Season-dependent effect of thermopulse on gonadal recrudescence in the female catfish, *Clarias batrachus*

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In the present study, the female *Clarias batrachus*, held under long photoperiod (13L:11D), were exposed to high water temperature either constantly (24 h) and/or in form of thermopulse of 6 h and 12 h durations, separately, at different times of the day/night cycle for six weeks during the early post-spawning and late post-spawning phases of its reproductive cycle. The effects of high water temperature (30±1°C) on gonadosomatic index (GSI), plasma levels of testosterone (T) and oestradiol-17β (E2) were observed. During the late post-spawning phase, thermopulse of 12 h duration given in the morning hour increased all the studied parameters most effectively as compared to that given at evening hour of the day/night cycle or even in comparison to the fish exposed to constant high temperature. Thermopulse of 6 h duration given in the morning or noon also raised these parameters compared to the controls, but the magnitudes of stimulation were moderate. However, exposures of the catfish to such photothermal regimes during the early post-spawning phase completely failed to bring any change in the studied parameters. These findings, thus, clearly indicate that treatment with high temperature under long photoperiod may stimulate gonadal activity in *C. batrachus*, provided given at appropriate season of the year. A diurnal basis of response to high temperature and the existence of a rigid gonado-refractory phase (perhaps just after the spawning) are also evident in the reproductive cycle of *C. batrachus*.

**Keywords**: Catfish, GSI, Oestradiol, Phase dependent, Reproduction, Testosterone, Thermopulse

One of the most ambitious means of increasing the productivity of fish is intensive breeding which requires perfect understanding of the mechanism involved in the regulation of reproductive physiology of the cultivable fish species. Fishes, occupying a diverse ecological niche, exhibit a well-defined temporal organization in the reproductive activity, suited to their habitats. Majority of fishes breed once a year, at the time most appropriate to ensure the maximum survival of their offspring. Extensive research during the last few decades have established that the annual rhythm of reproduction in fishes is entrained by the external factors, particularly photoperiod, temperature, rainfall etc., which change diurnally and seasonally. Environmental factors modulate the endocrine secretions, especially those from the hypothalamo-hypophyseal-gonadal axis, which ultimately control the reproductive events in fishes. In salmonids and gasterosteids, photoperiod plays a dominant role whereas in gobidi and cyprinodontiformes temperature is critical in controlling sexual activity. In cyprinids and perciform fishes, both photoperiod and temperature are reported to play a significant role in regulation of reproductive events. In goldfish, *Carassius auratus* long photoperiod along with warm temperature played a significant role in advancement of gonadal maturation. Rise in water temperature (10-14 ºC) results into increase in GSI, plasma levels of testosterone and oestradiol-17β in European bullhead, *Cottus gobio*. On the other hand, the responsiveness to these external zeitgebers in subtropical fish is very well phase-dependent. In *Heteropneustes fossilis*, the responsiveness of the ovary to long photoperiod and high temperature was found to be almost nil during the post-spawning phase. The hormonal gene expressions in the pituitary-gonadal axis has also been analysed in *Odontotesthes bonariensis* under high temperature condition. Thus, the responses of fish to different photothermal regimes are quite varied and appear to be species specific. Therefore, attempts have been made to evaluate the effect of high temperature on gonadal activity of a
Materials and Methods

The catfish, *Clarias batrachus* (45-55 g 17-20 cm in length) were collected during the early (1st week of October) and late post-spawning phases (1st week of January) of its annual reproductive cycle from ponds around Varanasi (25° 18’N; 83° 1’E), India. During the early post-spawning phase, ovaries of most of the fish were characterized by their very small size with full of oogonial nests whereas in the late post-spawning phase ovaries were flaccid and reduced in volume with dull colour. Fish were acclimated under natural photoperiod and temperature (early post-spawning phase- 11.5L:13.5D, 22±1 °C; late post-spawning- 11L:13D, 17±1 °C) in outdoor tanks (capacity 4000 ltr) for a fortnight prior to experimentation. They were fed with chopped goat liver at 2% of body weight on alternate days. On the day of start of the experiment, five females (identified after incision) were sacrificed for sampling as initial control (IC). Rest of the fish were divided in different batches (12 fish/batch) and held under long photoperiod (13L:11D), and were subjected to high temperature of 22±1 °C; late post-spawning phase- 11L:13D, 17±1 °C) in thermostatically-controlled environmental chambers (Aquar Envo, SVI, India) of 250 ltr capacity. Experiments lasted for six weeks during both the early as well as late post-spawning phases of the reproductive cycle. During these phases the following batches were maintained:

Initial control - (IC)
Natural photoperiod and temperature - (NPNT)
Natural photoperiod and high temperature (30±1 °C) constantly for 24 h of day/night cycle - (NPHT)

Long photoperiod (13L:11D) and natural temperature (early post-spawning phase- 22±1 °C and Late post-spawning phase – 17±1 °C) - (LPNT)
Long photoperiod (13L:11D) and high temperature (30±1 °C) - (LPHT)

6 h thermopulse of 30±1 °C given at 0600 h under long photoperiod (13L:11D)
6 h thermopulse of 30±1 °C given at noon 1200 h under long photoperiod (13L:11D)
6 h thermopulse of 30±1 °C given at 1800 h under long photoperiod (13L:11D)
6 h thermopulse of 30±1 °C given at 0000 h under long photoperiod (13L:11D)
12 h thermopulse of 30±1 °C given at 0600 h under long photoperiod (13L:11D)
12 h thermopulse of 30±1 °C given at 1800 h under long photoperiod (13L:11D)

At the end of experiments, 5 females from each batch were weighed and sacrificed. Blood was collected by caudal puncture in heparinized glass tubes. Plasma was obtained by centrifuging the blood samples at 3000 rpm in a cooling centrifuge. Ovaries were dissected out and weighed to the nearest mg to calculate gonadosomatic index (GSI). Plasma levels of testosterone (T, ng/ml) and oestradiol-17β (E2, ng/mL) were estimated by radioimmunoassay (RIA) technique. Data were expressed as mean ±SEM. Two-way ANOVA supplemented with Tukey’s test at 95% confidence limit was employed to ascertain the significant difference between means.

Results

During the late post-spawning phase, exposure of female *C. batrachus* to both long photoperiod and high temperature, separately, induced increase in all the studied parameters i.e. GSI, T, and E2, but the effect of high temperature on the studied parameters was more prominent as compared to the effects of long photoperiod (Table 1). The thermopulses of...
30±1 ºC (6 h and 12 h) were also effective in augmenting the gonadal activity. The 6 h thermopulses given at 0600 h and 1200 h increased GSI, T and E\textsubscript{2} moderately. Notably, 12 h thermopulse given in the morning at 0600 h induced the remarkable increase in GSI, T \& E\textsubscript{2} when compared to 6 h pulse given at 0600 h or 1200 h or even to the fish exposed to the constant high temperature under long photoperiod. During the early post-spawning phase, however, none of the photothermal manipulations could change the GSI and T in \textit{C. batrachus}. Estradiol was not detected in the catfish of any batch during this phase (Table 1).

