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Effects of Different Photoperiodic Regimes on the Body Mass, Behavioural and Stress Responses in Golden Mahseer, *Tor putitora*

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Abstract

Golden mahseer, *Tor putitora* is a teleostean cold-water fish, known to occur in the rivers of Himalayas. Light affects the physiology of the fish. Therefore, the study was carried out to evaluate the effects of different photoperiodic regimes as environmental cues on the development of body mass, behavioural and stress responses in *T. putitora*. Fishes were exposed in the different photoperiodic regimes such as 8 hours light: 16 hours dark (8L:16D), 16 hours light 8 hours dark (16L:8D) and natural light condition. The body mass, cortisol and behavioural profiling were studied. Ethovision (XT-13) was used to record the different behavioural responses of the fish after the exposure to various photoperiodic regimes. The difference in the behavioural profiling and scoring was recorded in the different groups of the juveniles of the fish. Effects of light: dark skeletons such as 8 hours light: 16 hours dark (8L:16D), 16 hours light 8 hours dark (16L:8D) and natural light condition (Control) exhibited remarkable differences in the biomass enhancement in the juveniles of the fish (P<0.05). Maximum amount of cortisol (0.93± 0.08ng/ml) was recorded in the group of the fish exposed to 16L:8D compared to natural (0.78± 0.21ng/ml) and the group exposed to 8L:16D photoperiodic regime (0.69± 0.11ng/ml). A significant difference (P<0.05) in behavioural profiling of the fish exposed to green, red and natural colour was noticed, where velocity/swimming speed of the fish was most affected by the green light spectra. The significant difference in the behavioural profiling and scoring was recorded in the different groups of the juveniles of the fish.

Keywords: Behavioural, Cortisol, Golden Mahseer,Photoperiodic Skeleton

1. Introduction

Environmental cues affect the physiological governing in fish during the timing of reproduction, circannual rhythmicity as well as behavioural imprinting in the brain. Development of various stages of the fish is affected by environmental and nutritional factors. Growth of fish is affected by the feeding rate, feeds, water quality, stock density and size (Trzebiatowski *et al.*, 1981, Kashyap *et al.*, 2015)[1,2]. Photoperiod acts as a zeitgeber for the growth performance, maturation and behavioural imprinting in fish. The study on the effects of different photoperiodic regimes on the organismal physiology was carried out by various researchers in fish (Kashyap *et al.*, 2015; Imsland, *et al.*, 1995; SILVA-GARCIA, 1996; Purchase *et al.*, 2000; Ruchin, 2004; Rad *et al.*, 2006; Taylor *et al.*, 2006; Valenzuela *et al.*, 2006; Bonnet *et al.*, 2007; Askarian & Kousha, 2009; Lee *et al.*, 2019)[2-12]. Fiszbein *et al.*(2010)[13] studied the photoperiodic role to evaluate the behavioural profiling and reproductive events of the cichlid fish, *Cichlasoma dimerus*. El-Sayed and Kawanna (2004)[14] have exposed the *Oreochromis niloticus* (Nile tilapia) fries to different conditions of the light regimes to study the specific growth rate, food conversion ratio, and weight gain and survival activity. Photoperiodic light intensities have been reported to play some roles in the enhancement of biomass and viability of juveniles of cultured Mexican cichlid fish, *Cichlasoma beani* (Aragón-Flores *et al.*, 2017)[15]. Mustapha *et al.* (2012)[16] studied the role of light: dark cycle on the body pigments of the fry of *Clarias gariepinus* (African catfish). Growth and survival

