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The impact of photoperiod to accelerate the development and maturation of platy fish *Xiphophorus maculatus*

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Abstract. This study was conducted to determine the manipulation capacity of photoperiods for alteration of growth, reproduction and coloration of platy fish. The experiment had four treatments (T1= 12 hrs (hours) L (Light): 12 hrs D (Dark), T2= 24 hrs L: 0hrs D, T3= 7 days12 hrs L 12 hrs D: 7 days 24 hrs D, T4= 7 days 24 hrs L: 7 days 24 hrs D). Each treatment had three replications with eight fish larvae reared for five months. At the end of the experiment, T2 had the highest mean weight (0.3587±0.009 g), length (2.4±0.0001 cm), and SGR (0.2990±0.0490). The T1 and T2 showed the highest survival rate (87.5 %). T2 had the best reproductive results (maturing in 113 days), the largest percentage of colorful fish (95.24%) and carotenoid content (0.0054 ±0.0003 mg. g^{-1}). A strong interaction was observed between the photoperiod, growth performance, and photoperiod and the coloration of platy fish (p<0.05). This study suggests that extended photoperiod can improve the growth performance and coloration of the platy fish.
Keywords: *Xiphophorus maculatus*, Carotenoid content, Ornamental fish

Introduction

Ornamental fish are becoming very popular worldwide because of their simple operating system and lower operating costs. There are over 30,000 fish species registered worldwide, with 800 of them being ornamental fish (Thomas 2020). However, its trade is expanding in Bangladesh (Mostafizur *et al.* 2009). Pigments are responsible for the vast spectrum of colors present in fish, which is an essential criterion for quality because they demand higher prices in the commercial market (Gupta *et al.* 2006). It is possible that additional light has a beneficial impact on fish growth over a long period, and photoperiod studies take more than 60 days to absorb effects of photoperiod (Barlow *et al.* 1995).Seasonal variations in photoperiod, temperature, rainfall, and other factors influence and organize developmental and maturational events (Vazquez *et al.* 2000).The importance of photoperiod in the commencement or suppression of reproductive timing and gonadal maturation differs by species (Singh and Zutshi 2020). The skin color is controlled by the pigments and microstructures found within the fish integument, which absorb, reflect, and scatter light (Fujii 2000). Carotenoids are lipid-soluble pigments that determine the market value of ornamental fish by controlling the color of their skin (Paripatananont *et al.*1999). They are pigments found naturally and range in color from yellow to red (Hill 2004). This experiment was done to determine the influence of photoperiod on the growth and maturation of Platy fish and to popularize the use of photoperiod in ornamental fish breeding.

Material and Methods

This study was conducted from March to July 2019 (total of 150 days) in the wet laboratory and the aquatic ecology laboratory of the Chattogram Veterinary & Animal Sciences University, Chattogram, Bangladesh. The fry of platy fish was collected from the ornamental fish shop Reyazuddin Bazar, Chattogram (22.3365°N, 91.8303° E). All the collected fry (n= 96) had the

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same age and from the same batch. Before starting the experimental treatments, fish fry was conditioned for three days and disinfected with 10 ppm formaldehyde for 10 minutes. The following experimental treatments were carried out during this study:

i. Treatment 1 (T1): 12 hours' light with 12 hours' dark in a day
ii. Treatment 2 (T2): 24 hours' light and no darkness in a day
iii. Treatment 3 (T3): Incessantly seven days 12 hours' light with 12 hours' dark condition, and next seven days 24 hours' dark condition
iv. Treatment 4 (T4): Incessantly seven days 24 hours' light and seven days 24 hours' dark rhythms

