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Growth and reproductive response in mango platy (*Xiphophorus maculatus*) to photoperiodic manipulation; effects on growth, gonadal development and fry production

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Abstract- Photoperiod is one of the important environmental factor, controlling different stages of fish growth to maturation. The present work was studied to evaluate the influence of photoperiod on the somatic growth, gonadal development of male and female fish and reproductive performance related to fry production in ornamental exotic fish, mango platy (*Xiphophorus maculatus*). After acclimatization for a week in lab condition the fish groups were exposed to long (18L: 6D) and short (10L: 14D) photoperiod for a period of 60 days and control fish group were kept in natural environmental laboratory condition. Throughout the study light intensity was kept constant at 1500 lx, dissolved oxygen (DO) ranged from 4.5 to 6.5mg/l, free ammonia varied between 0.72 to 0.78mg/l and pH was 7.0 to 7.5. Fish were fed with formulated diet twice a day (9.00hrs - 16.00hrs). Growth was analyzed in terms of weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF). Weight gain (1.24±0.076), FCR (54.03 ± 0.29), SGR (0.81±0.033) and GSI of female fishes (24.6±0.66) was higher in fish group exposed to long day photoperiod (18L:6D) when compared to those of short day photoperiod and control. Maximum number of yolky eggs and embryos were observed in the ovaries of female fish group exposed to long photoperiod. In view of the above, result of the present study suggests that long day photoperiod along with formulated feed may be recommended for healthy somatic growth and early fry production in *X. maculatus* without causing mortality and stress.
Keywords- Mango platy, *Xiphophorus maculatus*, Somatic growth, Gonadosomatic index (GSI), Fry production, Photoperiod.

1. INTRODUCTION

The ornamental fish demand in an aquaculture sector has increased since the 1990s. Among the ornamental fish producing countries, Singapore is the biggest exporter of many species [1]. Culture and breeding of ornamental fishes can be a promising alternate livelihood for many people as well as for unemployed youths. Nowadays ornamental fish culturist gaining importance because of their aesthetic value and attractive color patterns.

Mango platy *X. maculatus*, a small cyprinid fish belonging to the family – Poeciliidae, originated in Mexico, is an ornamental livebearer fish. Since it is a popular tropical fish and considered one of the most attractive aquarium fish having a life span of 5-6 years and their breeding season is March to November, techniques for regulating its growth and manipulating the reproductive cycle for any time of the year are essential. It has been observed that most of live-bearers prefer a vegetarian diet composition and it should be balanced with protein, carbohydrates, lipid, minerals and vitamins for their healthy growth rate [2].

The biological rhythm of animal including fish is dependent on environmental factor such as photoperiod, temperature as well as types and quality of food supply. Photoperiod provides the most reliable factor to manipulate the reproductive timing, inducing growth, physiological and immunological changes in many species of fish [3-4]. Increase in growth was reported in some species when exposed to for long photoperiods, either by increasing feed intake, inducing muscle mass development due to increased locomotor activity [5] or better nutrient use efficiency [6]. Photoperiod with temperature is commonly used to control the reproductive cycle and obtain larvae all year round in fish farms [7]. Photoperiod manipulation has been used successfully to improve the growth and maturation of many tropical fish species like- Nile tilapia, Eurpeon sea bass and Topmouth gudgeon [8-10]. Several studies have been demonstrated regarding growth performance and maturation due to increased photoperiod causing increase in growth [11], feed intake [12], maturation [13] and fry production [14]. Photoperiod manipulation is of economic value to several tropical aquaculture industries, but information regarding the application of these techniques on livebearer species is limited. Thus the optimal photoperiod required for better growth and reproductive performance of *X. maculatus* can be considered first step in the introduction of artificial lighting regimes for benefiting aquaculture industry.

The objectives of this study were to determine the effect of different photoperiodic regime (18L:6D and 10L:14D) on key growth parameters (weight gain, feed conversion ratio, specific growth rate, condition factor), viscerosomatic index and

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hepatosomatic index, maturation (gonadosomatic index) and fry production potential by influencing brood size of *X. maculatus* in laboratory condition.