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of the juveniles of Indian major carps after the exposure of photoperiodic regimes were also studied by Kashyap *et al.* (2015)[2]. Haematological and growth performances were affected by the photoperiodic manipulations in the juvenile of *Rutilus rutilus caspicus* (Shahkar *et al.,* 2015)[17]. Maaswinkel *et al.* (2013)[18] studied the shoaling behavioural responses in zebrafish after the exposure of alcohol. The different type of behavioural scoring of stress was studied in the zebrafish (Cachat *et al.,* 2010)[19]. Lee *et al.* (2019)[12] worked on the growth and behavioural aspect of zebrafish after the exposure of environmental signals. Some research workers reported that swimming nature has enhanced the biomass in fish through muscular growth and emphasized that the process of homeostasis provides immunity in relation to decreasing the confrontational behaviour (Davison, 1997; Palstra & Planas, 2013)[20,21]. Working on the coral reef fish breeding, Olivotto (Sarvi, 2012)[22] reported that the variable light regime exposure affected the feed conversion efficiency which caused the changes in the development and behaviour parameters. Light penetration affects the biomass by enhancing the consumption rate of food, strengthening the muscular mass via increasing movement activity (Boeuf & Le Bail, 1999)[23]. The effect of the variable light-dark cycle on the somatic growth and gonadal development (sexual maturity) has been studied by Rad et al. (2006)[7] in Nile tilapia. An impact of photoperiodic events on the consumption rate of foodstuffs concerning to biomass and reproductive events physiology in the red-eyed, *Poecilias phenops* (molly) was worked by Zutshi and Singh (2017)[24] during the artificial condition. The present study was carried out on the juveniles of hill stream *Tor putitora* (golden mahseer) which belongs to the family, Cyprinidae, is an inland water teleostean fish used as a middle feeder in pisciculture in the Himalayas foothills (Akhtar et al.,2017)[25]. *T. putitora* is confined in the south-east regions of the Himalayas (Nautiyal, 2014)[26] where the fish inhabits in the running water of rivers (Bhatt & Pandit, 2016)[27]. The population of the *T. putitora* is dwindling and belongs to the endangered category of IUCN. Therefore, the present scenario is demanding for the conservation of the fish. So, the present study was planned and carried out on the hypothesis that whether the photoperiodic events affect the development of biomass of juvenile fish and the impact of coloured lights on the behavioural patterning of the fish.

2. Materials and Methods

Juveniles of *Tor putitora* were collected from the River East Ramganga (29.54ºN, 80.104"E) in early morning hours, transported to the laboratory, acclimatized for environmental conditions of the aquarium, and experiment was carried out as per institutional ethics committee's guidelines. A total of 30 juveniles of *T. putitora*, weighing between 0.09-0.280g, were randomly selected and equally divided into three groups and kept in different aquaria (60×30×30cm) namely A, B and C for 96 days. The water volume of 20 litres was maintained in each aquarium which was equipped with an aerator supported by an air cum filter water pump. A total of 20% of the water volume of each aquarium, excretory materials and other debris were removed daily and the water level was maintained according to the required volume. Fish of all groups were fed twice a day with the paste of liver of goat at the rate of 0.05gm/day throughout the experimental period. Each group of *T. putitora* was exposed to either of the below mentioned photoperiodic regimes:

(a) Short photoperiod (8L: 16D) of 8 hours light (L^{on} at 8.00 am and L^{off} at 4.00 pm)

(b) Long photoperiod (16L: 8D) of 16 hours light (L^{on} at 4.00 pm and L^{off} at 8.00 am)

(c) Natural day length (12L: 12D)

Light-proof devices were used to separate the three different aquaria from each other, and artificial white fluorescent lamps of 5(W) LED (Panasonic), each was installed at a height of 10 cm above the water level of the aquarium. At the end of the experiment, all the juveniles of *T. putitora* were killed using clove oil as anaesthesia. The weight of each juvenile was recorded on an electronic balance sensitive up to 0.001 gm (Roy Electronics India, CBCAB300), and samples were immediately stored in -20˚C. The whole body of juvenile fish was homogenised thrice in phosphate buffer saline (1X PBS) with the help of homogeniser (Remi) at 3500 rpm for the complete extraction of cortisol. Yellowish supernatant was transferred into a tube (Eppendorf) and kept in 4˚C for the evaporation of ether, and dissolved in 1X PBS buffer and kept at 4˚C (Cachat *et al.,* 2010). Enzyme-linked immunoassay (ELISA) was performed for the quantification of cortisol using a cortisol assay kit (Oxford Biomedical Research). The intensity of ELISA reactions was measured in the ELISA plate reader (Systronics) at 450 nm.

