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Photo-thermal regulation of neuropeptide Y (NPY) expression in ovarian follicles and ovarian activity of the catfish, *Clarias batrachus*

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ABSTRACT

Authors have recently reported a gradual increase in neuropeptide Y expression in the ovarian follicles of *Clarias batrachus* with the progression of oogenesis, coinciding with increasing photoperiod and temperature. This indicates the involvement of photoperiod and temperature in controlling NPY expression. Therefore, a study was designed to investigate the role of photoperiod and temperature in regulation of NPY expression in ovarian follicles. The catfish were exposed to different photo-thermal regimes during the late-quiescence and late-recrudescence phases for one month, and the expression of NPY was analyzed along with other ovarian activities. Though the exposure of catfish to long photoperiod induced a marginal increase (1.5 fold) in NPY expression in follicular cells, the high temperature stimulated its expression more effectively (6–10 fold), irrespective of photoperiodic exposures. Exposure to long photoperiod and high temperature together induced NPY expression maximally in granulosa and thecal cells of fully grown oocytes, but exposure to low temperature decreased its expression significantly. The oogenic and steroidogenic activities were also promoted simultaneously after the exposure to high temperature and long photoperiod alone or in combination. However, the low temperature exposure suppressed the ovarian activities leading to atresia of advanced follicles. Thus it is suggested that photoperiod and temperature both affect NPY expression and ovarian recrudescence in fish but the influences of temperature seem to be more prominent.

1. Introduction

Fishes are mostly seasonal breeder. Seasonality in gonadal activities is primarily driven by annually changing environmental cues, particularly photoperiod and temperature, which regulate changes in the pattern and magnitude of neuroendocrine secretions of the hypothalamo-hypophyseal-gonadal axis (Miranda et al., 2009). Gonadotropin releasing hormone (GnRH), a principal hypothalamic neuropeptide, stimulates gonadotropin (GtH) secretion and thereby steroidogenesis and oogenesis (Miranda et al., 2009). Apart from GnRH, other neuropeptides also promote GtH secretion. Among the complex network of neuropeptides, neuropeptide Y (a 36 amino acid long peptide) has been recognized as an important neuropeptide in recent decades because on the one hand it shows powerful orexigenic activity, and on the other it plays crucial role in stimulation of hypothalamo-hypophyseal-gonadal axis in vertebrates including fishes (Pedrazzini et al., 2003; Shahjahan et al., 2014). Neuropeptide Y (NPY) immunoreactive cells and fibers are reported in the preoptic, tuberal area, and periventricular zone around third ventricle, inferior lobes of the brain, and all the three compartments of pituitary gland suggesting its potential role in regulation of GnRH-GtH secretion (Gaikwad et al., 2004), and thereby reproduction of *Clarias batrachus* (Subhedar et al., 2005). This peptide is also known to control the release of growth hormone (Peng et al., 1993) and daily food intake, and thereby influences the energy balance in fishes (Narnaware et al., 2000).

The presence of NPY peptide has also been reported in the mammalian ovary (Hulshof et al., 1994; Keator et al., 2010). Authors have recently demonstrated the expression of NPY peptide in the granulosa and thecal cells of ovarian follicles of *C. batrachus* (Priyadarshini and Lal, 2018). NPY expression increases steadily with the follicular growth and reaches its maximum in the fully grown ovarian follicles then after decreases in follicles undergoing final oocyte maturation and ovulation (Priyadarshini and Lal, 2018). The variation in NPY immunointensity in follicular cells in parallel to oogenesis calls for a study exploring factors that regulate seasonality in NPY expression in the ovarian follicles. Photoperiod and temperature are known potent regulators of seasonality in ovarian activities in fishes. The gonadal development in salmonid and gaterosteid fishes is dependent on the photoperiod (Lam, 1983) while other like gobiid and cyprinodontiforms respond to changes in water temperature (Lam, 1983; de Alvarenga and de Franc,

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2009) and some other such as cyprinids and perciforms rely on combined impact of photoperiod and temperature for their gonadal functions (Lam, 1983; Sundararaj and Vasal, 1976; Acharia et al., 2000). Acharia et al. (2000) have shown that treatment of catfish with high temperature and long photoperiod stimulates ovarian development by increasing sex steroid levels. Photoperiod and temperature influence fish reproduction by regulating secretion of hypothalamic GnRH and other neurohormones, and thereby GtH and sex steroids in fishes (Miranda et al., 2009; Fiszbein et al., 2010; Chaube et al., 2015). It was, thus, decided to evaluate the effects of photoperiod and temperature on ovarian NPY expression in the freshwater catfish, *C. batrachus* along with ovarian activities. This catfish is a seasonal breeder and breeds during monsoon, though ovarian development starts steadily with increase in photoperiod and temperature from February/March and become fully gravid by end of June/early July, then breeds in July/August (Priyadarshini and Lal, 2018).