2. MATERIALS AND METHODS

2.1 Experimental fish

The experimental fish *X. maculatus* was obtained from its brood stock in Ornamental Fish Research Center, Hebbal, Bengaluru. The test fish with a mean initial weight of 0.52±0.003g/fish and average total length of 3.5±0.001cm was selected for the conduct of experiment.

2.2 Experimental design

To achieve the objectives of the experiment, fish was subjected to two artificial photoperiod regimes (18L:6D and 10L:14D) and control group in natural light regime (laboratory condition). The experiment was conducted in the laboratory using fiberglass tanks with a capacity of 50L for each fish group. Water was aerated by a constant supply of air pump and 25% of water in each tank was renewed daily with fresh dechlorinated water so as to remove the feces and uneaten feed. The control tank was maintained in laboratory condition. The wooden chamber consisting of experimental groups of fishes to estimate photoperiodic effect was equipped by a florescent lamp of 28W suspended about 40cm over the water surface of each experimental tank. The light intensity was kept constant at 1500 lx, the temperature was maintained at 28^{o}C by thermostatically controlled 50W heaters (model no.RS008-A) in all treatments and the photoperiod regime was maintained by digital timer control.

The physico-chemical analysis of water (control and experimental) was conducted on weekly basis. The dissolved oxygen (DO) and free ammonia analyzed by standard method of APHA et al. [15] and pH by pH meter (model-361). The DO ranged from 4.5 to 6.5mg/l, free ammonia varied between 0.72 to 0.78mg/l and pH fluctuated as 7.0 to 7.5 in all the tanks including control as well as experimental throughout the period of experiment.

2.3 Experimental procedures

Each experimental tank was stocked with 4 fish (1male: 3female) with three replicas for 60 days. Mean initial body weights of 0.52 ± 0.003 g were selected as experimental groups and their replicates were stocked in each tank to avoid differences. The fish were acclimatized for 7days prior to start of the experiment at 26°C and were fed with formulated feed used during the period of study. Prior to the experiment day fish were starved for 24 hours and total length and weight measured. Fish were fed twice per day (09:00h and 16:00h), with a formulated feed for the whole experimental period at the rate of 15% (28days), 10% (20days) and 5% (12days) of body weight per day for 60 days [16]. The detail of feed formulation in % of inclusion is as follows-

Composition of the formulated feed:

S. No.	Ingredient	Contents (g)
1	Fish meal	30
2	Groundnut oil cake	10
3	Wheat flour	20
4	Rice brain	20
5	Vegetable oil	2
6	Vitamin	1
7	Minerals	4

Proximate composition of formulated feed on dry matter basis (%):

S. No.	Chemical composition	Formulated feed (%)
1	Crude protein	22.18
2	Moisture	11.23
3	Dry matter	88.77
4	Crude fiber	1.15
5	Ether extracts	9.30
6	Total ash	17.34
7	Nitrogen free extracts	50.03

2.4 Analysis of growth parameters

At the end of the experiment data were analyzed for the weight gain (WG), length gain (LG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) using the following formulae:

WG (%) = final body weight - initial body weight

LG (%) = final body length - initial body length
SGR (%) = 100 × [L_n (W_2)-L_n (W_1)]/time (days)
Where, W_1 and W_2 indicate the initial and final weight (g), respectively.
FCR = Feed delivered to group/ Live biomass gain of that group
CF = 100 X (W/L^3)
Where, W= wet body weight (g) and L = standard body length (cm)
VSI % = 100 X (wet weight of visceral organs and associated fat tissue (g)/ total body weight)
HSI % = 100 X [wet weight of liver (g)/wet body weight (g)]
Survival rate %= (Final fish number - Initial fish number) × 100/Initial fish number

2.5 Analysis of Gonadosomatic index (GSI)

At the end of the experiment fish were anesthetized by MS-222, and the gonads removed. For the female fish the ovaries were weighed and opened to count the number of yolky eggs and embryos present. Gonad weight of male and female fish was assessed in terms of gonadosomatic index (GSI) by using the following formula:
GSI (%) = (wet weight of gonad (mg) /wet total body weight) × 100

2.5 Data sampling and analysis

The data were analyzed and the results were expressed as mean (triplicates) ± SEM. The statistical significance of experimental and control groups was computed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-hoc test and the least significant difference was used to compare means at $P < 0.05$. The linear relationship was assessed by using linear regression and Pearson correlation coefficient. All statistical analysis was done by using GraphPad Prism ver. 6.00.