2.1 Effect of Colour Light on behaviour of Juvenile Fish

In order to study the behavioural profiling of the fish exposed to green, red and natural colour, the 30 juveniles of *T. putiora* weighing between 0.22-0.30g were divided into three groups in the three different aquaria (60×30×30cm). The first group (n=10) was a natural control while others were green and red light exposed groups. Both groups were treated with the photoperiodic regime of 8L: 16D (L^{on} at

8 a.m. and L^{off} at 4 p.m.). Specific growth rate (SGR) was computed using the formula:

SGR% =100× (Final body weight-Initial body weight)/ time (days)

The number of live juveniles of each aquarium was recorded and expressed as a percentage of survival rates. A total of 12 individuals of *T. putitora* were selected for the behavioural study of 300 sec at the rate of 25 frame/sec. The behavioural status of the fish was analyzed using automated video-tracking software, Ethovision XT 13 (Noldus, Info Tech, Wageninen, Holland). The behavioural scoring such as total distance travelled (overall activity), angular velocity, turn angle, swimming speed or velocity, movement, and meander of the fish were studied and recorded.

2.2 Statistical Analysis

Statistical method such as one-way analysis of variance (ANOVA)followed by post-hoc test of Newman-Keuls Multiple Comparison was used to differentiate the various groups of the fish. All analysis was done using the graph pad prism (version 5) software.

3. Results

The final average body mass of juvenile fish exposed to natural (12L:12D), short (8L:16D) and long (16L:8D) photoperiodic regime recorded were 0.1490±0.006g, 0.1592±0.002g and 0.1267±0.003g respectively while the specific growth rate of the fish under natural, short and long photoperiodic regime was 16%,15% and 12% respectively. One-way ANOVA analysis *(F_2, 15=13.80 at P<0.0004)* followed by post-hoc test of Newman-Keuls Multiple Comparison showed a significant difference in the body mass of the juvenile fish of three groups (Figure 1).

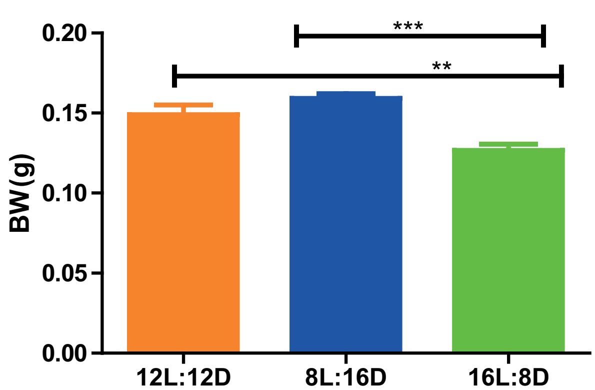

Figure 1. The final average body mass of juveniles of *T. putitora.*

The maximum mean value of cortisol hormone (0.93±0.08ng/ml) was recorded in the fish exposed to long photoperiodic regime compared to that of short (0.69±0.11 ng/ml) and natural (0.78±0.21ng/ml). However, no statistical difference was observed among the values of cortisol recorded among three groups of the fish (Figure 2).

