Effect of LED light spectra on reproductive performance of Koi carp (Cyprinus carpio)

Mukesh Kumar Bairwa*, Neelam Saharan, Kiran Dube Rawat, Virendra Kumar Tiwari and K. Pani Prasad

ICAR-Central Institute of Fisheries Education, Mumbai-400 061, Maharashtra, India. Received: 08-08-2015 Accepted: 10-06-2016 DOI:10.18805/ijar.v0iOF.8452

ABSTRACT
The present study aimed to evaluate the reproductive performance of Koi carp, Cyprinus carpio exposed to different light spectra using light-emitting diodes, LEDs: blue (peak at 450nm), green (530 nm), yellow (580nm) and red (630nm) within a visible light under long photoperiod (16h, light : 8h, dark) at ≈0.9 W/m² light intensity. White fluorescent light (simulated natural photoperiod, SNP) was used as control. The last 30 days of total experimental period (120 days), fishes from all the groups were uniformly exposed to green-LED light to assess the reproductive recovery. The highest levels of both male and female GSI, ova diameter, VTG and sex steroid hormones on 90th day were recorded in green-LED fish group. In case of male the highest concentration of 11-KT hormone was also found in green-LED fish group on 90th day. Yellow and red-LED fish groups showed significant lower level of above parameters compared to control (SNP) group. Yellow and red-LED fish group showed significant retrieval in gonadal maturation during the recovery period. Therefore, the present study indicates that green light accelerates gonadal maturation of koi carp and use of particular spectrum light likely to facilitates the more-energy efficient aquaculture practices.

Key words: Hormones, Light spectra, Light emitting diodes (LED), Reproductive performance, Sex steroid and Koi carp

INTRODUCTION
Among light factors, photoperiodic manipulation (periodicity) is emerging as an acceptable approach of practical application for regulating physiological functions in fish farming. Photoperiod has found to be influential on many physiological functions in fish species such as growth, reproduction and gonadal maturation, and are now widely used in aquaculture to alter spawning season, manipulate maturation and stimulate growth in captive condition (Purchase et al., 2000; Bromage et al., 2001; Randall et al., 2001; Gines, et al., 2003).

Recently, several studies were conducted on the effect of wavelength (spectrum) of light on physiological performances of fishes. It is known that the spectral composition of incident light changes differentially in underwater environments and there is a rapid attenuation with increasing depth (Lythgoe, 1994); the short or blue end of the visible spectrum becomes predominant in deeper waters, whereas red light only penetrates in shallow waters (McFarland, 1991 and Myrberg and Fuiman, 2002). Fishes are capable of detecting differences in the intensity and spectra of light by retinal and extra-retinal photoreceptors (Bayarri et al. 2002; Vera et al., 2010). Sensitivity to these differences varies among species. These determinants seem to affect behaviour, somatic growth, reproductive activity and the survival of juveniles and larvae (Volpato et al., 2004 and Bapary et al., 2011). Shin et al., 2013 and 2014 studied the effect of LED spectra on reproductive performance of yellowtail damsel fish, Chrysiptera parasema and gold fish, Carassius auratus respectively.

Among the ornamental fishes Koi carp (Cyprinus carpio) is a tropical fresh water fish belong to family Cyprinidae. Haniffa et al., 2007 reported induced breeding of koi carp and confirmed that Koi carp get maturity around 200-250 g, ova diameter ranging between 0.9 mm and 1.10 mm. and incubation period is 73.00 hrs. For energy savings and way to enhance the gonadal development the present study is proposed to evaluate reproductive performance of Koi carp, Cyprinus carpio (Linnaeus, 1758) exposed to different light spectra under long photoperiod (16L: 8D).