3. RESULTS

3.1 Growth parameters

To analyze the growth performance, the fish *X. maculatus* was exposed to different photoperiods. The result indicated that the fish exposed to long photoperiod (18L:6D) attained significantly higher weight gain (one - way ANOVA, df = 2; F = 8.546; $P < 0.0175$) and specific growth rate (one - way ANOVA, df = 2; F = 12.87; $P < 0.0067$) compared with control (Table 1), whereas feed conversion rate was significantly higher in 18L:6D (one - way ANOVA, df = 2; F = 104.3; $P < 0.0001$) compared with short photoperiod (10L:14D) and control (Table 1). Total length and condition factor was higher in long photoperiod but not significantly different from other treatments ($P>0.05$) (Table 1). No significant difference was observed in viscerosomatic index and hepatosomatic index between control and photoperiodic treatments (18L:6D and 10L:14D) (Table 1). Survival rate of fish exposed to the photoperiodic treatment was 100% and no mortality was observed during the 60 days of experiment in different photoperiods.

The linear regression indicated a significant relationship between mean body weight of fish and condition factor [linear regression, n = 3; r = 0.85; $P < 0.004$; Fig. 1 (a)], mean total length [linear regression, n = 4; r = 0.86; $P < 0.003$; Fig. 1(b)] and specific growth rate [linear regression, n = 4; r = 99; $P < 0.0001$; Fig. 1 (c)].

Table- 1 Growth performance of *X. maculatus* exposed to different photoperiods

Parameters	Control	10L:14D	18L:6D
Initial body weight (g)	0.50±0.00	0.50±0.00	0.50±0.00
Initial body length (cm)	3.50±0.00	3.50±0.00	3.50±0.00
Final body weight (g)	1.36±0.06	1.61±0.04	1.84±0.06
Final body length (cm)	4.40±0.08	4.50±0.05	4.70±0.04
Weight gain (%)	$0.86±0.07^{a}$	$1.01±0.04^{ab}$	$1.24±0.07^{b}$
Length gain (cm)	1.09±0.07	1.03±0.05	1.22±0.04
FCR (%)	$46.86±0.17^{a}$	$50.95±0.40^{b}$	$54.03±0.29^{c}$
SGR (%)	$0.59±0.04^{a}$	$0.71±0.02^{ab}$	$0.81±0.03^{b}$
CF	1.53±0.03	1.70±0.09	1.73±0.03
VSI (%)	0.33±0.22	0.13±0.001	0.12±0.03
HSI (%)	3.70 ± 1.30	2.40±0.09	1.66±0.40
Survival rate (%)	100.00 ± 0.00	100.00±0.00	100.00±0.00

Values are present as mean ± standard error (SEM) of three replicates. Significance was calculated by one-way ANOVA and post-hoc test was done with Tukey's multiple comparison using GraphPad Prism 6.00 and significance differences ($P<0.05$) between photoperiod treatments are presented by different superscripts

