Altered Reproductive Hormones and Calcium Homeostasis in *Cyprinus carpio* Exposed to Varied Spectral Exposure

Kruthika G S and K Renuka*

Pineal Research Lab, Department of Studies in Zoology, Davangere University, Davangere, Karnataka, India

*Pineal Research Lab, Department of Studies in Zoology, Davangere University, Davangere, Karnataka, India.

*Corresponding: renuckhaple@gmail.com, renukhaple@davangereuniversity.ac.in

ORCID: 0000000165736 49

Abstract

Light spectra exert profound influence on fish physiology, behaviors, and development, shaping processes that extend from circadian regulation to growth and reproduction. The present study investigated the effects of chromatic light on the reproductive axis and calcium metabolism in the common carp (*Cyprinus carpio*). Following acclimatization, fish were divided into five groups: white light as control, and red, green, yellow, and blue light treatments, maintained under a 12:12 light–dark cycle for 120 days. Reproductive status was assessed through ovarian weight, gonadosomatic index (GSI), and follicular kinetics, while circulating reproductive hormones and serum Ca^{2+} levels were analyzed.

Results demonstrated wavelength-specific modulation of endocrine and reproductive parameters. Yellow light significantly elevated FSH and induced maximal GSI, primarily due to previtellogenic follicle accumulation, suggesting enhanced follicle recruitment. Green light increased LH and progesterone, but produced the lowest GSI with maximal follicular atresia, indicating enhanced luteinization. Red light suppressed estradiol and vitellogenesis, reducing vitellogenic follicles. Blue light elevated progesterone while maintaining moderate GSI and balanced follicular development.
Collectively, these findings highlight the profound role of spectral manipulation in regulating reproductive endocrinology and ovarian dynamics. Chromatic light regimes may serve as a practical, non-invasive strategy to optimize brood stock management and improve reproductive performance in aquaculture.

Keywords: Light spectra, Follicular dynamics, Ovarian development, Calcium homeostasis, *Cyprinus carpio*

1. Introduction

Light is a fundamental environmental cue that regulates diverse physiological and behavioral processes in fishes, ranging from circadian rhythms to growth, reproduction, and metabolism. In aquatic ecosystems, spectral composition, intensity, and photoperiod act as critical signals that synchronize internal biological clocks with external environmental cycles, thereby influencing endocrine regulation and reproductive success. (Bromage *et al*., 2001).

The neuroendocrine control of reproduction in teleosts is mediated primarily through the brain–pituitary–gonadal (BPG) axis, which integrates photic inputs to regulate gonadotropin release and steroidogenesis (Renuka & Joshi, 2016). Light spectra, particularly chromatic variations, have been shown to modulate the secretion of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex steroids, thereby affecting oogenesis, spermatogenesis, and overall reproductive performance (Z Yaron and B Levavi-Sivan, 2011).

Despite growing evidence, systematic studies examining the combined effects of chromatic light on reproductive endocrinology and calcium metabolism in carp remain limited. Addressing this gap is crucial for both fundamental biology and applied aquaculture. The present study, therefore, investigates the impact of red, green, yellow, and blue light spectra, compared to white light control, on the reproductive axis and calcium metabolism in *Cyprinus carpio*. By integrating hormonal assays, gonadosomatic index (GSI), and follicular dynamics, this work aims to elucidate wavelength-specific mechanisms underlying ovarian development.

2. Materials and Methods:

Indian Common carp *Cyprinus carpio* were collected in the regenerative phase/reproductive phase in Shyagale, Davangere (D), and Karnataka (S). Fish were maintained in glass aquaria and acclimatized to laboratory conditions for 27 days before experimentation. During acclimatization, fish were fed a balanced diet daily, water was renewed every day, and chloramphenicol (5 mg/l) was added prophylactically to prevent bacterial infection.

Post-acclimatization, the animals were selected with an average length of 21 ± 1 cm and a weight of 87 ± 2 gms for experimentation.

