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Optimization of photoperiodism on growth and survival of *Clarias batrachus* (Linnaeus, 1758) larvae

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Abstract

Effect of photoperiod on growth and survival of *Clarias batrachus* (Linnaeus, 1758) larvae were studied under Five different photoperiodic conditions: 24L (hrs. light):0D (hrs. dark) (T1), 18L: 6D (T2), 6L: 18D (T3), 0L: 24D (T4)), 12L: 12D (C). Average body weight (542.60±11.89 mg), specific growth rate (SGR) (7.43±0.04) and percentage weight gain (PWG) (4010.6±50.10) obtained in T3 (6L: 18D) photoperiodic condition was found to be significantly ($p<0.05$) higher than other treatments. The allometric coefficient "b" obtained is higher for T3 (6L: 18D) and T4 (0L: 24D) which indicates more larval growth potential. The polynomial 2-parameter model indicated the best fit to explain and predict body weight and length of larvae with the increase in age. Higher growth and survival is recorded in 6L:18D which can be attributed to better feed intake, higher melatonin synthesis and suppression of swimming activity and stress in dark condition. The photoperiod (6L: 18D) may be recommended as a simple, low-cost technique to maximize the hatchery production of *C. batrachus* larvae.

Keywords: *Clarias batrachus*, larvae, photoperiod, growth, survival

1. Introduction

The Asian catfish, *Clarias batrachus* (Linnaeus, 1758) in India, popularly known as Magur is an economically important air-breathing catfish. It has good market demand, especially in North-Eastern parts, like in the states of Assam, Meghalaya, Tripura, and also in Andhra Pradesh, Uttar Pradesh, Karnataka, Maharashtra, Tamil Nadu, Bihar, Orissa and West Bengal which support significant natural fishery of this species [1]. The fish is revered as nutritious and therapeutic. *C. batrachus* is naturally distributed in two major riverine systems of India viz., Ganga and Brahmaputra and is also found in other freshwater bodies like bheels, wetlands and swamps in Northern and North-Eastern India and global distribution beyond India is in Nepal, Bhutan, and Bangladesh [2]. Magur is found in all types of waters but more so is in derelict and swampy waters. It can live out of water for quite some time and move short distances overland with the help of functional accessory respiratory organ [3, 4] It attains maturity within the first year of life and spawns typically from April to August, during onset of monsoon time in both open and confined waters [5, 6, 7]. However, the culture practice of this species has not received much importance throughout the country, probably due to inadequate supply of seed and due to lack of proper feeding management. Brood stock management, induced spawning using pituitary extracts, Ovaprim or Ovatide, stripping, incubation and hatching of fertilized eggs were demonstrated in the flow-through hatchery system by different workers [1, 8, 9]. Larval phase of this fish is considered as the most delicate and sensitive stage for handling and rearing in its life history. Even though the seed rearing technology is standardized to many extends the growth rate and survival rate of larvae at farm level is relatively low. More care should be given for the larval stocking and its diets (either live feeds or formulated diets). *C. batrachus* are bottom feeders, so these seem to suggest that restriction of light may be used in its culture to for reducing stress and better feeding, which enhance the growth and survival.

Photoperiod exposure is one of the most promising physical factor governing increase in growth rate and survival of fish larvae and photoperiod manipulation is one of the low-cost and straightforward techniques to obtain reasonable growth rate and survivability of fish larvae [10]. Studies on photoperiod also report the influence on body colouration and gonadal development activity for different fishes [11]. The constant change between day and night affect the physiology, feeding efficiency, and metabolism of the fish.

There are a particular threshold light intensity and light and dark regime for larvae to detect the prey, hunt, capture and metabolize it which can be species-specific and environment-dependent [12]. It is reported that photoperiod has an effect on fish feeding activity and play a decisive role in growth and survival [13]. Adamek *et al*. (2011) [14] found that biomass has increased in continuous dark condition while cannibalism and mortality have decreased due to light restrictions in larvae of *Clarius gariepinus*. However, the seed production and larval rearing of *C. batrachus* lacks the knowledge of the effect of photoperiod on the growth and survival of larvae in captivity. Thus this study aimed to experiment with the various photoperiodic conditions to know the growth and survival of *C. batrachus* larvae for improving the hatchery management practices.

