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Annual changes in serum calcium and inorganic phosphate levels and correlation with gonadal status of a freshwater murrel, *Channa punctatus* (Bloch)

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Abstract

Adult *Channa punctatus* murrels of both sexes (60-80 g) were collected locally from Ramgarh Lake during the second week of every month (10 individuals of each sex/month) throughout the year. Blood samples were collected and analyzed for serum calcium and phosphate levels by the methods of Trinder (1960) and Fiske and Subbarow (1925), respectively. Gonads were fixed to judge the state of maturation of the fish. Males exhibited no change in serum calcium levels throughout the year in correlation with testicular maturation. However, serum phosphate levels exhibited a rise in correlation with the increased gonadosomatic index. Females showed marked seasonal changes in serum calcium and phosphate levels which were associated with ovarian maturation (vitellogenesis).

Key words
- Calcium
- Inorganic phosphate
- Reproduction
- Teleost
- Vitellogenesis

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Introduction

Vitellogenesis involves hepatic formation of yolk proteins (vitellogenin) and raises blood vitellogenin content followed by deposition of two forms of yolk in the ovary: yolk vesicles (mucopolysaccharides) and yolk granules (phospholipoproteins). Several authors have correlated the increased blood calcium content with ovarian maturation (1-7). Estradiol has been shown to stimulate hepatic formation of yolk proteins (vitellogenins) and to raise serum vitellogenin levels and the protein-bound fraction of plasma calcium levels (8-19). However, few studies describe the relationship between blood phosphate level and gonadal maturation in fishes (7,20). Oguri and Takada (goldfish; 11), Woodhead (arctic cod; 12) and Singh and Srivastav (catfish; 7) have found no correlation between serum calcium level and testicular maturation.

In the present study an attempt was made to investigate changes in serum calcium and phosphate levels in relation to ovarian maturation (vitellogenesis) and testicular maturation in a freshwater murrel, *Channa punctatus*.

Material and Methods

Ten male and ten female adult *Channa punctatus* murrels (15-20 cm; 60-80 g) were collected during the second week of every month throughout the year. Body and gonad weights were recorded for each animal to assess the variation in gonadosomatic index (GSI): GSI = gonad weight/body weight x 100

Blood samples were collected under MS222 anesthesia by sectioning the caudal peduncle. Sera were separated by centrifugation and analyzed for calcium and phosphate levels according to the methods of Trinder (21) and Fiske and Subbarow (22), respectively. For histological studies gonads were fixed in Bouin's fixative. Sections were cut at 6 μm and processed with hematoxylin-eosin (HE).

To test the statistical significance, ANOVA, Student-Newman-Keuls and Dunnett multiple comparison tests were used.

Results

The annual gonadal cycle of *Channa punctatus* can be divided into five phases, as shown in Table 1.

Males

No change in serum calcium level was recorded throughout the year (Figure 1A) in relation to testicular cycle. However, serum phosphate level (Figure 1B) exhibited a rise corresponding to an increased GSI (Figure 1C).

Females

The lowest serum calcium levels were recorded during the resting phase (March and October), with values increasing thereafter and peaking in June and early January (Figure 1A) when the end of the prespawning phase occurs for both reproductive peaks. During the spawning and postspawning phases, serum calcium levels showed a progressive fall (Figure 1A).

Serum phosphate levels increased with increasing GSI (Figure 1B and C). The serum calcium and phosphate levels of male and female *Channa punctatus* during different reproductive phases are shown in Figure 2A and B.

ANOVA indicated that the seasonal differences in serum calcium and phosphate values of males were not significant, whereas

Table 1 - Annual gonadal cycle of *Channa punctatus*.

Reproductive phase	Testis	Ovary
Resting phase (March and October)	Primary germ cells, spermatogonia	Few immature oocytes
Preparatory phase (April-early May and November)	Primary spermatogonia and primary spermatocytes	Maturing oocytes containing increased amount of yolk
Prespawning phase (late May-early June and December)	Primary spermatocytes, secondary spermatocytes, few spermatids	Fully mature oocytes (with full amount of yolk), few maturing oocytes
Spawning phase (late June-July and late January)	Mostly spermatids, few empty lumina	Fully grown oocytes with few discharged follicles
Postspawning phase (August-September and February)	Empty lumen indicating discharge of spermatids, few primary germ cells	Discharged follicles and few immature oocytes