2. Materials and methods

2.1. Chemicals

NPY-polyclonal antibody (raised in rabbit against porcine NPY, cat. no. N9528) was purchased from the Sigma-Aldrich, St. Louis, USA. Goat anti-rabbit HRP-tagged secondary antibody (cat. no. 1140380011730) was procured from GeNei, Bangalore, India. ELISA kits for testosterone (cat. no. DKO002) and 17β-estradiol (cat. no. DKO003) were purchased from DiaMetra (Spello, Perugia, Italy). Other routine laboratory chemicals of analytical grade were acquired from Qualigens, SRL and HiMedia, Mumbai, India, through local authorized vendors.

2.2. Exposure of catfish to different photo-thermal regimes

The catfish, *C. batrachus* (weighing 90–100 g) were collected during the late-quiescence (January) and late-recrudescence phases (May) of its annual reproductive cycle from suburb of Varanasi (28°8′ N; 83°1′ E), India, and acclimated to laboratory conditions for two weeks under ambient photoperiod and temperature in 200 L cemented tanks. Fish were fed with chopped goat liver *ad libitum*; fish held at high temperature consumed more feed. After acclimation catfish were sorted out in a close weight range (90–95 g measuring 21–22 cm in length) and were divided into several groups; 15 fish in each group, and were exposed to different photo-thermal conditions [i.e. ambient photoperiod and ambient temperature (APAT- 11L:13D, 19 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 19 ± 1 °C), long photoperiod and ambient temperature (LPAT- 14.5L:9.5D, 19 ± 1 °C), short photoperiod and high temperature (SPHT- 9L:15D, 30 ± 1 °C), and long photoperiod and high temperature (LPHT- 14.5L:9.5D, 30 ± 1 °C)] during the late-quiescence phase (Feb. 4 - March 5, 2017), and [ambient photoperiod and ambient temperature, (APAT- 13.5L:10.5D, 30 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 30 ± 1 °C), ambient photoperiod and low temperature (APLT- 13.5L:10.5D, 16 ± 1 °C), and short photoperiod and low temperature (SPLT- 9L:15D, 16 ± 1 °C)] during the late-recrudescence phase (May 21-June 20, 2017) for one month. At the end of exposure, fish were anesthetized in ice-chilled water for 5–10 min, weighed and blood was collected through caudal puncture in separate glass tubes. Ovaries were dissected out aseptically and weighed to the nearest gram to calculate gonadosomatic index (GSI) using the formula: GSI = (weight of both the ovary in gram/body weight in gram) × 100. One of the ovaries of individual fish was stored at −70 °C for the analyses of 17β-estradiol (E2) and testosterone (T) contents and activities of 5-ene-3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD). The other ovary was fixed in Bouin's fluid and processed for paraffin embedding and sectioning (6 μm thick) for histological and immunohistochemical studies. Blood was centrifuged under refrigeration to collect serum and stored at −70 °C for steroid assay. All experiments were carried out as per the guidelines of Institutional Animal Ethics and Care of Banaras Hindu University, India (approval letter no. F.Sc./IAEC/2016-17/1135).

2.3. Ovarian histo-morphology and immunohistochemical localization of NPY

Paraffin ovarian sections of different groups were processed for hematoxylin and eosin staining for histological study and immunohistochemical localization of NPY as per the procedures described recently (Priyadarshini and Lal, 2018). Images were captured at 40× magnification by Leica DM2000 microscope attached with camera. Procedures of identification of different oocytes, their morphometric analyses and calculation of integrated density value (IDV) in term of arbitrary unit for NPY expression using software are described previously (Singh and Lal, 2017; Priyadarshini and Lal, 2018).

