

**Mortality rate of newly hatched larvae of Nile tilapia (*Oreochromis niloticus*) submitted to different photoperiods**

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Mortalidade de larvas recém-eclodidas da tilápia do Nilo (*Oreochromis niloticus*) submetidas a diferentes fotoperíodos

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ABSTRACT: Fish farming is a growing area in the aquaculture industry. Brazil is among the five largest tilapia producers in the world, with a production of 283 thousand tons in 2017. Tilapia has been intensively cultivated and improved through more efficient cultivation techniques. In this sense, the objective was to evaluate the mortality rate of newly hatched larvae of Nile tilapia reared three photoperiods (12Light:12Dark, 18L:06D, and 24L:0D) during 9 days of cultivation. For this purpose, a recirculation system with 9 water tanks was set up, with 3 boxes per group containing 930L of freshwater. After the embryos hatched, the larvae were transferred to nurseries (2 trays per tank, n = 64 larvae/tray), with 384 larvae per photoperiod. Water quality parameters were monitored and remained in the ideal ranges between treatments. However, temperature (p=0.004) and pH values (p= 0.009) for the 18L:06D photoperiod were lower than those observed for the 24L:0D photoperiod. A high mortality rate was observed in the first days of culture, specifically on the second day, for all photoperiods. However, there was a decrease in mortality throughout the experiment, with little variation in the mortality rate for the remainder of the experiment. In this context, it appears that the influence of the photoperiod on the survival of the fish seems to be related to the age of the animal.

KEYWORDS: Tilapia farming, Survival, Light stimulation.

RESUMO: A piscicultura é uma área em potencial desenvolvimento no ramo da aquicultura. O Brasil encontra-se entre os cinco maiores produtores de tilápia, no mundo, atingindo uma produção de 283 mil toneladas, em 2017. Tilápia tem sido intensamente cultivada e melhorada através de técnicas de cultivo mais eficientes. Neste sentido, o objetivo foi avaliar a taxa de mortalidade de larvas recém-eclodidas da tilápia do Nilo submetidas a três fotoperíodos (12Claro:12Escuro, 18C:06E e 24C:0E) durante 9 dias de cultivo. Para tanto, montou-se um sistema de recirculação com 9 caixas d'água, sendo 3 caixas por grupo contendo 930L de água doce. Após eclosão dos embriões, as larvas foram transferidas para berçários (2 bandejas por caixa d'água, n = 64 larvas/cada), com distribuição de 384 larvas por fotoperíodo. Os parâmetros de qualidade da água foram monitorados e mantiveram-se nas faixas ideais entre os tratamentos. Porém, os valores de temperatura (p = 0,004) e pH (p = 0,009) para o fotoperíodo 18L: 06D foram menores do que os observados para o fotoperíodo 24L: 0D. Observou-se alta mortalidade nos primeiros dias de cultivo, especificamente no segundo dia, para todos os fotoperíodos. Contudo, verificou-se uma diminuição da mortalidade ao longo do experimento, com dados pouco variáveis de mortes. Verifica-se, nesse contexto, que a influência do fotoperíodo, sobre a sobrevivência do peixe, parece estar relacionada a idade do animal.

PALAVRAS-CHAVE: Cultivo de tilápias, Sobrevivência, Estimulação luminosa.

INTRODUCTION

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Aquaculture is an ancient production model that originated in Asia. Currently, it is understood as a primary activity to address the population's nutritional deficiency. This is because it generates an excellent source of animal protein and because it creates job opportunities and revenues (ROCHA; RODRIGUES, 2015). This industry not only supplies protein-rich foods to a large and growing market but also indirectly reduces the pressure on the least sustainable fish populations from a production standpoint (BURGGREN *et al.*, 2019).

In 2014, 167.2 million tons of fish were produced, the majority of which came from fishing, with 93.4 million and 73.8 million coming from cultivation by aquaculture worldwide, respectively (FAO, 2016). In 2016, world fish production reached 171 million tons, with carp and tilapia as the main fish species in terms of production (FAO, 2018).

Tilapia are originally from Africa and arrived in Brazil around 1971 (MAINARDES-PINTO, 2000). They were initially introduced in dam reservoirs with the intention of restocking and consequent promotion for artisanal fishing and food security for local populations (EMBRAPA, 2015). There are several species of tilapia, and each has positive and negative characteristics according to the growing environment. Initially, in Brazil, *Tilapia rendalli* was introduced, which was later replaced by Nile tilapia (*Oreochromis niloticus*) which showed greater weight gain and growth in addition to good meat quality (DRUZIAN *et al.*, 2012). Tilapia are tolerant to heat, thus farming is especially successful in hot climates, with Nile tilapia usually grown in open ponds or hydrographic basins at temperatures of 30 to 35°C (BURGGREN *et al.*, 2019).

