PHOTO THERMAL INDUCED EFFECTS ON TESTES AND PITUITARY GONADOTROPIC CELLS DURING RESTING PHASE OF REPRODUCTIVE CYCLE IN A MURREL, *CHANNA PUNCTATUS* (BLOCH).

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ABSTRACT

Effects of various combinations of photoperiod and temperature (NL-NT, LD 15:9-28C, NL 28C and LD 15:9 NT) were studied on testicular activity and pituitary gonadotropic cells in *Channa punctatus* during resting phase of reproductive cycle. Long photoperiod (LD 15:9-28°C) and warm temperature (NL- 28°C) regimes were found to be more effective for testicular maturation and secretory activity of gonadotropic cells suggesting testicular maturation via brain- pituitary-testicular axis.

Key words: Photoperiod, Temperature, Testes, Gonadotropic cells, Resting phase, Channa punctatus.

INTRODUCTION

Like other vertebrates, in fish also hypothalamo-hypophysial system form a major link between environment and organ of reproduction. Environmental factors trigger internal reproductive mechanism into action via secretions of brain-pituitary gonadal axis which synchronize reproductive process in teleosts (Peter, 1981; Okuzawa et al., 1989; Munro, 1990; Sumptor, 1990; Bromage et al . 1993; Carrillo et al., 1993; Thomas and Arnold, 1993; Srivastava and Singh, 1993; Kumari and Dutt, 1995; Lin et al., 1996; Dabrowski et al., 1996; Goos et al., 1999; Kirschbaum, 2000; Amano et al., 2004; Quintana et al., 2004; Bhattacharya et al., 2005). Therefore, manipulation of environmental factors such as photoperiod and temperature attracted the attention of a number of fish culturists to modify the speed of maturation and spawning time in commercially important fish. Therefore, in the present investigation effects of various combinations of photoperiod and temperature were studied on the testes and gonadotropic cells of the pituitary gland in *Channa punctatus* during resting phase.

MATERIAL AND METHODS

Specimens of adult *Channa punctatus* (47.75g; 16cm) were collected from the local ponds at Ballia (25.522' N, 848' E) during resting phase (December) of the reproductive cycle and maintained in glass aquaria. They were acclimated to the laboratory conditions for 15 days under natural photoperiod and ambient temperature. The aquaria water was exchanged daily and the fish were fed *ad libitum* with wheat flour pellets and ground dried shrimps. Finally, healthy fish were selected for experiments. Specially constructed light proof aquaria (60x60x30cm) were used to expose the fish to different

photoperiod regimes. The top of each aquarium was covered with wooden lid which was fitted with a 20-W fluorescent cool daylight tubes controlled by electric timer (AMF, Vennerette, MK 11 AT 55). The light intensity above the surface of the water was 400 lux. The water temperature (±2°C) was maintained by thermostatic heaters. The fish were maintained to following experimental groups for 40 days.

Group A : Control : natural photoperiod and temperature (NL:NT)

Group B : 15h light : 9 h dark photoperiod at 28°C (LD 15:9 28°C)

Group C : 15h light : 9h dark photoperiod at natural temperature (LD 15:9-NT)

Group D : Natural light at 28°C (NL-28°C)

The water in the aquaria was renewed daily during light period with fresh dechlorinated water. The fish were fed daily *ad libitum* in the evening (16:00 to 18:00h). At termination of the experiment fish from each group (10 fish/group) were sampled, weighed and killed by decapitation. The brain along with pituitary gland and testes were quickly dissected out and fixed in aqueous Bouin's fluid. The testes were weighed to assess the variation in gonadosomatic index (GSI=testes weight / body weight x 100). After usual dehydration and embedding sections of testes and pituitary glands were cut at 5-6u. Testes were stained with hematoxylin/ eosin and pituitary glands were stained with Heidenhain's azan and Periodic acid Schiff with orange G (PAS-OG). Percentage of different types of maturation stages of testes were determined from the sections of the testes in each group. Nuclear measurements of pituitary gonadotropic cells were taken with the aid ocular micrometer. 100 nuclei were measured from each group. The student's 't' test was used for statistical calculations. The activity of gonadotropic cells has been determined by the changes occurred in the cytoplasmic granulation and the nuclear size.

To test the effects of photoperiod and temperature on testicular maturation, statistical analysis was done by the analysis of variance (Snedecor and Cochran, 1971). The mean difference between GSIs of different groups were compared with the help of critical difference.

