Effect of different photoperiods on the Growth and Physiology of Rohu *(Labeo rohita)*.

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By

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Department of Animal Sciences
Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad
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A thesis submitted in partial fulfillment of the requirement for the degree of

MASTER OF PHILOSOPHY

IN

FISHERIES AND AQUACULTURE

By

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CERTIFICATE

This dissertation "Effect of Different Photoperiods on the Growth and Physiology of Rohu *(Labeo rohita)*" submitted by **Mr. Zohaib Noor,** is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Fisheries and Aquaculture.

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Declaration

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

Zohaib Noor

Dedicated to:

My loving and caring parents,

And

My beloved siblings

CONTENTS

LIST OF ABBREVIATION

Abbreviations	Full Name
Ns	Non-significant
EDTA	Ethylenediaminetetraacetic acid
g/L	Gram per liter
g dL^{-1}	grams/deciliter
fL	femtoliters/Liter
pg	picograms
μL^{1}	microgram/Liter
mmol/L	mili mole/ Liter
FCR	Feed conversion efficiency
FCE	Feed conversion efficiency
SGR	Specific growth rate

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Zohaib noor

Abstract

The Current study was aimed to investigate the effect of diverse photoperiods on the growth performance, physiological indices and general morphology of indigenous fish species Rohu*(Labeo rohita)*.Three hundred healthy fingerlings of *L. rohita* having average initial body weight 6.95 ± 0.53 g were stocked in 12 glass aquaria, well equipped water heater, aerators and overhead fluorescent tube lights, at a stocking density of $4gL^{-1}$ (22-23 fish/ aquarium).Completely randomize experiment was designed consisting of four groups P1, P2, P3 and P4 exposed to four different photoperiod 6L/18D, 10L/14D 14L/10D, 18L/6D respectively for 90 days. During experimental duration, all water quality parameters i.e., temperature (26.5±0.5°C), total ammonia (<0.25 mg L^{-1}), and DO (6.0 ± 0.8 mg L^{-1}), levels were kept at optimum level while requisite photoperiods were maintained by fluorescent light tubes installed 13cm above the surface of the aquaria. At the completion of study, P1 group of fish showed significant ($P<0.05$) increase in the growth performance as compared to other experimental groups. Similarly, Feed conversion efficiency was comparatively higher in P1 group of fish reared under long day photoperiod. Manipulation of photoperiod also affects the circadian pattern and concentration of cortisol. Fish of P1 group exposed to 18L: 6D, showed a circadian-like pattern, highest levels of water-borne cortisol during 18-24 hrs with peak at 20:00 hr. A shift of peak level from 20 to 24hrs was observed in others groups exposed to different photoperiods. However, the maximum cortisol level was in a group of fish exposed to 18 hr prolonged scotophase. Similarly, significantly high glucose level was detected in the P4 group of fish exposed to prolong dark exposure compared to P1 exposed to continuous 18hr light duration. Similarly, complete blood count (CBC) indicated significantly higher ($P< 0.05$) values of HB (g dL^{-1}) MCV (fL), MCH (pg), LYM%, RBCs ($10^3 \mu L^{-1}$) and MCHC (%) in a P1group and Hct (%) in a P4 group of fish as compared to other groups. However, WBCs did not show any significant difference ($P>0.05$) among all experimental groups. Prolong scotophase also caused darker body colorations, deep yellowish spots on the body, damaging of skin and scales as well as narrowing the body structure. The results of this study indicate the negative impact of prolong scotophase and suggest long day photoperiod for gaining more production per unit area of this species.

Introduction

Introduction

The physical environment surrounding living organisms, changes periodically in accordance to distance between sun and earth. Adaptations to these changes occur on daily and annual basis which are compulsory for their survival (Laryushkina, 2000). From simplest organisms to complex vertebrates, environmental factors influence the activity of cells, organisms or populations. In aquatic ecosystem, the effect of abiotic factors on the growth, survival and physiological responses of fish have been extensively studied e.g. temperature shows a pivotal role in the growth of fish (Jobling, 2002; Ruyet, 2006), while some other environmental factors i.e., dissolved oxygen pH and photoperiod also greatly affect growth parameters (Loew and Sillman, 1998; Davies and Bromage, 2002; Bani et al., 2009). In fish, behavioral processes such as locomotion, skin pigmentation, thermoregulation, shoaling behavior, etc. are under the influence of external environmental factors. The same is also true for major physiological functions such as growth and reproduction (Mommsen, 1998; Boeuf and Falcón, 2002; Li et, 2013; Bajaj, 2017).

Photoperiod is the physiological and behavioral response of living organisms to 24 hours light interval (Goldman, 2001). In aquatic environment, fish being a sensitive organism, is effected by photoperiod as it interferes with its hormonal secretions like melatonin and thyroxin and brings alterations in its endocrine machinery, and thus work as a major exogenous influencing factor (Reinecke, 2010). Photoperiod have impact on rate of metabolism in different organisms. Enzymatic activities are known to elevate with increase in light durations and intensity which not only enhances metabolism but also increase solubility of different minerals and salts in cytoplasm. It also affects both innate and humoral immunity of sea bass (*Dicentrarchus labrax*) and seabream (*Sparus aurata L.*) by reacting with complement system, lysozyme and peroxidase activities (Esteban et al., 2005). Generally, photoperiod affects the feeding activity of fish, which in turn effects its survival, growth and social behavior (Boeuf and Falcon, 2001). Light precise by the photoreceptor cells of retina of the fish eyes effect pineal gland and yield melatonin in fish which in turn controls the production of growth hormone releasing factors (Moyle and Cech, 2000; Falcon et al., 2010; Reinecke, 2010).

Photoperiod may also affect the organism's activities through the influence on feeding strategy (Endal et al., 2000; Boeuf and Falcon, 2001). Every fish species require appropriate photoperiod for the growth which differ and depend upon species, age or developmental phase, and surrounding temperature (Jonassen et al., 2000).The behavioral and physiological rhythm of many species is directed by the 24hr light and dark cycle. May research work have suggested that these rhythmicity is controlled by biological clock, which is situated in the brain part called the supra chiasmatic nuclei in mammals. The24hr rhythmicity persist throughout the nature and exist for the 24hr. Additionally, these 24hr cycles can be coordinated with respect to outside signals and can persist even in the lack of such signals i.e., light source. The disturbance of the biological rhythms shows negative effects on the health and survival of the living organisms. The internal body clock comprises of set of genes sequence which transcript specific mRNA, which translate into specific proteins sequence, these proteins in turn control the various physiological processes of the body (Vitaterna et al.,2011). Living organism needs photoreceptive organs to synchronize rhythmic functions and behaviors in accordance to daily and annual cycles. Photoreceptive organs transduce the photoperiod information to target centers and produce output messages.