The total production of tilapia in Brazil reached 357 thousand tons in 2017, based on data published in 2018 by the Brazilian Association of Pisciculture, amounting to 51.7% of the total fish production in Brazil. This volume of production places Brazil among the world's four largest tilapia producers.

Tilapia larviculture corresponds to the most delicate period in the production chain, as it is from this stage that the best desired quality and animal production indexes are obtained (MEURER *et al.*, 2005). It is at this moment that the process of sexual

reversion takes place, a technique that produces a phenotypically monosex population, with the main purpose of controlling population density in the cultivation unit (BEARDMORE *et al.*, 2001).

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Density is an important factor in intensive fish farming, so it must be conducted in a planned manner, managed with technical and scientific criteria and guided by legal guidelines, to ensure the sustainable development of the activity (AYROZA; FURLANETO; AYROZA, 2006). Intensive cultivation, in aquaculture, is based on the offer of balanced feed and with high-density rates following regular and frequent monitoring of the physical-chemical parameters of the water. These elements are crucial for the success of intensive farming in fish farming (EL-SAYED, 2006).

Also, research on photoperiod has been identified as a possible way of improving fish farming (TAYLOR; MIGAUD, 2009; BIZARRO, 2013). Photoperiod corresponds to the duration of the light time over the 24-hour cycle (BROMAGE *et al.*, 2001), and it influences the development and survival of the animal, as light helps both in feeding strategy and in stimulating metabolic activities of several fish species, including Nile tilapia species (REYNALTETATAJE *et al.*, 2002). Research in Nile tilapia juveniles treated in different photoperiods has shown better results such as increased protein synthesis and decreased fat accumulation in photoperiod 18h light 6h dark (18L:6D) (VERAS *et al.*, 2014). A study carried out with fry showed better growth and increased mass in the larger light cycles, at 18L:6D and 24L:0D compared to photoperiods with extended dark periods (VERAS *et al.*, 2013).

In this context and considering some benefits of the photoperiod the objective of this research was to verify the mortality rate of newly hatched Nile tilapia (*Oreochromis niloticus*) larvae submitted to different photoperiods in an intensive cultivation system for 9 days.

MATERIAL AND METHODS

Location and ethics

To carry out this study, intensive cultivation systems were set up under different photoperiods at the Shrimp Laboratory of the Center for Human, Social and Agrarian

Sciences at the Federal University of Paraíba, Brazil (CCHSA / UFPB). This is located in the city of Bananeiras, Paraíba, Brazil, which, according to the 2010 Census of the Brazilian Institute of Geography and Statistics (IBGE), has an area of 258km², an altitude of 552m above sea level, a latitude of 6°39'6" and a longitude West 38°6'30" (IBGE, 2017).

The animals were kept under controlled conditions and all handling was carried out following the ethical principles required by the National Council for Animal Experimentation Control (CONCEA) and the experiments were performed after approval by the Animal Use Committee of the Federal University of Paraíba (CEUA-UFPB) under protocol number 7981110321/ 2021.

Experimental set-up

The experimental design was entirely random, with three photoperiod treatments in triplicate: 12 hours of light and 12 hours of the dark – 12L:12D; 18 hours of light and 6 hours of the dark – 18L:6D; and 24 hours of light and 0 hours dark – 24L:0D).

The *Oreochromis niloticus* embryos used in the experiment were acquired from the fish farm São José at Bananeiras, Paraíba, Brazil. The specimens were carried in plastic bags, with water and oxygen, to the laboratory.

Before seedling, the embryos were acclimatized for 30 minutes to the system water, in a thermal box, for gradual adaptation to the physical and chemical conditions of the water. Subsequently, they were washed and disinfected in a bath with 10% formalin solution for 30 seconds (SILVA *et al.*, 2015). Then they were transferred to a container with water from the system which, with the aid of a Pasteur pipette, underwent a thorough selection to remove residues from the washing solution and to obtain fertile eggs. The embryos were counted and distributed in polyethylene bottle incubators that were installed in the water tanks.

After hatching, considered the 1st day of the experiment, dead animals were removed and the hatched animals were measured and transferred to nurseries, according to the density described in Silva *et al.* (2015). Each of the three replicate water tanks

contained 2 nursery trays with 64 fish per tray), amounting 384 larvae per photoperiod. In the morning and afternoon, the nurseries were cleaned and dead animals were counted and removed from the tanks.