All the treatments had triplicates. Experimental fishes were cultured in rectangular transparent glass aquariums (n = 12 and L × W × H = 23.62 cm × 17.78 cm × 27.94 cm) filled with 8 liters of disinfected freshwater. A total of 8 fries were stocked in each aquarium. Six aquaria were illuminated with 12 hrs of sunlight and four 300 lux LED (light-emitting diodes) bulbs. Light intensity was measured with a lux meter (HTC Lx 101a). Dark condition was maintained at night. The black curtain and paper were used to create the dark artificial condition. Switching of photoperiods was maintained manually throughout the study period. Commercially available powder feed of the brand Super NOVA (moisture 12%, crude protein 26%, crude fat 5%, carbohydrate 35%, and fibre 7%) was providedat 4 - 5% of biomass of the fish and twice per day. Uneaten feed and faces were removed by siphoning every morning. Water quality was monitored regularly and maintained the ambient water temperature (24 - 27 ºC), pH 7 - 8, and 6.5 - 7.5 ppm dissolved oxygen (DO). About 40% of the water was exchanged once a week.

Growth performance: The growth performance (i.e., length, weight, SGR) and survival were recorded at every15days interval of stocking. The electric weight machine of the RADWAG AS 220/C/2 model was used to weigh the experimental fishes. The length of the fish was measured by measuring scale. There was no use of any anesthetics during sampling. Percent weight gain, SGR (%), and survival rate (%) (Roy *et al.* 2020) were calculated using the following formulas:

- Survival (%) = $\frac{\text{number of fish survived}}{\text{number of fish stocked}} \times 100$
- Specific growth rate ($\%day^{-1}$)=$\frac{\text{ln final weight}-\text{ln initial weight}}{duration in days} \times 100$
- Percent weight gain (%)=$\frac{\text{Mean fish final weight}-\text{Mean fish Initial weight}}{\text{rearing periods (total period)}} \times 100$

Reproductive performance: Reproductive performance was estimated based on the maturation period and the total number of newly born fish larvae. The number of fast-maturing and spawning fish was observed.

Pigmentation (total carotenoid content (TCC) in muscle and skin: The color enhancement was examined visually, and the following formula presented the percentage of fish coloration:

Percentage of coloration = (Number of total colored fish)/(Number of total fish)×100

Immediately after completing the experiment, the total carotenoid concentration (TCC) in the fish muscles tissue was analyzed. The carotenoid concentration in the skin and muscle of experimental fish was quantified following the protocol of Martínez *et al.* (2005). The fish skin and muscle were removed using the procedure described by Torrissen and Naevdal (1984).A sample of

1 gram of skin and muscle from the fish was taken and mixed with mortar pestles. These samples were transferred to 10 ml centrifuge tubes, and acetone was added to 10 ml. The samples were centrifuged at 5000 rpm for 5 minutes. The solutions were stored at 4 °C in the refrigerator for three days before being extracted three or four times until no more color was formed. The absorbance of the extracted liquids was measured using a spectrophotometer (Mecasys Co Ltd- Optizen Pop) (wavelength 470 nm for animals).The concentration of carotenoid was estimated and expressed as µg/g carotenoid using the following equation:

Carotenoid Value = (Abs * 10000 * V)/ (1900*W)

In where, Abs= Pigment absorption rate which measured by spectrophotometer, V= Total volume of the extract, W= Weight of sample.

Data analysis: All the data analyses for this study were performed using Microsoft Excel 365 and IBM SPSS ver. 23. The fishes' total length (TL) and body weight increment were compared using one-way analysis of variance (ANOVA) and following Tukey's test.

Results

The effect of different photoperiods on fish growth, reproduction, and coloration have been investigated, and the findings have been presented below:

Effects of photoperiod on growth performance: The effect of photoperiod on growth of Platy fish was analyzed from the final samples, and the results are presented in Table I. The initial average weight of fish was 0.0226 g. The final average weight of each treatment (T1, T2, T3, and T4) was 0.3161 ± 0.0063, 0.3587 ± 0.0090, 0.2453 ± 0.0187, 0.2634 ± 0.0117 (g), respectively (Table I). The initial length of the fish varied from 1.3 to 1.6 cm. The final average length of each treatment was T1, T2, T3, and T4, which were 2.2670 ± 0.0580, 2.4000 ± 0.0001, 2.1000 ± 0.0001, 2.133 ± 0.0577cm, respectively (Table I). The best SGR value found was 0.2990 ± 0.0490in T2. Under different photoperiodic conditions, statistical analysis indicated that mean length, mean weight and mean SGR of Platy fish of T2 were significantly higher ($p< 0.05$) than the other three treatments.

