Effects of photoperiod and temperature on kisspeptin1 (kiss1) expression in the gonads of *Clarias batrachus*

Ankur Singh · Bechan Lal · Ishwar S. Parhar

Abstract Photo-thermal regulation of reproduction in seasonally breeding vertebrates, including teleost fishes, is established. The photo-thermal cues are relayed to the higher brain centers, where from command, neurosecretions are released to the hypothalamus to activate the hypothalamo-pituitary–gonadal axis to secrete GnRH-FSH/LH-sex steroids in general. GnRH being a pivotal regulator of reproduction, plenty of studies have been carried out to investigate the regulators of GnRH secretion. Kiss is the recently established upstream regulator of GnRH. However, studies on the regulation of kiss are highly ambiguous in vertebrates, including fishes. Therefore, attempts were made to examine the role of photoperiod and temperature in the regulation of kiss1 expression in the gonad during the late-quiescence phase of the reproductive cycle of the catfish, *Clarias batrachus*, employing the techniques like histology, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA) along with analysis of data through analysis of variance (ANOVA) followed by a post hoc test and Duncan's multiple range test at $P < 0.05$ for comparisons amongst different groups. The study reveals that long photoperiod and high temperature stimulate the expression of kiss1 significantly ($P < 0.05$), while short photoperiod and low temperature suppress its expression in fish gonads ($P < 0.05$). Photoperiod and temperature were almost equally effective in regulating the kiss1 expression, but when long photoperiod and temperature are given in combination, their stimulatory role in kiss1 expression is amplified significantly ($P < 0.05$). The long photoperiod and high temperature also increase the steroid levels in gonads ($P < 0.05$) and stimulate gametogenesis.

Introduction

The ability of any species to survive and reproduce successfully in specific niches has been conferred through natural selection (Walton et al. 2011). Seasonal variation in several environmental factors affects food availability, weather, and temperature. Thus, different species have adopted a variety of changing temporal and spatial cues to cope with the demands of their survival and reproduction (Demas et al. 2003; Walton et al. 2011).

Several external environmental factors signal the most appropriate/suitable timing to initiate the

A. Singh · B. Lal (✉)
Fish Endocrinology Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, UP, India
e-mail: lalbhu@yahoo.co.in

I. S. Parhar
Brain Research Institute, School of Medicine and Health Sciences, Monash University, Sunway Campus, Subang Jaya, Malaysia

reproductive processes in seasonal breeders by activating or deactivating the pattern of secretions of the hypothalamus-pituitary–gonadal (HPG) axis. Amongst various external factors like photoperiod, temperature, pH, availability of food, rainfall, humidity, the photoperiod, and temperature are implicated as the most crucial environmental zeitgeber to signal the timing of reproduction in fishes and also to regulate the diurnal/hormonal endocrine rhythm (Baker and Baker 1936; Lam 1983; Duston and Saunders 1990; Rodríguez et al. 2001; Bromage et al. 2001; Biswas et al. 2002; Borg 2009; O'Brien et al. 2012). In general, fishes primarily and necessarily depend on photoperiod and temperature for the initiation of gonadal development (Lam 1983). In some fishes, gonadal development and maturation are dependent on day length, while others rely on the temperature, and in some fishes, day length and temperature are both crucial for the proper maturation of gonads (Sundararaj and Sehgal 1970; Sundararaj and Vasal 1976; Baggerman 1980; Poston 1978). In tropical and subtropical conditions, the temperature is more important than photoperiod to induce gonadal growth and maturation in fishes like catfish, carps, and various other cyprinids (Sundararaj and Sehgal 1970; Sundararaj and Vasal 1976; Munro 1990; Borg 2009). Alterations in photoperiod and temperature are relayed to the higher brain, where some neurosecretions reach the HPG axis to trigger and culminate breeding processes (Joy and Senthilkumaran 1995; Acharjee et al. 2017).

Considering the significant role of photoperiod and temperature in the regulation of reproductive activities, some earlier scientists have studied the effects of photoperiod and temperature on kiss expression in the brain/hypothalamus in vertebrates, including fishes. In short day length photoperiodic conditions, kiss1 expressing neurons/kiss content was decreased in the rostral part of the hypothalamus in mice, *Mus musculus*, and Syrian hamsters, *Mesocricetus auratus*; and increased in the brain of ewe, *Ovis aries*, and striped hamsters, *Cricetulus barabensis* (Chalivoix et al. 2010; Ansel et al. 2011; Li et al. 2015; Bohlen et al. 2018). However, a high level of kiss1 is reported in Syrian hamsters maintained under long days than the short day (Revel et al. 2006). Furthermore, in long day length photoperiodic conditions, higher levels of *kiss1/GPR54* mRNA have been shown in the brains of goldfish, *Carassius auratus*, and Atlantic salmon,

Salmo salar (Shin et al. 2014; Chi et al. 2017). Further, it has been reported that low temperature (15 °C) treatment resulted in a significant increase in brain *kiss1* mRNA level in comparison to control temperature (27 °C) treatment in male zebrafish, *Danio rerio* (Shahjahan et al. 2013). However, there is also a report that low and high temperatures both have significantly reduced the *kiss2* and *kiss2r* mRNA in grass pufferfish, *Takifugu niphobles* (Shahjahan et al. 2013). Further exposure of *Heteropneustes fossilis* to long photoperiod (16L) and high temperature (28 °C) caused a remarkable increase in the expression of kiss2 in the catfish brain (Chaube et al. 2020). Moreover, it has also been shown that photoperiodic regulation of kiss expression may vary with tissues, species, and sexes in striped hamsters, *Cricetulus barabensis* (Li et al. 2015). However, to date, no report of photo-thermal regimes on the gonadal expression of kiss1 is available, despite that gonadal *kiss* transcripts are reported in some fishes. Thus, in the present study, the role of photoperiod and temperature in kiss1 expression in *Clarias batrachus* gonad was investigated.

The *Clarias batrachus* is a highly consumer-preferred catfish because of its high nutritional value and hence highly cultured catfish in India. This fish is a seasonal breeder, and various aspects of its biology and reproductive processes are studied and published (Sinha et al. 1992, Acharia et al. 2000, Singh and Lal 2008; Priyadarshini and Lal 2018; Singh nee Priyadarshini and Lal 2018; Singh et al. 2021). The reproductive activities of *C. batrachus* are initiated with the escalation in photoperiod and temperature from February/March onwards, leading to a parallel increase in sex steroids and other orexigenic hormones, causing an increase in food intake (Sinha et al. 1992, Acharia et al. 2000, Singh and Lal, 2008, Priyadarshini and Lal 2018; Singh nee Priyadarshini and Lal 2018). The gonadal recrudescence is accelerated steadily with a further increase in photoperiod and temperature in subsequent months and attains full gonadal development in the month of June/July. Thereafter, with the approach of monsoon leading to a decline in the water temperature, the gametes (eggs and spermatids) undergo final maturation during the month of July/August and start breeding synchronously in closed proximate, i.e., release of mature gametes out of the body in the external aquatic environment to achieve fertilization and subsequent embryonic development.

