Asian Fisheries Science 5(1992):231-240.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1992.5.2.008

Effects of Constant Photoperiod-Temperature Regimes on the Testicular Activity of *Channa punctatus*

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Abstract

Channa punctatus exhibits two spawning periods in a year (early April and August-September). Effects of long (16 hours) and short (8 hours) daily exposure to light at constant temperature (30°C) on the testicular activity of *C. punctatus* were studied over one year. The 16-hour light regime stimulated testicular maturation, advanced maturity by two months and prolonged the spawning period. The 8-hour light regime inhibited testicular maturation and caused testicular regression. The analysis of variance of testicular GSIs revealed the significant contribution of both photoperiod and temperature for testicular maturation.

Introduction

The role of environmental factors in regulating the sexual cycle of male fish is not extensive (Bullough 1939; Harrington 1956, 1957; Wibe 1968; Breton and Billard 1977; Borg 1982a, 1982b; Bourlier and Billard 1984; Garg and Sundraraj 1985; Skarphedinsson et al. 1985). In the present study, an attempt has been made to study the effects of constant photoperiod-temperature regimes on the testicular activity during the annual reproductive cycle in *Channa punctatus*.

Materials and Methods

Samples of male *C. punctatus* were collected in July 1984 from local ponds. The experiments were conducted in Ballia (25.5°22'N, 84°8'E), where the average day length varies from 10.5 hours in

December to 13.5 hours in June and the average air temperature varies from 17°C in January to 38°C in June. Fish were acclimated to laboratory conditions under natural photoperiod and temperature prior to the initiation of the experiment.

Fish were maintained in light-proof 995-l plastic tanks under the following groups (200 fish/tank) for one year with monthly sampling:

Group A: Control: natural photoperiod and temperature.
Group B: 16 hours light: 8 hours dark photoperiod (LD 16:8) at 30°C.
Group C: 8 hours light: 16 hours dark photoperiod (LD 8:16) at 30°C.

Illumination was provided by 40-W fluorescent tubes. Light intensity was 400 lux at the water surface. The water temperature (± 2°C) was maintained by thermostatic heaters. The fish were fed daily with dry shrimps (*Macrobrachium lamarrei*) *ad libitum*.

Monthly sampling was done in each group (10 fish/group). Body and testes weights were recorded to assess the variation in gonadosomatic index (GSI = testes weight/body weight x 100). Testes were fixed in Bouin's fluid for histological studies. Sections were stained with hematoxylin/eosin.

To test the effects of photoperiod and temperature on testicular maturation, two-way analysis of variance (ANOVA) was used (Snedecor and Cochran 1971).

Results

Testicular Cycle

C. punctatus exhibited marked seasonal changes with two spawning periods over the years. The annual testicular cycle was divided into four phases viz.:

1. Preparatory phase (late November-February and May-June): in the early part of this phase, testicular lobules display resting germ cell (diameter 16.5 μm) and spermatogonia (primary and secondary diameter 12.6 and 8.7 μm, respectively). Later, lobules are characterized by growing spermatocytes (primary and secondary diameter 8.1 and 6.4 μm, respectively).

2. Prespawning phase (March and July): testicular lobules contain either spermatocytes or spermatids (diameter 2.7 μm).

Scattered spermatozoa (diameter 1 µm) were also encountered in few of the lobules.

3. Spawning phase (early April and August-September): testes display either empty lumina or lumina with spermatids/spermatozoa. Spermatozoa are thinly scattered in lobules of the testes and never form a dense cloud as previously reported in *C. striatus* (Swarup and Srivastava 1978) and *C. marulius* (Swarup and Srivastava 1979).

4. Postspawning phase (late April and October-early November): the lumina of the testicular lobules are mostly empty; some exhibit residual masses of spermatozoa. Regressed testes are characterized by disorganization of the testicular lobule, degeneration of germ cells, heavy vascularization, appearance of vacuoles and formation of collagenous capsules in the lobules.

GSI

The analysis of variance of testicular GSI indicates that the observed value of variance ratio (F2, 22=10.90646) between treatments was highly significant, showing the significant contribution of photoperiod and temperature on testicular GSI (Table 1).

Table 1. Analysis of variance of testicular GSI in *Channa punctatus*.