2.4. Measurements of steroids and activities of 3β-HSD and 17β-HSD

Testosterone and 17β-estradiol levels in serum and ovarian homogenate were measured using commercial available ELISA kits (DiaMetra, Italy). Detailed protocols are described earlier (Priyadarshini and Lal, 2018). Activities of 3β-HSD and 17β-HSD in ovary of different groups were determined following the protocols described elsewhere (Priyadarshini and Lal, 2018).

2.5. Statistical analyses

Data are expressed as mean ± SEM (n = 5). Data were analyzed through analysis of variance (ANOVA) followed by post-hoc test, Duncan's multiple range test at $P < 0.05$ for comparisons amongst different groups. All the statistical analyses were performed in SPSS16 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effect of different photo-thermal regimes on GSI and ovarian histology

During the late-quiescence phase, exposure of *C. batrachus* to short (9L)/or long photoperiod (14.5L) under ambient temperature (19 ± 1 °C) failed to bring any significant change in GSI (Fig. 1A). However, fish at high temperature (30 + 1 °C), irrespective of photoperiodic regimes (short- 9L or long photoperiod- 14.5L) exhibited significantly higher GSI as compared to the fish held under APAT (Fig. 1A). The exposure to high temperature under long photoperiod resulted in maximum increase in GSI (Fig. 1A) as compared to the fish maintained under ambient photoperiod and ambient temperature or fish held at other photo-thermal regimes (Fig. 1A).

During the late-recrudescence phase when ambient photoperiod and temperature were increased and fish ovary had already under gone in advanced stage, the exposure of such catfish to short photoperiod led to marginal reduction in GSI (Fig. 2A) but the treatment of fish with low temperature caused remarkable decline in GSI, irrespective of their photoperiodic exposures [short photoperiod (9L) and/or ambient photoperiod (13.5L)] (Fig. 2A).

During the late-quiescence phase, exposure of catfish to short or long photoperiod under ambient temperature failed to bring any histomorphometric changes in the ovary. Ovaries of the fish held under APAT, SPAT and LPAT were primarily populated by the perinucleolar oocytes or oocytes-I (Fig. 1A, B, C, D). There was marginal decline in number of perinucleolar oocytes in ovary of fish kept at LPAT with concomitant appearance of few oocyte-II in comparison to the SPAT group (Fig. 1A, C, D). However, ovary of the fish maintained at high temperature under short or long photoperiod exhibited the advancement of oogenesis, as the ovary of fish held at SPHT had considerable number of oocytes-II and occasional oocytes-III, in addition to the