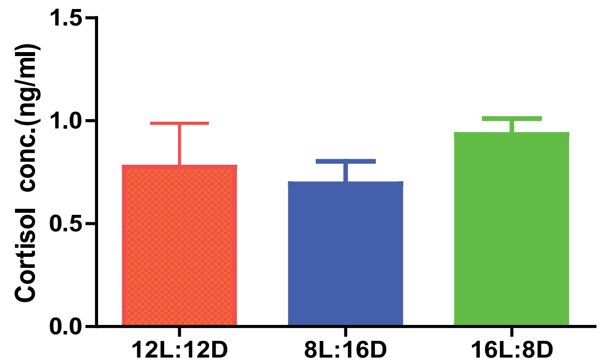

Figure 2. The average cortisol concentration in juveniles of *T. putitora.*

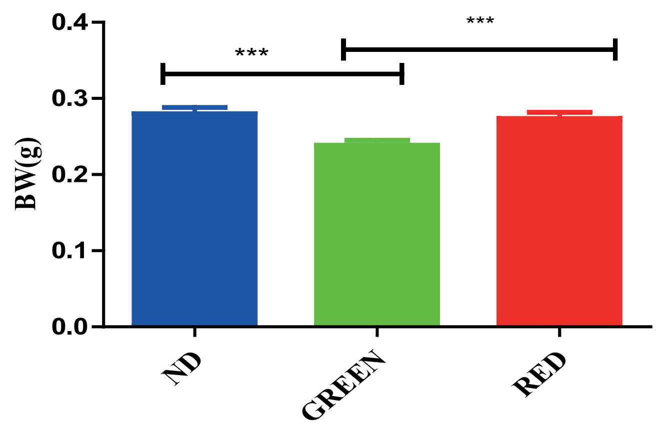

Figure 3. Body weight of juveniles after exposure to the different colour regimes.

The comparative average body mass of the juvenile fish exposed to natural photoperiod was higher (0.28±0.008g) as compared to that of green (0.24±0.006g) and red light (0.27±0.008g) exposed fish for the short photoperiodic regime. Similarly, a high value of specific growth rate was noticed in the juvenile fish of natural group (0.40±0.06%/day) as compared to that of green (0.38±0.035%/day) and red light (0.34±0.07%/day) exposed groups. The survival rate of juvenile fish was 100% in the group exposed to natural light while it was 90% in green and red light-exposed groups. One-way ANOVA analysis *(F_2, 14=16.09,* *p<0.0001)* followed by post hoc test of Newman-Keuls Multiple Comparison showed a significant difference among the values of the body mass of natural, green and red light-exposed groups at $P < 0.05$ (Figure 3). The velocity or swimming speed of the juvenile fish recorded during the behavioural study were 32.04 ± 1.12, 36.00 ± 1.68 and 38.39 ± 0.54 cm/sec in the fish exposed to natural, red and green lights respectively. The data of velocity was log-transformed for the normalization. The normalized data was subjected to one-way ANOVA analysis (F_2, 2841= 261.4 at $P<0.0001$). The post hoc Dunnett's multiple comparison test showed a significant difference among the values of the velocity of the fish exposed to natural, green and red lights at $P < 0.05$ (Figure 4). The maximum average distance was travelled by the fish exposed to green light (7.68±0.11 cm) compared to that of control (6.41±0.22 cm) and red light (7.20±0.34cm) exposed groups during the trial observation of 300 sec. The data of travelled distance was log-transformed for the normalization. The normalized data was subjected to one-way ANOVA analysis (F_2, 2841=261.7 at $P<0.0001$) by using the post-hoc Dunnett's Multiple Comparison test which showed a statistically significant difference at $P < 0.05$ (Figure 5). The value of body elongation of the fish was 0.70±0.0064, 0.75±0.003 and 0.70±0.004 in the juvenile fish exposed to natural, green and red light respectively which was significantly different from each other's at $P < 0.05$ (Figure 6). Maximum values of acceleration and meander were recorded in control and red groups respectively which were statistically significant ($P<0.05$). The details of acceleration and meander are given in (Figure 7 and 8).