2. Materials and Methods

2.1 Experimental Setup

The larvae for the experiment were obtained from, magur hatchery, ICAR-CIFE, Powarkheda Sub-centre, India by induce-breeding of *C. batrachus* following the standard method. A 50 days experiment was conducted for this study with one control and four treatments and each treatment with three replications. Completely Randomized Design (CRD) was the adopted design for the experiment of the study. The experimental setup consists of fifteen small plastic tubs (40 cm diameter and 14cm depth), each filled with 5L water. The water depth maintained was around 6cm. The light for the study was created through artificial lighting with the help of fluorescent light of 40 W and maintained the light intensity of 400 lux at the water surface and was taken as a constant throughout the experiment. The lights were provided on the top of each experimental units which were covered by black polyethylene sheets for different photoperiod manipulation. Each experimental units was exposed to the same ambient temperature of around 25 °C to 30 °C. The four photoperiod regimes and control experimented were as follows: T1 (24 hrs Light: 0 hrs Dark), T2 (18 hrs Light: 6 hrs Dark), T3 (6 hrs Light: 18 hrs Dark), T4 (0 hrs Light: 24hrs Dark), Control (C) (12hrs light: 12hrs Dark). Four days old larvae after yolk sac absorption were stocked in experimental units (tubs) randomly with a stocking density of 200 numbers per each tub. Aeration was provided round the clock in each tub. The average weight and length of larvae stocked were around 13.2 mg and 9 mm respectively. Mixed plankton was given as initial feed till 5 days with a frequency of 4 times per day (18:00, 22:00, 24:00 and 06:00 hours). From 5 to 15 days artemia was given with mixed plankton and after 15 days artificial feed with artemia was given as feed. About 30 minutes after feeding, the leftover feed was removed. Daily siphoning was carried out to remove wastes accumulated on the bottom of each tub. Water was completely drained out once in two days. Water quality was analysed in every 5 days and kept optimum in all units. Sampling was carried out by an interval of 10 days for growth estimation (length and weight) and for checking survivability.

2.2 Growth and survival analysis

Length-weight measurements were taken randomly from each experiment unit. The total length (TL) of the fish from tip of the snout to the tip of the caudal fin was measured using a meter scale graduated in mm (millimetre) with an accuracy of one millimetre. The total weight (TW) of fish was measured to the nearest mg (milligram) with an accuracy of 1mg. The weight gain, weight gain percentage and specific growth rate was calculated for each treatment using the following equations:

Weight gain (mg) = Average final weight-average initial weight

$$\text{Percentage weight gain} = \frac{\text{Average Final weight} - \text{Average Initial weight}}{\text{Average Initial weight}} \text{X } 100$$

$$\text{SGR / day (\%)} = \frac{(\ln \text{Final weight} - \ln \text{Initial weight})}{\text{Number of days}} \text{X } 100$$

$$\text{Total survival (\%)} = \frac{\text{Total number of an animal harvested}}{\text{Total number stocked}} \text{X } 100$$

Daily monitoring was done to note the number of larvae died and survivability was correlated by counting the larvae remaining in each experiment units in every 10 days. At the end of the experiment, all the culture tanks were dewatered, and the number of experimental animals in each tank was counted. Other physical characters like body colouration, clustering, swimming and aggressive behaviour were also noticed every day in different photoperiod conditions.

2.3 Length-Weight Relationship

The length-weight relationship of *C. batrachus* reared in different photoperiodic conditions was analyzed using log-transformed data of length and weight.

2.4 Regression Analysis of Body Weight and Length at Different Days

The data was made normal distribution using PROC UNIVARIATE of SAS 9.3. The regression models for both bodyweight and length at different ages were developed using SAS JMP 9.0. A total of five models were evaluated and 2 models were selected as per the fit, reduction in error sum of squares and coefficient of determination (R^2). The models selected for body weight were transformed fit to square and polynomial 2 parameter models while for body length were linear and polynomial 2 parameter model. The models selected were only compiled and presented here.