Fish farming includes control of photoperiod as a really good option to regulate fish physiological functions. In-fact, fish growth, development, primary and secondary sexual attributes and reproduction could be controlled and altered by using new photoperiod techniques (Boeuf and Le Bail, 1999; Purchase et al., 2000; Bromage et al., 2001; Randall et al., 2001; Rodriguez et al., 2001; Biswas and Takeuchi, 2002; Gines et al., 2003).Fish possess two types of photoreceptive organs i.e., retina of the lateral eyes and pineal gland. Melatonin is one of the different messages they elaborate in response to the alternation of light and dark (Falcón, 1999). During aquaculture practices large scale commercial production of fish e.g. Atlantic salmon has been made possible via photoperiodic manipulation (Žbjørnsson et al., 1995; Türker and Yıldırım, 2011). These manipulation techniques have been applied in adult Atlantic Salmon *Salmo salar* for controlling growth rate, incidence of early maturation and spawning time (ACRDP, 2012). Likewise, turbot maintained at 16L/8D and 24L/ 0D, continuous light showed enhanced growth performance after at least 3 months of exposure but not throughout the

6-month experiment (Imsland et al., 1995). Similarly, Cod fish could be commercially farmed by these techniques.

In temperate regions, photoperiod is associated with survival and increased growth of fish larvae, due to increase feeding efficiency via photo stimulation (Woiwode and Adelman, 1991; Boeuf and Baille, 1999). In fish like gilthead sea bream, *Lates calcarifer* (Barlow *et al.,* 1995) rabbit fish, the prolonged days increase larval survival by providing more opportunity to find more food. For instance in some fish like southern flounder (Tuckey and Smith, 2001), increase in photoperiod decrease the survival rate at larval stage, since more ingestion than maximum can stop cycle due to more ATP consumption (Tuckey and Smith, 2001). Long photoperiod associated growth is achieved by increased ATP conservation, obtained either by more sophisticated food processing or by storing energy that could be used for gonadal development or utilize for somatic growth by suppression of sexual maturation (Boeuf and Le Bail, 1999; Gines et al., 2004).The probable reason for the increase in feeding rate and strength maybe due the visual performance at high light intensity. Most marine fishes larvae feed by using visual cues and these are predatory in nature (Puvanendran and Brown, 2002; Pena et al., 2004). The hatchling have cone, retina, while rods are absent till the larvae grow sufficiently, therefore young larvae require high light intensity compared to advance larvae and visual structure Larvae cannot differentiate prey clearly when the visual stimulants are not enough due to the absence of light. Most bony fishes require 0.lux of light intensity for the visual detection, less than that hinder the fishes in locating the prey. Supplying light for the extended period or continuously to the cod *Gadus morhua*, a marine fish does not affect the yolk absorption, hence independent of light periodicity (Pena et al., 2004).

The light which enter into the water body have different wavelength, thus penetrate at different depth. Many fish species have peculiar colors, visions which are sensitive to colored light (Ruchin, 2004; Jauro and Usman, 2015). For example, the growth performance of silver carp larvae (*Hypopthalmichthys molitrix*) and young carp (*Cyprinus carpio L.)* improved with green light (Ruchin, 2004).Fish larvae can attain rapid and high weight gain when the light source and background coloration enhance feed intake (Henne and Watanabe, 2003; Jentoft et al., 2006; Strand et al., 2007).Light strength can affect the growth rate of the fish (Rajeswari et al.,2017;Oppedal et al., 1997),

while crossing the required intensity, the light intensity is not chief aspect for juvenile and adult growth performance (Boeuf and Le Bail, 1999).

Generally, prolong light source can improve the performance of the finfish juveniles e.g. barramundi, *Lates calcarifer* (Barlow *et al.*, 1995), largemouth bass, *Micropterus salmoides* (Petit et al., 2003), red sea bream, *Pagrus major* (Biswas et al., 2006), and Atlantic cod, *Gadus morhua* (Imsland et al., 2007). A "day length-prey abundance" association is operational for the optimization of production cycles, for example, Naess et al.(1996), reported that it's doable to produce juvenile halibuts from larvae, employing a 6-month delayed photoperiod and guarantee year-round production of juveniles. Hence, the combined outcome of food availability and light" is the most important factor effecting larval growth, thus allowing them optimal exploitation at trophic level. Purchase et al. (2000) studied the effect of photoperiod on the juvenile yellow tail flounder *Pleuronectes ferrugineus* and reported similar growth and survival rates under 24L/0D, 18L/6D and 12L/12D conditions. Similarly, growth rate of halibuts reared from 5 to 20 g was not affected by light regimes changing from 7 to 12hr L and from 12 to 18hr L as reported by Hallaråker et al.(1995). However, halibuts maintained for 5 months under different photoperiod conditions, showed high specific growth and survival rate under a 24L/0D cycle, whereas at 8L/16D cycle gave the poorest results; while intermediate values were obtained under natural conditions, Moreover, fish first maintained under short day length exhibited an increased growth rate 21 days after being transferred to continuous light (Simensen et al., 2000).

Environmental disturbances are usually thought to be possible sources of stress in fish (Barton, 1997; freeman, 2015). Plasma cortisol and lactate concentration are used as indicators of physiological stress reaction in sturgeon fish (Cataldi, 1998; Zuccarelli et al., 2008). According to wedemyer (1996) and Iwama et al. (2006), stress response in fish involves three stages, primary, secondary and tertiary. The primary stress responses in fish's involve the activation of natural alarm system and trigger the release of catecholamine, adrenaline/ noradrenaline from medulla of adrenal gland and corticosteroid hormones from adrenal cortex. In stress catecholamine helps the organism to show immediate physical reactions by accelerating heart rate while cortisol regulate the metabolism and increase blood glucose level through gluconeogenesis and deliver

energy to every cell. Iwama et al. (2006), reported the plasma levels of adrenaline and cortisol for Salomindes, <3 and 10nM respectively that shoot to 20-70nM and 150-500nM respectively in stress. The secondary response involves successful acclimation of animal with the use of energy that often results in suppression of growth process. The tertiary response appears when stressful condition surpasses the acclimation tolerance limits and fish become exhausted. Tertiary responses involve, mal-adaption, suppression of digestive process, retardation of growth and impairment of immune system that resulted in vulnerability to pathogen (Iwama et al., 2006). Photoperiod changes apparently influence steroid and corticosteroid hormone levels. However stress is not a forever event but a comprehensible consequence (Pickering and Pottinger, 1983; Maita *et al.*, 2004). Different stress determinants like LPO concentration, blood cortisol, water borne cortisol, glucose lactic acid, and antioxidant enzymes are used in fish studies as stress indicator (Barton, 2002; Iwama et al., 2004). Several studies indicated that photoperiod management tempt a significant increase in stress responses (Leonardi and Klempau, 2003), while others researchers have assessed the effect of photoperiod on the growth, stress and hematological parameters of fish (Semenkova and Trenkler, 1993; Ruchin, 2007).