RESULTS AND DISCUSSION

The experiment was started on 2nd December, 2003 (resting period) and terminated on 11th January, 2004.

Testes

Analysis of variance: The analysis of variance of testicular GSIs indicates that the observed value of the variance ratio ($F_{3, 36}$=3.5770796) between the control and experimental groups was highly significant (P<0.05) and shows marked contribution of photoperiod and temperature on testicular recrudescence and maturation.

Suitability of treatments for testicular GSI

The mean testicular GSI of all groups during resting phase were arranged and difference between consecutive values were estimated and compared with critical difference (Fig 1 and Table I). The mean testicular GSIs in all the groups of fish show insignificant difference (P<0.05) from each other as evaluated by critical difference (0.0265104). However, histologically each group exhibits marked differences from each other (Fig. 1).

Table I: Effects of various combinations of photoperiod and temperature on testicular GSI and pituitary gonadotropic cells during resting phase in *C.punctatus* (exposure time 40 days)

Photoperiod temperature	No. of fish	GSI (Mean±SE)	Gonadotropic cells		
			Nuclear Size (μm) (Mean ±S.E.)	Cytoplasm	Staining reaction with PAS/Azan
Control	10	0.100±0.003	2.50±0.12	Fine granulation	+
LD15:9-28°C	10	0.118±0.007	† 3.65±0.08	Dense granulation	+++
LD 15:9-NT	10	0.110±0.004	2.85±0.01	Increased granulation	++
NL-28°C	10	0.125±0.005	† 3.70±0.08	Dense granulation, vacuolisation	+++

Critical Difference (C.D.) at 0.05 level = 0.0265104

†. Significant ($P<0.05$)

