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Hypothalamo-neurosecretory system of the female sea bass, *Lates calcarifer* (Bloch), with special reference to gonadal maturation

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ABSTRACT

Hypothalamus of *Lates calcarifer* (Family : Centropomidae) consists mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO is a paired structure situated on either side of the third ventricle slightly above the optic chiasma. It is a highly vascularised structure and cytoarchitecturally divisible into dorsal pars magnocellularis (PMC) comprising larger neurons and ventral pars parvocellularis (PPC) with smaller neurosecretory cells. In the hypothalamus the NLT cells were found distributed uniformly in the infundibular floor adjacent to the pituitary stalk. Prominent phloxine-positive neurosecretory material (NSM) was encountered in PPC of matured female. Though neurosecretory cells of both NPO and NLT were positive to aldehyde fuchsin (AF), acid and chrome-alum-hematoxylin-phloxine (CAHP), cellular differentiation was more marked in the latter. Both NPO and NLT contributed beaded axons to form the neurohypophysial tract. Herring bodies (HB) of varying sizes were encountered in the anterior neurohypophysis (ANH). Neurosecretory cells of both NPO and NLT exhibited fluctuations in their secretory activity in response to the gonadal maturation.

Introduction

Lates calcarifer, popularly known as sea bass is distributed in the tropical and the subtropical coastal waters of the Indo-Pacific (Grey, 1987; Bensam and Nammalwar, 1991). It is a highly carnivorous fish belonging to family Centropomidae. The demand to consumers as an excellent tablefish coupled with its fast growing and euryhaline nature, are considered as valuable attributes by the aquaculturists (James and Marichamy, 1987). Though the fish is distributed throughout the coastal waters of India it does not contribute much to the total fish landings except in certain areas of the northeast like Chilka Lake and Hooghly-Matlah estuaries (Kasim and James, 1987). The sea bass exhibits complex sexuality involving protandric hermaphroditism making it an interesting specimen for academic studies too (Moore, 1979). *Lates calcarifer* is reported to breed from mid-October till December along the Tuticorin coast (Lal, 1991).

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Hypothalamus is a strategic point in vetebrate brain where various neural stimuli converge. It comprises groups of neurosecretory cells which control the secretion of the pituitary tropic hormones by elaborating releasing (RH) and / or inhibiting (IH) hormones (Ball, 1981; Maksimovich, 1987). Hypothalamus also contains receptors specifically sensitive to the hormones which in turn regulate its activity through feedback mechanism (Maksimovich, 1987). Though there are increasing evidences that the hypophysial functions in fishes are also mediated by hypothalamic neurohormones the regulatory mechanisms are not properly defined (Peter *et al.*, 1991). So far, the hypothalamus of sea bass is not described. An attempt has therefore, been made to record the hypothalamo-neurosecrtory centres of the female *Lates calcarifer* with special reference to the gonadal maturation.

Material and methods

Live specimens of the sea bass, *Lates calcarifer* (Bloch), were collected from the commercial catches caught in gill net and shore seine operations at Tuticorin coast (Gulf of Mannar). The region experiences rainfall during October-November due to the northeast monsoon. Average monthly temperature ranges between 25 and 31°C. The shore is partly muddy and sandy. The area adjoining Tambraparani estuary and Korampallam are swampy, infringed with thick mangrove vegetation. Sea bass though distributed throughout this region, is more concentrated near Punnaikkayal.

Female specimens (total length range 855-1050 mm; total weight range 8.0-11.0 kg) were collected during August 1988 to December 1988 out of which sixteen specimens with ripe ovaries were used for the study. The brains along with pituitary were surgically removed from the live fish and fixed immediately in aqueous Bouin's solution. A small piece of ovary was also dissected out and fixed in Zenkers-acetic (for 7 hrs) and neutral buffered formalin (for 24 hrs). After 24 hrs the tissues were thoroughly washed in running tap water dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. To maintain the original shape and avoid breakage of yolky oocytes, double embedding (with celloidin and paraffin) of the ovary was done (Khoo, 1979). Serial sections were cut at 6-8 μ and stained in aldehyde fuchsin (AF), Mallory's triple and chrome-alum-hematoxylin-phloxine (CAHP) whereas ovarian sections were stained in hematoxylin-eosin (H & E).