Excessive cortisol is produced during acute stress, which then causes leukocyte lysis (Ivanov, 2003). The levels of corticosteroid hormone and steroids also vary during photoperiod alterations but do not always lead to stress (Pickering and Pottinger 1983; Biswas et al., 2004). However, in some fish this might lead to stress and activates stress response (Leonardi and Klempau, 2003). Different studies indicated the role of photoperiod in increasing cortisol cycle in different species i.e., cortisol peak changes in accordance to photoperiod shift, both shifts occurs correspondingly (Srivastava and Meier, 1972; Redgate, 1974; Bani et al., 2009; Navarro et al., 2014). Cortisol concentrations in fish have been implicated in the regulation of a broad array of physiological function that affect energy metabolism, growth, reproduction, hydro mineral balance and the immune system (Wendelaar Bonga, 1997; Mommsen,1999; Norris and Hobbs, 2006). Generally, cortisol affects the energy metabolism in fish by increasing their liver glucose production, and by stimulating proteolytic and lipolytic capacity (De boeck et al., 2001; Aluru and Vijayan, 2007). Along with its catabolic

effects, cortisol suppresses somatic growth in fish through its effect on growth hormones, insulin like factor I axis (Kajimura et al., 2003; Peterson and Small, 2005; Pierce et al., 2005) and on the neuroendocrine pathways that regulate food intake (Bernier, 2006).

Increase in the level of plasma glucose vary in different species, mainly dependents on fish's ability to store glycogen in liver(Pottinger et al., 2002; wright et al., 2007).While after the stress, glucose profile (to attain peak level and return back to basal level) show variations with the cortisol level(pottinger, 1998; Flodmark et al., 2002).

Various blood parameters like Red blood cells (RBCs), hemoglobin (HB), and hematocrit (HCT) indicates the carrying capacity of oxygen in blood stream, MCH and MCHC are particularly important in anemia diagnosis and status of stress response (Zhou et al., 2009), while Leukocytes indicates body's primary means of fighting infection (Banaee et al., 2008). Normal fish tries to maintain blood cells in certain limits. But some time one or more parameters is influenced by intrinsic and extrinsic factors (Masopust, 2000). Blood can readily reflect the changes happening in the body as it is actively involved in metabolism (Golovina, 1996; Ivanov, 2003). The overall fish health condition was indexed by white blood cell count (Golovina, 1996; Mordovian, 2006). Moreover high light intensity improves the carp physiological state and less intensity have inverse effect (Mordovian, 2006). The welfare indicators ought to provide data on potential problems and additionally the sources of weakened welfare (Rousing et al., 2001).The fish welfare is concerned with the well-being of fish, as being safe, nourished and healthy, and not in any kind of stress and pain.

Environmental stress can also be responsible for color change of fish. Light intensity effects the pigment distribution by interacting with hormone regulation (Van der Salm, et al., 2004). Fish are rich in pigments like melanin, carotenoids and purines (Moyle and Cech, 2004). Carotenoids exist in yellow to red range and are naturally occurring (Hill, 2002). They are lipid soluble pigments, which makes fish skin color in ornamental fish. The more attractive the color the more valuable fish (Paripatananont et al., 1999). Chromatophores are small vesicles, containing color pigments, present in the skin of fishes, reptiles and amphibians, which maintain the skin colors (Zarnescu, 2007). This color is visible as a result of light scattering, absorption and reflection by fish

pigmental architecture buried in fish skin (Fujii, 2000). Chromatophore can experience pigment dispersion or aggregation due to light intensity (Fujii, 2000).

For improving production efficiency of aquaculture system, it is necessary to improve the culture conditions, like physical environment (temperature, pH, salinity, dissolve oxygen, photoperiod and light intensity) and basic nutritional status such as composition of diet, its nutritional value, feeding frequency and feeding ration (Mohseni et al., 2006). Growth and survival of fish are under the direct influence of culture condition, thus best culture condition increase per unit area production. Hence, for optimum fish production, knowledge about environmental fluctuation in local conditions and their impact on survival growth and health is necessary.

Indian major carps are the common culturable fish species in most of Asian countries. These species include rohu *(L. rohita),* catla *(Catla catla)* and *mrigal (Cirrhinus mrigala).* Rohu, *L. rohita* is a main Indian major carp that is cultured all over the world because of its fast growth rate, high economic value and customer demands (Mohapatra et al., 2012; Ibrar et al.,2017). However, there's no data on the impact of photoperiod manipulation on the growth performance and survival of this species. Therefore, the present study was aimed to investigate the effect of photoperiod manipulation on general morphology, survival, growth performance and hematological indices of juvenile *L.rohita*, reared in the glass aquaria under control conditions.

The present study was conducted with the aim to examine the effect of different photoperiods on

- Survival and growth performance of the *L. rohita.*
- Hematological indices and stress response of *L. rohita.*
- General morphology and body colorations of *L. rohita* under different photoperiod regimes.

Materials & Methods

Materials and methods

The present study was conducted in the fisheries and aquaculture research station Quaid-i-azam university, Islamabad, Pakistan.

Collection and maintenance

Three hundred healthy fingerlings of Rohu (*L. rohita*), average body weight 6.95 ± 0.53 g, were procured from Rawal fish hatchery, Islamabad and transported to Fisheries and Aquaculture Research Station, Quaid-i-Azam University, Islamabad in the oxygen filled polythene bags (36cm length × 24cm width; 10 L water), by using live hauling technique and were stocked in the circular fiberglass tanks (volume, 500L). After few days, they were re-housed into 12 glass aquaria (60 cm L ×30 cm W× 30 cm H) 54 L water capacity, covered with black light proof sheet from all the sides, at a stocking density of 4g/L (22-23 fish/ aquarium) . All aquaria were well equipped with aerator and water heater, to keep a stable temperature of 26.5°C and had permanent volume marks for maintaining water volume and fluorescent tube at top for maintaining photoperiod

To each aquarium, DE chlorinated water was supplied through main pipeline from water tank. The pipeline at the top of each aquarium had tap with regulator to control rate of flow of water, while for outflow of water from experimental aquarium, Tygon® tubing and a regulator was connected. Experiment was conducted in semi-static condition. Fish were fed 40% prepared feed, twice a day at 09:00 and 16:00 hr. Every day uneaten food and faces were removed by siphoning and about 20% water in each aquarium was also replaced by addition of fresh water from water reservoir. During experiment, the fish were maintained in the well-controlled laboratory conditions and all the water quality parameters were checked daily by multi-parameter (Multi-parameter Hanna HI 9147, Hanna Instruments Inc., UK), and found within the range suitable for rearing of *L. rohita* (pH, 6.9 - 7.1, DO near to saturation, >6.4 mgL^{-1} and total ammonia, <0.25 mgL^{-1}) .

Feed Formulation

Feed having 40% crude protein was formulated and prepared by using ingredients mentioned in Table. 1

Table 1. 40% crude protein basal diet formulation

Ingredients	Amount (g/ kg)
Fish meal	250
Soybean meal	130
Sunflower meal	50
Gluten meal 60%	500
Wheat bran	10
Rice bran	10
Wheat flour	10
Vitamin-mineral premix[a]	10
Canola oil	20
DCP*	10

*dicalciumphosphate

[a] (Vitamin premix contains vitamins, amino acid and minerals premix kg-^{1})

Experimental design

Experiment was conducted in replicate of three by using different photoperiods and similar temperature, pH and DO. Twelve glass aquaria having uniformed size fish were divided into four groups and reared under four different photoperiods

P1, 18hrs light and 6hrs dark (18L: 6D)

P2, 14hrs light and 10hrs dark (14L: 10D)

P3, 10hrs light and 14hrs dark (10L: 14D)

P4, 6hrs light and 18hrs dark (6L: 18D)

Particular photoperiod regime was maintained by fluorescent tube at the top of each aquarium about 13cm above the level of water. Fish were exposed to respective photoperiod for 90days. During experimental period, feed was given twice a day at the

rate of 5% body weight. Daily fecal matter and undigested feed were collected through siphoning, filtered and dried for calculation of FCR. Moreover, 20-25% water daily exchanged with fresh water.