2.5 Statistical Analysis

One-way ANOVA (Analysis of Variance) and DNMRT (Duncan's New Multiple Range Test) was performed to determine differences between the means taking at 5% significance levels using SPSS version 16.0. Regression analysis of body weight (BW) and body length (BL) with sampling days were analysed using SAS Institute 2010, SAS/STAT user guide, VERSION 9.3. SAS INSTITUTE, CARY, NC, USA (1-940) for windows.

3. Results and Discussion

3.1 Growth Performance and Survival Rate

Growth parameters of *C. batrachus* larvae in different photoperiodic conditions during the experimental period showed a changing trend. The initial weight, final body weight (BW), the specific growth rate (SGR), percentage weight gain (PWG) and survival rate were calculated for different photoperiodic treatments (Table 1). After 50 days, the highest mean BW was found in T3 (6L: 18D) which is significantly ($p<0.05$) different from all other treatments. SGR was found to be highest for T3, followed by T4 and C. Lowest SGR value is for T1 (6.57±0.03). The highest value of PWG was for larvae reared in treatment T3 (6L: 18D) which was about 4010.6±50.10 and is significantly ($p<0.05$) different from other treatments followed by T4 (3492.9±61.03). Survival was higher (56%) for treatment T3 (6L: 18D) and treatment T4 (0L: 24D) as compared to control and other treatments. Lowest (50.8%) survival% was found to be for T1 (24L: 0D). A significant difference ($p<0.05$) in BW was obtained from 30 days onwards. Highest BW (89.33±4.34 mg) was observed for T3 (6L: 18D) photoperiodic condition (Table 2).

Jorgensen and Jobling (1989) [15] reported that Arctic Char (*Salvelinus alpinus*) reared in complete darkness showed higher feed intake and enhanced growth rate. Adamek *et al.* (2011) [14] reported that *C. gariepinus* juvenile reared in continuous light condition shows lesser body weight compared to the dark condition, which could be due to high metabolic activity in the light condition which leads the decrease in growth rate. In this study, highest growth performance was obtained in 6hrL:18hrD photoperiod condition and is presumably due to increased feed consumption, since less feed consumption is observed in 18hrL:6hrD and 24hrL:0hrD photoperiods. The increased feed consumption in a longer dark period could be due to the nocturnal habit of Clariid catfishes. Examining the growth rates of Nile tilapia (*Oreochromis niloticus*) in terms of SGR, 24L: 0D photoperiod exposure was found to be less effective [16]. High SGR, under total darkness, was reported by Jauro *et al.* (2015) [17] in *C. gariepinus* juveniles. In this study, the lowest growth rate was seen for 24hrL:0hrD photoperiodic condition. Experiment conducted in *Sparus auratus* larvae shows that the levels of hormones involved in growth, metabolism and development, i.e. growth hormone (GH) and prolactin (PRL), are deficient and it has been demonstrated that the number of GH cells and GH mRNA expression does not increase until 30dph [18]. In quickly developing larvae, when GH and PRL supply is still insufficient, melatonin may play a role in the stimulation of cell proliferation and differentiation processes, as it has been postulated in *Danio rerio* [19] which supports a decisive role of melatonin in larval development. In this study higher growth in T3 could be due to high melatonin secretion during exposure of more darkness rather than light exposure. It is reported that larvae and juveniles of African catfish reared in dark condition has higher survival% compared to light condition [20, 21, 22]. In this present study, higher survival got in dark conditions (T3 and T4) but it is not significantly higher than the light conditions (T1 and T2). Survival of early-stage cod larvae raised with continuous (24-hour) light exposure was significantly higher than that of larvae reared with 12 or 18 hours photoperiod [23]. These studies reveal that the effect of photoperiod on growth and survival are strictly species-specific. Mortality was more in the first two weeks of post-hatching, especially in 10 to 12 days of post-hatch. This may be due to starting of aerial respiration on the 10th day. Higher SGR is obtained in 20 to 40 days of larval rearing and it could be due to better acceptance of feed and well developed digestive organs. Faruque *et al.* (2010) [24] also reported that growth and survival of *C. gariepinus after* 15 days is higher than the early larval stages. For *C. batrachus* photoperiod of 6hrL:18hrD is more beneficial in terms of growth and survival than the complete darkness.