MATERIALS AND METHODS
Experimental design and set up: Three month old Koi carp, Cyprinus carpio (n=300, average weight 16.24±0.243 g) procured from the local market (Mumbai, Maharasatra, India) during September. These fishes were stocked in fifteen blue colour (inner side with white colour) rectangular shape tanks with 100 L capacity. Fishes were stocked by following completely randomized design (CRD). Fishes were allowed for 2 weeks acclimatization. During the acclimatization period, the fish were exposed to light from a white fluorescent bulb (27W); in addition this type of light exposure was used for the control group

*Corresponding author’s e-mail: mukeshbairwa2@gmail.com
(Simulated Natural Photoperiod, SNP Group). There were four treatments along with control (White fluorescent light) in triplicate manner. The fishes were exposed to Blue-LED (peak at 450nm), Green-LED (peak at 530nm), Yellow-LED (peak at 580nm) and Red-LED (peak at 630nm) procured (Dasin LED Co. kyunggi, Korea).

The last 30 days of total experimental duration of 120 days, fishes from all the groups were uniformly exposed to green-LED spectrum to assess reproductive recovery. Commercial feed was given throughout the experiment (34.5% protein). The fishes were fed commercial feed twice daily (09.00 and 17.00) at the rate of 3.5% of body weight in first 60 days and 3.0% of the body weight in rest period. The water quality parameters were monitored weekly during the experiment period.

**Estimation of reproductive growth:** Formulae used for the calculation of gonado-somatic index and relative fecundity are following:

\[
\text{Gonado-somatic index (GSI)} = \frac{\text{Weight of Gonad (g)}}{\text{Weight of whole fish (g)}} \times 100
\]

Relative fecundity = \(\frac{\text{No. of eggs in whole fish} \times 100}{\text{Body weight of female fish}}\)

**Sex steroid hormone and vitellogenin assays:** E\(_2\), T and 11-KT concentration were analyzed by EIA kit procured from the Cayman Chemicals, according to the manufacturer’s instructions. Vitellogenin concentration in serum sample was estimated by EIA kit procured from Biosense Laboratories, Norway.

**Gonadal histology:** Histology for gonads was done by following the method given by (Mukherjee, 1988 and Humason, 1967). These permanent prepared slides were examined and photographed under a light microscope.

**Statistical analysis:** The data were statistically analyzed by statistical package SPSS version 16 in which data were subjected to one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981).

**RESULTS AND DISCUSSION**

**Gonado-somatic Index (GSI):** In present study, both male and female GSI in yellow-LED, red-LED and SNP fish groups were not significantly \((P < 0.05)\) different from each other.
other but significant lower \( (P > 0.05) \) than blue and green LED fish groups. On 90\(^{th}\) day same pattern was followed by the male GSI among different light spectra (Fig. 1a, 1b). After one month recovery period under green LED (120\(^{th}\) day), there was significant increment in male GSI in yellow-LED, red-LED fish groups compared to the same on 90\(^{th}\) day \( (P < 0.05) \). During the entire experiment the highest Male GSI was recorded in green-LED fish group whereas the lowest in red-LED fish group.

**Ova diameter and relative fecundity:** After 60 day exposure to different light spectra ova diameter of green-LED fish group was significant high \( (P < 0.05) \) compared to other light spectrum groups (Fig.-2A). On 90\(^{th}\) day same pattern was recorded among different light spectrum group. During this experiment ova diameter in red-LED fish group was the lowest whereas highest in green-LED fish groups. The range of ova diameter for koi carp was 1.03-1.16 mm during this experiment.

Relative fecundity did not show any significance \( (P > 0.05) \) difference among the different spectrum groups on 90\(^{th}\) day as well as on 120\(^{th}\) day (Fig.-2B). The maximum relative was recorded in green-LED fish group whereas minimum in red-LED fish group.

**Steroid hormones and vitellogenin:** Steroid hormone (Testosterone, Estradiol and 11keto-testosterone) and vitellogenin concentration in blue-LED, green-LED fish groups were similar to each other and significantly higher than other spectrum fish groups on 60\(^{th}\) day (Fig.-3A, 3B, 4A and 4B). On 90\(^{th}\) day in yellow-LED, red-LED fish groups did not show significant difference \( (P > 0.05) \) from 60\(^{th}\) day in same spectrum group. However, it has been decreased (testosterone, estradiol and vitellogenin) or increased (11 keto-testosterone) in blue-LED, green-LED and SNP fish groups.