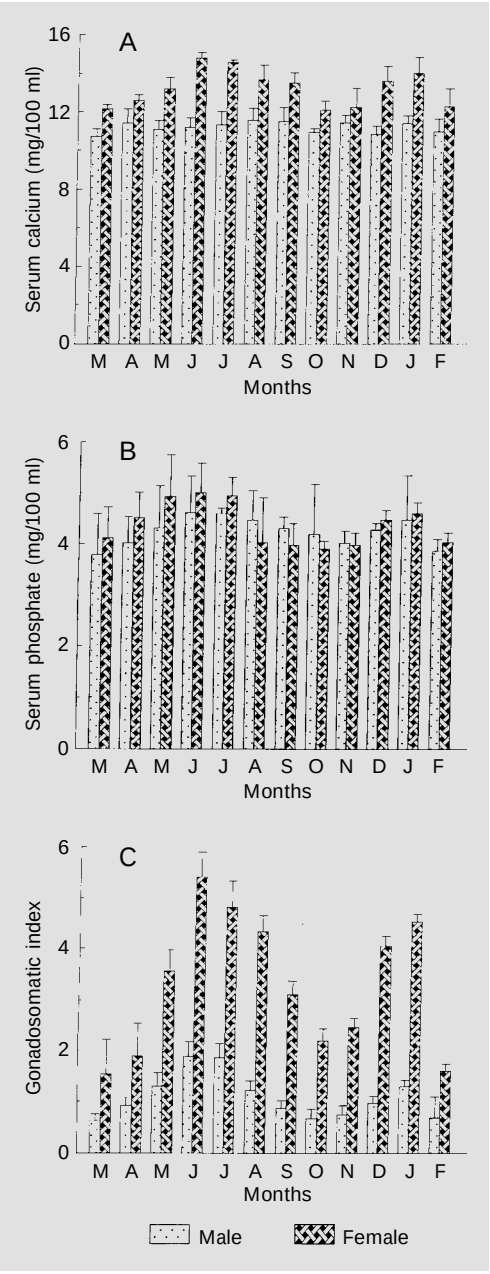

Figure 1 - Seasonal changes in serum calcium (A) and phosphate (B) levels and gonadosomatic index (C) of male and female *Channa punctatus*. Each value represents the mean ± SD of ten specimens.

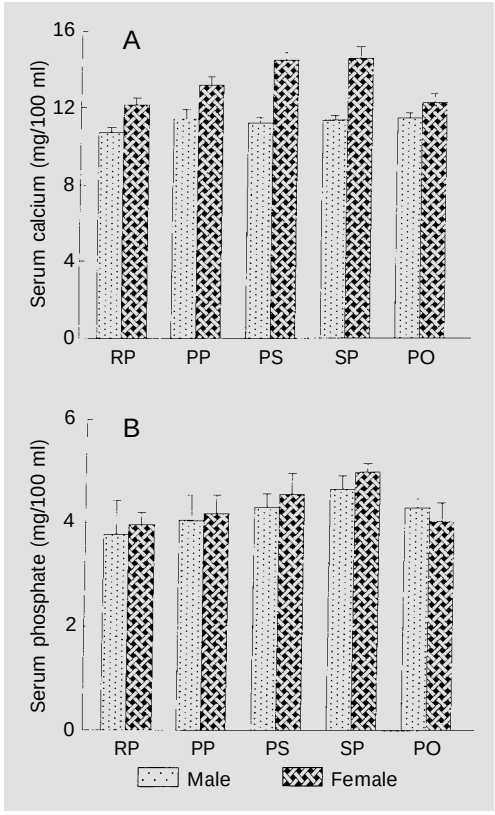

Figure 2 - Serum calcium (A) and phosphate (B) levels of male and female *Channa punctatus* during different reproductive phases. Each value represents the mean ± SD of ten specimens. RP = Resting phase; PP = preparatory phase; PS = prespawning phase; SP = spawning phase; PO = postspawning phase.

in females the serum calcium ($P<0.03$; $F = 5.162$) and phosphate values ($P<0.05$; $F = 4.74$) were significant. According to the Student-Newman-Keuls test, the serum calcium and phosphate levels of males and females showed no significant seasonal differences. According to the Dunnett multiple comparison test (considering resting phase as control), the serum calcium levels of preparatory, prespawning and spawning phases in females were significantly different from controls ($P<0.05$).