3.2 Length-Weight Relation

Length-weight relation of *C. batrachus* larvae reared in different photoperiods is given in Table 3. The larvae reared in treatment T3 (6L: 18D) shows the higher exponential value (2.6±0.03) compared to other treatments. Hossain *et al.* (2006) [25] stated that the length/weight relationship of fishes depends on habitat, seasonal changes, sex, ontogenetic changes, gonadal maturity, diet, and stomach fullness. The calculated allometric coefficient “b” varied within the range of 2.55 to 2.60. These values are within limits (2 and 4) reported by Tesch (1971) [26] for most fishes.

Le Cren (1951) [27] reported that conditional factor depends on shape, length and other seasonal changes of cultured fish. The “b” value of the present study was lower <3, which shows negative allometric growth pattern as given by Hossain *et al.* (2006) [25]. Yosuf *et al.* (2011) [28] reported that pangasid catfishes were expressing negative allometric growth because of their elongated and vertically flat body shape. Walking catfish of similar body shape with the flattened head also joins in this context. Cherif *et al.* (2008) [29] mentioned that b value of the fishes is influenced by the body shape and fatness. The calculated “b” value is high which indicates that at a given length fish having more growth potential. So, it can be concluded that fishes reared in 6L: 18D (T3) photoperiodic conditions having more growth potential than other treatments.

3.4 Regression Analysis of Body Weight and Length at Different Days

Age-body weight relation of *C. batrachus* larvae reared in different photoperiods is given in Table 4(transformed fit to square model and polynomial 2-parameter model). The error sum of square (ESS) obtained for transformed fit to square and polynomial 2-parameter model are within the range of 65975.4-157574 and 30713.1-64784.8 respectively. ESS is lower for the polynomial 2-parameter model. The R^2 value is higher for the polynomial 2-parameter model (97-98%) than transformed fit to Square model (95%). Age-body length relation of *C. batrachus* larvae reared in different photoperiods are given in Table 5 (linear model and polynomial 2-parameter model). The ESS obtained for the linear and polynomial 2-parameter model are within the range of 231.96-400.5 and 106.94-252.41 respectively. ESS is lower for Polynomial model. The R^2 value is higher for the Polynomial 2-parameter model (97-98%) than the linear model (95-97%). So in this present study, ESS is less for the polynomial 2- model as compared with the transformed fit to square model for body weight and linear model for body length. Also the R^2 value, also known as the coefficient of determination is higher for the polynomial 2- model. So it can be concluded that the polynomial model is the best fit to explain and predict body weight and length of *C. batrachus* larvae with an increase in age. Imai *et al.* (2002) [30] also reported that the growth trajectory of *Tribolodon nakamurai* was non-linear, which will be a supporting fact. Further, the hatchery managers can utilize the model for predicting the growth of larvae under different ages and also will be useful for calculating biomass at the various growing period of the larvae to optimize feeding.

Table 1: Average body weight (mg) and SGR (%) day^{-1} PWG, SURVIVAL (%) (Mean ± S.E) of *C. batrachus* larvae observed during 50 days experimental period in different treatment group

	12L:12D (C)	**24L:0D (T1)**	**18L:6D (T2)**	**6L:18D (T3)**	**0L:24 D(T4)**
Initial average weight (mg)	13.2	13.2	13.2	13.2	13.2
Final average weight (mg)	$390.17^{c}\pm12.26$	$354.00^{c}\pm5.89$	$375.40^{c}\pm11.67$	$542.60^{a}\pm11.89$	$474.27^{b}\pm12.01$
SGR %	$6.77^{c}\pm0.06$	$6.57^{cd}\pm0.03$	$6.69^{cd}\pm0.06$	$7.43^{a}\pm0.04$	$7.16^{b}\pm0.05$
PWG	$2955.8^{c}\pm52.94$	$2581.8^{d}\pm44.38$	$2743.9^{cd}\pm38.46$	$4010.6^{a}\pm50.10$	$3492.9^{b}\pm61.03$
Survival (%)	$52.16^{a}\pm2.33$	$50.83^{a}\pm1.01$	$51.66^{a}\pm2.01$	$56.50^{a}\pm1.80$	$56.33^{a}\pm1.85$