After exposing to green-LED spectrum under recovery period yellow-LED, red-LED and SNP groups showed significantly \( (P < 0.05) \) decreased concentration of testosterone, estradiol and vitellogenin while increased concentration on 11keto testosterone concentration.

**Histology:** Gonadal section from koi carp female on 90\(^{th}\) day shows the advance stages of oocytes in green, blue and SNP group compare to other treatment groups (Fig.5). Most of the oocytes in green-LED in tertiary yolk stage (TYS) whereas secondary yolk stage (SYS) in blue and SNP group. It shows the advance gonadal development in green-LED group. On 90\(^{th}\) day oocytes in red-LED group were

---

**Fig 2:** Changes in ova diameter (A) Relative fecundity (B) of koi carp, Cyprinus carpio reared under Blue-LED, Green-LED, Yellow-LED, Red-LED and simulated natural photoperiod (SNP). The fishes were reared under long photoperiod (16L: 8D). Mean values with different letters are significantly different at different time points (Days) in fish exposed to same light spectrum \( (P < 0.05) \). The numbers indicate significant differences between different light spectra within the same time point \( (P < 0.05) \). All values are mean± SD (n=6). * Fishes from all spectrum groups were exposed to green light spectrum from 91\(^{st}\) to 120\(^{th}\) day.
in Peri-nucleus stage (PNS), which proves the slow gonadal development in this treatment. Green light exposure to all treatment under recovery period for 30 days geared up the gonadal development (Fig.6). Oocytes from all the treatments showed advance stages of gonadal maturation but oocytes from red-LED light were not in same stages (mixed stages).

Gonado-somatic index (GSI) is an important parameter to determine the maturity stage of fishes. During reproductive phase, gonadal weight increases relative to the fish weight, with corresponding increase in GSI values. In present study for both male and female, the significantly higher GSI (p<0.05) were recorded in blue and green-LED fish groups. Higher GSI indicated the better maturation in fishes. Histological study also revealed that oocytes were in advance stage and uniform in size in fishes exposed to blue and green-LED lights compared to other spectrum groups. These results can be attributed to the higher maturity both blue and green LED fish groups. After 30 day recovery period under green-LED light GSI in both sex showed significant increment in GSI, vitellogenin and sex steroids in yellow-LED, red-LED fish groups compared to 90th day in same group. This indicates the partial recovery in gonadal maturation process in these groups and support green light exposer is better for gonadal maturation in Koi carp.

Green-LED fish group was recorded the highest ova diameter in this experiment. Large ova diameter indicates the advance maturation in particular group. Higher vitellogenin concentration in green-LED fish groups supports the present finding. Relative fecundity did not show significant difference among the different spectrum groups on 90th day as well as on 120th day. It reveals that higher GSI in female was due to larger size ova but not due to increase in numbers of oocytes.

In present study three major sex steroid hormones i.e. Testosterone, 11-Keto testosterone (in male) and estradiol (in female) were estimated. With this Vitellogenin protein concentration (yolk precursor protein) in female blood serum is also estimated. In this experiment testosterone concentration in blue-LED, green-LED and SNP fish groups reached at the peak level at 60th day and after that it started decreasing. However fishes did not show this pattern (decreasing after peak level) in yellow and red-LED fish groups upto 90th day. This indicates that process of

![Fig 3: Serum concentration of testosterone (A) 11-keto testosterone (B) in koi carp, Cyprinus carpio reared under Blue-LED, Green-LED, Yellow-LED, Red-LED and simulated natural photoperiod (SNP). The fishes were reared under long photoperiod (16L: 8D). Mean values with different letters are significantly different at different time points (Days) in fish exposed to same light spectrum (P < 0.05). The numbers indicate significant differences between different light spectra within the same time point (P < 0.05). All values are mean± SD (n=6). * Fishes from all spectrum groups were exposed to green light spectrum from 91th to 120th day.](image-url)
Fig 4: Serum concentration of estradiol (A) Vitellogenin (B) in koi carp, *Cyprinus carpio* reared under Blue-LED, Green-LED, Yellow-LED, Red-LED and simulated natural photoperiod (SNP). The fishes were reared under long photoperiod (16L: 8D). Mean values with different letters are significantly different at different time points (Days) in fish exposed to same light spectrum (*P* < 0.05). The numbers indicate significant differences between different light spectra within the same time point (*P* < 0.05). All values are mean± SD (n=6). * Fishes from all spectrum groups were exposed to green light spectrum from 91<sup>th</sup> to 120<sup>th</sup> day.

