Journal of Environmental Biology **January 2007,** 28(1) 137-139 (2007)

Tissue cholesterol and serum cortisol level during different reproductive phases of the female freshwater fish *Notopterus notopterus* (Pallas)

D.S. Shankar and R.S. Kulkarni*

Fish Endocrinology Research Unit, Department of Zoology, Gulbarga University, Gulbarga – 585 106, India

(Received: October 30, 2004 ; Revised received: June 29, 2005 ; Accepted: August 5, 2005)

Abstract: *In the present study tissue cholesterol and serum cortisol changes during two reproductive phases have been correlated in the freshwater fish Notopterus notopterus. The reproductive cycle of N. notopterus has two phases such as breeding phase (April – August) and non breading phase (October – December). The cholesterol content of the ovary and liver increased during breeding phase. The serum cortisol estimated by radio immuno assay (RIA) technique indicates that the level of the hormone was high during breeding phase compared to non breading phase. The increase in cholesterol during breeding phase may be because of increase in cortisol synthesis needed for ovarian growth and vitellogenesis. The gonadosomatic index (GSI) also increases during breeding phase.*

Key words: *Cortisol, Cholesterol, Reproductive phases, Notopterus notopterus*

Introduction

Cholesterol in the steroidogenic tissue has been shown to be associated with reproduction and its fluctuation in relation to maturity has been reported in the fish, *Etroplus suratensis* (Premjith *et al.*, 1992; Diwan and Krishnan, 1986), suggesting cholesterol being the precursor for the synthesis of steroid hormone influencing the maturation phenomenon.

Glucocorticoids can either inhibit or stimulate reproductive physiology depending on the timing of the annual cycle of species (Brann and Mahesh, 1991). The hormone cortisol plays a pivotal role in teleost stress response but relatively few species have been studied in depth with respect to the levels of cortisol in the blood under different stages of development and environmental conditions (Pottinger *et al.*, 2000). In addition, the level of cortisol in the blood of both unstressed and stressed fish is subject to modulation by a number of internal and external factors, such as sexual maturity (Pottinger *et al.*, 1995) genetic pedigree (Pottinger and Carrick, 2000), water temperature (Sumpter *et al.*, 1987) and salinity adaptation (Shrimpton *et al.*, 1994). The seasonal studies carried out in fish adrenal tissue in relation to reproductive activity indicate that the adrenal cells are hyperactive during breeding phase of the fish and also it is reported that cortisol is shown to interfere with reproductive function in mature and maturing rainbow trout (Chakraborti *et al*., 1987).

In the present study correlation of cholesterol content in relation to cortisol hormone level during breeding and non breading phases of the reproductive cycle in the female fish *Notopterus notopterus* has been undertaken.

Materials and Methods

Freshwater fish, *N. notopterus* was selected for the present study and was collected from a water body situated about 40 km from Gulbarga. About 50 live *N. notopterus* were collected and brought to the laboratory during breeding (April-August) and non breading (Oct.-Dec.) phases with the help of local fisherman. In the present investigation ovarian somatic index (OSI), hepatosomatic index (HSI), ovarian cholesterol and serum cortisol were measured during two breeding phases of the reproductive cycle.

Ovarian somatic index (OSI) and hepatosomatic index (HSI): The total ovarian, liver and body weights were taken by using the Anamed electronic balance for the determination of OSI and HSI by using following formula:

$$\text{Gonadosomatic index (OSI)} = \frac{\text{Weight of ovary}}{\text{Weight of fish}} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\text{Weight of liver}}{\text{Weight of fish}} \times 100$$

Cholesterol estimation: Cholesterol, in the ovary and liver was estimated by using the methods of Liberman and Burchard as described by Peters and Vanslyke (1946).

Hormone radioimmunoassay (RIA): Serum cortisol level was measured by Coat-A-Count method using a solid phase radioimmunoassay kit with ^{125}I cortisol (TC 02) purchased from DPC® diagnostic products corporation, 5700 west 96th street Los Angeles.