*Mean values in the same row with different superscripts vary significantly for each sampling day ($P<0.05$). One way ANOVA was used following Duncan multiple range test in SPSS 16.0

Table 2: Mean body weight (mg) (Mean ± S.E) of *C. batrachus* larvae observed at an interval of 10 days

Days	**12L:12D(C)**	**24L:0D(T1)**	**18L:6D(T2)**	**6L:18D(T3)**	**0L:24D(T4)**
10	$18.46^{a}\pm0.58$	$17.26^{a}\pm2.42$	$18.06^{a}\pm0.98$	$20.20^{a}\pm2.71$	$18.73^{a}\pm2.17$
20	$28.30^{a}\pm0.97$	$26.73^{a}\pm2.22$	$27.40^{a}\pm1.24$	$36.90^{a}\pm5.45$	$33.36^{a}\pm4.45$
30	$70.26^{bc}\pm3.63746$	$64.63^{c}\pm2.04$	$67.86^{c}\pm1.53$	$89.33^{a}\pm4.34$	$80.88^{ab}\pm5.49$
40	$253.73^{c}\pm7.38$	$231.93^{c}\pm7.13$	$245.43^{c}\pm9.70$	$340.17^{a}\pm10.48$	$298.60^{b}\pm11.04$
50	$390.17^{c}\pm12.26$	$354.00^{c}\pm5.89$	$375.40^{c}\pm11.67$	$542.60^{a}\pm11.89$	$474.27^{b}\pm12.01$

*Mean values in the same row with different superscripts vary significantly for each sampling day ($P<0.05$). One way ANOVA was used following Duncan multiple range test in SPSS 16.0

Table 3: Length/weight relationship of *C. batrachus* larvae exposed to different photoperiods

Group	**Regression Equation**	**a ± S.E**	**b ± S.E**	**r**	**n**	**R^2**
12L:12D(C)	W = -1.47 + 2.57 L	-1.47±0.08	2.57±0.07	0.97	75	0.95
24L:0D(T1)	W = -1.45 + 2.56 * L	-1.45±0.12	2.56±0.10	0.95	75	0.92
18L:6D(T2)	W = -1.46 + 2.55 * L	-1.46±0.09	2.55±0.09	0.97	75	0.94
6L:18D(T3)	W = -1.48 + 2.60 * L	-1.48±0.05	2.6±0.03	0.97	75	0.95
0D:24L(T4)	W = -1.49 + 2.58 * L	-1.49±0.09	2.58±0.07	0.94	75	0.96

* W= log (bw), L= log (l); log (w) and log (l) are the natural logarithm of body weight and length: S.E: standard error; a: intercept; b: slope; r: correlation coefficient; R^2: coefficient of determination; n: no of samples; bw: body weight; l: length.

Table 4: Regression analysis of body weight at different days by Transformed Fit to Square model and Polynomial 2-parameter model of *C. batrachus* larvae exposed to different photoperiods

Model	**Treatments**	**Equations for body weight at different sampling intervals**	**a± SE**	**b±SE**	**ESS**	**R^2 (%)**
Transformed Fit to Square	12L:12D(C)	weight = -15.06 + 0.15*(Age)^2	-15.06±4.51	0.15±0.003	80929.3	95
	24L:0D(T1)	weight = -12.63 + 0.14*(Age)^2	-12.63±4.08	0.14±.003	65975.4	95
	18L:6D(T2)	weight = -14.45 + 0.15*(Age)^2	-14.45±4.40	0.15±0.003	76966.7	95
	6L:18D(T3)	weight = -27.07 + 0.21*(Age)^2	-27.07±6.31	0.21±.004	157574	95
	0D:24L(T4)	weight = -21.93 + 0.19*(Age)^2	-21.93±5.47	0.19±0.004	118608.5	95