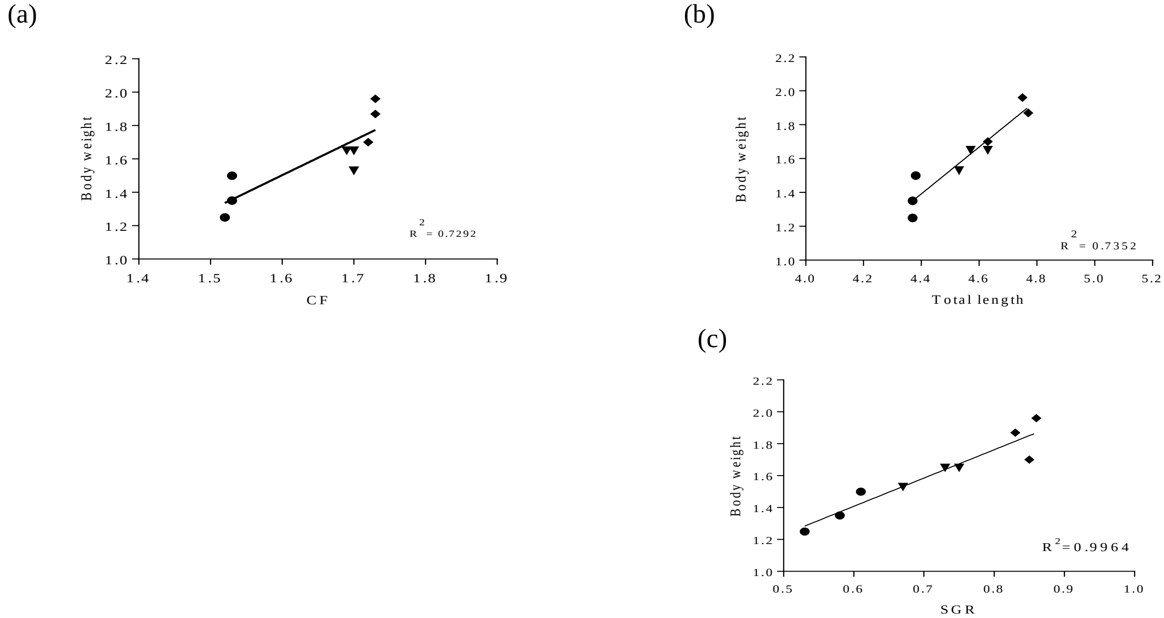

Figure 1. The linear relationship assessed by linear regression and Pearson correlation coefficient using GraphPad Prism ver. 6.00, between body weight of *X. maculatus* and (a) condition factor (CF) (b) total length (c) specific growth rate (SGR). The curves were fitted by: (a) y = 2.084*X - 1.832; (b) y =1.384*X - 4.698; (c) y = 2.118*X + 0.1133.

3.2. Gonadosomatic index (GSI)

In the present study photoperiod is an important factor for the gonadal development and GSI of the *X. maculatus*. The gonadal development in fish showed significant variation when exposed to different photoperiod (18L:6D and 10L:14D). The mean GSI of males did not showed significant difference in those exposed to photoperiod treatment (18L:6D and 10L:14D) and control (Table 2) whereas those of females differed significantly between long photoperiod (18L:6D) and control as well as in short photoperiod (10L:14D) and control (Table 2). The Mean GSI of females under long photoperiod (18L:6D) was significantly higher than those of under short day photoperiod (10L:14D) (one - way ANOVA, df = 2; F = 103; P < 0.0001) (Table 2).

A significant relationship indicated by linear regression between mean body weight and GSI of female [linear regression, n = 3; r = 0.85; $P < 0.004$; Fig. 2 (a)] and also between mean body length and GSI of female *X. maculatus* [linear regression, n = 3; r = 0.71; $P < 0.03$; Fig. 2 (b)].

Table- 2 Gonadosomatic index (GSI) of *X. maculatus* under different photoperiods

Parameters	Control	10L:14D	18L:6D
Sex ratio (female : male)	3:1	3:1	3:1
Male GSI (%)	2.03±0.67	1.03±0.22	1.63±0.32
Female GSI (%)	10.20±0.49^{a}	18.90±0.92^{b}	24.60±0.66^{c}

Values are present as mean ± standard error (SEM) of three replicates. Significance was calculated by one-way ANOVA and post-hoc test was done with Tukey's multiple comparison using GraphPad Prism 6.00 and significance differences (P<0.05) between photoperiod treatments are presented by different superscripts