Then after, the catfish undergo gonadal reorganization and quiescence from September onwards with the decline in photoperiod and temperature and remain quiescent till the photoperiod and temperature restart increasing from January end to February onwards.

Materials and methods

Reagents

Kiss1 antibody and its respective peptides (Code-PAS3809 and Code-AS1560) were validated before its routine use (see Singh et al. 2021). The secondary antibody biotin-labelled goat anti-rabbit-IgG was purchased from GeNei (cat No. 1110280011730) Bangalore, India. The ABC Kit (elite kit PK-6100) was obtained from Vector Laboratories, Inc., Burlingame, CA, USA. Triton X-100 and 3, 3′-diaminobenzidine tetrahydrochloride hydrate (DAB) were procured from Sigma-Aldrich, India. ELISA kits for 17β-estradiol (cat no. DKO003) and testosterone (cat no. DKO002) were purchased from DiaMetra, Italy, while other routinely used chemicals were acquired from Merck, SRL, and HiMedia (AR Grade) through authorized vendors.

Collection and exposure of catfish to different photo-thermal regimes

The adult catfish were collected during the first week of the early-quiescence phase (January) of its annual reproductive cycle from the suburbs of Varanasi, India, and were acclimated in 200 L cemented tanks for 14 days under ambient photoperiod and temperature. Throughout the experiment, catfish were fed with chopped goat liver ad libitum. After 2 weeks of acclimation, catfish were then sorted out in a close weight range (80–85 g) and divided into several groups with 25 fish in each and were subjected for 30 days under different photo-thermal regimes during the early-quiescence phase (January) to late-quiescence phases (February), i.e., ambient photoperiod and ambient temperature (APAT—11L:13D, 19 ± 1 °C), ambient photoperiod and high temperature (APHT—11L:13D, $30° \pm 1$ °C), short photoperiod and ambient temperature (SPAT—9L:15D, 19 ± 1 °C), short photoperiod and high temperature (SPHT—9L:15D, $30° \pm 1$ °C), long photoperiod and ambient temperature (LPAT—14.5L:9.5D, 19 ± 1 °C), and long photoperiod and high temperature (LPHT—14.5L:9.5D, $30° \pm 1$ °C) in thermostatically controlled aqua-environmental chambers (Aqua-Envo, SVI, India). At the end of the experiment, fish were cold anesthetized group-wise in ice-chilled water for 5–8 min, weighed after wiping out with a soaked towel, and blood was collected group-wise in separate tubes. Briefly, blood was collected in glass tubes through the caudal puncture and centrifuged at $1370 \times g$ in a refrigerated centrifuge to collect the serum. The gonads (ovary and testis) were dissected out aseptically and weighed to the nearest gram to calculate the gonadosomatic index (GSI). One lobe of the ovaries and testes was fixed in Bouin's fluid for 18 h for histological and immunohistochemical studies, while the other lobe was extirpated, snap frozen, and stored at -80 °C for the estimation 17β-estradiol and testosterone.

Histology and immunohistochemical localization of kiss1 in ovary and testis

The 6 μm thin ovarian and testicular sections were cut group-wise by Leica semi-motorized rotary microtome (RM 2245) and were processed for routine hemotoxylin and eosin (H&E) staining for histological as well as immunolocalization according to Singh et al. (2021). In brief, the testicular sections were spread on coated slides (1% gelatin). The sections after deparaffinization and hydration using descending ethanol series were stained with Ehrlich's hematoxylin solution for 45 min. Thereafter, slides were subjected to bluing under tap water for 45 min following differentiation in 1% acid water for 5 s. The slides were then kept under running water for 1 h. Thereafter, the sections were then stained with 1% eosin solution for 5 min. Subsequently, sections were dehydrated and cleared in xylene. Finally, the stained sections were mounted with a few drops of DPX. After appropriate drying, slides were examined using a Leica microscope (LEICA DM 2000, Leica Microsystems, Germany).

The testicular sections, after hydration, were washed with phosphate buffer saline (PBS, 0.05 M, pH 7.5). The sections were then dropped in a coupling jar containing blocking solution (5% normal goat serum, 10% TX-100, and 1% H_2O_2) for 1 h, thereafter incubated with polyclonal antibody against

kiss1 (1:1200) overnight at 4 °C in a moist chamber. The control experiment includes (1) omission of the primary antibody from the reaction, and (2) kiss1 antibodies were adsorbed by preincubated peptides at 25 μg/mL, respectively. These control slides resulted in a loss of immunoreactivity.

The following day, the sections were washed and subsequently incubated with biotinylated secondary antibody (1:200) for 2 h at room temperature in a moist chamber. Sections were then processed with the Vectastain ABC kit according to the manufacturer's instructions for 1 h. Then, slides were incubated with 3, 3′-diaminobenzidine tetrahydrochloride hydrate (0.025% DAB and 0.066% hydrogen peroxide in PBS) as a chromogen to visualize kiss1 binding sites. The reaction was stopped by washing the slides in distilled water several times. The slides were then mounted and viewed under a microscope. The densitometric analyses for kiss1 in the gonadal tissues were performed as described elsewhere (Singh et al. 2021; Priyadarshini and Lal 2018; Singh nee Priyadarshini and Lal 2018; Singh and Lal 2016, 2017; Kumar and Thakur 2012; Kumar and Thakur 2014) by Alpha EaseFC software (Alpha Innotech Corp., USA) which is expressed in terms of integrated density value (IDV) unit area^{-1}.