Effect of photoperiod on HPI-axis

For evaluation of impact of different photoperiods on HPI-axis and on glucose level, water samples from each aquarium and plasma from some fish of each group were collected at the end of experiment.

Water sampling

For measuring water-borne cortisol, 3 days before water sample collection, aquarium flow through system was started and constant rate of flow i.e., 20± .1 mL min^{-1} was maintained in each aquarium. This setup was arranged in order to avoid any handling stress.

All fish were starved about 24hr before collection of water samples. On the day of experiment, without disturbing fish, water from each aquarium was exchanged with fresh water from main tank by controlling rate of flow. After 2hrs, at 2:00PM, 500mL water from each aquarium was collected in glass bottle through long outflow Tygon® tube, without approaching too closely to experimental aquaria. The rate of flow was recalibrated and further water samples at 2hrs interval, up to 24hrs were collected by adopting same procedure. To minimize the likelihood of any intrusion from background, water from main water reservoir was also collected for cortisol analysis. Throughout the experiment, flow rate was periodically checked by recording the time to fill the graduated beaker and adjusted the inflow or outflow of water accordingly. Moreover, volume marks on aquaria also helped in maintaining the constant rate of flow. After sample collection, water was immediately filtered with the help of whatman filter paper in order to remove the suspended particulate.

At the end water collection, clove oil ($0.1mlL^{-1}$) was added in each aquarium and fish were collected for glucose analysis and growth performance.

Water borne cortisol (Non-invasive technique)

Free cortisol was extracted by using method reported by Zuberi et al. (2011). Briefly 3 mL size solid phase extraction cartridges (pore size , 40–63 μm, standard PP Merck) were fitted to vacuum manifold (24- port). The cartridge were activated with 2 ml 100% methanol followed by two consecutive wash with de-ionized water. After that water samples were passed through the columns by creating vacuum in manifold with the help of vacuum pump fitted with it. After passing water samples, the column was washed with 5 mL DI water and free cortisol was extracted by using 6 ethyl acetate in 2 successive washes. The ethyl acetate was collected in the 10 mL glass test tube and solvent was evaporated at 45°C under light stream of nitrogen gas. The resultant residue was dried and stored till further analysis.

Growth performances

After 90 days rearing under respective photoperiod, fish of each aquarium weight collectively and their average weight was calculated. After calculating the average weight of fish in each aquarium, the mean ± SE, weight of fish in each group was calculated. For evaluating growth performance, following standard formulas were used

Percentage weight gain: After 90 days, percentage weight gain of the different groups was measured with the help of given formula.

% WG = (Wf – Wi) / Wi × 100

Where,

Wi = body weight of fish at the start of experiment

Wf = body weight of fish at the end of experiment

Specific Growth Rate (SGR): It was calculated by adopting following given formulas.

SGR (%) = (lnWf - lnWi) / No. of days of experiment × 100

Where,

In Wf= Natural log of body weight at the end of experiment

In Wi = Natural log of body weight at the start of experiment

Feed conversion ratio (FCR) = $\frac{\text{dry feed consumed (g)}}{\text{Net wet weight gain (g)}}$

Blood glucose level:

All the fish were analyzed for the blood glucose concentration with the help of Glucometer ACCU-CHEK®Softclix. Drop of blood drawn from the caudal vein of the fishes were placed on the glucose strip and inserted in to the glucometer. The glucose level in mg dL^{-1} was displayed on the digital screen was noted and converted into $mmolL^{-1}$.

Hematological parameters:

For the analysis of the blood parameters blood were drawn from the caudal vein of the fish by inserting near the lateral line system, 1mL heparinized syringe and stored in the EDTA tubes to prevent coagulation, blood was then analyzed by properly caliberated top standing hematology analysis machine (HMA-730), Nayab Clinical Laboratory Islamabad. For getting sufficient blood for hematological analysis, blood of 4 fish from similar tank was pooled. Thus, from each group 12 samples were collected.

Statistical Analysis

Results of growth of rate, hematological indices, and glucose and cortisol concentration were expressed as Mean ± SE. For statistical evaluation, the resultant data was analyzed by using SPSS and Statistix version 8.1and 16.0 respectively. For comparison among groups one way analysis of variance followed by LSD test was used. Moreover, comparison was considered statistically significant when values of $P < 0.05$.

Results

Results

% Survival

The P1 group of fish showed considerably higher ($P< 0.05$) % survival in comparison to other groups of fish. However, P2 and P3 groups of fish showed comparatively similar but significantly higher % survival as compared to P4 group of fish reared under (6L/ 18D photoperiod.

Growth performance parameters

The initial body weight of all the fishes did not show any significant differences, while after 90 days i.e., at the end of experiment, P1 (18L/ 6D) group of fish showed significantly higher weight ($P=0.001$) followed by P2 (14L: 10D) group of fish. However, P4 (6L/18D) group of fish, body weight was significantly less as compared to others groups. Other growth related parameters like % weight gain, specific growth rate (%) and feed conversion efficiency (%) also demonstrated similar trend. Like growth rate parameter, FCE% decreased with increase in dark period, thus P1(18L/ 6D) and P4 (6L/ 18D) groups showed highest and lowest FCE% respectively (Table 2).

Hematological indices

Hematological indices are shown in the Table 2. A *complete blood count* (*CBC*) of all groups of fish indicated significantly ($P< 0.05$) higher and lowest (HB (g dL^{-1}) MCV (fL) and RBCs ($10^3 \mu L^{-1}$) in a P1 (18L/8D) and P4 (6L/18D) group of fish respectively. Hct (%) showed an increasing trend with decreasing the photoperiod, while WBCs of all groups did not show any significant difference ($P>0.05$). Furthermore, except P1, LYM% of all groups of fish also showed statistically similar ($P>0.05$) values (Table 3).

Cortisol

Daily water borne cortisol concentrations (ngL^{-1}) of *L rohita* after rearing different photoperiods for 90 days is presented in the Table 4. Throughout day (in 24hrs period), water-borne cortisol concentration showed significant variation ($P<0.05$) in all groups. When comparison was made between groups, the concentration of cortisol at different time of day also showed significant differences ($P< 0.001$). Fish in P1 groups exposed to 18L/ 6D, showed a circadian-like pattern in water borne cortisol, highest levels during 18 to 24hrs with peak at 22:00 hr. A shift of peak level from 18 to 22hrs was observed in others groups exposed to different photoperiods (P2,14L/10D; P3, 10L/14D and P4, 6L/18D). Moreover, the maximum cortisol level was prolonged in a group of fish exposed to 18hrs scotophase (6L/ 18D) (Table.4)

Blood glucose

The effect of various photoperiod on blood glucose level is present in Table 5. The P4 group of fish reared under long scotophase (6L/ 18D) showed significantly higher level of blood glucose as compared to other groups. The scotophase and glucose level revealed positive relationship i.e., glucose level increase with an increase of dark period, therefore P1 group of fish had lower level of glucose.