\+ Positive; ++ fairly positive; +++ strongly positive

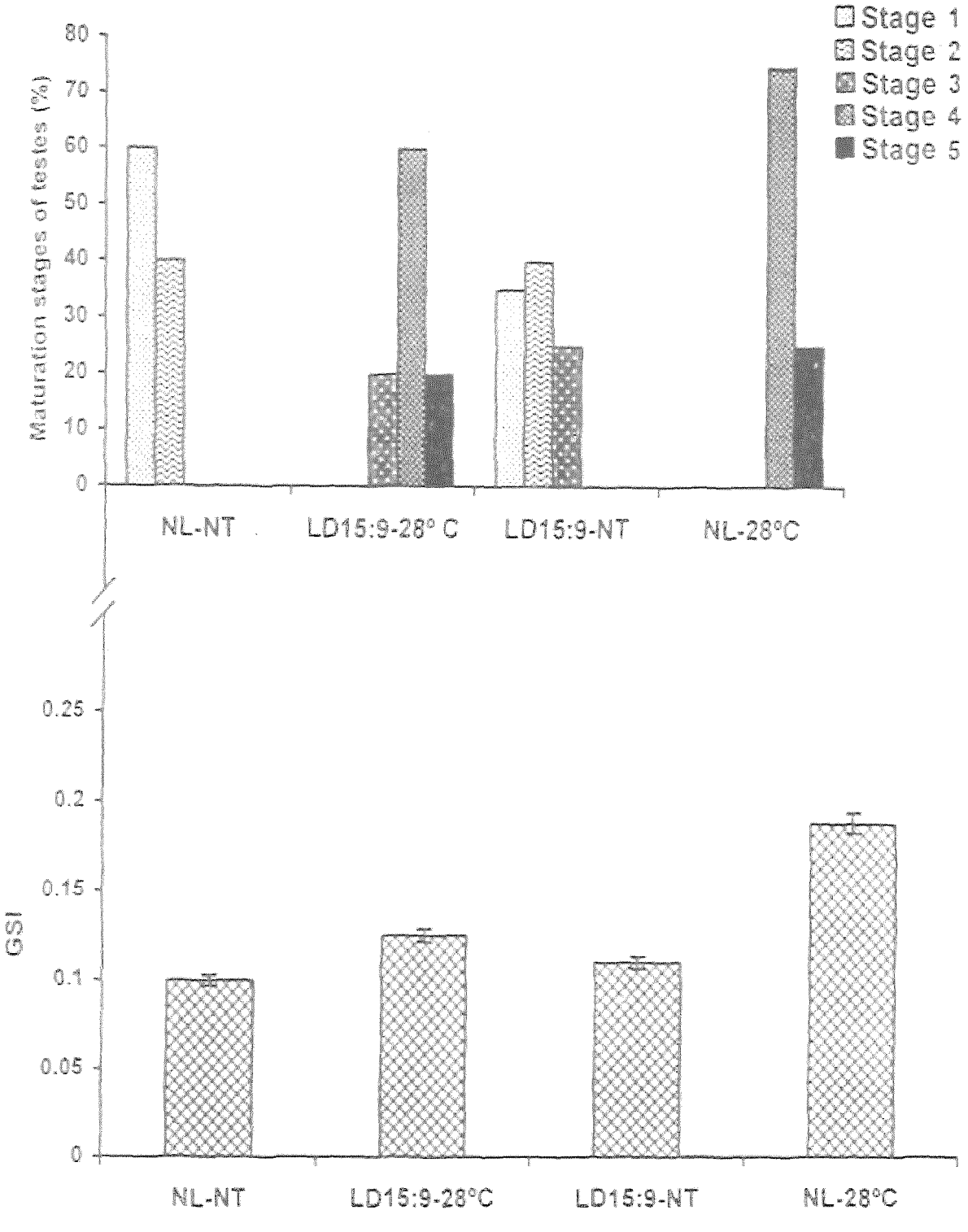
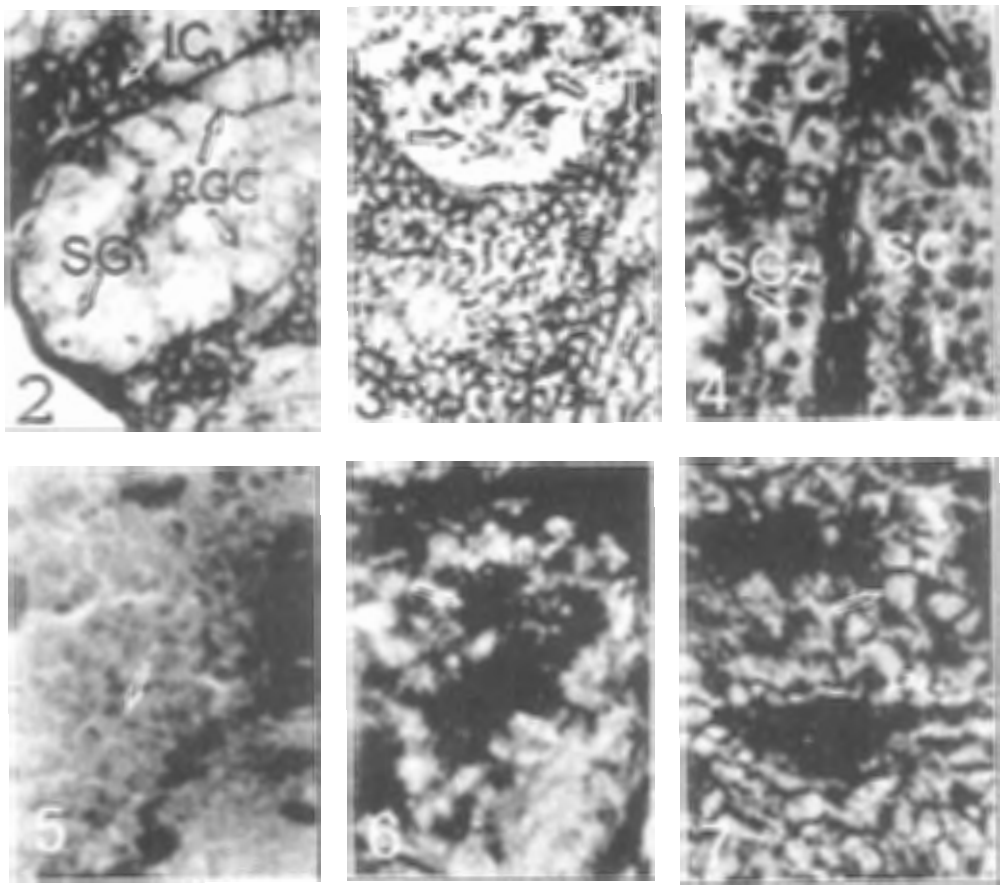


FIG. 1

LEGENDS

Fig. 1 : Effects of various combinations of photoperiod and temperature on testicular GSI and percentage distribution of maturation stages of testes in *C.punctatus*.