Estimation of 17β-estradiol and testosterone

Serum and gonadal 17β-estradiol (E2) and testosterone (T) levels were measured by enzyme-linked immunosorbent assay using a commercial kit (DiaMetra, Segrate, Milan, Italy) as per the manufacturer's described procedure. The sensitivity of this kit, as per the manufacturer, is 0.008 ng/mL and 0.10 ng/mL, which is within the detectable limits of estradiol and testosterone levels in this catfish species. The intra- and inter-assays variations are 5.4% and 6.8% for 17β-estradiol and 6.4% and 7.2% for testosterone, respectively. Briefly, the ELISA plate was supplemented with 25 μL of various standards, gonadal homogenate (10%) prepared in phosphate buffer (PB, 0.01 M, pH 7.3), and female and male serum. Thereafter, 200 μL of estradiol conjugate and 100 μL of testosterone conjugate were added to each ELISA plate well and mixed properly by mild shaking. The plate was then incubated at 37 °C for 1 h in the dark. Thereafter, the contents of each well were carefully

drained by flicking, followed by washing with 300 μL wash buffer supplied with the kit. Subsequently, 100 μL of 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate was added to each well and incubated for 30 min (estradiol) and 15 min (testosterone) in the dark. The reaction was stopped by adding a stop solution. The enzyme complex was measured quickly at an optical density of 450 nm against blank using the ELISA Plate Reader (Multiskan, Thermo Labsystem).

Statistical analyses

The data in relation to 17β-estradiol, testosterone, and kiss1 IDV are expressed as mean $\pm$ SEM ($n=5$). The concentration of 17β-estradiol or testosterone is expressed as ng/g ovary or testis and ng/mL serum. Data were analyzed through analysis of variance (ANOVA) followed by a post hoc test and Duncan's multiple range test at $P < 0.05$ for comparisons amongst different groups. All the statistical analyses were performed using SPSS16 software (SPSS Inc., Chicago, IL, USA).

Results

Effects of different photo-thermal regimes on gonadosomatic index in female and male catfish

During the quiescence phase, exposure of the catfish to APHT and LPAT caused a modest increase in the GSI compared to APAT and SPHT exposed catfish (Figs. 1B and 3B). However, the exposure of fish to the LPHT increased the GSI to a greater extent (Figs. 1B and 3B).

Effects of different photo-thermal conditions on ovarian morphology

The quiescence ovary consists primarily of perinucleolar oocytes and a few oocyte-I. The ovaries of the catfish exposed to LPAT display a relatively higher number of oocyte-I with developing follicular cells as compared to the ovary under APAT, APHT, SPAT, and SPHT (Fig. 1). The ovary of the fish held under APHT also exhibited a moderate increase in an oocytes-II, while fish held under SPHT displayed relatively more growing oocytes-I than the fish maintained under APAT, APHT,
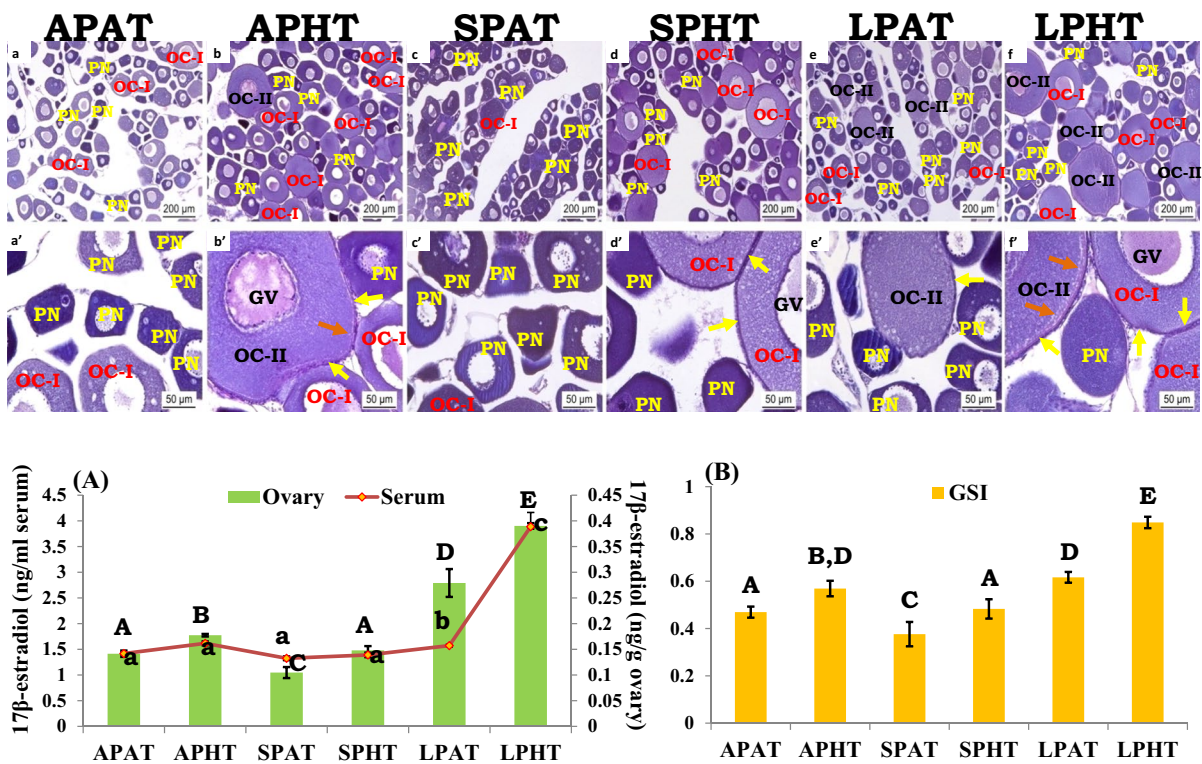
Fig. 1 Images of hematoxylin/eosin stained transverse sections of *Clarias batrachus* ovary after their exposure to different photo-thermal regimes [ambient photoperiod and ambient temperature (APAT—11L:13D, 19 ± 1 °C), ambient photoperiod and high temperature (APHT—11L:13D, 30° ± 1 °C), short photoperiod and ambient temperature (SPAT—9L:15D, 19 ± 1 °C), short photoperiod and high temperature (SPHT—9L:15D, 30° ± 1 °C), long photoperiod and ambient temperature (LPAT—14.5L:9.5D, 19 ± 1 °C), long photoperiod and high temperature (LPHT—14.5L:9.5D, 30° ± 1 °C)], 17β-estradiol in ovary and serum

(A) and changes in gonadosomatic index (B) during the early-quiescence phase. Note: perinucleolar oocytes (PN), oocytes-I (OC-I), oocytes-II (OC-II), germinal vesicle (GV), cortical alveoli (yellow arrow), granulosa cell (orange arrow). Each bar represents mean ± SEM (*n* = 5). Means bearing the same superscripts do not differ from each other while means bearing different superscripts are different from each other statistically at *P* < 0.05 (Duncan's multiple range test). Superscripts A, B, C, D, and E and a, b, and c are used for 17β-estradiol in ovary and serum, while superscripts A, B, C, D, and E are used for GSI in panel **B**

and SPAT (Fig. 1). Exposure of catfish to LPHT resulted in the development of a large number of growing follicles with oocyte-II in comparison to the ovarian conditions under other photo-thermal regimes (Fig. 1).