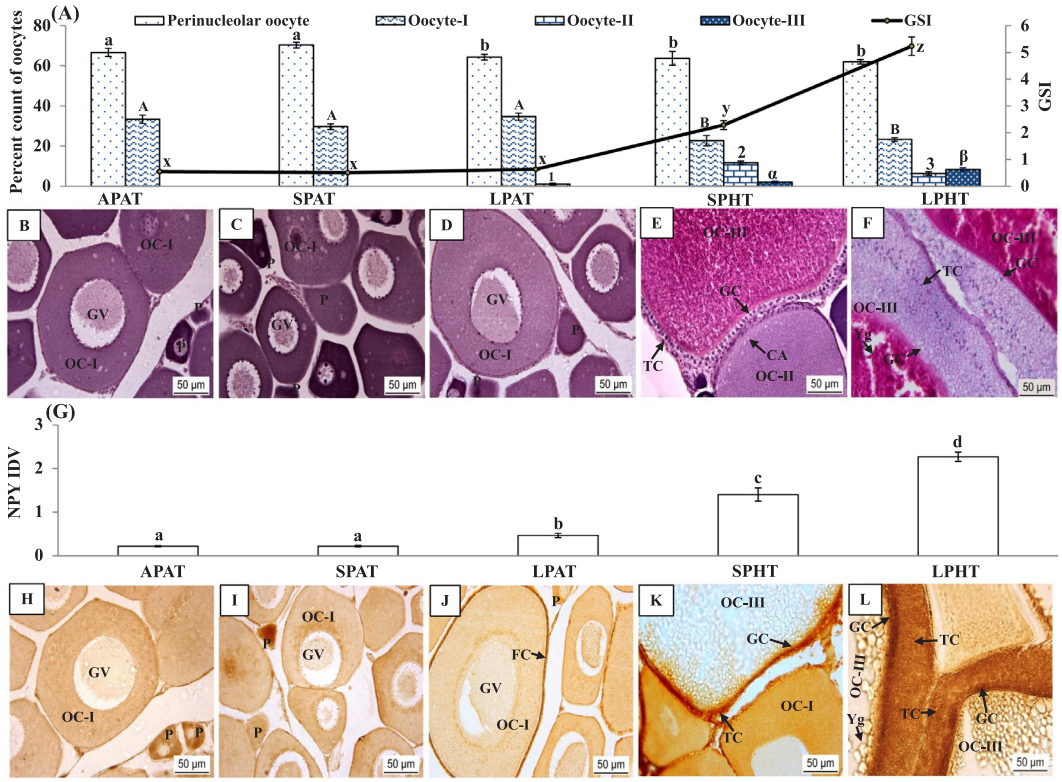

Fig. 1. Representative images of hematoxylin/eosin-stained transverse sections and their morphometry (A-F), and NPY expression (G-L) in the ovary of *Clarias batrachus* after their exposure to different photo-thermal conditions [ambient photoperiod and ambient temperature (APAT- 11L:13D, 19 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 19 ± 1 °C), long photoperiod and ambient temperature (LPAT- 14.5L:9.5D, 19 ± 1 °C), short photoperiod and high temperature (SPHT- 9L:15D, 30 ± 1 °C) long photoperiod and high temperature (LPHT- 14.5L:9.5D, 30 ± 1 °C)] during the late-quiescence phase. Different oocytes count in percentage as well as gonadosomatic index (GSI) are given in panel A. Expression of NPY was quantified in the term of integrated density value (IDV) as arbitrary unit and is presented in panel G. 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 are used for perinucleolar oocytes, whereas A & B for oocytes-I and 1, 2 & 3 for oocytes-II and α & β for oocytes-III in panel A. Superscripts x, y & z are used for GSI in panel A. Superscripts a, b, c and d are used for NPY expression in term of IDV in panel G. Note- Perinucleolar oocytes (P), Oocytes-I (OC-I), Oocytes-II (OC-II), Oocytes-III (OC-III), Germinal vesicle (GV), Cortical Alveoli (CA), Granulosa Cell (GC), Thecal Cell (TC), Yolk Granule (Yg), Follicular Cells (FC).

perinucleolar oocytes and oocytes-I (Fig. 1A, E). The catfish kept at LPHT exhibited more developed ovary; it was populated by fully grown oocytes-III also, in addition to oocytes-II, oocytes-I and perinucleolar oocytes (Fig. 1A, F).

However, exposure of advanced recrudescing *C. batrachus* to short photoperiod (9L) under ambient temperature (30 ± 1 °C) not only arrested the advancement of oogenesis but led to degeneration of oocytes-III when compared with fish ovary of APAT group. Ovary also exhibited a few atretic follicles (Fig. 2A, C). The ovary of fish maintained at low temperature under short (9L) or ambient photoperiod (13.5L) showed high occurrence of atretic follicles and drastic decline in oocytes-II and III (Fig. 2A, D, E).

3.2. Effect of different photo-thermal exposures on NPY expression in ovarian follicles

Though the maintenance of catfish at LPAT (14.5L:9.5D,19 ± 1 °C) (Fig. 1G, J) during the late-quiescence phase induced NPY expression marginally in the follicular cells of the oocytes-I in comparison to the ovarian follicles of fish held at APAT (11L & 19 ± 1 °C) and SPAT (9L & 19 ± 1 °C) (Fig. 1G, H, I), the treatment of catfish with high temperature (30 ± 1 °C) either under short (9L) (Fig. 1G, K) or long photoperiod (14.5L) (Fig. 1G, L) stimulated intense NPY expression; maximal expression was observed in follicular cells of ovarian follicles of fish held under LPHT (Fig. 1G, L). On the contrary, exposure of recrudescing catfish to low temperature (16 ± 1 °C) under ambient photoperiod (13.5L) and short photoperiod (9L) caused reduction in NPY expression (Fig. 2F, I, J).