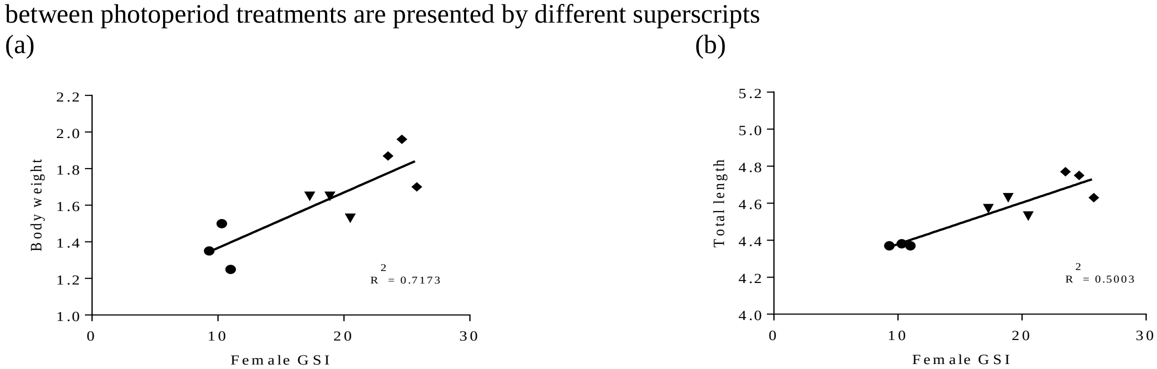

Figure 2. The linear relationship assessed by linear regression and Pearson correlation coefficient using GraphPad Prism ver. 6.00, between body weight of *X. maculatus* and (a) female gonadosomatic index (GSI), and (b) between total length and GSI of female fish. The curves were fitted by (a) y = 0.03029*X + 1.064 and (b) y = 0.02239*X + 4.155.

3.3. Reproductive performance and fecundity

The number of yolky eggs of *X. maculatus* showed significant difference (P<0.05) when exposed to different photoperiod regimes. Females of *X. maculatus* exposed to long photoperiod regime (18L:6D) had significantly more yolky eggs and embryos within their ovaries than that of short photoperiod (10L:14D) and control. Fecundity was estimated by including yolky eggs along with the embryos which will give rise to subsequent brood. Since maximum spawning was observed in fish under long day photoperiod the fecundity of fish was 54. Although the fish under short day photoperiod had slightly lower fecundity (50), the specimens contained maximum of undeveloped eggs along with very few yolky eggs and embryos in the ovaries (Table 3) (Fig. 3). Maximum number of females observed was without eggs and embryos in control group.

The photoperiodic regime of long and short photoperiod successfully influences growth, gonadal development and reproductive performance in female and male of *X. maculatus* in laboratory condition.

Table- 3 Reproductive performance of female *X. maculatus* under different photoperiods

	Control	Photoperiod	
		10L:14D	18L:6D
Number of fish with yolky eggs & / or embryos	1	2	4
Percent of total sample	10%	25%	50%
Mean standard length (range)	4.45 cm	4.53cm	4.72 cm
Mean number of yolky eggs and embryos	36	50	54
Number of females without eggs or embryos	4	3	3

Total number of female fishes in each treatment was – 03/replicates (total, 09)

4. DISCUSSION

The aim of this objective was to investigate photoperiod regulation of somatic growth and gonadal development in *X. maculatus*. Therefore, the photoperiod regimes reported to most successfully influence maturation in *X. maculatus* in laboratory condition. Photoperiod was used to simplify seasonal transfer with the help of long and short day regime to investigate growth, gonadal development and reproductive performance in terms of physiological parameters. A complete understanding and manipulation of photoperiod has been recorded by various scientists to improve the productivity of ornamental aquaculture. According to Barimani et al. [11], photoperiodic effects are species specific which is related to environmental adaptation. It has been observed that freshwater fish are more sensitive to photoperiod than those of marine species [17]. There is a lacunae in the studies related to the effect of photoperiod manipulation on gonadal maturation and fry production in the *X. maculatus,* although similar investigation have been reported in several other species.