Effects of different photo-thermal regimes on 17β-estradiol (E2) in serum and ovary

The ovarian E2 level in the catfish under APHT increased marginally compared to APAT and SPHT regimes (Fig. 1A), while ovarian E2 was decreased in SPAT-exposed fish. The LPAT exposure also increased E2 levels in the ovary and serum (Fig. 1A). However, the treatment of fish with LPHT increased

the circulating as well as ovarian E2 to a greater extent than the catfish held under other photo-thermal conditions (Fig. 1A).

Effects of different photo-thermal regimes on kiss1 expression in ovary

Marginal expression of kiss1 was observed in the APAT and SPAT during the quiescence phase. However, the exposure of catfish to LPAT induced an increase in kiss1 expression and was similar to APHT and SPHT (Fig. 2). The maximum expression of kiss1 was noted in the follicular cells of the oocytes
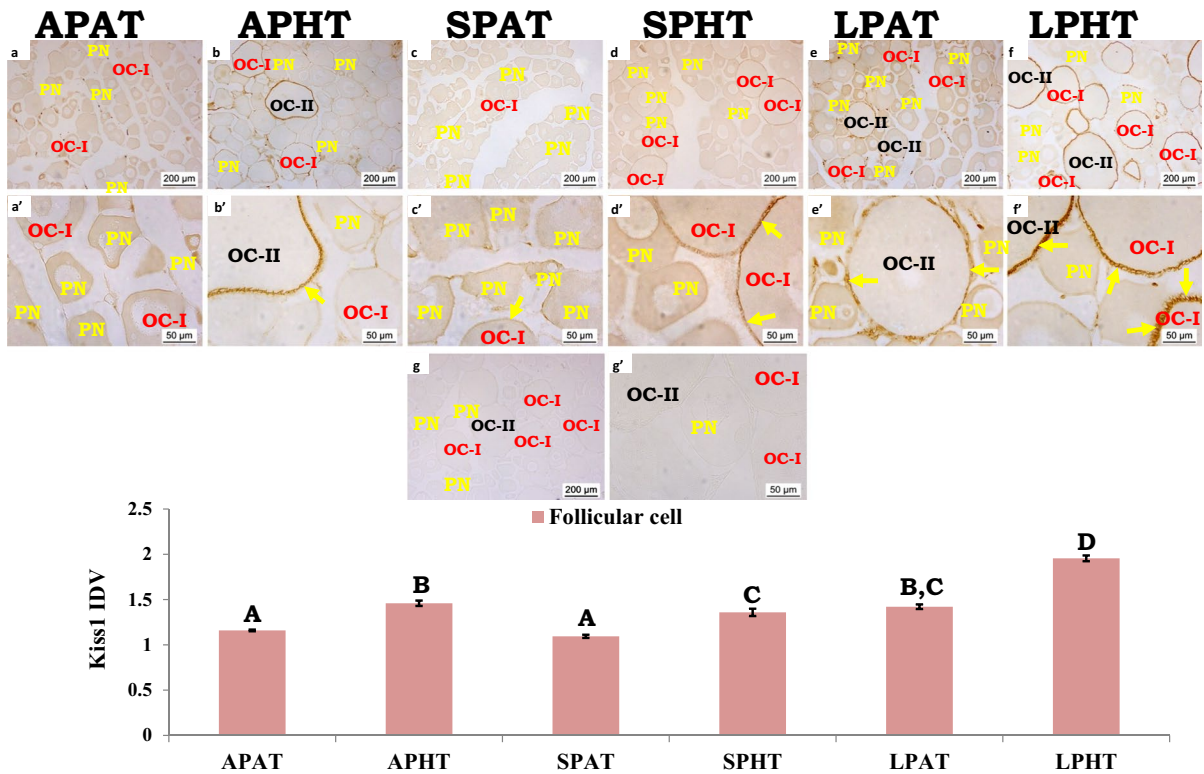
Fig. 2 Images of kiss1 immunohistochemistry in the ovary of *Clarias batrachus* after their exposure to different photo-thermal regimes [ambient photoperiod and ambient temperature (APAT—11L:13D, 19 ± 1 °C), ambient photoperiod and high temperature (APHT—11L:13D, 30° ± 1 °C), short photoperiod and ambient temperature (SPAT—9L:15D, 19 ± 1 °C), short photoperiod and high temperature (SPHT—9L:15D, 30° ± 1 °C), long photoperiod and ambient temperature (LPAT—14.5L:9.5D, 19 ± 1 °C), long photoperiod and high temperature (LPHT—14.5L:9.5D, 30° ± 1 °C)], during the early-quiescence phase. Note: oocytes-I (OC-I), oocytes-II (OC-II), granulosa cell (yellow arrow). Each bar represents mean ± SEM (*n* = 5). Means bearing same superscript do not differ from each other, while means bearing different superscripts are different from each other statistically at *P* < 0.05 (Duncan's multiple range test). Superscripts A, B, C, and D are used to express kiss1 expression

in the ovary of the catfish exposed to LPHT (Fig. 2). Immunoreactivity was absent in pre-adsorbed control (Fig. 2g and g').

Effects of different photo-thermal conditions on testicular histology

The quiescent testis displays poorly developed interstitium and smaller seminiferous tubules. Under the SPAT condition, the testicular histology was almost similar to the quiescent testis (Fig. 3). The catfish exposed to APHT revealed dividing germ cells in the germinal epithelium as well as the lumen of the seminiferous tubules. While under SPHT condition, the seminiferous tubules