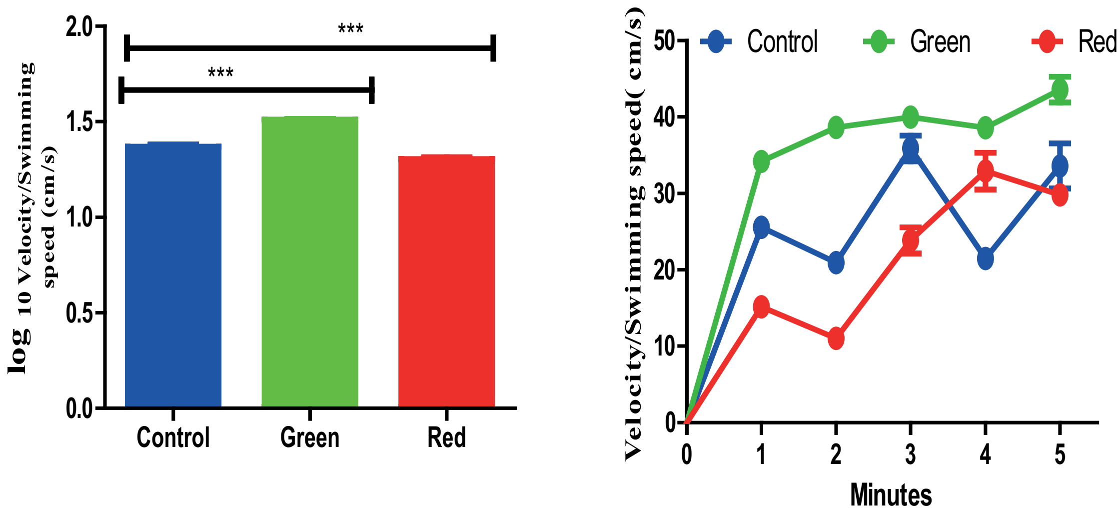

Figure 4. Velocity or swimming speed of the fish after exposure to the different colour regimes.

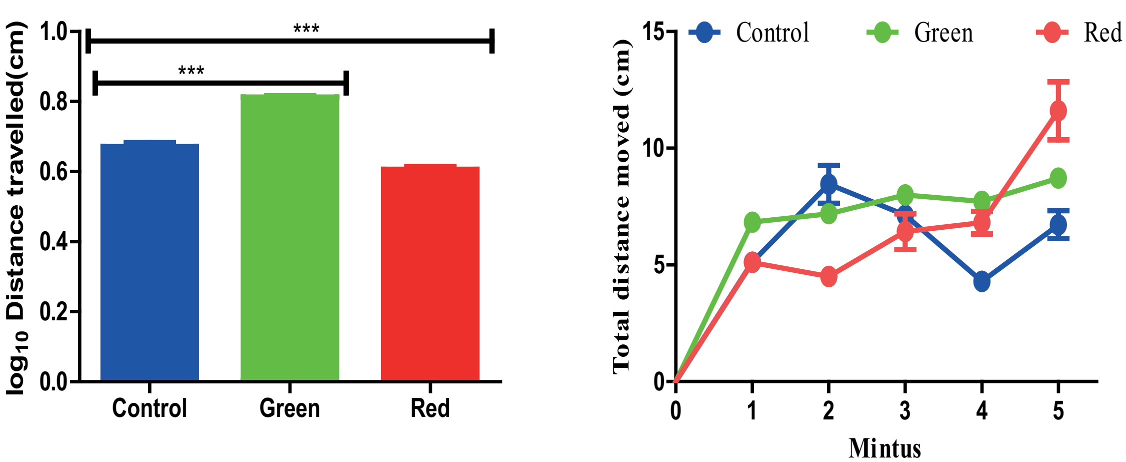

Figure 5. The average distance travelled by fish during the trial observation.