Table I. Effect of photoperiod on the growth performance of Platy fish

Growth Parameter	Treatment			
	T1	T2	T3	T4
Mean weight ±SD (g)	0.3161 ± 0.0063^{b}	0.3587 ± 0.0090^{a}	0.2453 ± 0.0187^{c}	0.2634 ± 0.0117^{c}
Mean length ±SD (cm)	2.2670 ± 0.0580^{b}	2.4000 ± 0.0001^{a}	2.1000 ± 0.0001^{c}	2.133 ± 0.0577^{c}
Mean SGR ±SD	0.0837 ± 0.0175^{c}	0.2990 ± 0.0490^{a}	0.1768 ± 0.0452^{b}	0.1862 ± 0.0071^{b}

Effects of photoperiod on body coloration: The effect of photoperiod oncoloration of Platy fish was analyzed from the final samples, and the results are presented in Fig 1. The final mean carotenoid content of each treatment (T1, T2, T3, and T4) was0.0045±0.0019, 0.0054±0.0003, 0.0036±0.0003, 0.0042±0.0002(mg. g^{-1}), respectively (Fig 1). Under different photoperiodic conditions, statistical analysis indicated that mean length, mean weight and mean SGR of Platy fish of T2 were significantly higher ($p< 0.05$) than the other three treatments. The study showed T2 had the highest carotenoid (0.0054 ± 0.0003 mg. g-1)) and the lowest (0.0036±0.0003mg. g-1) was found in T3. T2 was statistically significantly different from the other three treatments, and there was no difference not only in T3 and T4 but also in T1 and T3 in terms of mean carotenoid content.

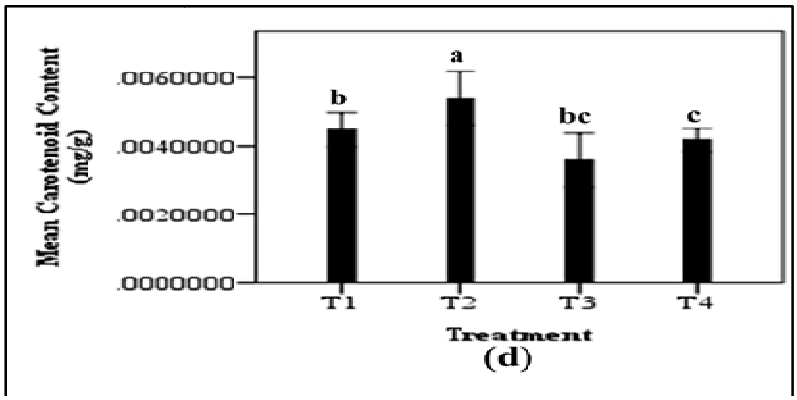

Fig. 1. Effects of photoperiod on mean carotenoid content (mg/g) of Platy fish.

Effect of photoperiod on survival rate, percentage of red-colored fish, percent weight gains of fish: The experiment's highest survival rate was 87.5% in T1 and T2 (Fig. 2). The highest weight gain was 0.19% in T2, and the lowest was 0.15% in T4 (Fig. III). The best visual coloration was 95.24% in T2, and the least was 58.82% in T3(Fig. II).