in the testis showed dividing spermatogonial stem cells in the germinal lining and a few advanced germ cells within the cysts in the lumen of seminiferous tubules. The interstitial cells (probably Leydig cells) were also distinctly visible in such testis (Fig. 3). Similarly, the testis of the catfish exposed to LPAT exhibited division in spermatogonial cells in the germinal epithelium. The cysts had dividing germ cells at different stages (Fig. 3). The testis of the LPHT-exposed fish displayed maximally enlarged seminiferous tubules with a large number of advanced germ cells, some of which were also evacuating the seminiferous tubules (Fig. 3).
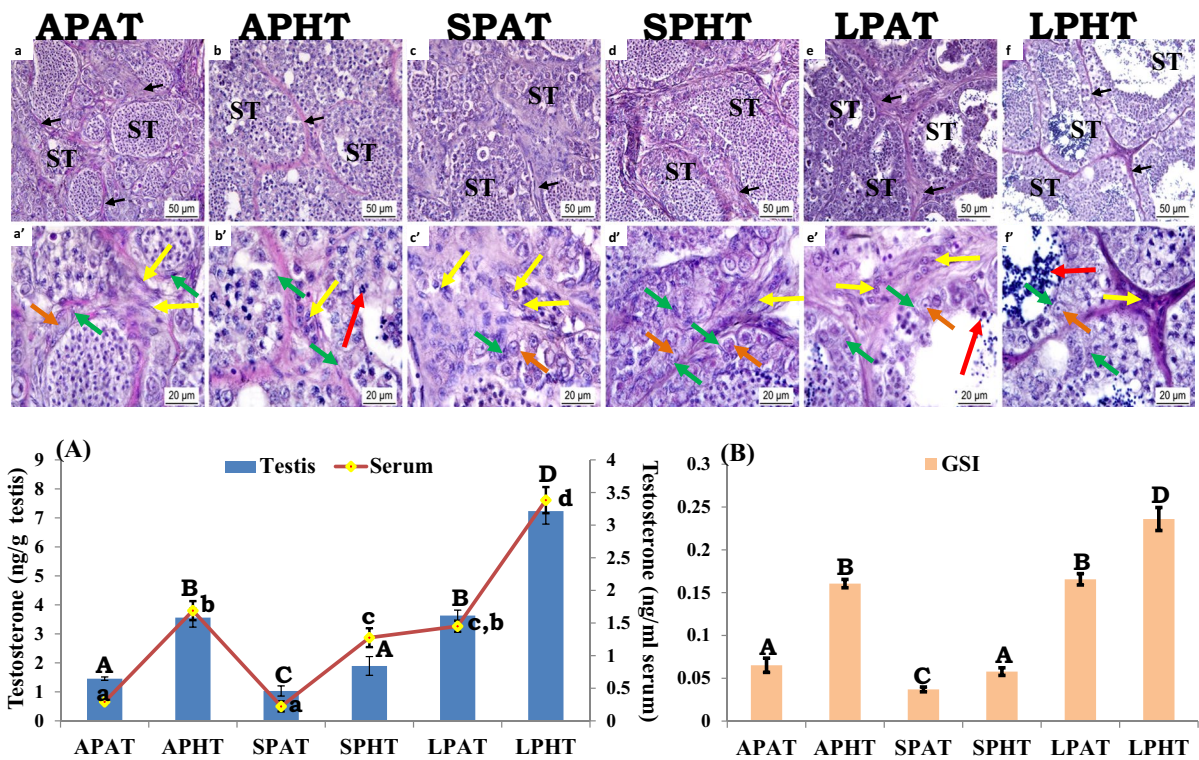
Fig. 3 Images of hematoxylin/eosin stained transverse sections of *Clarias batrachus* testis after their exposure to different photo-thermal regimes [ambient photoperiod and ambient temperature (APAT—11L:13D, 19 ± 1 °C), ambient photoperiod and high temperature (APHT—11L:13D, 30° ± 1 °C), short photoperiod and ambient temperature (SPAT—9L:15D, 19 ± 1 °C), short photoperiod and high temperature (SPHT—9L:15D, 30° ± 1 °C), long photoperiod and ambient temperature (LPAT—14.5L:9.5D, 19 ± 1 °C), long photoperiod and high temperature (LPHT—14.5L:9.5D, 30° ± 1 °C)], testosterone in testis and serum (**A**) and changes in gonadosomatic index (**B**) during the early-quiescence phase. Note: interstitium (black arrow), seminiferous tubule (ST), spermatogonial stem cells (orange arrow), interstitial cells (yellow arrow), advance germ cells (red arrow), sertoli cells (green arrow). Each bar represents mean ± SEM (n = 5). Means bearing the same superscript do not differ from each other, while means bearing different superscripts are different from each other statistically at $P < 0.05$ (Duncan's multiple range test). Superscripts A, B, C, and D and a, b, c, and d are used for testosterone in testis and serum, while superscripts A, B, C, and D are used for GSI in panel **B**

Effects of different photo-thermal regimes on testosterone in serum and testis

The level of testicular testosterone was low in the quiescent testis, which declined when exposed to the SPAT regime. The testosterone levels in the testis and serum were increased considerably under APHT and LPAT regimes (Fig. 3A). The testosterone levels in the testis and serum were increased maximally under LPHT conditions when compared to other photo-thermal regimes (Fig. 3A).