Body coloration and Morphological changes

A prominent difference in the body coloration of fish was noticed when they exposed to different photoperiods. Fish exposed to the prolong darkness had darker body coloration with dark and yellow spots appeared on their bodies compared to the other groups of fish. Along with that, the fin color turned dark yellowish as compared to fish exposed to prolong light duration as shown in the figure encircled areas (Fig. 4)

A prominent change in the body morphology of fish exposed to long scotophase (6L/ 18D) was also observed. The fish showed increase head to body ratio, easily slough out scales from the skin, narrow body posterior. However, P1 groups of fish having longer light expose period showed normal body color compared to other group of fish (Fig. 4).

Table 2. Growth performance of Rohu *(L. rohita)* after rearing on different photoperiods for 90 days

	Treatment			
Growth parameters	P1 (18L:6D)	P2(14L:10D)	P3 (10L:14D)	P4(6L:18D)
Initial mean BW (g)	5.1 ± 0.04^{a}	5.13 ± 0.01^{a}	5.14 ± 1.7^{a}	5.11 ± 0.01^{a}
Final mean BW(g)	18.51 ± 0.02^{a}	16.38 ± 0.02^{b}	14.79 ± 0.01^{c}	12.41 ± 0.03^{d}
Mean weight gain (%)	270.77 ± 0.04^{a}	209.39 ± 0.03^{b}	184.46 ± 0.05^{b}	144.34 ± 0.02^{d}
WG (g)	13.13 ± 0.01^{a}	11.25 ± 0.01^{b}	9.54 ± 0.05^{c}	7.34 ± 0.02^{d}
FCR	1.38 ± 0.01^{d}	1.69 ± 0.01^{c}	1.91 ± 0.05^{b}	2.52 ± 0.05^{a}
FCE (%)	71.42 ± 0.01^{a}	58.47 ± 0.02^{b}	50.56 ± 0.02^{c}	38.55 ± 0.04^{d}
Survival (%)	83.33 ± 0.88^{a}	76.66 ± 3.33^{b}	76 ± 2.08^{b}	65.33 ± 7.85^{c}
SGR(%BW/day)	1.21 ± 0.04^{a}	0.89 ± 0.02^{b}	0.85 ± 0.02^{b}	0.71 ± 0.02^{c}

Data represented as Mean ± SE (n=3). Means having different letter within the rows are statistically different ($P<0.05$). One-way ANOVA followed by Tukey test, all mean pair wise comparison. P1, 18L/6D; P2, 14L/ 10D; P3, 10L/ 14D and P4, 6L/ 18D.

Table 3. Hematological indices of *L. Rohita* after rearing on different photoperiods for 90 days

	Photoperiods			
Blood parameters	P1 (18L:6D)	P2(14L:10D)	P3 (10L:14D)	P4(6L:18D)
Hct (%)	17.26 ± 0.41^{d}	20.2 ± 0.24^{c}	22.96 ± 0.54^{b}	26.86 ± 0.54^{a}
HB (g dL^{-1})	6.38 ± 0.23^{a}	3.3 ± 0.26^{b}	4.6 ± 0.41^{c}	2.7 ± 0.26^{d}
MCV (fL) $10L^{-15}$	91.6 ± 2.85^{a}	88.33 ± 2.33^{b}	71.56 ± 0.51^{c}	67.7 ± 0.73^{d}
MCH (pg)	20.53 ± 0.45^{a}	21.6 ± 0.36^{a}	17.06 ± 0.05^{b}	12.36 ± 0.36^{c}
MCHC (g dL^{-1})	19.83 ± 0.34^{a}	18.43 ± 0.23^{a}	14 ± 0.55^{b}	12 ± 0.41^{c}
WBCs(10^{3} μL^{-1})	1.568 ± 0.15^{a}	1.159 ± 0.02^{a}	0.708 ± 0.001^{a}	1.398 ± 0.09^{a}
RBCs(10^{6} μL^{-1})	4.306 ± 0.10^{a}	2.2 ± 0.033^{b}	1.67 ± 0.16^{c}	0.94 ± 0.01^{d}
PLT(10^{3} μL^{-1})	11.33 ± 0.50^{a}	12.83 ± 0.58^{bc}	16.66 ± 0.50^{ab}	19 ± 0.33^{c}
LYM%	85.93 ± 1.04^{a}	72.7 ± 0.56^{b}	71.66 ± 0.38^{b}	70.66 ± 0.69^{b}

Data represented as Mean ± SE (n=15), Means having different letters within the row are statistically different ($P<0.05$). One way ANOVA followed by LSD test, all means pair wise comparison. P1; 18L/ 6D; P2, 14L/ 10D; P3, 10L/ 14D and P4, 6L/ 18D.

Table 4. Water borne cortisol (ng L^{-1}) of *L. rohita* after 90 days rearing on different photoperiods.

	Treatment				Comparison within groups
Sampling Time(hr) (PM-AM)	P1 (18L:6D)	P2(14L:10D)	P3 (10L:14D)	P4(6L:18D)	**P-value**
		Cortisol (ng L^{-1})			
2	22.15 ± 0.08^{F}	21.70 ± 0.01^{G}	23.61 ± 0.02^{I}	26.33 ± 0.01^{G}	0.001
4	22.5 ± 0.01^{D}	22.07 ± 0.03^{F}	23.65 ± 0.01^{HI}	26.33 ± 0.08^{G}	0.001
6	22.90 ± 0.03^{C}	22.41 ± 0.01^{DE}	23.70 ± 0.02^{GH}	26.35 ± 0.02^{G}	0.001
8	22.55 ± 0.02^{D}	22.30 ± 0.02^{EF}	23.71 ± 0.02^{G}	26.33 ± 0.01^{G}	0.001
10	22.96 ± 0.02^{C}	22.45 ± 0.01^{DE}	24.64 ± 0.02^{F}	26.58 ± 0.02^{F}	0.001
12	23.12 ± 0.01^{B}	22.51 ± 0.03^{CDE}	24.71 ± 0.02^{E}	27.26 ± 0.02^{E}	0.001
14	22.26 ± 0.02^{E}	22.53 ± 0.02^{CDE}	24.74 ± 0.02^{E}	27.59 ± 0.02^{D}	0.001
16	22.50 ± 0.02^{D}	22.58 ± 0.01^{CDE}	24.81 ± 0.01^{D}	27.70 ± 0.01^{D}	0.001
18	23.13 ± 0.02^{B}	22.70 ± 0.02^{BCD}	25.83 ± 0.02^{B}	27.94 ± 0.01^{C}	0.001
20	23.31 ± 0.02^{A}	23.26 ± 0.03^{B}	25.80 ± 0.01^{B}	28.13 ± 0.01^{B}	0.001
22	23.17 ± 0.03^{B}	23.16 ± 0.02^{BC}	25.12 ± 0.01^{C}	28.18 ± 0.01^{B}	0.001
24	23.17 ± 0.03^{B}	23.42 ± 0.02^{A}	26.12 ± 0.01^{A}	28.32 ± 0.02^{A}	0.001