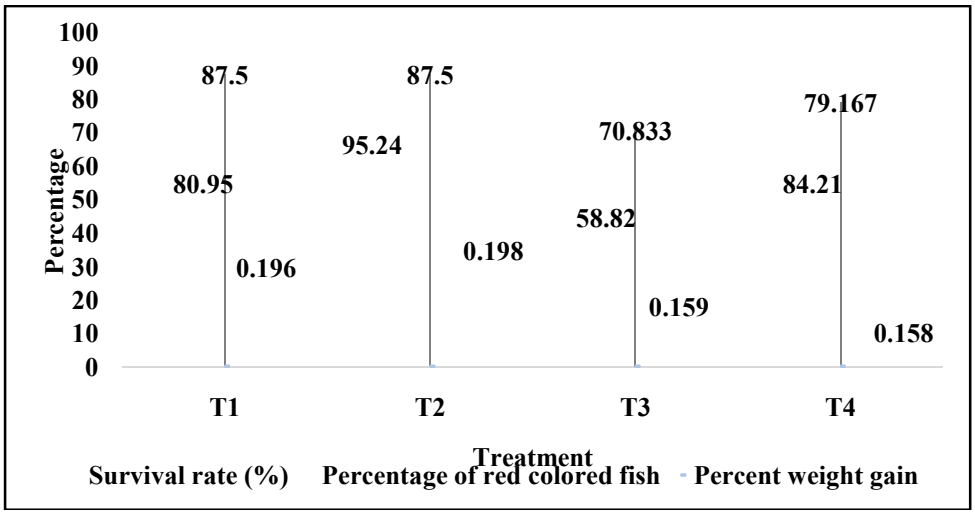

Fig 2. The effect of photoperiod on survival rate, percentage of red-colored fish, percent weight gains of fish.

Effect of photoperiod on reproductive performance: Among four treatments, T2-treated (0hrs D: 24 hr L) brood fish gave birth early. It only took 113 days (average) to attain sexual maturity. T2-treated fish gave birth total 25platy fish throughout the research(Table II).

Table II. Reproductive performance of Platy fish

Treatment	Maturation period (days)	Fry Number (newly born platy fish fry)
T1	126	16
T2	113	25
T3	134	8
T4	140	6

Discussion

Effect of photoperiod on the growth pattern: Ten samplings were done during the research period where the1st sampling showed a higher weight gain due to the peak growing period. From second to fourth sampling, lower growth rate and lower weight gain occurred because time was taken to adjust the new physiological phase. Moreover, fourth sampling to seventh sampling showed a higher weight gain in all experimental fish in which T2 showed higher weight than others because of a suitable environment, genetic process, maturity, and gestation period. After the 7th sampling, lower weight gain was found in all experimental fish during the sampling time due to fluctuation in temperature and weather change. The higher weight gain was found later in the9thsampling due to the pregnancy period of platy fish (Table I). The higher photoperiod treated fish had a higher length than the lower ones (p<0.05). The specific growth rate was used to deal with the growth of aquatic organisms under experimental conditions. The highest SGR value was recorded in T2 (0.2990±0.0490) among four treatments. It was suggested that their growth was hampered because developing juveniles were inactive during the hours of darkness. Some previous investigations on other species coincided with the conclusions of this study. According to Barimani *et al.* (2013), for young French and Iranian rainbow trout, the continuous (24 L:0 D) and long photoperiod (16 L:8 D) photoperiods were suggested for faster growth and lower feed conversion rates, respectively. According to Biswas *et al.* (2005), the rapid growth performances of red sea bream under 16L:8D and 24L:0D photoperiods were linked to improved appetite, greater food intake, higher feed conversion efficiency, and higher digestibility. Veras *et al.* (2014) examined that the weight and length of larvae exposed to the longest photoperiods (24 L:0 D and 20 L: 4 D) improved significantly. As a result, a photoperiod of 20 L:4 D with twice-daily feeding is recommended by them. Fish reared under the 24 hr L:0hr D light regime feed for longer periods than those reared under the shortest photoperiod, resulting in higher growth and development rates. As a result, the current study concluded that the photoperiod had a direct effect on the growth and development of Platy fish. The highest survival rate was found in both T1 and T2, and the lowest was in T3. According to Howell *et al.* (2003), mortality of black seabass during the experimental period did not vary significantly between treatments and was unrelated to the photoperiod. So, it was proved that photoperiod has no negative effect on the survival rate of Platy fish.