Experimental chromatic conditions:

Fish were randomly assigned to five experimental groups (n =10 per group): White as control, Red, Green, Yellow, and Blue light. All fish were maintained under a controlled photoperiod of 12 h light: 12 h dark (lights on at 06:00 h and off at 18:00 h) using an automatic timer for 120 days. Body mass and total lengths were recorded at the beginning and end of the experiment. Post experimentation, the animals were sacrificed for hormone assay and histology separately. Animals with the same weight were sacrificed (n=5), and the whole body was squashed with methanol and centrifuged at 3000 rpm for 12minutes. The supernatant was used for hormone analysis; the estimated hormones are growth hormone (GH), follicular stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone.

Tissue Collection and Histological Procedures

At the end of the experimental period, fish were sacrificed (n=5), and ovaries were dissected and weighed using an electronic balance (Sartorius). Ovarian tissues were fixed in Bouin's fluid, processed by standard histological procedures, embedded in paraffin wax, sectioned at 5 µm thickness, and

stained with haematoxylin and eosin for microscopic examination.

Statistical Analysis

Gonadosomatic index (GSI) was calculated using standard formulae. Follicular kinetics was quantified to assess ovarian development among experimental groups. Data were analyzed using one-way analysis of variance (ANOVA).

Results

Hormone Profiles

Plasma Hormone Levels: GH remained low and invariant across all light treatments (~0.01ng/ml). Yellow light exerts a profound stimulatory effect on both gonadotropins (FSH and LH) in *Cyprinus carpio*. Under yellow light exposure, FSH levels rise sharply to ~4.8 mIU/ml, significantly higher than all other treatments ($P<0.05$) (Figure 1a). Similarly, LH levels peaked at ~4.0 mIU/ml under yellow light, while remaining below 1.0 mIU/ml in all other spectra. In contrast, red, green, blue, and control (white) lights maintained low baseline values for both hormones (Figure 1b). White light maintained the highest estrogen levels (~3200 pg/mL), while all colored spectra (red, yellow, green, blue) significantly ($P<0.05$) suppressed estrogen (<500 pg/mL). Fish maintained under green and blue light exhibited significantly ($P<0.05$) elevated progesterone concentrations (~2.2 ng/ml). In contrast, white and red light maintained moderate baseline values (~1.0 ng/ml). Notably, yellow light resulted in the lowest progesterone levels (~0.6 ng/ml).

Ovarian development and follicular kinetics

Fish maintained under yellow light exhibited the highest GSI (~6), and blue light also supported elevated GSI (~5). In contrast, green light produced the lowest GSI (~2). The control (white light) maintained moderate values (~4 grams), while red light showed slightly reduced GSI (~3.8) (Figure 2a).

Fish maintained under yellow light exhibited the highest number of pre-vitellogenic but the lowest vitellogenic follicle count and reduced atretic follicles. In contrast, white light (control) supported maximal vitellogenic activity with moderate pre-vitellogenic and atretic counts. Blue light promoted intermediate vitellogenic and elevated pre-vitellogenic follicles, alongside moderate atresia. Red light maintained moderate vitellogenic but low pre-vitellogenic follicles, coupled with higher atresia. Green light produced the lowest vitellogenic and pre-vitellogenic counts, while showing the highest atretic follicle numbers (Figure 2b, c, d).

Serum calcium

Fish maintained under white light exhibited the highest calcium levels (~7.2 mg/dl). Calcium concentrations decreased under red light (~6.8 mg/dl) and reached their lowest under yellow light (~6.0 mg/dl). Levels rose again under green light (~6.7 mg/dl), indicating partial recovery, but dropped under blue light (~6.1 mg/dl), and remaining lower than control (Figure 4).