Discussion

In the present study, in agreement with reports by others, no correlation was found between serum calcium level and testicular maturation (6,7,9,11,20). In contrast to these reports, Woodhead and Woodhead (10) and Woodhead (12) have suggested a positive correlation between blood calcium level and testicular maturation in arctic cod and sea cod. Moreover, administration of testosterone propionate to male goldfish and killifish had no effect on blood calcium levels (23,24).

Bjornsson et al. (15) have reported that female rainbow trouts have higher levels of

protein-bound calcium than males from September to February. Balbontin et al. (20), however, recorded fluctuations in the serum calcium levels of both sexes of hake (*Merluccius gayi gayi)* throughout the year and reported that calcium levels remained relatively constant from December to August in killifish. The authors suggested that this difference between male hake and killifish may correspond to different patterns of sex hormone secretion.

In male *Channa punctatus*, serum phosphate levels seemed to increase with increasing GSI even though the statistical results were not significant. A similar correlation has also been reported earlier by Balbontin et al. (20) for killifish and by Singh and Srivastav (7) for *Heteropneustes fossilis*.

A marked seasonal variation in serum calcium level was observed in female *Channa punctatus*, associated with ovarian maturation (i.e., different phases of vitellogenesis), in agreement with earlier observations (5-7,9-12,15,20,25). Bjornsson and Haux (26) reported that free plasma calcium levels are not affected, but the increase in total plasma calcium is due to the appearance of the calcium-containing yolk protein precursor vittellogenin in plasma.

Nagler et al. (27) have reported a linear relationship between increasing levels of indirect indicators such as serum total phosphoprotein phosphorus, alkali-labile phosphoprotein phosphorus, total calcium and increasing level of serum vitellogenin in mature female rainbow trout (*Salmo gairdneri*). The enhanced secretion of estrogen during the sexual maturation of females increases the calcium level (6,28). Bailey (23), Ho and Vanstone (8), Fleming et al. (9), Chan and Chester Jones (14) and Woodhead (13) detected increased serum calcium levels in female fish after the administration of estradiol. In female fish estradiol administration increases serum calcium level, as also observed in males of *Carassius auratus* (23), *Fundulus kansae* and *Fundulus catenatus* (9), *Oncorhynchus nerka* (8), *Gadus morhua* (13) and *Fundulus heteroclitus* (24). Serum calcium levels fell during the spawning and postspawning phases in *Channa punctatus*, in agreement with the earlier investigations of Fontaine et al. (25), Swarup et al. (6) and Singh and Srivastav (7).

We conclude that there is no correlation between the serum calcium levels of male *Channa punctatus* and testicular maturation; however, serum phosphate increased with increasing GSI. In females, the serum calcium and phosphate levels exhibited a progressive increase with ovarian maturation.

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Announcement

1999 Award in honor of Fred L. Soper (1893-1976) for publications in the field of Inter-American Health

This is an announcement and call for submission of nominations for the 1999 award in honor of Fred L. Soper, former Director of the Pan American Health Organization (the World Health Organization Regional Office for the Americas) from 1947 to 1958.

In addition to his service with PAHO/WHO, Dr. Soper played a major role in the fight against yellow fever and other infectious diseases in Brazil as part of his work with the Rockefeller Foundation in the 1920s and 1930s and in the control of typhus in North Africa and Italy during the Second World War. He was one of the truly major figures of the century in inter-American health.

The Award is presented annually to the author or authors of an original scientific contribution comprising new information on, or new insights into, the broad field of public health, with special relevance to Latin America or the Caribbean or both. This may consist of a report, an analysis of new data, experimental or observational, or a new approach to analyzing available data. Preference is given to studies involving more than one discipline and to papers related to infectious disease, a life-long concern of Dr. Soper.

Only papers already published in scientific journals listed in the Index Medicus or in the official journals of the Pan American Health Organization are eligible for consideration. Furthermore, the Award is limited to contributions by authors whose principal affiliation is with teaching, research or service institutions located in the countries of Latin America and the Caribbean (including the Centers of the Pan American Health Organization).

The Award Fund is administered by the Pan American Health and Education Foundation (PAHEF), which receives voluntary contributions designated for the purpose and holds them in a separate fund. The Award consists of a suitable certificate and a monetary prize of US$1000.00. The winner(s) of the Award each year is nominated by an Award Committee, composed of representatives designated by PAHO and by PAHEF; final selection is made by the Board of Trustees of PAHEF.

Papers submitted by or on behalf of their authors may be considered for the Fred L. Soper Award. For purposes of the 1999 Award, only papers published during calendar year 1998 will be considered; all submissions must be received by 31 March 1999 at the following address:

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