3.3. Effect of different photo-thermal exposures on steroids level in ovary and serum

During the late-quiescence phase, exposure of fish to short photoperiod at ambient temperature did not change the T and E2 levels in ovary and serum (Fig. 3A & B) but long photoperiod at ambient temperature induced marginal increase in the ovarian E2 (Fig. 3A). However, treatment of fish with high temperature under short photoperiod (9L) induced considerable increase in ovarian and circulating T and E2 (Fig. 3A & B) but high temperature under long photoperiod (14.5L) provoked tremendous increase in these steroids (Fig. 3A & B) in comparison to fish held at APAT and other regimes (SPAT, LPAT).

During the late-recrudescence phase, exposure of *C. batrachus* to short photoperiod at ambient temperature caused marginal decline in E2 without altering the T levels in ovary (Fig. 4A) and serum (Fig. 4B). But the treatment of fish with low temperature either under ambient photoperiod (13.5L) or short photoperiod (9L) induced maximum decline in their ovarian and serum levels (Fig. 4A & B).

3.4. Effect of different photo-thermal exposures on activity of steroidogenic enzymes

Changes in the activities of ovarian 3β-HSD and 17β-HSD in

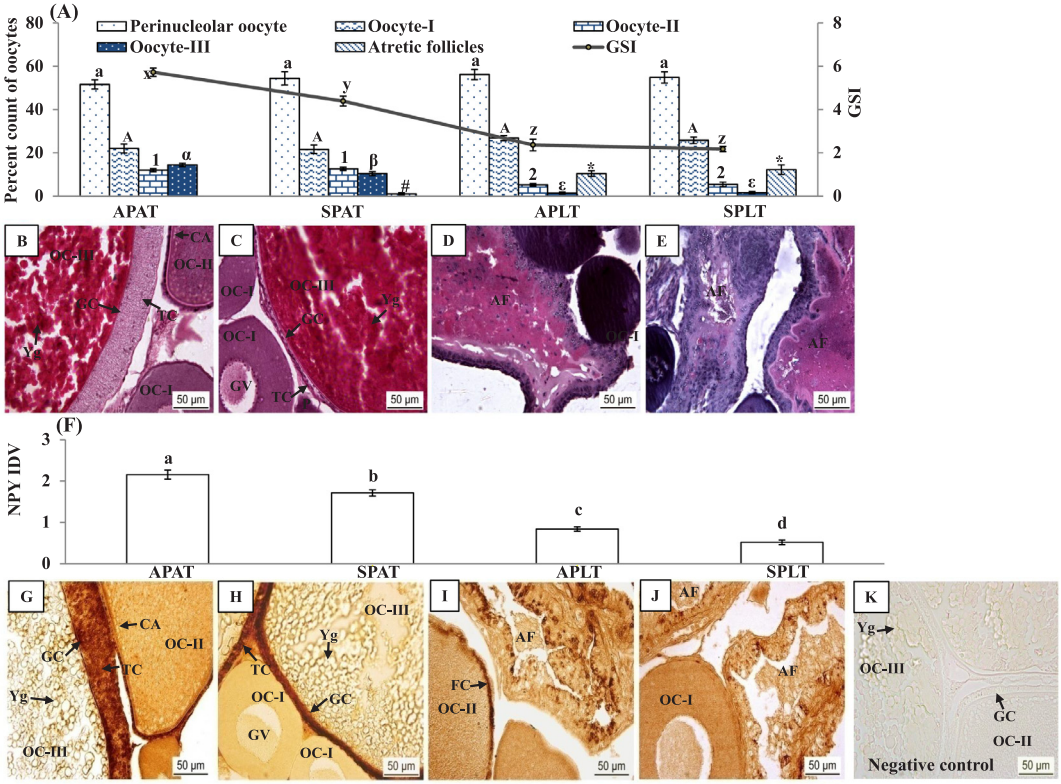

Fig. 2. Representative images of hematoxylin/eosin-stained transverse sections and their morphometry (A-E), and NPY expression (F-J) in the ovary of *Clarias batrachus* after their exposure to different photo-thermal conditions [ambient photoperiod and ambient temperature, (APAT- 13.5L:10.5D, 30 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 30 ± 1 °C), ambient photoperiod and low temperature (APLT- 13.5L:10.5D, 16 ± 1 °C), short photoperiod and low temperature (SPLT- 9L:15D, 16 ± 1 °C)] during the late-recrudescence phase. Figure K represents pre-absorbed control ovarian section. Different oocytes count in percentage and gonadosomatic index (GSI) are given in panel A. Expression of NPY was quantified in the term of integrated density value (IDV) as arbitrary unit and is presented in panel F. 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). Superscript a is used for perinucleolar oocytes, whereas A for oocytes-I, 1 & 2 for oocytes-II, α, β & ε for oocytes-III, and # & * for atretic follicles. Superscripts x, y & z are used for GSI in panel A. Superscripts a, b, c & d are used for NPY expression in term of IDV in panel F. Note- Perinucleolar oocytes (P), Oocytes-I (OC-I), Oocytes-II (OC-II), Oocytes-III (OC-III), Germinal vesicle (GV), Cortical Alveoli (CA), Granulosa Cell (GC), Thecal Cell (TC), Yolk Granule (Yg), Atretic follicles (AF), Follicular Cells (FC).

response to exposure to different photo-thermal regimes followed the changes in ovarian T and E2 levels during both reproductive phases (Figs. 3C and 4C).

4. Discussion