Effects of different photo-thermal exposures on kiss1 expression in testis

During the quiescence phase, the kiss1 expression was low in the catfish testis (Fig. 4), which was further reduced when exposed to SPAT and SPHT conditions (Fig. 4). A marginal increase in its expression was observed under the APHT regime as compared to the APAT condition. In LPAT- and LPHT-exposed catfish, the expression of kiss1 in the testis was increased considerably (Fig. 4). Kiss1 expression was
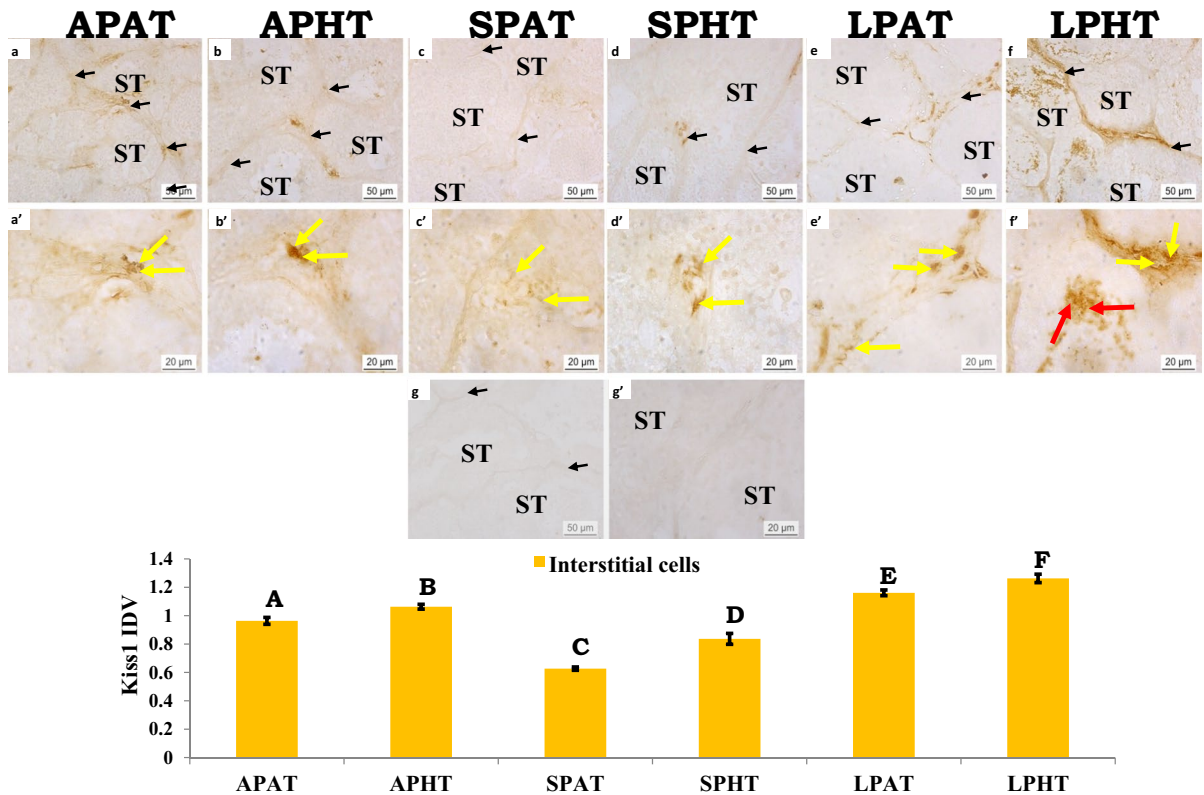
Fig. 4 Images of kiss1 immunohistochemistry in the testis of *Clarias batrachus* after their exposure to different photo-thermal regimes [ambient photoperiod and ambient temperature (APAT—11L:13D, 19 ± 1 °C), ambient photoperiod and high temperature (APHT—11L:13D, 30 ± 1 °C), short photo-period and ambient temperature (SPAT—9L:15D, 19 ± 1 °C), short photoperiod and high temperature (SPHT—9L:15D, 30 ± 1 °C), long photoperiod and ambient temperature (LPAT—14.5L:9.5D, 19 ± 1 °C), long photoperiod and high temperature (LPHT—14.5L:9.5D, 30 ± 1 °C)]. Note: inter-stitium (black arrow), seminiferous tubule (ST), interstitial cells (yellow arrow), advance germ cells (red arrow). Each bar represents mean ± SEM (*n* = 5). Means bearing the same super-script do not differ from each other, while means bearing dif-ferent superscripts are different from each other statistically at *P* < 0.05 (Duncan's multiple range test). Superscripts A, B, C, D, and F represent kiss1 expression in testis

highest in the testis of the catfish kept under LPHT condition as compared to other regimes (Fig. 4). Interestingly, under the LPHT regime, a distinct kiss1 expression was observed in the advanced germ cells also (Fig. 4).

Discussion

The results of the present study suggest that the photo-thermal regimes influence the expression of kiss1 in the fish gonads. So far, no such study is avail-able in vertebrates for the comparison and discussion of the present findings, except the only one report by Li et al. (2015), wherein they have studied the effect of only photoperiod on the gonadal expression of kiss1. They have shown the sex-specific effects of photoperiod on kiss1 expression in ovaries and testis of the striped hamster. These authors have observed that long and short photoperiod treatments have caused a significant reduction in kiss1 expression in the ovaries of female striped hamsters, while expo-sure of striped male hamsters to long photoperiod stimulated the *kiss1* mRNA level in testis, but short photoperiod exposure resulted in a drastic reduction in kiss1 expression.

However, some researchers have studied the effects of photoperiod and temperature on brain/hypo-thalamic expression of kiss1 in some vertebrates, including fishes, but the results are highly varied and

inclusive. The effects of photoperiod and temperature appear to be species, sex-, and tissue-specific. Bohlen et al. (2018) have recorded a lesser number of kiss1 expressing neurons in the rostral part of mice hypothalamus kept under short day length. But the short day exposure to ewe and striped hamsters increased the kiss1 levels in the diencephalon and hypothalamus, respectively, in comparison to other photoperiodic exposures (Chalivoix et al. 2010; Li et al. 2015). However, a high level of kiss1 is reported in Syrian hamsters maintained under long day than the short day (Revel et al. 2006). Ansel et al. (2011) have also shown less brain kiss1 in short day exposed-Syrian hamsters. Similarly, long day exposure of goldfish, *Carassius auratus*, and Atlantic salmon, *Salmo salar*, resulted in a higher level of *kiss1/GPR54* mRNA expression in the brain (Shin et al. 2014; Chi et al. 2017). Further, Shahjahan et al. (2013) have reported that low temperature (15 °C) treatment of male zebrafish resulted in a significant increase in brain *kiss1* mRNA level in comparison to the zebrafish held at control temperature (27 °C), while the same authors have shown that treatment of grass puffer fish with low and high temperature has reduced the brain *kiss2* and *kiss2* mRNA significantly (Shahjahan et al. 2017). Chaube et al. (2020) have recently reported that exposure of the catfish, *Heteropneustes fossilis*, to long photoperiod (16L) and high temperature (28 °C) induced a considerable increase in kiss2 expression in the brain.

In the absence of studies on the regulation of kiss1 in gonads, as well as based on the reports on the effects of photoperiod and temperature on brain kiss1 expression in other vertebrates, it is suggested that gonadal kiss1 expression in the catfish, *C. batrachus*, is regulated by the rearing photo-thermal conditions; long photoperiod and high temperature increase, while short photoperiod and low temperature decrease the kiss1 expression in the ovary and testis in fish, although the mode, mechanism, and signaling system involved in the regulation of gonadal kiss expression remain to be elucidated.

Further, the regulation of oogenesis, spermatogenesis, and steroidogenesis by photo-thermal conditions in the seasonally breeding fishes is well established (Lam 1983). The continuous light/long photoperiod exposure increases sex hormones which induce sexual maturation in Atlantic salmon (*Salmo salar*) (Taranger et al. 1999), Atlantic salmon, *Salmo*

salar (Duston and Saunders 1990), and Atlantic cod, *Gadus morhua* (Hansen et al. 2001). Peñaranda et al. (2016) have reported the direct role of temperature in the regulation of steroid production and activities of steroidogenic enzymes in fish gonads. It is also well known that steroids regulate gametogenesis in fishes (Sisneros et al. 2004; Singh and Lal 2019; Agarwal et al. 2020; Singh et al. 2021). The high levels of circulating and gonadal steroids were observed when the catfish were exposed to long photoperiod and/or high temperatures alone as well as in combination coinciding with the development of advanced germs cells (oocytes/III in ovarian follicles and spermatocytes/spermatids in testis). The maximum steroids and gonadal development were recorded during long photoperiod along with the high temperature. These findings are in concurrence with earlier reports by Singh and Lal (2019) in the same species following exposure to different photo-thermal conditions, as well as the reports on other fishes.