Water recirculation system and photoperiod regime

The water recirculation system was divided into three photoperiod regimes, each containing three 1000L polyethylene water tanks, which were fed by a 2000L reservoir and another 500L allocated inside the cultivation room and connected to the system. Chemical, physical and biological filtration of the water in the 2000L reservoir was carried out. The second reservoir (500L) fed the entire system with the aid of a 1800L/h peripheral water pump (Ferrari®, Acquapump).

For temperature control and monitoring, each box was equipped with a thermostat and a submerged thermometer. The oxygen was maintained using a radial air blower (model 2RB3107AA01) with a power of $\frac{3}{4}$ HP with a flow rate of 2.1m³/min. Silicone hoses with porous stones were installed into each tank to diffuse the air from the blower. A digital oximeter (Instrutherm®, MO-900, Brazil) and the pH-meter (Tecnopon®, LUCA-210, Brazil) were used to monitor oxygen and pH levels. Ammonia concentration was analyzed using a bench-top photometer (Alfakit® AT-100PB), and the alkalinity level by the titration method, both were measured weekly.

LED lamps (12W, LLUM Bronzearte, Slim LED) were used as a light source as described by Veras (2011) and were controlled by digital timers to obtain three photoperiods. The lights of the experimental periods 12L:12D and 18L:6D were switched on at 7 am and 1 am, respectively. The lights in the 24L:0D tanks remained on across the experiment.

Mortality rate

For mortality rate in the tanks during the 9 days interval, a casualty in each tank was recorded every day. Dead larvae were removed with the aid of a Pasteur pipette.

Statistical analysis

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To compare the averages of the physical-chemical parameters of the water, we used the ANOVA test, and to verify possible differences between the photoperiods, we applied the *posthoc* Tukey test. We verified the average of the absolute number of deaths using the Chi-square test. Statistically significance was set at 0.05. Statistical analyzes were performed using the IBM SPSS Statistics 21 program.

RESULTS AND DISCUSSION

Water parameters

Water quality parameters were measured daily and the results are described in Table 1. The parameters remained within the recommended range for Nile tilapia cultivation. However, the tanks in the 18L:06D photoperiod had a higher temperature ($p = 0.004$) and lower pH values (0.009) compared to the 24L:0D photoperiod.

Table 1. Water parameters (mean \pm standard deviation) during the experiment.

Water parameter	Mean \pm standard deviation				p-value	Recommended range
	12L:12D	18L:6D	24L:0D			
Temperature (°C)	27.99 \pm 0.86 ^{ab}	27.56 \pm 0.62 ^b	27.24 \pm 0.45 ^a	0.004	25-30	
Dissolved oxygen (mg/L)	6.12 \pm 1.18 ^a	5.99 \pm 1.05 ^a	5.98 \pm 0.85 ^a	0.889	>5	
pH	8.10 \pm 0.12 ^{ab}	8.14 \pm 0.14 ^a	8.24 \pm 0.19 ^b	0.009	6,0-8,5	
Ammonia (mg/L)	0 ^a	0 ^a	0 ^a	1	<0,1	
Alkalinity (CaCO ₃ /L mg/L)	53.33 \pm 1.15 ^a	53.33 \pm 2.30 ^a	53.33 \pm 1,15 ^a	0.296	>20	

The p-value for ANOVA test. Same superscript letters mean statistically equal means according to the Tukey post-test. Different letters mean statistically different means.

The temperature of thermal comfort for the development of tilapia is between 25 and 30 °C, with adaptation to temperatures lower than 14 °C or higher than 33 °C. Although tilapia is a rustic species, which means it is more resistant to handling,

variations in water quality and density (SEBRAE, 2016), Tilapia cultivation performs better with dissolved oxygen concentrations above 5 mg L^{-1} (MERCANTE *et al.*, 2007). The pH for the well-being of the species should be in the range of 6.0 to 8.5. pH values 4.5 and above 10.5 significantly contribute to high mortality rates (KUBITZA, 2000).

Ammonia and alkalinity were also analyzed (Table 1). Both parameters were maintained under recommended concentrations, with no statistical differences between the photoperiods. No toxic ammonia concentrations were detected, which must be lower than 0.1 mg/L and the alkalinity greater than $20 \text{ mg of CaCO}_3/\text{L}$ (EMBRAPA, 2016).