Discussion:

The present study demonstrates that spectral light exerts profound and wavelength-specific effects on the endocrine regulation, ovarian development, and mineral metabolism of *Cyprinus carpio*. Among the tested spectra, yellow light emerged as a potent modulator of gonadotropin secretion, markedly elevating both FSH and LH levels. This dual stimulation suggests that yellow light promotes early follicle recruitment and ovulatory readiness, consistent with the observed increase in pre-vitellogenic

follicles and elevated gonadosomatic index (GSI). However, the concomitant suppression of vitellogenic follicles, reduced estrogen levels, and minimal progesterone secretion under yellow light indicate a divergence between gonadotropin activation and steroidogenic output. Such a pattern implies that while yellow light enhances follicular recruitment, it may delay or inhibit vitellogenesis and luteinization, thereby altering the normal progression of ovarian maturation.

In contrast, white light maintained maximal estrogen levels and supported the highest vitellogenic follicle counts, reflecting robust vitellogenesis under natural illumination. This aligns with previous findings that photoperiod and broad-spectrum light sustain estradiol-mediated ovarian growth. The moderate GSI and balanced follicular distribution under white light further reinforce its role in maintaining physiological reproductive rhythms.

Blue and green lights exhibited distinct steroidogenic responses, with both spectra significantly elevating progesterone concentrations. This suggests enhanced luteinization and late-stage follicular maturation, consistent with elevated GSI under blue light and increased atresia under green light. Blue light also promoted recruitment of pre-vitellogenic follicles, indicating a balanced stimulation of both early and late ovarian stages. In contrast, green light suppressed follicle recruitment and vitellogenesis while maximizing atresia, highlighting its inhibitory role in reproductive performance.

Red light maintained low gonadotropin and steroid levels, coupled with reduced GSI and increased atresia, suggesting suppression of both recruitment and vitellogenesis. This inhibitory effect is consistent with reports that longer wavelengths may disrupt photoneuroendocrine signaling pathways in teleosts.

Serum calcium levels also varied with spectral exposure, with white light supporting maximal calcium availability, essential for vitellogenesis and steroid biosynthesis. The lowest calcium levels under yellow light further corroborate the suppression of vitellogenesis in this treatment, while intermediate values under red, green, and blue lights reflect partial modulation of mineral metabolism.

These findings highlight the complex interplay between spectral cues, endocrine regulation, and ovarian physiology. Yellow light uniquely stimulates gonadotropins and follicle recruitment but suppresses vitellogenesis and steroidogenesis, whereas white light sustains estrogen-driven vitellogenesis. Blue and green lights enhance progesterone synthesis, with blue supporting balanced ovarian growth and green promoting degeneration. Red light exerts an overall inhibitory effect. These results underscore the importance of spectral manipulation as a non-invasive tool in aquaculture, offering potential strategies to synchronize ovarian development, optimize broodstock conditioning, and regulate reproductive cycles in carp culture.

Previous studies in teleosts have consistently demonstrated that photoperiod and spectral quality of light exert species-specific and stage-dependent influences on reproductive endocrinology (Lam, 1983; Nagahama, 1994; Bromage *et al*., 2001; Pani Prasad, 2021). The present findings in *C. carpio* extend these observations by showing that gonadotropin stimulation under yellow light does not necessarily translate into vitellogenic progression, underscoring the complexity of endocrine regulation under varying spectral environments.

Similar wavelength-specific effects have been reported in *Channa punctatus*, where red light stimulated vitellogenesis, green light suppressed ovarian activity, and blue light produced intermediate responses, underscoring the broader role of spectral cues in tropical teleost reproduction (Renuka & Joshi, 2016).

The elevation of FSH and LH under yellow light, coupled with suppressed estrogen and progesterone, parallels earlier reports that gonadotropin release can be uncoupled from steroidogenic output depending on photic cues (Peter & Yu, 1997; Hollander Cohen *et al*., 2021). This suggests that spectral light may differentially modulate hypothalamic GnRH signaling and dopaminergic inhibition, resulting

in selective activation of gonadotropins without downstream steroidogenesis.