Though photo-thermal stimulation of oogenesis and steroidogenesis in sub-tropical/sub temperate fishes is well established (Sundararaj and Vasal, 1976; Acharia et al., 2000), the regulation of ovarian NPY expression by the photo-thermal regimes under which fish are held, is a notable finding of the present study.

Despite known presence of NPY transcript and/or peptide in gonad of mammals (Hulshof et al., 1994; Keator et al., 2010) and fishes (Sudhakumari et al., 2017; Priyadarshini and Lal, 2018), no attempt has been made to investigate the factor(s) that regulate expression of gonadal NPY in vertebrates including fishes. It is, therefore, difficult to compare the present results on photo-thermal regulation of ovarian NPY in *C. batrachus*. Nevertheless some attempts have been made to evaluate the role of photoperiod and temperature on NPY expression in the brain of vertebrates including fishes (Kehoe and Volkoff, 2008). Some studies clearly demonstrate photo-thermal regulation of expression of brain orexigenic NPY but these studies pertain to food intake (Mercer et al., 1995). Campos et al. (2010) have reported that brain NPY mRNA increases in fasting flounder, *Paralichthys orbignyanus* held at higher temperature, and thus, suggest the influence of temperature on NPY transcript. However, Kehoe and Volkoff (2008) have shown that forebrain NPY transcript remained unaltered in cod, *Gadus morhua* held at different water temperature, although food intake was lowered at low temperature. The temperature-induced changes in ovarian NPY of *C. batrachus* might be due to enhanced food uptake as observed at high temperature, though other mode of thermal regulation of ovarian NPY cannot be ruled out, as tissue-specific differential regulation of NPY expression has earlier been suggested (Sucajtys-Szulc et al., 2008). Terado et al. (2006) have distinctly demonstrated that food deprivation induces appreciable increase in arcuate NPY mRNA in mouse hypothalamus but not in the testis suggesting that NPY expression is differently regulated than that of the hypothalamic NPY. To our knowledge, this is the first time that NPY expression has been evaluated in ovary after exposing fish to different temperature and photoperiod regimes during different phases of the reproductive cycle. The present study suggests the temperature as a potent regulator of NPY expression in fish ovary; high temperature stimulates and low temperature inhibits its expression. Photoperiod may be less important in regulation of ovarian NPY expression in catfish. However, the mode and mechanism of photo-thermal regulation of ovarian NPY expression remains to be elucidated.

Moreover, exposure of *C. batrachus* to both long photoperiod (14L) and high temperature (30 °C) separately or in combination resulted in stimulation of oogenesis and steroidogenesis, as was evident from the increase in GSI, number of advanced oocytes, levels of E2, T and