Model	**Treatments**	**Equations for body weight at different sampling intervals**	**a± SE**	**b±SE**	**c ± SE**	**ESS**	**R^2 (%)**
Polynomial 2-parameter model	12L:12D(C)	weight = -127.81 + 7.44*Age + 0.24*(Age-25)^2	-127.81±4.58	7.44±0.12	0.24±0.009	37564.0	97
	24L:0D(T1)	weight = -114.73 + 6.7 4*Age + 0.22*(Age-25)^2	-114.73±4.11	6.74±0.11	0.22±0.008	30713.1	97
	18L:6D(T2)	weight = -123.67+ 7.19*Age + 0.23*(Age-25)^2	-123.67±4.42	7.19±0.12	0.23±0.008	35545.8	97
	6L:18D(T3)	weight = -185.23 + 10.34*Age + 0.34*(Age-25)^2	-185.23±5.97	10.34±0.16	0.34±0.01	64784.8	98
	0D:24L(T4)	weight = -159.65 + 9.02*Age + 0.30*(Age-25)^2	-159.65±5.20	9.02±0.14	0.30±0.01	49237.5	98

*a: intercept, b: slope, ESS: Error Sum of Square, R^2: coefficient of determination

Table 5: Regression analysis of body length at different days by Linear and Polynomial 2-parameter model of *C. batrachus* larvae exposed to different photoperiods

Model	**Treatments**	**Equations for body length at different sampling intervals**	**a± SE**	**b±SE**	**ESS**	**R^2(%)**
Linear model	12L:12D(C)	length = 6.58 + 0.53*Age	6.58±0.32	0.53±0.01	265.4	96
	24L:0D(T1)	length = 6.58 + 0.55*Age	6.58±0.36	0.55±0.01	342.19	95
	18L:6D(T2)	length = 6.39 + 0.64*Age	6.39±0.40	0.64±0.01	400.5	96
	6L:18D(T3)	length = 6.73 + 0.60*Age	6.73±0.31	0.60±0.008	231.96	97
	0D:24L(T4)	length = 6.56 + 0.56*Age	6.56±0.36	0.56±0.01	332.64	96

Model	Treatments	Equations for body length at different sampling intervals	a± SE	b±SE	c ± SE	ESS	R^2 (%)
Polynomial 2-parameter model	12L:12D(C)	length = 5.14+ 0.52*Age + 0.005*(Age-25)^2	5.14±0.24	0.52±0.006	0.005±0.0004	106.94	98
	24L:0D(T1)	length = 5.32+ 0.55*Age + 0.004*(Age-25)^2	5.32±0.34	0.55±0.009	0.004±0.0007	221.77	97
	18L:6D(T2)	length = 4.99+ 0.64*Age + 0.005*(Age-25.10)^2	4.99±0.37	0.64±0.01	0.005±0.007	252.41	97
	6L:18D(T3)	length = 5.88+ 0.60*Age + 0.003*(Age-25)^2	5.88±0.31	0.60±0.008	0.003±0.0006	177.22	98
	0D:24L(T4)	length = 5.19+ 0.55*Age + 0.005*(Age-25)^2	5.19±0.32	0.55±0.009	0.005±0.0006	191.42	97

*a: intercept, b: slope, ESS: Error Sum of Square, c: physical maxima, R^2: coefficient of determination

4. Conclusion

Photoperiod mediated growth in fish is species-specific depending on the daily activity rhythms. The present finding can be a management measure to optimize the magur larval rearing protocols using 6L: 18D photoperiodic conditions. Also, providing shelter as well as quantification of shelter in the rearing tanks must be evaluated to explore the growth parameters in regular photoperiod along with the evaluation of the minimal intensity of light thresh-hold for the *C. batrachus* larvae which is under question.

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