hours photoperiod (treatment T3) for 60 days. Legend: SG1, primary spermatogonia; SG2, secondary spermatogonia; SPD, spermatids; LU, lumen of seminiferous tubules; Z, spermatozooids; \*testicular interstitial space.

**Table 1.** Morphological characteristics of Nile tilapia males following exposure to different photoperiods for 60 days.

Treatment*	Final length (cm)	Weight of gonads (g)	Gonadosomatic index (%)	Final weight (g)
T1(0L:24D) photoperiod)	17.33 ± 0.85 <sup>a</sup>	1.07 ± 0.21 <sup>a</sup>	1.09 ± 0.36 <sup>a</sup>	108.0 ± 11.11 <sup>a</sup>
T2 (12L:12D)	18.75 ± 0.48 <sup>a</sup>	0.85 ± 0.26 <sup>a</sup>	0.57 ± 0.16 <sup>a</sup>	101.5 ± 10.98 <sup>b</sup>
T3 (24L:0D)	19.00 ± 0.41 <sup>a</sup>	0.56 ± 0.20 <sup>a</sup>	0.38 ± 0.13 <sup>a</sup>	139.33 ± 4.48 <sup>a</sup>

Within a column, mean values (± standard deviations) bearing superscript lowercase letters are significantly different according to the Tukey test ( $P < 0.05$ ). \*(Light:Dark cycle - LD).

**Table 2.** Volumetric ratios of the components of the testicular parenchyma following exposure of Nile tilapia males to different photoperiods for 60 days.

Variable	Treatment*		
	T1 (0L:24D) photoperiod)	T2(12L:12D)	T3 (24L:0D)
Germinal epithelium (%)	62.03 ± 2.2 <sup>b</sup>	64.00 ± 1.8 <sup>b</sup>	80.28 ± 3.6 <sup>a</sup>
Tubular lumen (%)	18.53 ± 4.1 <sup>b</sup>	5.00 ± 3.7 <sup>a</sup>	5.00 ± 5.70 <sup>a</sup>
Spermatozooids (%)	7.73 ± 5.3 <sup>a</sup>	12.79 ± 9.39 <sup>b</sup>	4.00 ± 2.8 <sup>a</sup>
Tubular compartment (%)	88.29 ± 28.74	81.79 ± 32.0	89.28 ± 43.75
Connective tissue (%)	7.6 ± 0.9 <sup>a</sup>	11.14 ± 6.43 <sup>a</sup>	8.35 ± 1.55 <sup>a</sup>
Blood vessels (%)	0.74 ± 0.30 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
Leydig cells (%)	3.3 ± 1.85 <sup>a</sup>	7.70 ± 3.64 <sup>a</sup>	2.37 ± 4.4 <sup>a</sup>
Intertubular compartment (%)	11.71 ± 3.46	18.21 ± 5.70	10.72 ± 4.30

Within a row, mean values (± standard deviations) bearing superscript lowercase letters are significantly different according to the Duncan test ( $P < 0.05$ ). \*(Light:Dark cycle - LD).

## Discussion

This study demonstrated how sexual maturation in male Nile tilapia may be suppressed under long photoperiods light associated with a stimulation of growth parameters, although not significant. Photoperiod is usually considered as one of the major synchronizer of sexual maturation and breeding in fish (BROMAGE et al., 2001). Photoperiod manipulation is widely employed to control reproduction of a variety of fish species (BROMAGE et al., 2001).

Although male Nile tilapia of the different treatments showed similar morphological characteristics, fish exposed to 24 L:0D exhibited a higher length and weight as well as a lower gonadal weight and gonadosomatic index, which can be related to inhibition of gonadal maturation and increased energy invested in somatic growth. Several studies have shown that exposure of fish to long photoperiods or to continuous light tends to suppress gonadal maturation with a redirection of energy towards somatic growth (GINÉS et al., 2004), while Rad et al. (2006) reported that Nile tilapia fry maintained under continuous light exhibited reduced gonadal maturation and GSI values followed by increased somatic growth. European sea bass and gilthead seabream exposed to long photoperiods, and Atlantic cod exposed to have showed delayed or disrupted sexual maturation and increased growth (KISSIL et al., 2001). Nevertheless, other authors like Biswas et al. (2005) reported a higher growth rate in Nile tilapia exposed to 6L:6D (12 hours cycle) than long photoperiods, explained by the probable inhibition of reproduction.

The delayed development and maturation of the gonads, with concomitant reduction in GSI, during exposure to long photoperiods or to continuous light have been reported for diverse species of temperate fish and salmonids (UNWIN et al., 2005; TARANGER et al., 2006). Additionally, Milla et al. (2009) observed that GSI values were reduced in the redfin perch (*Perca fluviatilis*) following three months of exposure to a 12 hours photoperiod.

In the present study, the increased somatic growth observed in Nile tilapia maintained under continuous light was associated with a reduction in GSI value, although not significant. The reduction in the tubular lumen and increase in the germinal epithelium in males exposed to T3 may indicate that these animals were in the initial maturity stages. During the initial maturation, the amounts of spermatogonial cysts and spermatocytes are high, with considerable increase of the latter. In addition,

there is a progressive decline in the tubular lumen owing to the proliferation of spermatogonia (ANDRADE et al., 2010). In contrast, the germinal epithelium in males exposed to T1 was underdeveloped and the percentage of tubular lumen was increased, indicating that the testes were at a more advanced maturity stage, probably an intermediary phase in comparison with T3. According to Andrade et al. (2010) the proliferation of primary spermatogonia diminishes during the intermediary maturity stage and the tubular lumen is more expanded in comparison with the initial phase. With regard to the intertubular compartment of the testicular parenchyma, the arrangement pattern of the components differs among fish species (FAWCETT et al., 1973; RUSSELL, 1996), hence the lack of significant differences between the treatments in the present study may reflect the intra-specific pattern of Nile tilapia. This dynamic nature of the spermatogenesis process was previously reported by Navarro et al. (2010) for the same species of fish.

## Conclusion

The results of the present study show that exposure of Nile tilapia to continuous light (24L:0D) enhances somatic growth and delays gonadal maturation, even though no significant reduction in GSI values were observed. Thereby, the photoperiod manipulation in Nile tilapia culture systems can improve production, and consequently increase the return on investment, as appropriate light regimes may reflect in increased growth associated with inhibition of gonadal maturation.

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