If not maintained within the recommended range, water quality significantly affects the animals' cultivation performance (EMBRAPA, 2016). The success of aquaculture enterprises depends strongly on the quality of the water in which the animals live (ARIDE *et al.*, 2007). The relationships between physical and chemical patterns need to be well understood to be able to properly manage the cultivation units and to contribute to the improvement of this activity (MERCANTE *et al.*, 2007). Thus, it is necessary to constantly control the quality of the water in which they are kept, as this will be a determining factor for better performance and growth of the animals. Failure to maintain the water parameters within the recommended range usually results in stress to the fish which increases the possibility of the occurrence of diseases and low survival rates (SEBRAE, 2015). In the present experiment, temperature, dissolved oxygen, pH, toxic ammonia and alkalinity remained within the recommended range for the cultivation of tilapia, and at values comparable between groups, which allowed the analysis of mortality based on the difference in photoperiod with a slight influence and environmental factors.

Mortality rates

There was an increase in mortality in the first days of tilapia life (Table 2), but no statistical differences were observed between the means of the photoperiods. Throughout the days, mortality remained similar between photoperiods until the end of the experiment, with low variation.

Table 2. Average absolute mortality of Nile tilapia submitted to 9 days under 12L:12D, 18L:06D, and 24L:00D photoperiod regimes.

Days post-hatch	12L:12D	18L:06D	24L:00D	Chi-square p-value
1	128 ± 0	128 ± 0	128 ± 0	0,99
2	13 ± 3	12 ± 4	19 ± 7	0,38
3	3 ± 2	2 ± 1	2 ± 1	0,87
4	3 ± 2	2 ± 2	1 ± 1	0,61
5	8 ± 7	7 ± 6	3 ± 4	0,31
6	0 ± 0	0 ± 1	0 ± 1	1,00
7	0 ± 0	0 ± 1	0 ± 1	1,00
8	0 ± 1	1 ± 2	1 ± 1	0,61
9	0 ± 0	0 ± 1	0 ± 1	1,00
Sum	155 ± 4	154 ± 4	155 ± 4	1,00

The data show that the initial period after the hatch is a critical phase for cultivation, which shows the importance of improving management techniques to result in lower mortality rates and better growth of tilapia in production plants. In line with the data, a mortality rate of around 89% and 7%, respectively, were reported in post-larvae and fingerlings of tilapia submitted to 24h light photoperiod (BEZERRA *et al.*, 2008).

Following the same perspective, tilapia fry submitted to different photoperiods (0L, 6L, 12L, 18L, and 24L) did not show statistical differences with the survival rate, with a high mortality rate for all groups (VERAS, 2013), thus evidencing a possible influence of the photoperiod to the age of the animal, which seems to be more influenced by the time of exposure to light (EL-SAYED; KAWANNA, 2004).

In the early stage of Nile tilapia development, they are particularly vulnerable and require intensive care for better animal development and growth. It is important to emphasize that in the production systems other factors affect the productivity of tilapia larvae, which are integrated with the reproductive peculiarities of the species, such as weight rank, nutrition, handling and stocking density of the breeders. Also, the environmental parameters are important during this phase of development such as temperature, salinity, hardness, pH, water flow and animal handling (MARENCONI; WILD, 2014).

Tilapia is a very resistant species surviving to a comparatively wide range of environmental conditions. However, in the initial phase of their development, they may have low survival rates, due to their greater fragility in this stage of life due to the conditions of artificial incubation (ÁVILA; ROMAGOSA, 2004). Thus, concerning the perspective of cultivation, a dynamic balance of all these factors must be sought in a viable and sustainable manner and, thus, meet the social, environmental and economic needs of the fish cultivation (ARANA, 2004; MERCANTE et al., 2007).

Therefore, fish farmers must take special care during the post-hatch stage, so that economic success can be achieved. The periodic analysis and maintenance of the climatic, biotic, abiotic and management factors in the ideal range are of importance to obtain a lower mortality rate and higher fish growth rate (LEIRA et al., 2017). Research aiming at the influence of the photoperiod in the development, survival and zootechnical performance of fish will contribute to the improvement of rearing techniques for the production of animals of commercial interest. And thus, offer a quality of fish products, especially Nile tilapia, which has great aquaculture potential (SANTOS et al., 2020).

CONCLUSIONS

The photoperiods 12L:12D, 18L:06D and 24L:0D did not statistically affect the survival of Nile tilapia larvae during the 9 days of the experiment. However, a high mortality rate was observed in the first days of the animal's life, especially on the 2nd day of life. However, there was a decrease in mortality for the remainder of the experiment, with little variability in death rates, to the early days of cultivation. Thus, given the data described and reported in the literature, the influence of the photoperiod on the biology of the fish seems to be linked to the age of the animal.

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