Consistent with Bromage & Cumaranatunga (1988) and Bromage *et al*., (2001), who demonstrated that photoperiod manipulation alters egg production in salmonids, our results confirm that ovarian development in carp is highly sensitive to light environment. However, unlike simple photoperiod extension, spectral manipulation produced divergent outcomes: white light sustained vitellogenesis, blue and green lights enhanced luteinisation, while red and green lights promoted atresia.

These findings resonate with earlier ecological studies (de Vlaming, 1972; Lam, 1983) that emphasized light as a synchronizer of reproductive cycles, but they further refine the concept by demonstrating that wavelength specificity, not just duration, is critical in shaping ovarian dynamics. The persistence of ovarian activity under inhibitory spectra also supports the view that photoperiodic regulation in teleosts involves distributed photoreceptive systems (retinal and pineal), rather than reliance on a single sensory organ, as suggested by Rasquin (1958) and Joy & Khan (1989).

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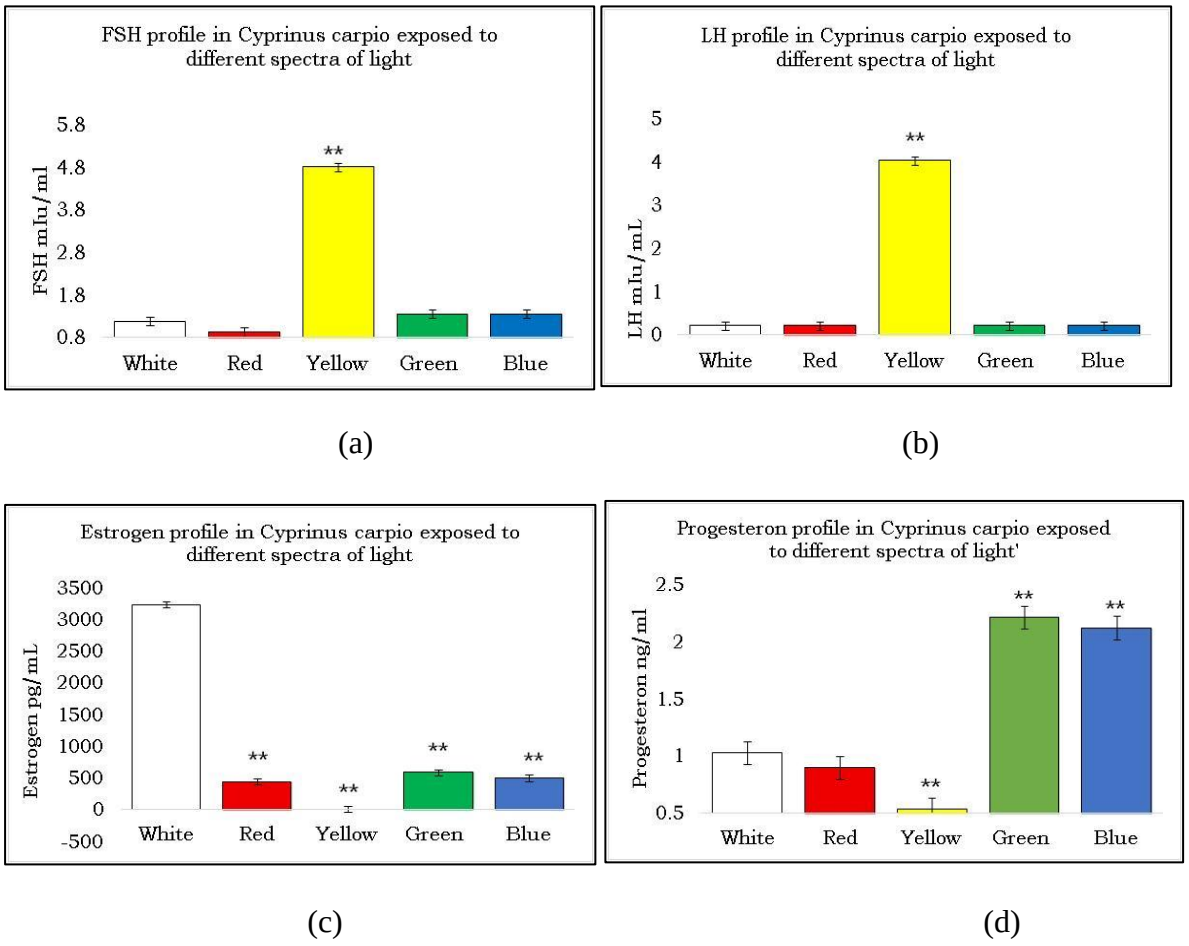
Figures:

Figure 1: Hormone profile in *Cyprinus carpio* exposed to varied spectra.

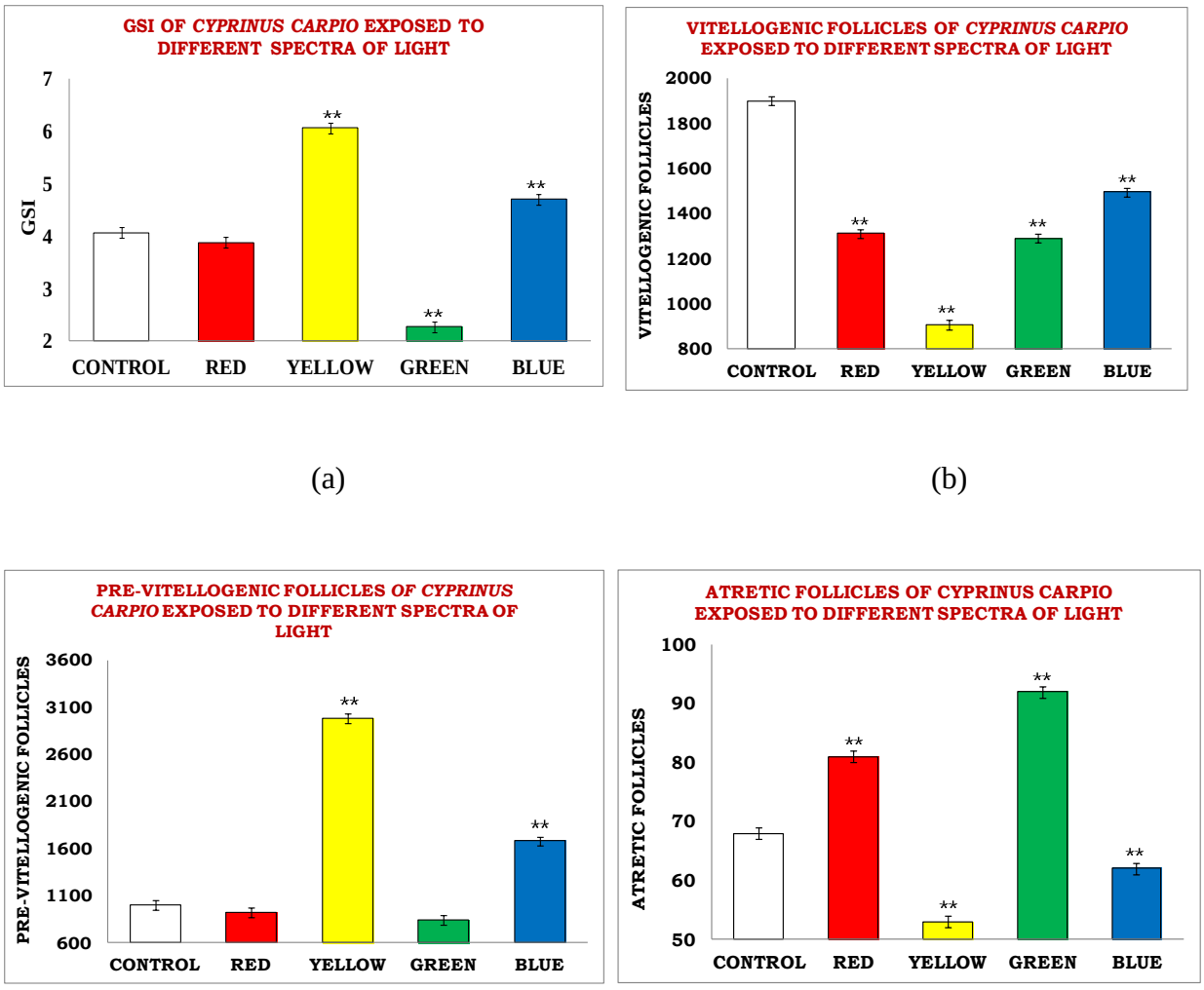


(a) (b)

(c) (d)

Legend to figure 1: Hormone profiles (a) FSH (follicular stimulating hormone), (b) LH (luteinizing hormone), (c) Estrogen, (d) Progesterone in *Cyprinus carpio* exposed to red, yellow, green and blue spectra.