Standard gonad weight (GS), used as an index in place of gonosomatic index (GSI) (Davis, 1985) was calculated as : GS = GES x (GO/GEO): where GES is expected gonad weight at standard length, GEO expected gonad weight at observed length and GO observed gonad weight. Oocyte diameter was measured along its horizontal axis using ocular micrometer. Oocytes were measured at random till the count reached 100 cells. The data obtained was used to work out largest oocyte diameter.

Results

The salient features of ovaries of sea bass, *Lates calcarifer* corresponding to various stages of development are summarised in Table 1. Sea bass females with ovaries in recovering and maturing phases (Stage 2, 3) were observed during February. However, the female possessing vitellogenic oocyte (stage 4)

TABLE 1. *Salient features of ovaries of* L. calcarifer *(n = 311) corresponding to various stages of maturity*

Stage	Macroscopic characteristics	Histological characteristics	Largest oocyte diameter (μm)	Standard gonad weight Gs (g)
Stage –1 (newly formed)	The ovaries are deep reddish in colour, recently derived from testes after sex inversion.	Ovigerous lamellae are packed with chromatin nucleolus and early perinucleolus stage oocytes with a few at late perinucleolus stage.	110	78.1041
Stage –2 (developing virgin/ recovering spent)	The ovaries are thick walled, translucent, with pinkish-red hue.	Chromatin nucleolus stage oocytes present but perinucleolus stage oocytes dominate.	140	82.9406
Stage –3 (maturing)	The ovaries increase in size, translucent and cream in colour	Perinucleolus stage oocytes are present; oocytes entering gonadotropin dependent phase (cortical alveoli stage) predominate the population.	160 - 270	168.1038
Stage –4 (mature)	The ovaries are markedly larger in size, with thinner wall. Yellow hue start appearing	Ovigerous lamellae are packed with yolky oocytes visible to the naked eye. Most of the oocytes are at early and late vitellogenic stages, forming the leading clutch. Previtellogenic stages also coexist with this clutch.	260 - 500	406.7025
Stage –5 (ripe)	The ovaries are deep yellow, in colour, with thin walls, distended and occupying most of the body cavity	The oocytes undergo final maturation, migration of germinal vesicle and break down, coalescence of oil droplets.	450 - 700	628.7930
Stage –5A. (involuting)	The ovaries are deep red in colour, progressively decrease in size and have watery consistency.	Vitellogenic oocytes at various stages of atresia, often surrounded by blood vessels.		
Stage –6 (spent)	The ovaries are flaccid, reduced in size, with purple yellow colour. The wall becomes tough and wrinkled.	Post ovulatory follicles; previtellogenic oocytes dominate along with a few residual yolky oocytes.	140	127.4911

started appearing only in July. The ripe females (stage 5) were seen in late September till December with peak in November. The spent fish (stage 6) were encountered in late October till early January.

Hypothalamo-neurosecretory complex of *Lates calcarifer* consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and their axonal tracts. NPO was a paried structure situated on either side of the third ventricle

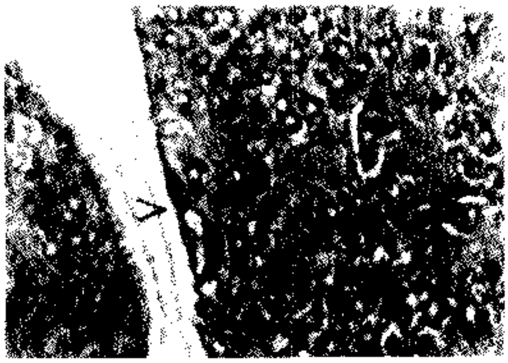

Fig 1 Nucleus preopticus (NPO) of female *Lates calcarifer*. Note the paired structure on either side of the third ventricle (V). Mallory's triple x 240.