Sources of variations	D.F.	Sum of squares	Mean sum of squares	F ratio
Treatments	2	0.022993	0.0114965	10.90646*
Months	11	0.0185149	0.00168331727	1.5967175**
Error	22	0.023191	0.0010541363	
Total	35	0.0646989		

*Significant at 0.01 level of significance.
**Insignificant.

The value of the variance ratio (F 11,22 = 1.5967175) between months was insignificant, indicating insignificant effects of seasons on testicular GSI (Table 1).

The mean testicular GSI values in the two treatments (0.15875 and 0.11767) were not significantly different. However, the mean

testicular GSI in the LD 16:8 treatment was significantly different from the control (0.09658), whereas the mean GSI in the LD 8:16 treatment was not.

The monthly GSI values for the LD 16:8 treatment were significantly higher than the control except in the last two months (June-July); while for the LD 8:16 treatment, insignificant differences from the control were found in August, February-March as well as in June-July (Fig. 1).

Marked histological differences were observed in the testicular activity of the three groups of fish (Table 2). Testes of all fish in the control as well as of experimental groups were in the spawning phase in August which was observed till September in the majority of the fish in the control group and in the LD 16:8 treatment. Spent or regressed testes were encountered during October in the control group and in LD 16:8 treatment; and September-January in the majority of fish in LD 8:16 treatment. Testicular recrudescence was observed in November-March in the control group, November-December in LD 16:8 treatment and February-March in LD 8:16 treatment. All the fish in the control group and in LD 8:16 treatment exhibited spermatozoa in late March and early April; whereas in LD 16:8 treatment, spermatozoa were observed during January-April. Spawning/spent testes were encountered in late April and early May in the control group as well as in LD 8:16 treatment and January-May in LD 16:8 treatment. Testicular recrudescence was again observed during late May-July in the control group and in LD 8:16 treatment and June-July in LD 16:8 treatment. In July, testes of all the fish in the control and treated groups were characterized by spermatozoa (prespawning phase). However, the rate of testicular maturation and formation of spermatozoa was higher and faster in the LD 16:8 treatment than in the controls and LD 8:16 treatment.

Testicular regression was observed any time during the year in fish maintained under both the fixed photoperiod groups, but was more frequently observed in the LD 8:16 treatment.

Distribution Of Germ Cells In The Testes

The distribution of germ cells is illustrated in Fig. 2. Early spermatogenic cells (spermatogonia) were frequently observed throughout the year in all the groups. However, their percentage varied greatly in the three groups. In the control group and the LD:

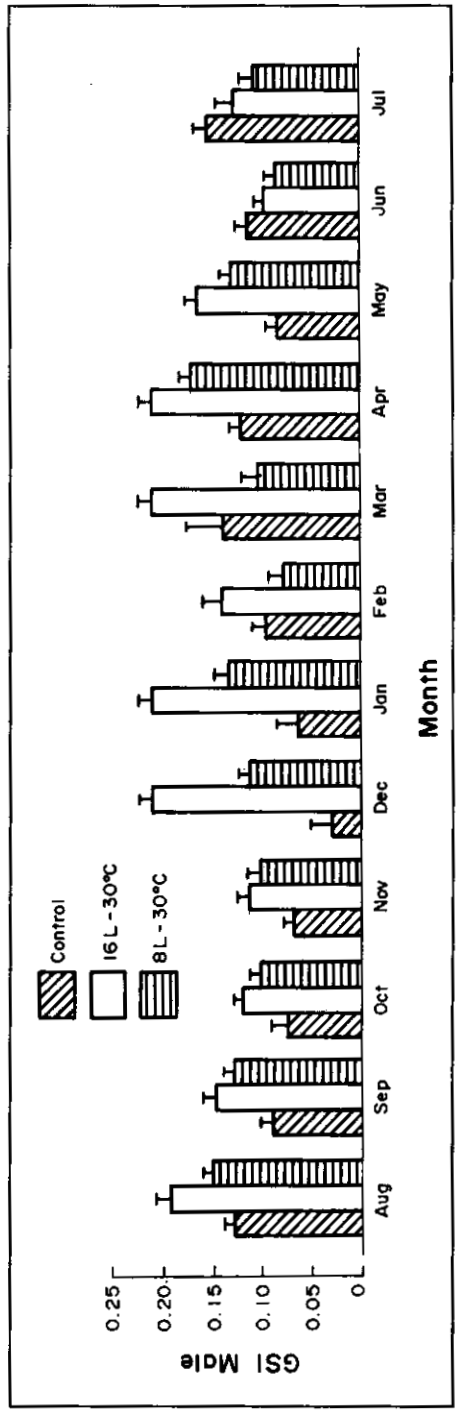

Fig. 1. Effects of constant photoperiod-temperature regimes on the testicular GSI in *Channa punctatus* during annual reproductive cycle (Vertical bars represent ±SE).