Effect of photoperiod on reproductive performance: Light is one of the most important environmental parameters because it synchronizes fish growth from embryo to sexual maturity (Guo *et al.* 2012,Villamizar *et al.* 2011). In T2, Platy's reproductive rate was highest, and it took less time than other treatments to breed. This study started brood reproduction after the 7th sampling in T2R1 and T2R2 aquaria. It was shown in this research that the T2 treated Platy took the shortest time, which was 113 days on average among four treatments. T2 also produced 25 platy fish fries, which was the best among others. Some research justified this study. The long photoperiod regime also successfully stimulates spawning in goldfish, so the long photoperiod warm temperature regime is a powerful tool to modify gonadal growth (Sarkar and Upadhyay

(2011). The results showed that fish subjected to a long photoperiod gained much more weight, had a higher specific growth rate and had a higher gonadosomatic index than those exposed to a short photoperiod and a control condition(Singh and Zutshi 2020).Seasonal variations in photoperiod, temperature, food sources, rainfall, and other factors influence and organize developmental and maturational events (Vazquez *et al.* 2000).

Effect of photoperiod on coloration: According to the findings of this study, fish grown under higher light levels showed brighter colors than those under lower photoperiodic levels. Environmental stress causes color changes in fish, and lighting may be a key component in regulating pigment distribution via hormone modulation (Salm *et al.* 2004). The pigments and microstructures within the fish integument absorb, reflect, and scatter light, resulting in the color of the skin (Fujii 2000). Photoperiod enhanced enzyme activity and respiration rate in fish, affecting their metabolic rate. A shorter photoperiod decreases photosynthesis and increases carbohydrates, whereas a longer photoperiod causes photochemical reactions and color pigmentation (Boeuf and Bail 1999). According to the carotenoid content analysis, fish reared in low light (250-500 lux) had significantly higher carotenoid content than fish reared in medium or high light(Rajeswari *et al.*2007).As a consequence of the current study's findings, it was stated that a greater light intensity (T2- 24 hrs' L: 0 hrs' D) was more suitable for boosting the skin color of platy fish, which might be recommended for the species' continued development.

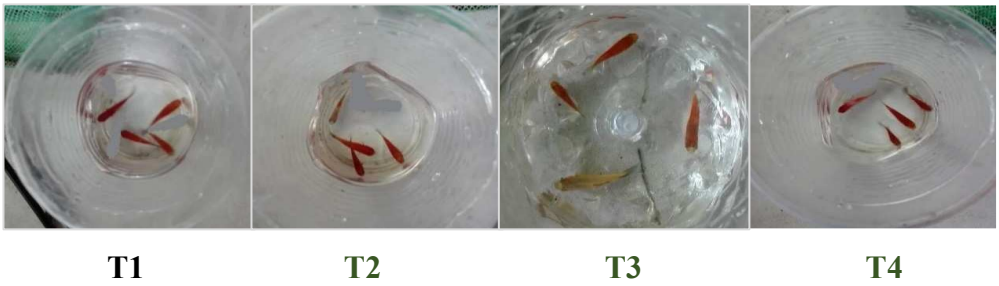

Fig 3. The effect of photoperiod on growth and body coloration of platy fish.

The impact of photoperiod on fish physiology must be observed because it is a significant environmental factor. The purpose of this study was accomplished, which was to determine the ability of photoperiod to manipulate the physiological performance of ornamental platy fish. Now-a-days, feeding a carotenoid diet has become very popular to enhance the coloration of fish. Besides, photoperiod may be subsidiary to create pigmented fish for the ornamental fisheries sector. Continuing this form of research will help create an effective policy for the faster and more sustainable growth of aquarium fish production.

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