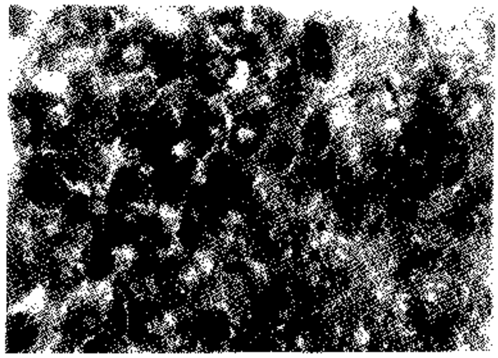

Fig.3 NPO pars parvocellularis (PPC) of maturing (stage 4) female *Lates calcarifer* comprises small neurosecretory cells. Mallory's triple, x 600.

slightly above the optic chiasma (Fig. 1). The closely placed neurosecretory cells of NPO were positive to aldehyde fuchsin (AF), acid fuchsin (in Mallory's triple stain) and chrome-alum-hematoxylin-phloxine (CAHP), however, cellular differentiation was more marked in the last one. It is a highly vascularised structure and cytoarchitecturally divisible into a dorsal pars magnocellularis (PMC) comprising large neurones (Fig. 2, 4) and ventral pars parvocellularis (PPC) with smaller neurosecretory cells (Fig. 3, 5). Thus, a progressive reduction in the size of neuronal cells was discernible from dorsal to ventral aspect of NPO. Most of the PMC and PPC neurones were bipolar.

NPO neurosecretory cells (both PMC and PPC) of the females possessing vitellogenic oocytes (stage 4) stained readily during July-August owing to the accumulation of neurosecretory material (NSM) (Fig. 2, 3). However, during spawning season (October-November), the neurosecretory cells of ripe female (Stage 5) exhibited declined staining

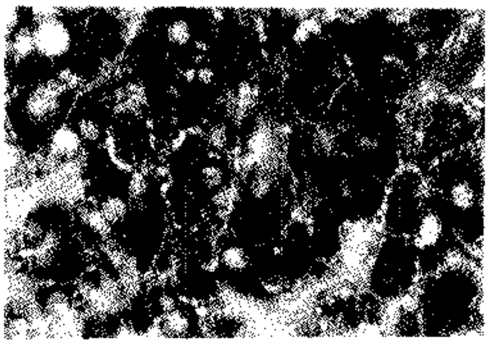

Fig. 2 NPO pars magnocellularis (PMC) of maturing (stage 4) female *Lates calcarifer* exhibiting darkly stained large neurosecretory cells. Mallory's triple x 600.

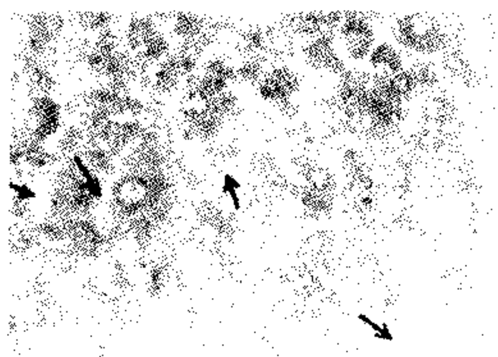

Fig. 4. PMC of ripe (stage 5) female *Lates calcarifer* exhibiting decreased staining response and vacuolation (arrow). Mallory's triple, x 600.

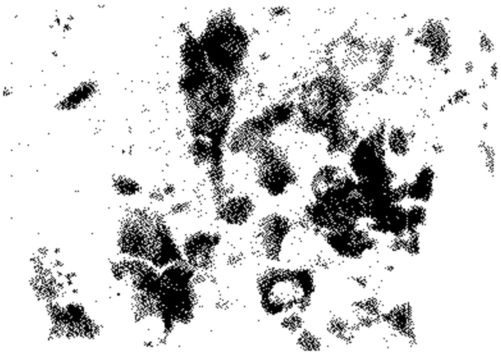

Fig. 5. PPC of ripe (stage 5) female *Lates calcarifer* depicting vacuolation. CAHP x 600.

Fig. 7. Nucleus laterali tuberis (NLT) of maturing (stage 4) female *Lates calcarifer* exhibiting acid fuchsin-positive neuro-secretory cells (arrow) and infundibulum (broken arrow). Mallory's triple x 240.

affinities and vacuolation probably due to release of the NSM (Fig 4, 5). Interestingly, a phloxine-positive globule-like NSM was encountered in the PPC of matured female *Lates calcarifer* (Fig. 6).