The present findings have aquacultural importance. The reproductive efficiency of this catfish species can be enhanced by manipulating the photo-thermal conditions of the aquatic rearing ambience under controlled conditions, leading to high production of *C. batrachus*, which is consumer-preferred and highly cultured catfish in India.

Thus, it may be summarized that long photoperiod and high temperature both are almost equally effective in increasing the gonadal kiss1 expression in this catfish, but when long photoperiod and high temperature are coupled, their stimulatory effects on kiss1 expression are further amplified. In addition, the long photoperiod and high temperature also stimulate sex steroid production leading to gonadal development and maturation. However, the mode, mechanism, and signaling system involved in photo-thermal regulation of kiss1 expression in the catfish gonads are yet to be resolved.

Declarations

References

Acharia K, Lal B, Singh TP, Pati AK (2000) Circadian phase dependent thermal stimulation of ovarian recrudescence in Indian catfish, *Clarias batrachus*. Biol Rhythm Res 31:125–135. https://doi.org/10.1076/0929-1016(200004)31:2;1-U;FT125

Acharjee A, Chaube R, Joy KP (2017) Effects of altered photoperiod and temperature on expression levels of gonadotrophin subunit mRNAs in the female stinging catfish *Heteropneustes fossilis*. J Fish Biol 90:2289–2311. https://doi.org/10.1111/jfb.13305

Agarwal D, Gireesh-Babu P, Pavan-Kumar A, Koringa P, Joshi CG, Gora A, Bhat IA, Chaudhari A (2020) Molecular characterization and expression profiling of 17-beta-hydroxysteroid dehydrogenase 2 and spermatogenesis associated protein 2 genes in endangered catfish, *Clarias magur* (Hamilton, 1822). Anim Biotechnol 31:93–106. https://doi.org/10.1080/10495398.2018.1545663

Ansel L, Bentsen AH, Ancel C, Bolborea M, Klosen P, Mikkelsen JD, Simonneaux V (2011) Peripheral kisspeptin reverses short photoperiod-induced gonadal regression in Syrian hamsters by promoting GNRH release. Reproduction 142:417–425. https://doi.org/10.1530/REP-10-0313

Baggerman B (1980) Photoperiodic and endogeneous control of reproduction in teleost fishes. In: Ali MA (ed) Environmental physiology of fishes. Plenum Publishing Corp, New York, pp 537–567

Baker JR, Baker Z (1936) The seasons in a tropical rain-forest (New Hebrides). Part 3. Fruit-bats (Pteropidae). Zool J Linn Soc 40:123–141

Biswas AK, Endo M, Takeuchi T (2002) Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia *Oreochromis niloticus*: part II. Fish Sci 68:543 - 553. https://doi.org/10.1046/j.1444-2906.2002.00460.x

Bohlen TM, Silveira MA, Buonfiglio D, do C, Ferreira-Neto HC, Cipolla-Neto J, Donato J, Frazao R, (2018) A short-day photoperiod delays the timing of puberty in female mice via changes in the kisspeptin system. Front Endocrinol (lausanne) 9:1–9. https://doi.org/10.3389/fendo.2018.00044

Borg B (2009) Photoperiodism in fishes. In: Nelson RJ, Denlinger DL, Somers DE (eds) Photoperiodism: the biological calendar. Oxford University Press, New York, pp 371–398

Bromage N, Porter M, Randall C (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197:63–98. https://doi.org/10.1016/s0044-8486(01)00583-x

Chalivoix S, Bagnolini A, Caraty A, Cognié J, Malpaux B, Dufourny L (2010) Effects of photoperiod on kisspeptin neuronal populations of the ewe diencephalon in connection with reproductive function. J Neuroendocrinol 22:110–118. https://doi.org/10.1111/j.1365-2826.2009.01939.x

Chaube R, Sharma S, Senthilkumaran B, Bhat SG, Joy KP (2020) Expression profile of kisspeptin2 and gonadotropin-releasing hormone2 mRNA during photo-thermal and melatonin treatments in the female air-breathing catfish *Heteropneustes fossilis*. Fish Physiol Biochem 46:2403–2419. https://doi.org/10.1007/s10695-020-00888-4

Chi L, Li X, Liu Q, Liu Y (2017) Photoperiod regulate gonad development via kisspeptin/kissr in hypothalamus and saccus vasculosus of Atlantic salmon (*Salmo salar*). PLoS ONE 12:1–15. https://doi.org/10.1371/journal.pone.0169569

Demas GE, Bartness TJ, Nelson RJ, Drazen DL (2003) Photoperiod modulates the effects of norepinephrine on lymphocyte proliferation in Siberian hamsters. Am J Physiol Regul Integr Comp Physioll 285:R873–R879. https://doi.org/10.1152/ajpregu.00209.2003

Duston J, Saunders RL (1990) The entrainment role of photoperiod on hypoosmoregulatory and growth-related aspects of smolting in Atlantic (*Salmo salar*). Can J Zool 68:707–715. https://doi.org/10.1139/z90-103

Hansen T, Karlsen Ø, Taranger GL, Hemre GI, Holm JC, Kjesbu OS (2001) Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. Aquaculture 203:51–67. https://doi.org/10.1016/S0044-8486(01)00610-X

Joy KP, Senthilkumaran B (1995) A serotonergic control of pituitary-gonadal activity in the female catfish, *Heteropneustes fossilis*, under normal and long photoperiods. In: Goetz FW, Thomas P (eds) Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, The University of Texas at Austin, Austin, Texas, USA, Fish Symposium 95, Austin, pp 67–70

Kumar A, Thakur MK (2012) Presenilin 1 and 2 are expressed differentially in the cerebral cortex of mice during development. Neurochem Int 61:778–782. https://doi.org/10.1016/j.neuint.2012.07.001

Kumar D, Thakur MK (2014) Perinatal exposure to bisphenol-A impairs spatial memory through upregulation of neurexin1 and neuroligin3 expression in male mouse brain. PLoS ONE 9:e0110482. https://doi.org/10.1371/journal.pone.0110482

Lam TJ (1983) Environmental influences on gonadal activity in fish. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology behavior and fertility control, vol 9B. Academic Press, New York, pp 65–116