Data represented as Mean ± SE, Means having different letter within the column are significantly different ($P<0.05$). Oneway ANOVA followed by LSD test, all mean pair wise comparison. P1(18L/ 6D), P2(14L/ 10D), P3(10L/ 14D) and P4(6L/ 18D)

Table No. 5. Blood glucose ($mmolL^{-1}$) in *L. rohita* after 90 days rearing under different photoperiods

Photoperiods	**Glucose ($mmolL^{-1}$)**
18D/6L	3.67 ± 0.05^{D}
14D/10L	4.22 ± 0.03^{C}
10D/14L	4.92 ± 0.08^{B}
6D/18D	5.30 ± 0.03^{A}

Data represented as Mean ± SE, (n=15). Different letters within column show mean significance difference from one another ($P<0.05$).

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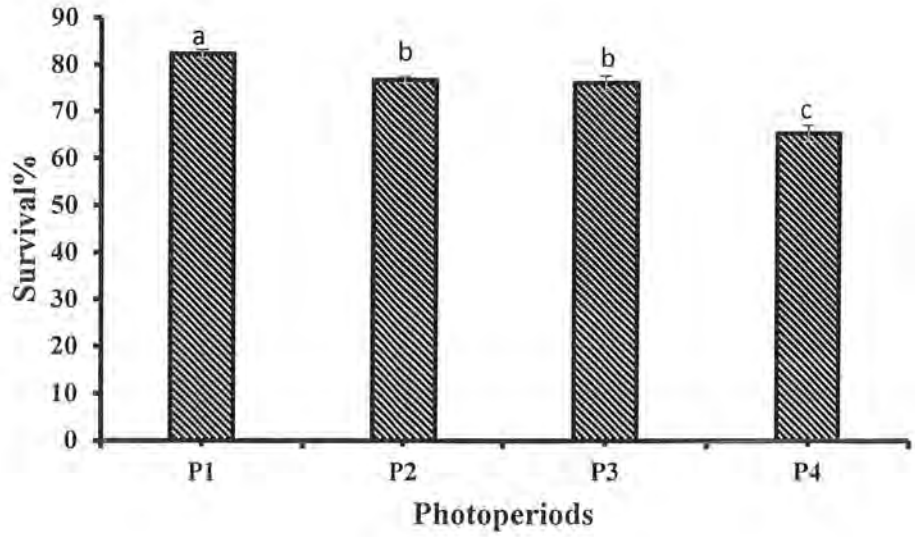

Figure 1: Survival rate % of fingerlings of Rohu *(L rohita)* after 90days exposure to different photoperiods. P1, 18L/6D; P2, 14L/ 10D; P3, 10L/ 14D and P4, 6L/ 18D. Each bar showing the values as Mean ±SE .Means having dissimilar letter are statistically different ($P<0.05$). One way ANOVA followed by Tukey test, all mean pair wise comparison.

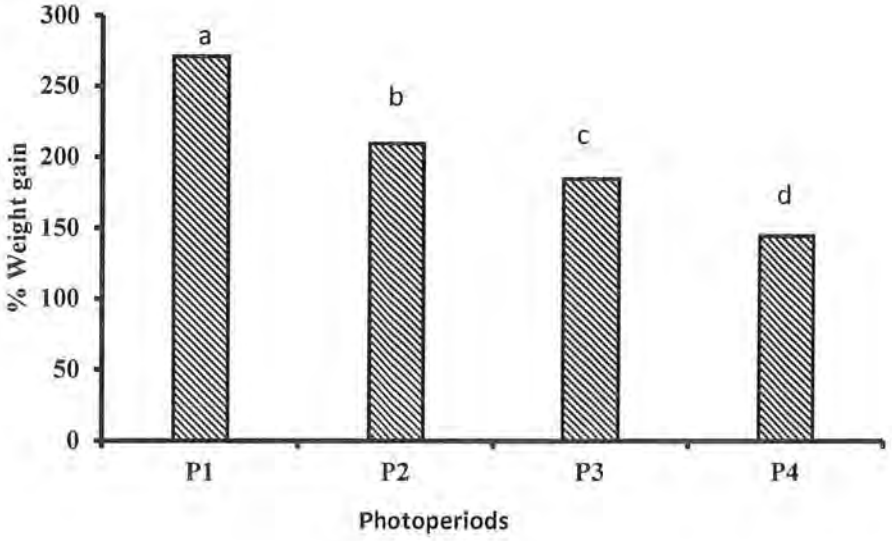

Figure 2: % increase in body weight of fingerlings of Rohu*(L. rohita)* after 90days exposure to different photoperiods. P1, 18L/6D; P2, 14L/ 10D; P3, 10L/ 14D and P4, 6L/ 18D.
Each bar showing the values as Mean ±SE (n=3). Means followed by the dissimilar letter are statistically different ($P<0.05$).

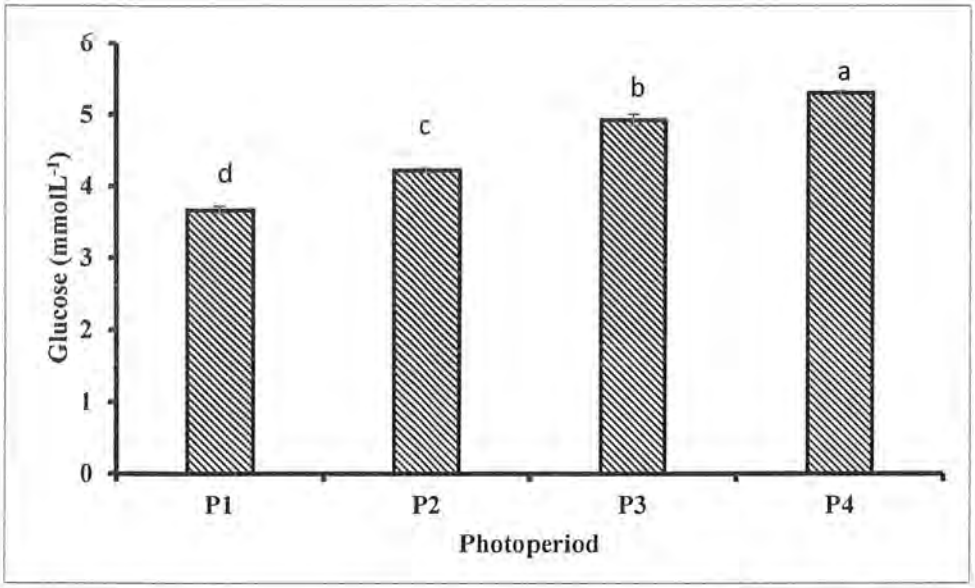

Figure 3: Glucose (mmolL^{-1})of Rohu*(L rohita)* after 90days exposure to different photoperiods. P1, 18L/6D; P2, 14L/ 10D; P3, 10L/ 14D and P4, 6L/ 18D.
Each bar represent the values as Mean ±SE (n=15). Means having different letter are statistically different ($P<0.05$).