Table 2. Effects of constant photoperiod-temperature regimes on testicular histology during the annual reproductive cycle in *Channa punctatus*.

Months	Percentage of testes in different phases								
	Control			LD 16:8, 30°C			LD 8:16, 30°C		
August	Sp.	100	(10)	Sp.	100	(10)	Sp.	100	(10)
September	Sp.	80	(8)	Sp.	60	(6)	Sp.	30	(3)
	S.	20	(2)	S.	40	(4)	S.	70	(7)
October	S.	100	(10)	Sp.	30	(3)	S.	100	(10)
				S.	70	(7)			
November	Pr.	100	(10)	Pr.	100	(10)	Pr.	10	(1)
							S.	90	(9)
December	Pr.	100	(10)	Pr.	100	(10)	Pr.	30	(3)
							S.	70	(7)
January	Pr.	100	(10)	Ps.	40	(4)	Pr.	30	(3)
				Sp.	60	(6)	S.	70	(7)
February	Pr.	100	(10)	Sp.	100	(10)	Pr.	100	(10)
March	Ps.	100	(10)	Sp.	100	(10)	Ps.	100	(10)
April	Sp.	40	(4)	Sp.	80	(8)	Sp.	60	(6)
	S.	60	(6)	S.	20	(2)	S.	40	(4)
May	Pr.	80	(8)	S.	100	(10)	Pr.	40	(4)
	S.	20	(2)				S.	60	(6)
June	Pr.	40	(4)	Pr.	20	(2)	Pr.	100	(10)
	Ps.	60	(6)	Ps.	80	(8)			
July	Ps.	100	(10)	Ps.	90	(9)	Ps.	100	(10)
				Sp.	10	(1)			

Pr. = Preparatory phase; Ps. = Prespawning phase; Sp. = Spawning phase; S. = Spent/regressed; figures in parentheses indicate number of fish.

8:16 treatment, their percentage was generally greater than in the LD 16:8 treatment. Advanced stages (spermatocytes, spermatids and spermatozoa) were predominantly observed during August, March-April and June-July in the control group; August-November, January-April and June-July in LD 16:8 treatment; and August-September, March-April and June-July in LD 8:16 treatment.