Fig 5: Changes in cross section of the ovary histology of koi carp, *Cyprinus carpio* on 90<sup>th</sup> day, reared under simulated natural photoperiod, SNP (A), blue (B), green (C), yellow (D) and red (E) LEDs. The fishes were reared under long photoperiod (16L: 8D). Scale bar=10 µm PNS: peri-nucleous stage, PYS: primary yolk stage, SYS: secondary yolk stage, TYS: tertiary yolk stage.
Fig 6: Changes in cross section of the ovary histology of koi carp, *Cyprinus carpio* after recovery period (fishes from all spectrum groups were exposed to green light spectrum from 91th to 120th day). Here simulated natural photoperiod, SNP (A), blue (B), green (C), yellow (D) and red (E) LEDs. The fishes were reared under long photoperiod (16L: 8D). Scale bar=10 µm PNS: peri-nucleous stage, PYS: primary yolk stage, SYS: secondary yolk stage, TYS: tertiary yolk stage.

Testosterone formation was affected by different spectrum used during this experiment. Shin *et al.* (2014) support the above statement who reported the significantly high expression of GPR54 (specific receptor which helps Kiss 1 gene to regulates GnRH expression), in gold fish reared under green-LED light compared red-LED and white fluorescent bulbs exposrer. Decline of testosterone concentration in yellow and red-LED fish group after recovery period support revoke of hormonal pathway (conversion of testosterone to estradiol and 11-keto testosterone) under green-LED light.

In this experiment the highest 11-keto testosterone concentration (11-KT) was estimated in green-LED fish group which was significantly higher than SNP fish group. It indicates that males in this group were more mature than SNP fish groups. Yellow and red-LED fish group showed significant low concentration of 11-KT up to 90th day. However after recovery period (120th day) significant increment in 11-KT concentration was recorded in these groups. This resulted to attain the peak level of 11-KT in these groups, which was already got by other spectrum fish groups on 90th day itself. Estimated male GSI support the above results. Variation in sex steroids hormone concentration in different spectrum fish group indicates the effect of light spectrum on hormonal pathway. Shin *et al.*, (2014) found the significant higher expression of LH and FSH (regulate testosterone formation) in gold fish reared under green-LED support above hypothesis.

Both estradiol and vitellogenin concentration in female fish also followed the same pattern as 11-KT in male and support the advance maturation in females in green light whereas blockage or delay in yellow and red-LED light. Shin *et al.* (2013) reported higher concentration of vitellogenin and plasma estradiol in yellow tail damsel fish, *Chrysiptera parasema* exposed to green-LED compared to blue and red-LED light exposrer. Present results of hormonal assays support the shin *et al.*, 2014 who suggested that green spectrum regulates the HPG axis and enhances sexual maturation in gold fish (cyprinids).

In conclusion, present study supports the better reproductive performance of Koi carp under green light spectrum. This study also found the negative effect of yellow and red light spectrum on gonadal maturation over white fluorescent light (SNP). No variations in relative fecundity indicate that light spectrum affect only ova quality (ova diameter) but not quantity (fecundity). Present finding may help the aquaculturist to enhance the reproductive performance (advancement) of Koi carp and other cyprinids by photoperiodic manipulation without any extra expenditure. Further studies will be required to understand the mechanism that regulates the hormonal pathway through light spectrum in fish.
ACKNOWLEDGEMENT
This research was done in Ph.D. programme at Central Institute of Fisheries Education (CIFE), Mumbai, under Indian Council of Agriculture Research, New Delhi, India. Authors express their obligations to Director and Vice Chancellor of CIFE, Dr. W.S. Lakra for his valuable guidance and support during the research work.

REFERENCES