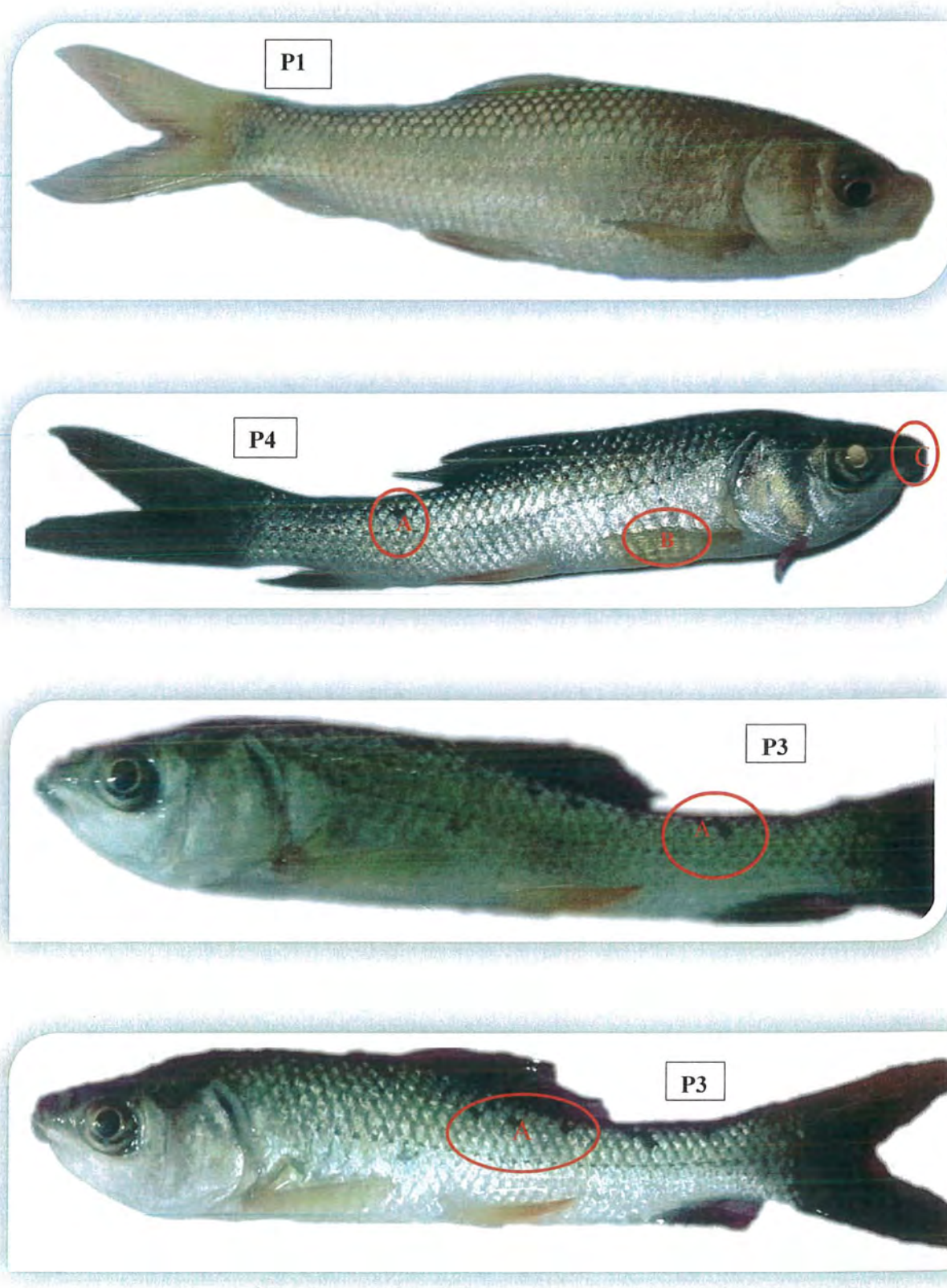

Figure 4: Showing body coloration and morphological changes observed when fish were exposed to different photoperiods, P1: Normal body colorations and morphology *(L.rohita)*.Encircle areas (A): sloughed scales (B) Deep yellowish colours scales (C) De shaped body, i.e. snout.

Discussion

Discussion

In our study, 90 days rearing on different photoperiods i.e., increasing dark period significantly affect the growth performances of *L. rohita*. Along with growth, photoperiod manipulations also revealed significant effect on the physiology and general morphology of *L .rohita* i.e., hematological indices, cortisol level, glucose and body colorations.

The effect of photoperiods on growth performances have been investigated in many fish species. For instance, Gilthead sea bream reared under different photoperiod regimes i.e., (8L/16D), (16L/8D), (12L/12D), (24L/0D), and natural (day light) displayed better growth performance during long day lengths. However these changes appeared only after a long exposure time i.e., 45–145 days, may be due to increase protein sparing, thus leading to a higher growth performance in fish (Silva-Garcia, 1996; Biswas et al., 2005). Similarly, in our study, P1 (18L/6D) group of fish showed maximum final weight gain and specific growth rate as compared to P2 (14L/10D), P3 (10L/14D) and lowest in the P4 (6L/18D) group. Likewise, Kashyap et al. (2015) reported maximum weight gain, specific growth rate in *Catla catla* due to increase feed conversion rates after being subjected to the continuous light sources (Kashyap et al., 2015). Yagci and Yigit (2009) also found high growth rates in turbot and mirror carp due to high feed conversion rates instead of differences in feed intake. Fish reared under prolong and constant photoperiods have low body lipid concentration as fatty acids being used to compensate increase energy demand due to increase physical activities (Ginés et al., 2004; Biswas et al., 2005). Similarly, Biswas et al. (2005, 2006) revealed that long day provide enough time for feeding activities and efficient digestion, most likely due to improve nutrient retention. According to Taylor et al. (2005) muscle growth stimulators i.e., GH and IGF-1 level tend to increase with respect to increase photoperiod and temperature in juvenile *Oncorhynchus mykiss*. Similar effect of long photoperiod might occurred in the *L. rohita* when exposed to prolong photoperiods like (18L/6D) and 14L/ 10D, while rearing under short photoperiod (6L/18D) showed low weight gain. High feed conversion efficiency was observed by Kashyap et al., (2015) in the *catla catla* subjected to (24L/0D). Similarly we observed increase in FCE% with increase in photoperiod.