Statistical analysis: In all the cases six observations were made and the datas were expressed as arithmetic means with their standard deviation and subjected to student 't' test as described by Snedecore and Cochran (1967) and Fisher (1963).

Results and Discussion

The freshwater fish *N. notopterus* exhibits seasonal growth of gonads with two phases such as breading and non

***Corresponding author:** E-Mail: raghavendrakulkarni@rediffmail.com, Tel.:+91-8472-445513(O) + 91-8472-270479 (R) Fax: +91 08472 445632 / 445450.

Table - 1: Changes in GSI, tissue cholesterol and serum cortisol level in the female fresh water fish *N. notopterus* during four reproductive phases

Reproductive phases	GSI (OSI)	HSI	Ovarian cholesterol	Hepatic cholesterol	Serum cortisol μg/dL
Breeding phase	3.67***± 0.021	0.690***±0.018	7.931**±0.479	7.06**±0.322	10.58***±0.201
Non breeding phase	0.418^{NS}± 0.141	0.487^{NS}± 0.101	3.60***± 0.538	6.73***± 0.300	5.16*± 0.021

The values are expressed in mg/50g net weight of cholesterol in the tissue. Serum cortisol hormone are expressed 25 μg/dL of blood
All values are expressed as mean ± standard error (SE)n=6, * p<0.05, ** p<0.01, *** p<0.001, differs from the values of the each phases respectively

breading phases. The GSI of fish studied during two phases of the reproductive cycle increased and reached maximum during breeding phase while it reduced in non breeding phase (Table 1). The HSI of fish studied during two phases of the reproductive cycle (Table 1) indicates that during breeding phase HSI increased significantly while it reduced during non breeding phase.

A comparison made between GSI and HSI in a fish shows that increase of OSI and HSI during breeding phase indicates preparation of gonads for growth.

Ovarian hepatic cholesterol: The cholesterol content of the ovary and liver increased during breeding phase and maximum tissue content of cholesterol noticed during this phase (Table 1) may be because of increase in cortisol synthesis needed for ovarian growth and vitellogenesis during this phase. The ovarian cholesterol increased during breeding phase (April – Aug.) and hepatic cholesterol showed almost similar increase during breeding phase and reduced during non breeding phase indicating its availability for steroid hormone production needed for oocyte vitellogenesis.

Serum cortisol level: The cortisol estimation during two breeding phases of the reproductive cycle are presented in Table-1. The results indicated that in female fish cortisol level significantly increased during breeding phase and decreased during non breeding phase. The increase in cortisol during breeding phase in female fish relates to its involvement in the reproductive activity. In the breeding phase the fish starts developing gonad, in the ovary the vitellogenesis gets geared up utilizing hepatic contents and the oocytes begin growing to bigger size accumulating yolk. Thus this vitellogenic activity needs increased metabolism, since cortisol is a metabolic hormone, its increase during breeding phase indicates increased metabolic activity probably resulting in the diversion of energy for the vitellogenesis.

Studies pertaining to corticosteroid action on the gonadal responses are limited and the hormone cortisol produced from the fish interrenal gland (Kulkarni and Sathyanesan, 1982) is identified as an important metabolic hormone (Mommsen *et al*., 1999). The peak of seasonal activity of the interrenal tissue reportedly corresponds to the reproductive period (Verma and Mishra, 1992). These authors have noted hyperplasia of the interrenal tissue at the time of breeding in different fishes. In sockey salmon, *Oncorhynchus kisutch* the development of interrenal hypertrophy is directly related to ovarian maturation and suggested the existence of pituitary-interrenal-ovarian axis in the maturation of atleast few teleostean oocytes (McBride and Fagerlund, 1976). Sundararaj and Goswami (1971) have shown that the concentration of plasma cortisol increased approximately four fold after injection of either ovine luteinizing hormone (LH) or salmon gonadotropin (SG-GLOO) in the sexually regressed as well as gravid catfish. Overiectomy in the sexually regressed catfish does not prevent the LH induced rise in plasma cortisol levels, it therefore, appears highly probable that the plasma cortisol in the fish studied by the above authors is of interrenal origin and its synthesis and release are stimulated by gonadotrophins secreted by the pituitary gland. In the fish *N. notopterus* the adrenocortical cells and chromaffin cells are embedded inside the anterior portion of the kidney and exhibit seasonal activity showing active hypertrophied cells during breeding period.