Nucleus lateralis tuberis (NLT) of *Lates calcarifer* consists of neurosecretory cells distributed uniformly in the infundibular floor adjacent to the pituitary stalk (Fig. 7). These cells were negative to aldehyde fuchsin (AF) but stained readily with acid fuchsin and chromo-alum- hematoxylin-phloxine (CAHP) (Fig. 8). Based on the distribution and size of neurosecretory cells NLT was divisible into pars anterior (Fig. 9), pars posterior (Fig. 10) and pars inferior (Fig. 7). NLT is a highly vascu--larised structure and a few neuro- secretory cells were seen close to the blood vessels (Fig. 11, 12).

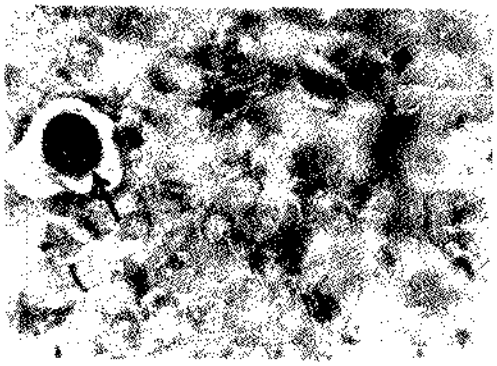

Fig. 6. PPC of matured (stage 5) female *Lates calcarifer*. Note the decreased staining response of the neurosecretory cells and a prominent phloxine-positive globule like neurosecretory material (NSM) (arrow). CAHP x 600.

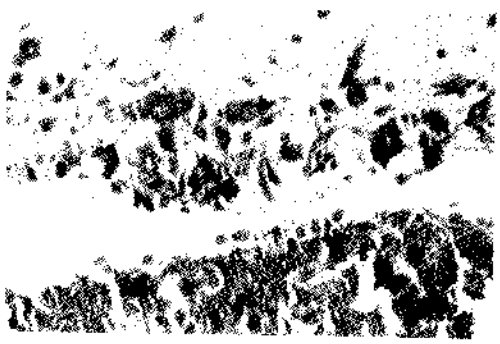

Fig. 8. NLT of maturing (stage 4) female *Lates calcarifer* stained readily with CAHP. Note the variously shaped neurosecretory cells distributed in the infundibular floor close to the pituitary (P). CAHP x 260.

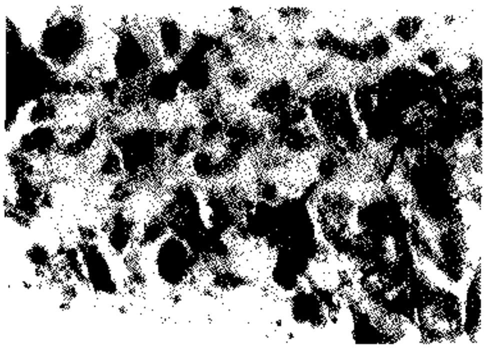

Fig. 9. NLT pars anterior of maturing (stage 4) female *Lates calcarifer* showing the darkly stained neurosecretory cells laden with NSM (arrow). CAHP x 600.

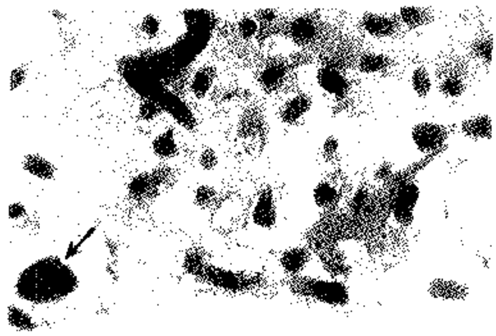

Fig. 11. NLT pars anterior of ripe (stage 5) female *Lates calcarifer* exhibiting active neurosecretory cells with uniform cytoplasm. CAHP x 600.

NLT neurosecretory cells of the female *Lates calcarifer* with vitellogenic oocytes (stage 4) exhibited accumulation of NSM during July-August, however, in ripe females (stage 5), these cells were hypertrophied possessing less NSM (Fig. 11, 12). A prominent phloxine-positive NSM was noticed in the pars anterior of matured *Lates calcarifer* (Fig. 11).