In our study, highest survival rates were obtained in the P1 group of *L. rohita* reared under (18L/6D) followed by P2 (14L/10D), P3 (10L/14D) and lowest in the P4 (6L/18D).Comparable results were reported by Yagci and Yigit, (2009) in the juvenile *C. carpio* reared under the continuous light source. Similarly *Catla catla* exhibited 100% survival in all the fish reared under (16L/8D), (24L/0D) except (0L/24D) (Kashyap et al., 2015). However, in early developmental stage of *Miichthys miiuy* at (0L/24D) photoperiod regime, Shan et al. (2008) observed significantly lower survival% initially and then 100% mortality after the seventh day of hatching. Contrary to that African catfish *Clarias gariepinus* fingerlings under a photoperiod of (0L/24D) showed better survival rates (Adewolu et al., 2008). The increased survival rates mainly depends on feeding habit of African catfish as it prefer to live in river bed where the light penetration is low (Feiden et al., 2006), thus eat in the dark condition (Adewolu et al., 2008). Variances in the survival % of various species can be credited to the great difference in the preferred photoperiod, that is species-specific and depends on the different developmental stages of fish (Britz and Pienaar, 1992; Silva-Garcia, 1996; Boeuf and Bail, 1999; Adewolu et al., 2008).

Effect of photoperiod on level of the daily cortisol release has been revealed by many investigators (Pavlidis et al., 1999; Navarro et al., 2014;Çevik and Aslan, 2015). Like in many vertebrates, in fish cortisol secretion has a circadian rhythm and is under the control of the hypothalamic-pituitary-adrenal-axis, while physiological and psychological stress factors can modulate the synthesis of cortisol (Johansson et al., 2003; Kılıçoğlu and Gülcan, 2007). Among external factors light also effect the cortisol secretion by regulating corticotropin-releasing hormone. In majority of fish, cortisol secretion is diurnal, maximum and minimum levels are found at night and in the morning respectively (Kılıçoğlu and Gülcan, 2007). In the present study, manipulation of photoperiod showed significant effect on daily rhythmicity and concentration of cortisol of *L. rohita* (Table 4). Fish exposed to the prolong light period (18L/6D) i,e., with 6hrs of scotophase showed peak at 4 hrs, while there was a shift in peak level from 4 to 8hrs, when fish exposed to prolong scotophase (6L/18D). Similar circadian changes also observed in common dentex, *Dentex dentex* (Pavlidiset al., 1999) , hamsters (Pawlak et al., 2009) female lambari (Navarro et al., 2014). These result confirm the concept of

daily rhythmicity in the level of cortisol in the fresh water fish (Boujard and Leatherland, 1992) and change in specific pattern of the cortisol with day light (Spieler and Noeske, 1984; Boujard and Leatherland, 1992) and also with dark (Ranceet al.,1982; Pickering and Pottinger, 1983).

In the present study, we observed that photoperiodic changes slightly modulate cortisol rhythm and also affect hormone levels. The increases in cortisol level are the indicator of stress and indicate the increase demand of energy. It appears that in *L. rohita*, the prolong scotophase, act as stressor and activate the hypothalamic-pituitary-adrenal-axis. We collected water samples at the end of the study. Our results showed significantly higher level of water-borne cortisol in P4 group of fish reared under exposure of 6L/18D followed P3 and P4 groups of fish exposed to 10L/14D and 14L/10D photoperiod.

In addition to cortisol, glucose level also showed significant increase with increase of scotophase, thus P1 and P4 groups of fish reared under 6hrs and 18hrs dark regime showed lowest and highest levels of blood glucoseres pectively (Table 5). Blood glucose has long been used as secondary response indicator to stress in fish (Silbergeld, 1974; Donaldson, 1981; Wedemeyer and McLeay, 1981; Martínez-Porchas et al., 2009). The result of present study confirmed that photoperiod management i.e., increasing scotophase caused a significant acute stress response in *L.rohita* as indicated by increase in concentration of different stress indicators (glucose and cortisol). In fish, chronic stress, generally cause higher increase in cortisol and glucose level (Leonardi and Klempau, 2003). In presnt study, diel change in water borne cortisol was determined, while glucose concentration was calculated at the end experiment. Both results indicated that photoperiod manipulation especially increase in scotophase cause major chronic stress response in the fish. Like our results, Pavlidis et al. (1999) also observed increase in glucose level, when Dentex *dentex* exposed to (8L/16D) photoperiod. Moreover, the glucose concentration of female lambari subjected to prolong photoperiod (24L/0D) was also significantly lower when compared to fish exposed to (0L/24D) and (12L/12D). In contrary to our results juvenile red sea bream did not show any significant change in plasma glucose levels while subjecting to different photoperiods (Biswas et al., 2006).

Hematological indices are good markers of the overall health state of fish in pisciculture (Golovina, 1996; Ivanov, 2003). Under optimal light conditions (12, 16, and 24 light), the general physiological state of the fish could be considered as normal according to the hematological indices (Ruchin, 2007). During traumatic situations, immature and mature erythrocytes released from the spleen and increase the hematocrit levels (Kita and Itazawa, 1990; Pierson et al., 2004). We did not observe any significant raise in the hematocrit in the fish reared under 6L/18D and 10L/14D photoperiods. This might be due to fact the erythrocyte numbers did not increase during these photoperiods. In our study hematocrit was low in the *L. rohita* subjected to short photoperiod (6D/18L) while highest in the fishes reared under prolong photoperiod (18L/6D). Martem and yanov (1995) reported that stress decrease the number of blood erythrocyte in fish . The same response was observed in group of fish reared under prolong dark period (6L/18D) .Likewise hemoglobin concentrations were also low in a group of fish reared under short photo period i.e., 18 hrs dark period. Some other investigators including Bani et al.(2009) observed the development of anemia in fish towards 24 hrs dark period (00L/24D), while increase in concentration of hemoglobin with the application of long day photoperiod

Like other vertebrates, fish cannot synthesize carotenoids, but they have the capability to change color (Matsuno, 2001). We observed that fish reared under (6L/18D) and (10L/14D) had dark pigmented body, and dark patches on the ventral and dorsal surface when monitored with visual inspection (Fig.4). It seems that absence of light is responsible for the dark pigmentation because melatonin production is increased in the dark (Hisar et al. 2005). Like our results Mustapha et al. (2012) also observed similar results when reared fish under continuous darkness (0L/ 24D) . The dark color of the fish adds economic value to the fishes and consumption in the African countries. Adewolu et al. (2008) have also noted that fish cultured in total darkness (0L/ 24D) had darker skin coloration than those in (12D/12L). Fish that were reared under prolong day light (18L/ 6D) and (14L/10D) showed brighter and normal body colorations. The results of the color augmentation study suggested that the fish reared under short day length show brighter color than other light levels (Rajeswari et al., 2017). For instance *C. gariepinus* under 24 hr continuous light showed a lighter appearance, as compared to fish exposed to (12L/12D) photoperiod and had usual dark body color on the dorsal side.

The results indicated that the day length and darkness have significant effect on the growth parameters, general morphology and physiology of *L.rohita.* The prolong photoperiod showed positive impact, increase growth rate , improve health status and general morphology of *L. rohita* while increase darkness lower the growth rate and showed negative impact on hematological indices and body color and shape of fish on the growth, health status and color of fish, thus , suggesting that *L. rohita* should be cultures in the long photoperiods.

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