4.1 Effect of photoperiod on growth

The results showed that growth performance were higher in *X. maculatus*, exposed to the long day photoperiod (18L:6D). Similar results have been reported for several fish species, *Rutilus rutilus caspicus* [18], *Oncorhynchus mykiss* [19] and *Oreochromis niloticus* [8], suggesting that growth can be induced in many fish species with the help of photoperiodic exposure. In the present investigation positive correlation observed between total length and condition factor (CF) with its body weight, during long day photoperiod suggests that long day photoperiod improves the growth performance in ornamental fish. In similar lines SGR also showed significantly higher values in fish exposed to 18L. These results are in confirmation with Biswas et al., [12] (*Pagrous major*) and Hernandez et al. [20] (*Diplodus puntazzo*). A significant increase in FCR under long photoperiod were parallel found in other species such as, *Beluga sturgeon huso huso* [21], *Micropterus salmoides* [22] and *Sparus aurata L*. [23]. Such increase in FCR may be attributed to increase in swimming activity due to exposure to long day photoperiod with an increase in the food intake or stimulation of appétit, since long photoperiods induces secretion of growth promoting hormones. This intern might improve digestion resulting in increased somatic growth [10]. Good amount of food intake was observed during short photoperiod regimes and also in control group under natural light indicating that these fishes are not completely dependent on exposure to light regime for feeding, but the feed intake of the fishes was greater when exposed to long day light period than control and short photoperiod.

The results of this research demonstrated that photoperiodic manipulation of extended day length 18L:6D allow *X. maculaus* to stay active and consume more food and thereby increase growth. These results may help towards a long photoperiod regime for the aquaculture industry to increase growth as well as reproduction of *X. maculaus*.

4.2 Effect of photoperiod on reproductive performance

GSI is one of the functional indicators to investigate the reproductive biology of fish that demonstrates gonadal development. Swain et al. [2], reported that the platys' takes 6-8 weeks to mature. In the present study the GSI values of the female fishes exposed to long day photoperiod showed an increase when compared to short day photoperiod and control. These results clearly demonstrated that the timing of maturation cycle in female fishes showed alteration in response to the photoperiodic regimes. Similar results on gonadal development was recorded in many species of fishes when exposed to 16L:8D such as, Pejerrey *Odontesthes bonariensis,* [24]; (14L:10D) Damselfish, *Chrysiptera cyanea* [25] and (19L:5D)

Goldfish, *Carassius auratus* [26] showed higher GSI. Keeping the positive reports of such scientists in view, the present study on fishes reared under a photoperiod regime (18L:6D) with a constant light intensity of 1500 lx, is indicative of having induced an early development in gonads compare to those reared in natural condition. Further a positive correlation of GSI with length and weight of fish indicated that photoperiodic regime have positive effect on growth as well as on gonadal development of fish.

Female fish, *X. maculatus* exposed to long photoperiod showed more number of yolky eggs and embryos in their ovaries than those exposed to short photoperiod and control group. This indicated that long photoperiod would be necessary to induce significant increase in egg production and embryo development in this species. Similar results that photoperiod affect the frequency of spawning, the total fertility rate, relative fecundity and egg production, were also reported in Poecilia sphenops, live-bearer (exposed to 12.9h light regime) [27]; in Tilapia, egg- layer (during 18L:6D) [8] as well as in *Betta splendens*, egg-layer (during 16L:8D) [14]. Howell et al. [28] recorded an advance of 2 months of the maturation in *Centropistis striata* when subjected to 15L: 9D for 12 months. Duston et al. [29], confirmed the negative effects on the sexual maturation of *Salvelinus alpines* due to decrease in photoperiodic light regime from 16L: 8D to 8L:16D. This indicates that long and short photoperiods interfere in reproductive periodicity of many species.

Photoperiod is one of the most important abiotic factors that affect the maturation and quantity of eggs [3, 30, 31]. Low fertility was observed in temperate fish, *Perca fluviatilis*, when subjected to long photoperiods like 16L:8D [32]. Similarly, in tropical fish, *B. splendens* photoperiod showed an effect on fertility, even though these fishes didn't have any fixed reproductive timing. Very long photoperiods (18L:6D) and also continuous light exposure (24L:0D), showed higher percentages of mortality in some fishes like, *Clarias gariepinus* and *Wallago attu* [33-35] whereas during the present experimental period 0% mortality was observed when subjected to long as well as short photoperiod.

5. CONCLUSION

This work provides an initial path to the application of different photoperiodic regime to induce somatic growth and gonadal maturation in *X. maculatus*, as well as providing novel research and may aid optimization of manipulated photoperiod techniques for the aquaculture industry. Further studies on GnRH and growth hormone are therefore required to better understand how photoperiod regimes regulate maturation and growth in *X. maculatus*.

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