The neurohypophysial tract (NHT) enters the pituitary (hypophysis) through infundibulum (Fig. 7, 14). Varying sizes of acid fuchsin-positive herring bodies were encountered in the anterior neurohypophysis (ANH) (Fig. 13, 14) of the ripe *Lates calcarifer*

Discussion

The basic cytoarchitectural pattern of hypothalamo-neurosecretory system of female *Lates calcarifer* is almost similar to those described for other teleostean species (Viswanathan and

Fig. 10. NLT pars posterior of maturing (stage 4) female *Lates calcarifer*. Note the accumulation of NSM in the neurosecretory cells. CAHP x 600.

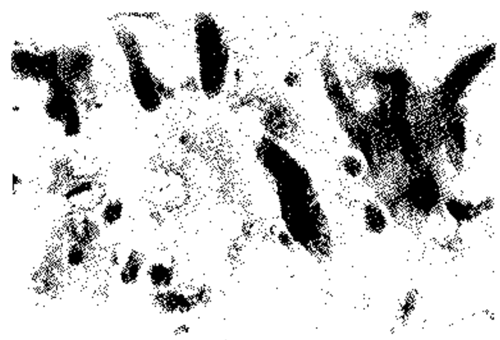

Fig. 12. NLT pars posterior of ripe (stage 5) female *Lates calcarifer* showing hypertrophied active neurosecretory cells and extensive vascularization. CAHP x 600

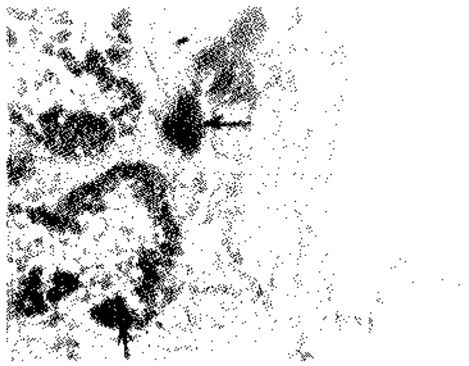

Fig. 13. Anterior neurohypophysis (ANH) of the matured (stage 5) female *Lates calcarifer* depicting acid fuchsin-positive herring bodies (arrow). Mallory's triple x 260.

Sunderaraj, 1974; Thomas and Sathyanesan, 1982; Prakash *et al.*, 1984; Maksimovich, 1987; Pandey, 1993). Generally, the neurosecretory cells of NPO stain with aldehyde fuchsin (AF) and chrome-alum-hematoxylin-phloxine (CAHP) (Maksimovich, 1987) but these are also stainable to acid-fuchsin in the sea bass (Fig. 1-6). Similar staining response of the NPO cells has also been observed in *Notopterus chitala* (Prakash *et al.*, 1984), *Rastrelliger kanagurta* (Pandey, 1993) and *Decapterus tabl* (Pandey and Mohamed, 1993).

Nucleus preopticus (NPO) is reported to play an important role in the spawning activities and its secretion does influence gonadal maturation among teleosts (Maksimovich, 1987; Pandey and Mohamed, 1993). We observed accumulation of NSM in PMC (Fig. 2) and PPC (Fig. 3) of maturing (Stage 4) female *Lates calcarifer* whereas depletion of the NSM and vacuolation in the neurosecretory cells of PMC (Fig. 4) and PPC (Fig. 5) were noticed in the ripe female (Stage 5) during peak spawning phase (October-November). NPO neurosecretory cells of *Channa punctatus*, *Tor tor*, *Heteropneustes fossilis*, *Coregonus autumnalis migratorius*, *Glossogobius giuris*, *Cirrhinus mrigala*, *Colisa fasciata* and *Labeo bata* also exhibited depletion of NSM during spawning season (Viswanathan and Sundararaj, 1974; Rai and Pandey, 1986; Maksimovich, 1987; Das and Sinha, 1988). However, Sokolowska *et al.* (1978) could not observe much difference in the contents of Gomori-positive neurosecretion in NPO cells of male *Anguilla anguilla* treated with human chorionic gonadotropin (HCG: 100 IU/fish) and testosterone (5mg/fish) for 7 weeks and remarked "The neurohormones (which are carried by Gomori-positive neurosecretion) in male cells do not play a role in reproduction". Occurrence of colloid (globule)-like NSM has been recorded in NPO of maturing (stage 4) and matured (stage 5) specimens of *Porichthys notatus*, *Channa punctatus*, *phoxinus phoxinus*, *Clarias batrachus*, *Heteropneustes fossilis*, *Tor tor*, *Glosso- gobius giuris*, *Scophthalamus maeoticus*, *Notopterus chitala*, *Rastrelliger kanagurta* and *Decapterus tabl* (Zolotiniskiy, 1980;