Figure 2: GSI and follicle count in *Cyprinus carpio* exposed to varied spectra.

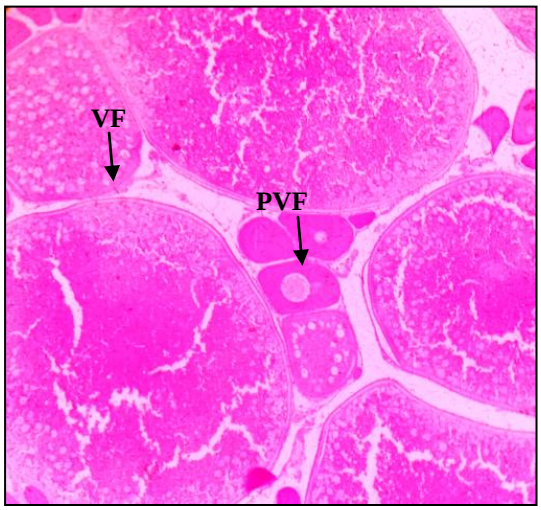


(a) (b)

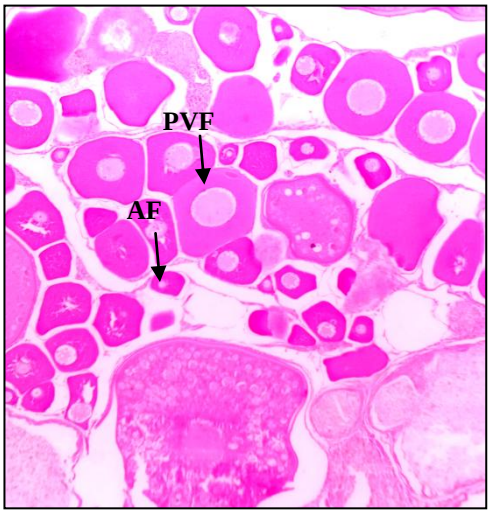
(c) (d)

Legend to figure 2: (a) GSI (Gonadosomatic index), (b) VF (Vitellogenic follicles), (c) PVF (Pre-vitellogenic follicles), (d) AF (Atretic follicles) in *Cyprinus carpio* exposed to red, yellow, green and blue spectra.