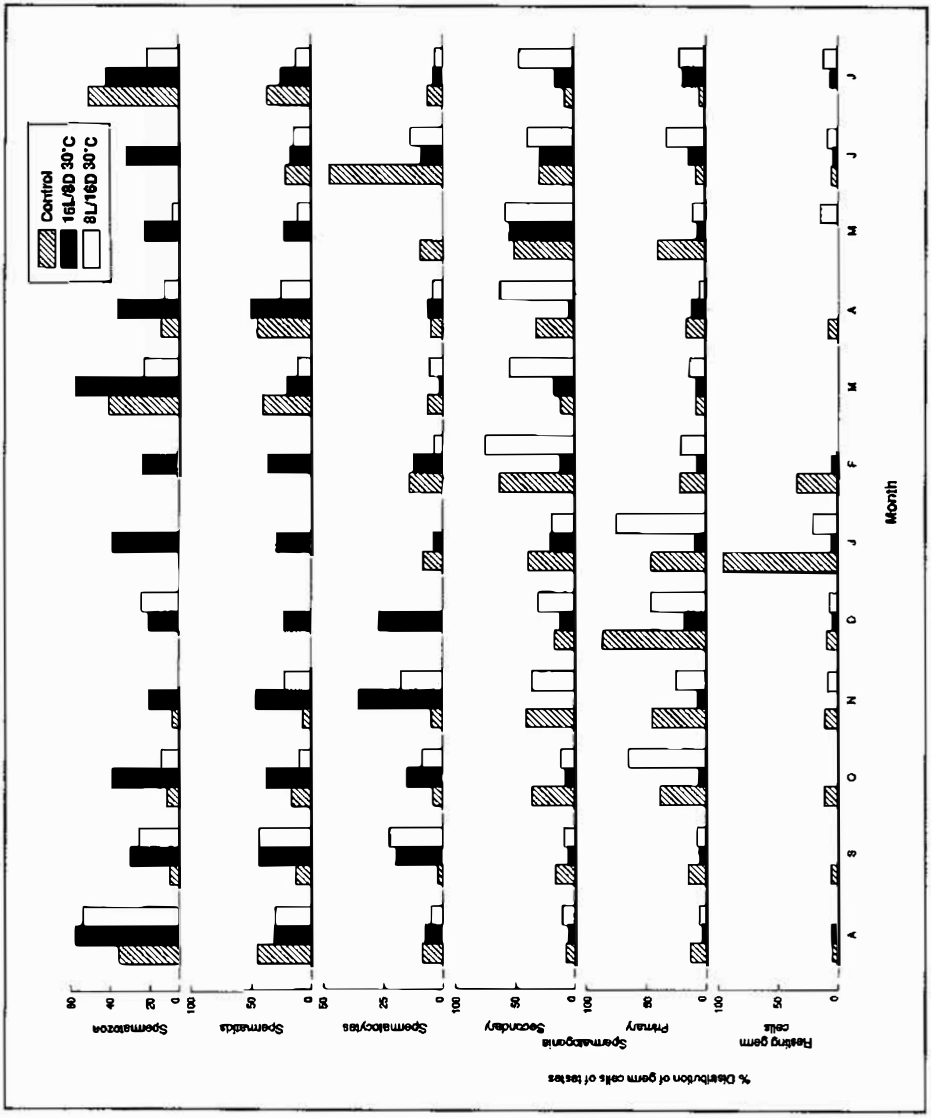

Fig. 2. Effects of constant photoperiod-temperature regimes on the percentage distribution of germ cells in *Channa punctatus*.

Discussion

The differences in mean GSI values of the LD 16:8 treatment and the control suggest that the long light regime in combination with warm temperature provides superior conditions for testicular maturation than short light regime at warm temperature regime and ambient conditions (control). However, the effect of different seasons (months) on testicular GSI was insignificant.

Histological studies also revealed stimulatory effects of the long light regime at high temperature on testicular maturation and stimulation of spermatogenic activity in *C. punctatus*, resulting in a longer spawning period. Similar photoperiod-induced changes have been reported by many workers (Kaya and Hasler 1972; de Vlaming 1975; Borg 1982b; Garg and Sundararaj 1985). The short light regime in combination with warm temperature also induced testicular maturation, but for a shorter period and finally resulting in testicular regression. In this study, a high frequency of regressed testes in the fish maintained in the latter treatment was observed any time during the year. This agrees with the observations of de Vlaming (1975) who also found that *Notemigonus*, a short photoperiod in combination with high temperature, suppressed both male and female gonads. Little information is available on the role of environmental factors controlling gonadal regression in teleosts. Breton and Billard (1984) stated that the effect was "probably due to suppression of early GtH cycle and also to direct effect of temperature on the gonad affecting steroidogenic activity."

As in female *C. punctatus* (Srivastava and Singh 1991), the spawning periods of male fish also remain undisturbed under constant photoperiods at warm temperature regimes. However, males matured two months earlier in the long light regime than in both the control group and in the short light regime. It seems that the seasonal cycle of sexual maturation in male fish is also regulated by an endogenous cycle. However, it is of different duration in males and females. Such sexual variation in endogenous timing in males and females was also suggested in brook trout, *Salvelinus fontinalis* (Pyle 1969; Poston and Livingston 1971).

The effects of constant photoperiods at warm temperature regimes on testicular activity in *C. punctatus* could be regulated by gonadotropin secretion as the activity of the gonadotropic cells of the pituitary gland is significantly greater in fish exposed to a long

light-warm temperature regime than those maintained on a short light-warm temperature regime (Srivastava and Singh, unpubl. data).

Acknowledgement

The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for financial assistance.

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Manuscript received 8 April 1991; revised ms received 16 October 1991; accepted 22 April 1992.