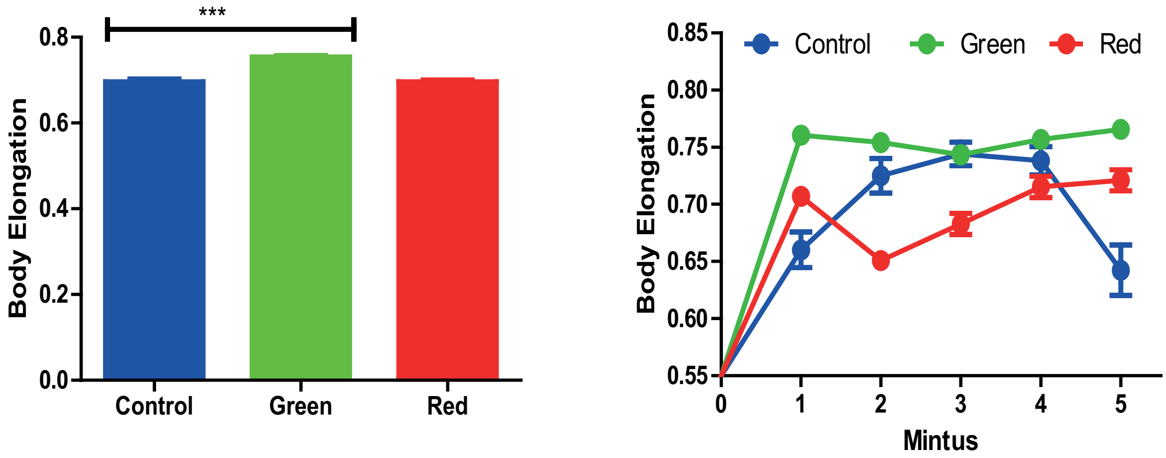

Figure 6. Body elongation of the fish during the observational scaling.

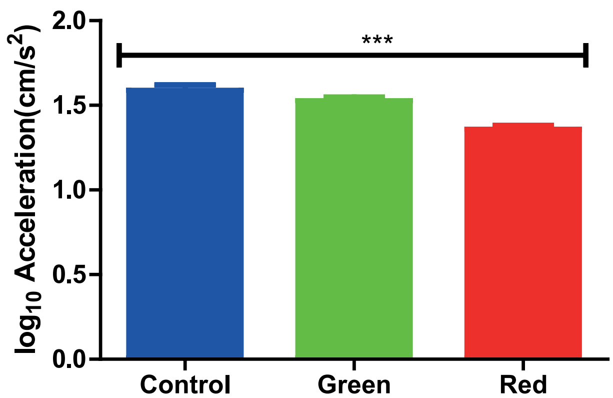

Figure 7. Acceleration of fish during the behavioural observation.