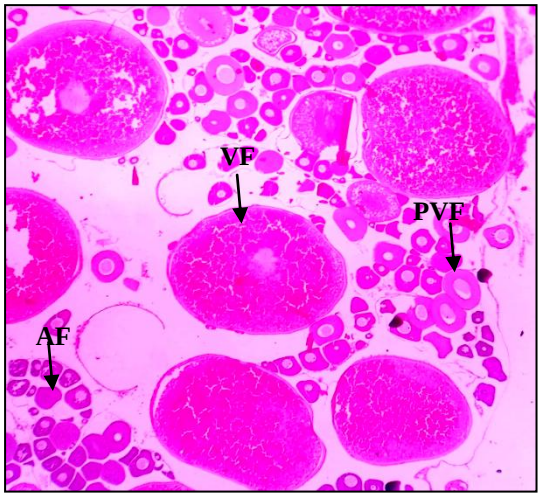
Figure 3: Microphotographs of ovarian double-stained sections (5µ thickness) post experimentation in *Cyprinus carpio*. (4x)



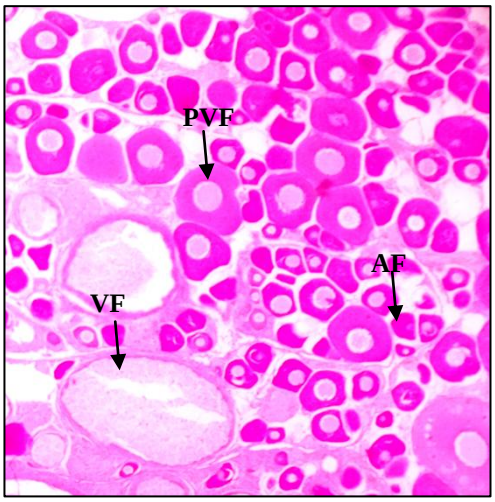
(a)



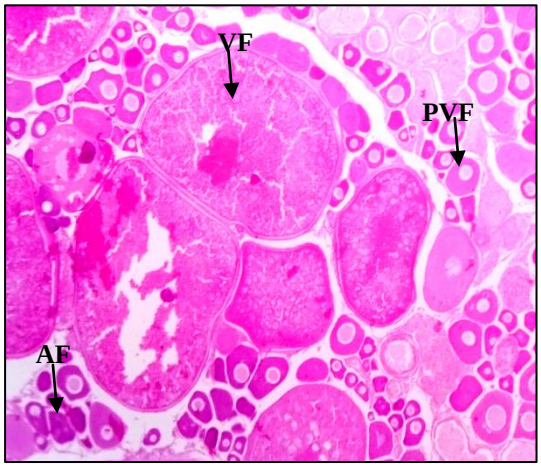
(b)



(c)



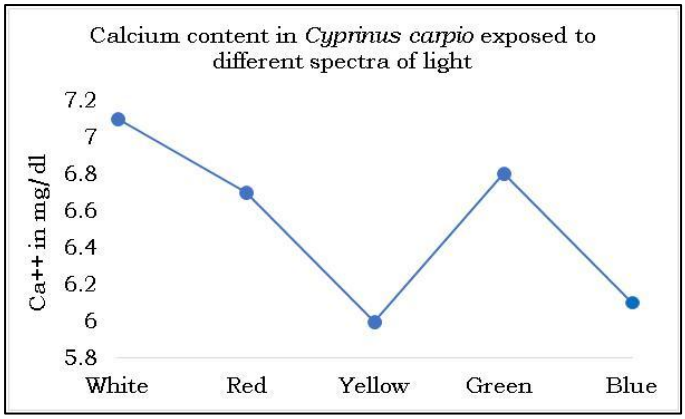
(d)



(e)

Legend to figure 3: Microphotographs of ovarian double-stained sections (5µ thickness) in *Cyprinus carpio* exposed to varied spectra (a) white (control), (b) red, (c) yellow, (d) green, (e) blue. Abbreviations- PVF (Pre-vitellogenic follicles, VF (Vitellogenic follicles), AF (Atretic follicles).

Figure 4: Calcium profile in *Cyprinus carpio* exposed to varied spectra.



Legend to figure 4: serum calcium content in *Cyprinus carpio* exposed to red, yellow, green and blue spectra.