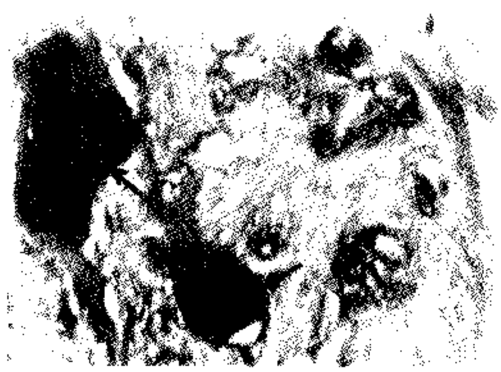

Fig. 14. Magnified view of the anterior neurohypophysis (ANH) of the ripe female *Lates calcarifer* to show the prominent herring bodies (arrow). Mallory's triple x 600.

Prakash *et al.*, 1984; Maksimovich, 1987; Pandey, 1993; Pandey and Mohamed, 1993). Similar structure was noticed in the NPO (PPC) of matured female *Lates calcarifer* also (Fig. 6).

Nucleus lateralis tuberis (NLT) has been reported to be the second neurosecretory centre in the teleostean hypothalamus, however, there exist stray reports of its absence among a few species (Kobayashi *et al.*, 1959; Maksimovich, 1987). Since this structure has been implicated to play a major role in the gonadal maturation of teleosts, it is unlikely that the same might be completely absent in some species (Maksimovich, 1987). Kobayashi *et al.* (1959) have also remarked that season or age factors may be responsible for the absence of stainable granules in the NLT. The neurosecretory cells of NLT pars anterior (Fig. 9), pars posterior (Fig. 10) and pars inferior (Fig. 7) of *Lates calcarifer* are readily stainable with acid fuchsin and CAHP. Fluctuations in the secretory activity of pars anterior (Fig. 9) and pars posterior (Fig. 10) with ovarian development suggest their involvement in the maturation of the sea bass. *Clarias batrachus, Tor tor, Heteropneustes fossilis, Colisa fasciatus and Decapterus tabl* also exhibited enhanced secretory activity in NLT neurosecretory cells during spawning phase of reproductive cycle and/or sex steroid administration (Viswanathan and Sundararaj, 1974; Rai and Pandey, 1986; Maksimovich, 1987; Pandey and Mohamed, 1993). We found colloid-like neurosecretory material (NSM) in NLT pars anterior and pars posterior of *Lates calcarifer* at stage 4 and 5 of maturity. Such structures have also been recorded in NLT pars posterior of matured *Decapterus tabl* (Pandey and Mohamed, 1993). Zolotnitskiy (1980) also noticed similar structures in the form of different-sized drops in lateral nucleus of the Black sea turbot, *Scophthalamus maeoticus.*

Herring bodies (HB) of varying sizes were seen in the anterior neurohypophysis (ANH) of the ripe female *Lates calcarifer* (Fig. 13, 14). A similar distribution of herring bodies have also been observed in *Porichthys notatus, Clarias batrachus, Phoxinus phoxinus, Glossogobius giuris, Scophthalamus maeoticus, Rastrelliger kanagurta* and *Decapterus tabl* (Zolotnitskiy, 1980; Pandey, 1993; Pandey and Mohamed, 1993). It is assumed that HB have accumulated neurosecretory material (NSM) which help to increase the surface area for the release of biologically active principles in the blood circulation (Zolotnitskiy, 1980; Maksimovich, 1987; Pandey and Mohamed, 1993).

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