Recently Haider (1997) has reviewed hormonal control of oocyte maturation in fish showing 17a, 20b-dihydroxy-4-pregnen-3-one (17a, 20b-DP) has been found to be the most effective maturation inducing substance in most of the teleostean species studied so far. In the Atlantic salmon, *Salmo solar* histological evidences has been presented implying seasonal changes in pituitary output of adrenocorticotropic hormone ACTH and interrenal secretion of corticosteroids and changes in plasma concentrations have been reported which is associated with smolting (Thorpe *et al*., 1987). The estimation of cortisol in *N. notopterus* also indicates that the hormone undergoes seasonal changes in the output in relation to reproductive phases. It is known that liver is involved in the regulation of ovarian growth by contributing the biochemical components to the ovary and providing energy for vitellogenesis. The correlative changes between liver weight and gonadal activity has been shown to be associated with energy requirement of the ovary for the development of oocytes (Htun -Han, 1978). It has been also shown that GSI and HSI have relationship and this relationship is directly related to gonadal activity (Patil and Kulkarni, 1996). The results on comparison between GSI and HSI during different phases in *N. notopterus* in the present study shows that when GSI is increased the HSI at also increases during breeding phase of the fish.

Cholesterol content in the steroidogenic tissue has been shown to be associated with reproduction of female hormone (Premjith *et al*., 1992). In *Heteropneustes fossils* (Rishi and Kaul, 1984) lowest cholesterol levels was observed during maximal gonadal activity and highest during breeding phase

indicating that the cholesterol content is gonadotrophin dependent. The lower levels of cholesterol were attributed to its utilization in steroid hormone synthesis. Diwan and Krishnan (1986) have reported that the value of serum cholesterol in male and female *Etroplus suratensis* showed fluctuations in relation to maturity. The cholesterol was highest when GSI value was minimal and lowest when GSI was maximum. In the female the recorded value of gonadal cholesterol though high but was not highest when GSI was lowest. The authors have suggested that the cholesterol being the precursor for the synthesis of steriod, which in turn influences the maturation phenomena. The hepatic and ovarian cholesterol contents in *N. notopterus* during two reproductive phases studied in the present investigation suggests that there is an existence of positive cholesterol relationship between ovary and liver for oocyte growth in *N. notopterus*.

Acknowledgments

Author D.S. Shankar is grateful to the Gulbarga University and ICAR for providing the financial assistance.

References

Brann, D.W. and V.B. Mahesh: Role of corticosteroids in female reproduction, *FASEB J.,* **5**, 2691 (1991).

Chakraborti, P.K., M. Weisbart and A. Chakraborti: The presence of corticosteroid receptor activity in the gills of the brook trout, *Salvelinus fontinalis*. *Gen. Comp. Endocrinol.*, **66**, 323 (1987).

Diwan, A.D. and L. Krishnan: Levels of cholesterol in blood serum and gonads in relation to maturation in *Etroplus suratensis* (Bloch). *Indian J. Fish.,* **33(2)**, 241 (1986).

Fisher, R.: Statistical methods for research workers, 6th Edition (Edinburgh). (1963).

Haider, S.: Hormonal control of oocyte maturation in Fish. *Ad. Fish Res*., **2,** 285 (1997).

Htun-Han.: The reproductive biology of the dab *Limanda limanda* L. in north sea: Gonadosomatic index, hepatosomatic index and condition factor. *J. Fish. Biol.,* **13,** 369 (1978).