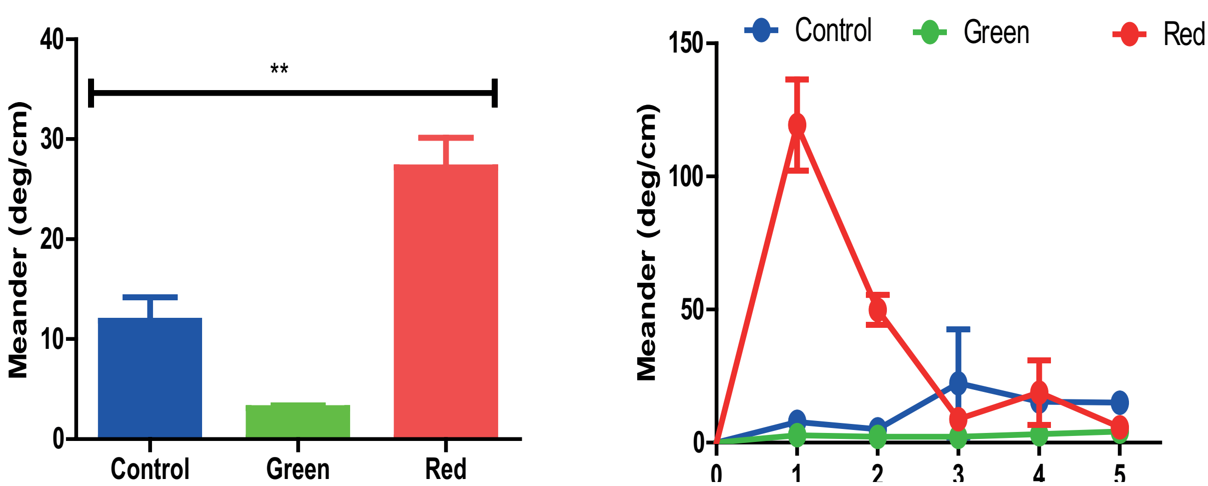

Figure 8. Meander(Mean±SE) in the fish after exposure to the different colour regimes.

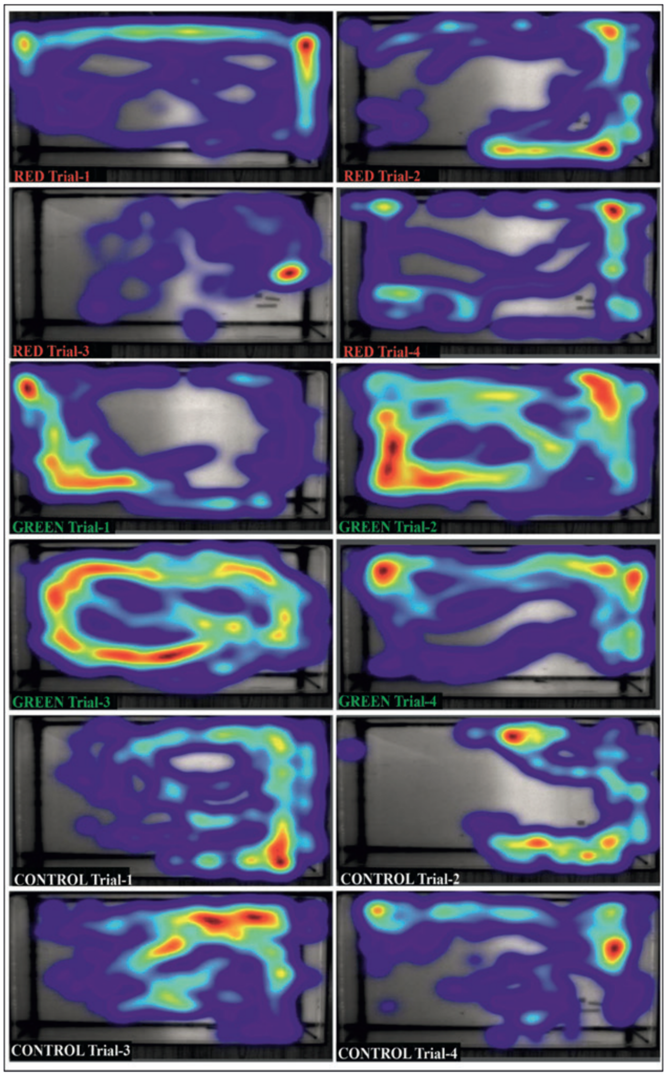

Figure 9. Heat map of activity pattern of the juveniles of *T. putitora.*

4. Discussion

Requirement of photoperiod is an extremely variable factor in fish which is related to adaptation to environmental conditions, and specific to species and age (SILVA-GARCIA, 2020; Boeuf & Le Bail 1999)[4,23]. The present findings showed that the juvenile growth of *T. putitora* is affected by the treatments of various photoperiodic regimes; and short day light (8L:16D) was found to be suitable for the growth of the fish. Similarly, Arvedlund *et al.* (2000)[28] also reported slow growth under continuous light (24L:0D) compared to 16L:8D in *Amphiprion melanopus*. A number of researchers (Fuchs, 1978; Barahona-Fernandes, 1979; Barlow *et al.,* 1995)[29-31] reported different optimum photoperiodic regimes for different fish species. In contrast to the present findings, the growth performance of the juveniles of Caspian roach, *Rutilus rutilus caspicus* was maximum in the 24h light exposure as compared to another photoperiodic skeleton (Shahkar *et al.,* 2015)[17].