Photomicrographs of transverse sections of the testes and gonadotropic cells (C_3) of pipuitary gland of *C. punctatus* showing:

Fig.2 : Resting germ cells (RGC) and primary spermatogonia (SG_1) and interstitial cells (IC) in controls. H/EX1000.

Fig.3 : Partially empty lumen with spermatids (ST), spermatozoa (arrows) and interstitial cells (IC) in LD 15:9 28C group. H/E X 1000.

Fig.4 : Lumen with few secondary spermatogonia (SG_2) and spermatocytes (SC) in LD 15:9 NT group. H/E X 1000.

Fig.5 : Poor cytoplasmic granulation (arrows) of C_3 cells in controls. Heidenhain's azan X 1000.

Fig.6 : Heavy cytoplasmic granulation (arrows) in LD 15:9 - 28C group. PAS X 1000.

Fig.7 : Degranulation and vacuolisation (arrows) in NL -28C group. PAS X 1000.

Histological studies and distribution of germ cells

On the basis of distribution of testicular germ cells six stages of testes have been recognized.

1. **Quiescent stage**: seminiferous lobules are small with thick interlobular septa and mostly consist of resting germ cells and few primary and secondary spermatogonia;

2. **Mitotic stage:** having more spermatogonia and mitotic figures are observed in many spermatogonia;

3. **Meiotic stage**: seminiferous lobules are larger and consist of spermatocytes;

4. **Prespawning stage:** seminiferous lobules are very large and contain spermatocytes, spermatids and scattered spermatozoa in their lumen;

5 **Spawning stage**: seminiferous lobules show either empty lumina or lumina with spermatids or spermatozoa;

6. **Post spawning stage**: seminiferous lobules are small and mostly empty or containing residual masses of spermatozoa.

The testicular histology of all the fish of control group revealed the testes in stage 1 and 2 of spermatogenesis (Fig.1 & 2). In the testes of fish exposed to LD15:9 28°C and NL-28°C regimes spermatogenic responses were maximum and testes were mostly in stage 4 and 5 of spermatogenesis (Fig. 1 & 3). Testes of fish exposed to LD 15:9 NT regime were in stage 1, 2 and 3 (Fig.1, 4 & Table I).

Pituitary gonadotropic cells

The gonadotropic cells in the control group exhibit homogeneous cytoplasm stained lightly with PAS and azan with distinct nucleus (Fig. 5 and Table I). Fish exposed to LD15:9 28°C and NL-28°C regimes showed marked degree of hypertrophy in the gonadotropic cells with heavy cytoplasmic granulation and stained darkly with PAS and azan (Fig. 6 and Table I). Nucleus also show significant increase in size. Few cells show beginning of degranulation and vacuolisation (Fig. 7). However, gonadotropic cells of fish exposed to LD15:9-NT regimes also exhibited hypertrophy in comparison to control group but less than LD15:9- 28°C and NL-28°C regimes (Table I).

In the present study, testicular activity shows a close correlation with gonadotropic cells and seems to be influenced by photoperiod and / or temperature. In the fish exposed to LD15:9-28°C and NL-28°C regimes, the gonadotropic cells exhibited increased synthetic and secretory activity as evident by heavy granulation, degranulation and vacuolisation of their cytoplasm which is in conformity with prespawning and spawning stages of testes, whereas, fish exposed to LD15:9 - NT regimes, the gonadotropic cells and testicular activity also displayed stimulatory influence to some extent.

The synthetic and secretory activity of gonadotropic cells correlates well with the testicular activity, *i.e.,* prespawning and spawning phase of testicular cycle. Increased/ decreased activity of testes and gonadotropic cells under long/ short photoperiod at warm temperature regimes were also reported in many teleosts (Peter, 1981; Billard, 1985; Srivastava and Singh 1992, 1993; Kumari and Dutt, 1995; Dabrowski et al., 1996; Lin et al., 1996; Alok et al.1998; Amano et al., 2004; Quintana et al., 2004; Bhattacharya et al., 2005).

It seems that in *C.punctatus* long photoperiod and/or high temperature augmented synthesis and release of gonadotropins resulting testicular maturation via brain pituitary testicular axis as also suggested in many teleosts (Peter, 1981; Breton and Billard 1984; Razani et al., 1988; Goos et al., 1999; Amano et al., 2004; Quintana et al., 2004).

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