Kulkarni, R.S. and A.G. Sathyanesan: Histochemistry and histoenzymology of the adrenal of the teleost fish, *Mystus vittatus* (Bloch.). *Biol. Bull. India*, **3(3),** 131 (1982).

McBride, J.R. and U.H.M. Fagerlund: Sex steroids as growth promoters in the cultivation of juvenile coho salmon (*Oncorhynchus kisutch*). *Proc. World Maricult. Soc.,* **7,** 145 (1976).

Mommsen, T.P., M.M. Vijayan and T.W. Moon: Cortisol in teleosts: Dynamics, mechanisms of action and metabolic regulation. *Rev. Fish Biol. Fish*, **9**, 211 (1999).

Patil, M. and R.S. Kulkarni: Annual biochemical changes of ovary and liver in relation to vitellogenesis in the female fish, *Purtius sarana sarana* (Ham.). *J. Parasit. Appl. Anim. Biol.*, **5(2)**, 61 (1996).

Peters, J.P. and D.D. Vanslyke: Quantitative clinical chemistry, Williams and Wilkins Co., Baltimore, 1, (1946).

Pottinger, T.G. and T.R. Carrick: Contrasting seasonal modulation of the stress response in male and female rainbow trout. *J. Fish Biol.,* **56**, 667 (2000).

Pottinger, T.G., P.H.M. Balm and A.D. Pickering: Sexual maturity modifies the responsiveness of the pituitary – Interrenal axis to stress in male rainbow trout. *Gen. Comp. Endocrinol.*, **98**, 311 (1995).

Pottinger, T.G., T.R. Carick., A. Appleby and W.E. Yeomans: High blood cortisol levels and low cortisol receptor affinity: Is the Chub, *Leuciscus cephalus*, a cortisol resistant teleost? *Gen. Comp. Endocrinol.*, **120**, 108 (2000).

Premjith, S., S.K. Pillay and P. Natarajan: Variation in phospholipid and cholesterol content of muscle and gonads of *Tilapia mossambica* in relation to growth and development. *In:* Current trends in fish and fishery biology and aquatic ecology (*Eds*: A.R. Yousuf, M.K. Raina and M.Y. Qadri). University of Kashmir, Srinagar, India, pp. 41-45 (1992).

Rishi, K.K. and M. Kaul: Seasonal gonadosomatic index, histological and cholesterol changes in the testes of the catfish, *Heteropneustes fossilis* and effect of exogenous hormones/releasing hormones on the cholesterol contents during prebreeding season. *Int. J. Acad. Ichthyol. AISI.,* **5**, 95 (1984).

Shrimpton, J.M., N.J. Bernier and D.J. Randall: Change in cortisol dynamics in wild and hatchery reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. *Can. J. Fish Aquat. Sci.,* **51**, 2179 (1994).

Snedecore, G.W. and W.G. Cochran: Statistical methods. 6th Edn. *Oxford and IBH Publ. Co*., Delhi, Bombay, Calcutta (1967).

Sumpter, J.P., J. Carragher, T.G. Pottinger and A.D. Pickering: The interaction of stress and reproduction in trout. *In:* Reproductive physiology of fish, (*Eds:* D.R. Idler, L.W. Crim and J.M. Walsh), Memorial Univ. Press, St. John's, Newfoundland, Canada 299 (1987).

Sundararaj, B.I. and S.V. Goswami: Effects of deoxycorticosterone and hydrocortisone singly and in various combinations *in vitro* maturation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.*, **17,** 570 (1971).

Thorpe, J.E., M.G. McConway., M.S. Miles and J.S. Muir: Diel and seasonal changes in resting plasma cortisol levels in juvenile Atlantic salmon, *Salmo salar* L. *Gen. Comp. Endocrinol.*, **65,** 19 (1987).

Verma, G.P. and S.K. Misra: Morphological and histochemical aspects of interrenal tissue of a teleost, *Channa guchua* (Ham.). *Proc. Nat. Acad. Sci.,* **52,** 147 (1992).