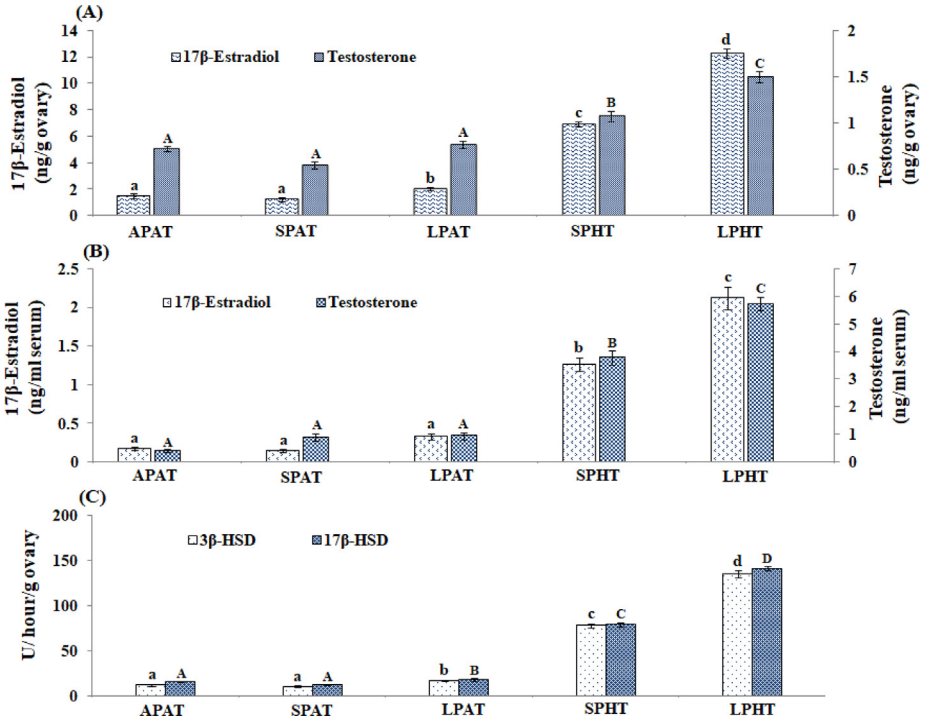

Fig. 3. Changes in 17β-estradiol and testosterone levels in ovary (A) and serum (B) and activities of 3β-HSD & 17β-HSD (C) in ovarian tissue of *Clarias batrachus* after their exposure to different photothermal regimes [ambient photoperiod and ambient temperature (APAT- 11L:13D, 19 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 19 ± 1 °C), long photoperiod and ambient temperature (LPAT- 14.5L:9.5D, 19 ± 1 °C), short photoperiod and high temperature (SPHT- 9L:15D, 30 ± 1 °C) long photoperiod and high temperature (LPHT- 14.5L:9.5D, 30 ± 1 °C)] during the late-quiescence phase. 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 & d are used for 17β-estradiol, and A, B & C for testosterone in figure's panels A and B. While a, b, c & d are used for comparison in activities of 3β-HSD and A, B, C & D for 17β-HSD in panel C.

activities of steroidogenic enzymes. Stimulation was much greater when the long photoperiod and high temperature was given together, suggesting their combined role in ovarian recrudescence. However, temperature seems be more important, as the high temperature promoted ovarian recrudescence more prominently, regardless of photoperiodic regimes, as is clear from the fact that high temperature under short photoperiod also initiated the ovarian development considerably. These findings are in agreement with earlier studies in sub-tropical/sub-temperate monsoon breeding fish species. Photoperiod and temperature both are reported to affect ovarian recrudescence, but temperature is found more important cue in the catfish, *Heteropneustes fossilis*. Sundararaj and Vasal (1976) have reported that exposure of *H. fossilis* to long photoperiod (14L) during late-quiescence phase (February) stimulates vitellogenesis, thereby ovarian recrudescence, but the response depends on the temperature, being greater at high temperature (> 25 °C). At 30 °C, vitellogenesis was augmented regardless of photoperiod. Acharia et al. (2000) have also shown that high temperature (30 °C) treatment under long photoperiod (13L) or short photoperiod (11L) elevated E2 and T levels in *C. batrachus*; though increases in E2 and T were maximum under long photoperiod and high temperature. A number of other studies have also established that long photoperiod and high temperature stimulate gonadal recrudescence in fish by augmenting the secretions of hypothalamo-hypophyseal-gonadal axis (Miranda et al., 2009; Fiszbein et al., 2010; Chaube et al.,