The cortisol concentration was highest in the juvenile fish exposed to 16L:8D photoperiodic regime in the present study as compared to other photoperiodic regimes. Pavlidis *et al.* (1992)[32] had worked on the common *Dentex dentex* (Dentax) and also reported the highest value of cortisol in the fish exposed to 16L:8D photoperiodic regime. Audet *et al.* (1986)[33] working on *Gasterosteus aculeatus,* reported and emphasized that the level of cortisol remains high in the fish exposed to the short photoperiodic regime.Light differentiated into six distinct spectra (V, B, G, Y, O, R) in decreasing wavelength (Singh, 2006; Heydarnejad *et al.,* 2017)[34,35]. Different light spectra act as environmental cues and affect the growth and reproduction and coordinate the different stages of life in fish (Zutshi & Singh, 2017, 2020)[24,36]. Water has optical properties which lead to the different light-absorbing pattern at different degrees. Psychologists reported that the different colour spectra show different properties of warm and cool in their nature, which affect the brain through absorbance of wavelength. Red and orange are warmer while the blue and green are relatively cool. Fujii (2000)[37] has emphasised that the intensity of light regulates the chromatophore activity in fishes because of aggregation or dispersion of pigments, Effect of different colours of the artificial light on the growth of juveniles of common carp (*Cyprinus carpio*) showed that the red light gives better growth performance as compared to blue, white, yellow, green and dark (Abdul-Nabi Nasir & Willaim Farmer, 2017)[38]. In the present study, a high growth was recorded in the juveniles fish exposed to red light compared to green colour exposed groups.The movement tracking analysis to quantify the behavioural changes in the fish in every 0.20 sec was carried out of using Ethovision (XT 13) where swimming speed, distance travelled elongation and meander (turning movement) were recorded in order to study their effects on the growth of the fish. Fish species have their own daily cycle of activities based on the endocrine signalling, maturation and behavioural status. Most of the animals including fish are having light-sensitive circadian clocks which provide internal synchronization by regulating melatonin, known as time-keeping hormone that affects the rhythmic physiological functions of fish (Bromage *et al.,* 2001; Villamizar *et al.,* 2009)[39,40].The behaviours are affected by colour lights in fish which are species-specific (Villamizar *et al.,* 2009; Volpato *et al.,* 2013; Villamizar *et al.,* 2011)[40-42]. The scoring of behavioural parameters such as velocity or swimming speed, travelling distance, body elongation, acceleration and meander were carried out for the first time in the juveniles of *T. putitora* for the quantification of their behavioural profiling after the exposure of different light spectra. In our study, swimming speed, distance travelling and body elongation were maximum in the group of fish exposed to green light compared to that of natural and red light exposed groups, which indicated that the rate of exploration was supported by green light in the fish. Measuring behavioural, the different activity pattern was showed in heat maps (Figure 9). Endocrine responses and neuro-phenotyping studies were carried out by Cachat et al. (2010, 2011)[19,43] in adult zebrafish where they studied the body elongation pattern as well as 3D swimming trajectory. The sensitivity of Zebrafish towards the spectra was also carried out by Stewart (Stewart *et al.,* 2015)[44] where meander in the group of fish exposed to red light was higher as compared to others indicating that the fish made more trajectories for themselves for the capturing of food because of the impact of light on the brain of the fish. The information on the function of the clock and light-sensitive neuron within the brain, till to this date, is unknown in *T. putitora*. Therefore, additional studies are required for understating the impacts of colours for generating effective signals which change the fish behavioural activity by conveying the message to the brain.

5. Conclusion

Photoperiodic regime of short day is effective for growth enhancements in the juveniles of *T. putitora*. The coloured lights affect the behavioural changes in the fish.

6. Acknowledgements

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7. Conflict of Interest

There is no conflict of interest among the authors.

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