**Discussion**

The present study demonstrates a season-dependent induction of gonadal recrudescence by high temperature under long photoperiodic regime in a freshwater catfish, \textit{C. batrachus}. Precocious ovarian maturation has also been demonstrated in \textit{H. fossilis} on exposure to high temperature of 30 ºC for 60 days\textsuperscript{4}. The failure of stimulation of gonadal activity by the similar photothermal manipulations during the early post-spawning phase in present catfish suggests that the induction of reproductive activity by the changing photoperiod and temperature is not possible in just-spawned catfish, perhaps due to the existence of a fixed gonado-refractory period in its reproductive cycle. The exposure of post-spawned \textit{C. batrachus} to the gradual declining pattern of natural photoperiod and temperature during the early post-spawning phase (October and November) appears necessary to abolish the inherent refractory phase. Because the exposures of \textit{C. batrachus} to the same high temperature under long photoperiodic regime during the late post-spawning phase (January and February) increased GSI as well as rise in levels of T \& E\textsubscript{2} in plasma, and so induced gonadal activity. During this phase, catfish had experienced the increasing trend in natural photoperiod and temperature for a considerable period of time before exposed to long photoperiod and high temperature either constantly or in form of thermopulses. Gonado-refractory phase has also been reported in \textit{H. fossilis}\textsuperscript{4}. These authors have rather reported degeneration in gonadal morphology following the treatment with constant high temperature (30 ºC) during the post-spawning phase. But in their experimental condition, exposure of \textit{H. fossilis} to high temperature covered both early as well as late post-spawning phase and hence the delineation of the period for responsiveness to high temperature cannot be inferred. Further, relatively less gonadal stimulation by long photoperiod under natural temperature during the late post-spawning phase establishes importance of photoperiod also in promoting ovarian activity in this sub-tropical catfish. In fact, close interaction between photoperiod and temperature (due to photoperiod-linked increase in temperature in nature) makes it difficult to delineate their distinctive effects on gonadal recrudescence. Nevertheless, long photoperiod potentiate the effects of warm temperature on gonadal development in fishes. In fact, every seasonally breeding temperate/subtropical fish, whether monsoon breeder or autumn or spring breeder, needs a set of proximate factors, most importantly photoperiod and temperature, to trigger gemetogenesis. Although the nature and degree of response to these proximate factors vary widely in fishes depending on their ecological niche and their inherent physiological make-up. Warm temperature under long photoperiod has been reported to augment the gonadal development in other teleosts also, such as \textit{Notropis bifreniatus}\textsuperscript{9}, \textit{Gasterosteus aculeatus}\textsuperscript{10-12}, \textit{Oryzias latipes}\textsuperscript{13,14}, \textit{Tilapia}\textsuperscript{15}. However, in \textit{Gillichthys mirabilis} low temperature regardless of photoperiod has been shown to stimulate gonadal activity\textsuperscript{16}. Thus, the understanding of pattern and direction of photothermal exposure of the fish prior to experimental condition is of paramount importance before the application of any altered photothermal conditions for the gonadal recrudescence in fishes. In \textit{Trichogaster trichopterus}\textsuperscript{17} exposure to higher temperature of 27 ºC resulted in a higher percentage of vitellogenic oocytes as well as higher mRNA levels of brain GnRH compared to that at 23 ºC. Significant increases in GSI and plasma E\textsubscript{2} level coinciding with the higher expression of GnRH gene have been demonstrated under long photoperiod in \textit{Odentothetes bonariensis}\textsuperscript{18}. Recently, the elevated water temperature has been shown to stimulate the expression of LH and FSH genes in pijerrey fish, \textit{Odontotesthes bonariensis}\textsuperscript{8}. Thus, in the light of these reports, it may be suggested that the photothermal stimulation of gonadal activities in \textit{C. batrachus} might have been mediated through neuro-endocrine pathways.

Moreover, it is also notable that the thermopulse of 30±1ºC for 12 h given in the morning is equally effective in stimulating gonadal activities to that of the stimulation obtained after exposure to constant high temperature during the late post-spawning phase. Morning or noon hour pulse of 6 hrs are also effective,
but of lesser magnitude. Evening hour thermopulses were not effective in ovarian activation. Thus, these findings further suggest that there is a thermo sensitive window in 24 h day/night cycle, during which this catfish respond to temperature effectively. The other part of the day/night cycle is non-sensitive to temperature. Perhaps, this diurnal basis of thermal stimulation of gonadal activity allows the temporal interaction of thermocycle with photocycle, which determines the nature and degree of impact on gonadal recrudescence in fish. Though the diurnal basis of photo-stimulation of gonads is very well established in Gasterosteus aculeatus, Orizias latipes, Rhodeus oscellatus, Oncorychus mykiss, basis of timed thermal gonadal stimulation yet not clear in fishes. However, the possible mechanism lying behind this circadian rhythm of photo-thermal interaction may be the timed stimulation of neuroendocrine secretions of hypothalamo-hypophyseal-gonadal axis within the day night cycle. Confirmation of this possibility invites further studies. Further, during the early post-spawning phase the oestradiol could neither be measured in the circulation of the control C. batrachus nor in those exposed to different photothermal conditions, however, testosterone could be detected. Presence of considerable amount of plasma testosterone during this phase, suggests the role of testosterone in some other physiological activities.

Lastly, it can be summarized that the present fish do require high temperature and long photoperiod as proximate factors to trigger the gonadal activity. An inherent gonado-refractory period also exits in just-spawned catfish, C. batrachus during which this fish does not respond to proximate factors. The time-measuring mechanism involved in thermal responses appears to be based on circadian rhythm sensitivity to temperature.

References
13. Yoshioka H, On the effects of environmental factors upon the reproduction of fishes. 1. The effects of day-length on the reproduction of the Japanese Killifish, Orzias latipes, Bull Fac Fish Hokkaido University, 13 (1962) 123.
14. Yoshioka H, On the effects of environmental factors upon the reproduction of fishes. 2. Effects of short and long day-length on Orzias latipes during the spawning season, Bull Fac Fish Hokkaido University, 14 (1963) 137.