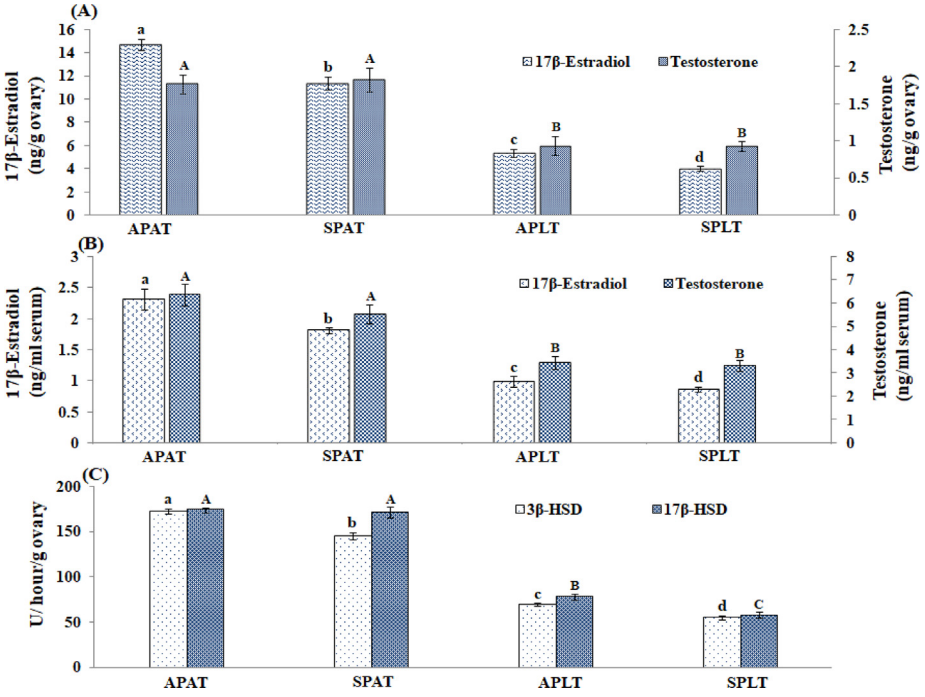

Fig. 4. Changes in 17β-estradiol and testosterone levels in ovary (A) and serum (B), and activities of 3β-HSD & 17β-HSD (C) in ovarian tissue of *Clarias batrachus* after their exposure to different photothermal regimes [ambient photoperiod and ambient temperature, (APAT- 13.5L:10.5D, 30 ± 1 °C), short photoperiod and ambient temperature (SPAT- 9L:15D, 30 ± 1 °C), ambient photoperiod and low temperature (APLT- 13.5L:10.5D, 16 ± 1 °C), short photoperiod and low temperature (SPLT- 9L:15D, 16 ± 1 °C)] during the late-recrudescence phase. 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 & d for 17β-estradiol, and A & B for testosterone in figure's panels A and B. Whereas a, b, c & d are used for comparison in activities of 3β-HSD and A, B & C for 17β-HSD in panel C.

2015). Moreover, the temperature-induced NPY expression might also be involved in the stimulation of follicular development in catfish ovary at higher temperature regime, as the NPY is reported to enhance the secretions of GnRH (Gaikwad et al., 2004), and LH and GH (Wu, et al., 2012), and FSH (Di Yorio et al., 2015) and sex steroids (Priyadarshini and Lal, 2018) and thus, the reproductive activity in fishes (Subhedar et al., 2005).

Though the exposure of catfish to short photoperiod during the late-recrudescence phase (when ambient photoperiod (13.5L) and temperature (30 °C) both were already increased, and ovary had undergone faster development) caused marginal decrease in steroids levels and activities of steroidogenic enzymes, and arrested the development of oocytes-II. But lowering of temperature (16 ± 1 °C) either at ambient photoperiod (13.5L) or short photoperiod (9L) induced atresia of the advanced follicles more frequently than the control fish held at ambient photoperiod (13.5L) and temperature (30 °C). Activities of 3β-HSD and 17β-HSD, levels of E2 and T were drastically lowered. The developed oocytes- II and III started undergoing atresia very fast. Unfortunately, such studies investigating role of photoperiod and temperature in ovarian degeneration are very few and contradictory. Nevertheless, present results clearly indicate that maintenance of recrudescing fish at low temperature may not only lead to arrest of ovarian development but to degeneration. Low temperature has been reported to cause gonadal regression in *Tilapia* sps. (Terkatin-Shimony et al., 1980).

Thus, it may be summarized that photoperiod and temperature both affect NPY expression and ovarian recrudescence in fish but temperature is apparently more important cue.

Conflict of interest

Authors declare that they have no conflict of interest.

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