Li SN, Xue HL, Zhang Q, Xu JH, Wang S, Chen L, Xu LX (2015) Photoperiod regulates the differential expression of *KiSS-1* and *GPR54* in various tissues and sexes of striped hamster. Genet Mol Res 14:13894–13905. https://doi.org/10.4238/2015.October.29.10

Munro AD (1990) Tropical freshwater fish. In: Munro AD, Scott AP, Lam TJ (eds) Reproductive seasonality in teleosts: environmental influence. CRC Press, Boca Raton, FL, pp 145–239

O'Brien CS, Bourdo R, BradshawWE HCM, Cresko WA (2012) Conservation of the photoperiodic neuroendocrine axis among vertebrates: evidence from the teleost fish, *Gasterosteus aculeatus*. Gen Comp Endocrinol 178:19–27. https://doi.org/10.1016/j.ygcen.2012.03.010

Peñaranda DS, Morini M, Tveiten H, Vílchez MC, Gallego V, Dirks RP, van den Thillart GEEJM, Pérez L, Asturiano JF (2016) Temperature modulates testis steroidogenesis in European eel. Comp Biochem Physiol -Part A Mol Integr Physiol 197:58–67. https://doi.org/10.1016/j.cbpa.2016.03.012

Poston HA (1978) Neuroendocrine mediation of photoperiod and other environmental influence on physiological response in salmonids a review. Technical Paper 96. In: Technical Papers of the U.S. Fish and Wildlife Service, United States Department of the Interior Fish and Wildlife Service, Washington, DC, pp 1–14

Priyadarshini, Lal B (2018) Seasonal ovarian immunolocalization of neuropeptide Y and its role in steroidogenesis in Asian catfish, *Clarias batrachus*.Gen Comp Endocrinol 255:32–39. https://doi.org/10.1016/j.ygcen.2017.10.002

Revel FG, Saboureau M, Masson-Pévet M, Pévet P, Mikkelsen JDD, Simonneaux V (2006) Kisspeptin mediates the photoperiodic control of reproduction in hamsters. Curr Biol 16:1730–1735. https://doi.org/10.1016/j.cub.2006.07.025

Rodríguez L, Zanuy S, Carrillo M (2001) Influence of daylength on the age at first maturity and somatic growth in male sea bass (*Dicentrarchus labrax*, L.). Aquaculture 453:159–175. https://doi.org/10.1016/S0044-8486(00)00555-X

Shahjahan M, Kitahashi T, Ogawa S, Parhar IS (2013) Temperature differentially regulates the two kisspeptin systems in the brain of zebrafish. Gen Comp Endocrinol 193:79–85. https://doi.org/10.1016/j.ygcen.2013.07.015

Shahjahan M, Kitahashi T, Ando H (2017) Temperature affects sexual maturation through the control of kisspeptin, kisspeptin receptor, GnRH and GTH subunit gene expression in the grass puffer during the spawning season. Gen Comp Endocrinol 243:138–145. https://doi.org/10.1016/j.ygcen.2016.11.012

Shin HS, SongJA CJY, Kim NN, Choi YJ, Sung SN, Park MS, Min BH, Choi CY (2014) Effects of various photoperiods on kisspeptin and reproductive hormones in the goldfish *Carassius Auratus*. Animal Cells Syst (seoul) 18:109–118. https://doi.org/10.1080/19768354.2014.902863

Singh AK, Lal B (2008) Seasonal and circadian time-dependent dual action of GH on somatic growth and ovarian development in the Asian catfish, *Clarias batrachus* (Linn.): role of temperature. Gen Comp Endocrinol 159:98–106. https://doi.org/10.1016/j.ygcen.2008.08.001

Singh VK, Lal B (2016) Nitric oxide (NO) stimulates steroidogenesis and folliculogenesis in fish. Reproduction 153:133–146. https://doi.org/10.1530/REP-16-0482

Singh VK, Lal B (2017) Pro-steroidogenic and pro-spermatogenic actions of nitric oxide (NO) on the catfish, *Clarias batrachus*: an *in vivo* study. Gen Comp Endocrinol 242:1–10. https://doi.org/10.1016/j.ygcen.2016.05.001

Singh P, Lal B (2019) Photo-thermal regulation of neuropeptide Y (NPY) expression in ovarian follicles and ovarian activity of the catfish, *Clarias batrachus*. Gen Comp Endocrinol 279:114–119. https://doi.org/10.1016/j.ygcen.2019.02.012

Singh A, Lal B, Parhar IS, Millar RP (2021) Seasonal expression and distribution of kisspeptin1 (kiss1) in the ovary and testis of freshwater catfish, *Clarias batrachus*: a putative role in steroidogenesis. Acta Histochem 123:151766. https://doi.org/10.1016/j.acthis.2021.151766

Singh nee Priyadarshini, Lal B (2018) Seasonal ovarian immunolocalization of neuropeptide Y and its role in steroidogenesis in Asian catfish, *Clarias batrachus*. Gen Comp Endocrinol 255:32–39.https://doi.org/10.1016/j.ygcen.2017.10.002

Sinha N, Lal B, Singh TP (1992) Seasonal effects on diel changes in circulating thyroid hormones in freshwater catfish, *Clarias batrachus*. J Interdisciplinary Cycle Res 23:277–286. https://doi.org/10.1080/09291019209360186

Sisneros JA, Forlano PM, Knapp R, Bass AH (2004) Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. Gen Comp Endocrinol 136:101–116. https://doi.org/10.1016/j.ygcen.2003.12.007

Sundararaj BI, Sehgal A (1970) Effects of a long or an increasing photoperiod on the initiation of ovarian recrudescence during the preparatory period in the catfish, *Heteropneustes fossilis* (Bloch). Biol Reprod 2:413–424. https://doi.org/10.1095/biolreprod2.3.413

Sundararaj BI, Vasal S (1976) Photoperiod and temperature control in the regulation of reproduction in the female catfish *Heteropneustes fossilis*. J Fish Res 33:959–973. https://doi.org/10.1139/f76-123

Taranger GL, Haux C, Hansen T, Stefansson SO, Björnsson BT, Walther BT, Kryvi H (1999) Mechanisms underlying photoperiodic effects on age at sexual maturity in Atlantic salmon, *Salmo salar*. Aquaculture 177:47–60. https://doi.org/10.1016/S0044-8486(99)00068-X

Walton JC, Weil ZM, Nelson RJ (2011) Influence of photoperiod on hormones, behaviour, and immune function. Front Neuroendocrinol 32:303–319. https://doi.org/10.1016/j.yfrne.2010.12.003