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CORRELATIVE SEASONAL CHANGES IN THE HYPOTHALAMIC NUCLEI, ADENOHYPOPHYSIAL CELLS AND GONADS OF A TROPICAL PERCH *COLISA FASCIATA* (BL. & SCHN.).

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Suresh Chandra Rai and Kamleshwar Pandey (1986) Correlative seasonal changes in the hypothalamic Nuclei, adenohypophysial cells and gonads of a tropical perch *Colisa fasciata* (Bl. & Schn.). *Bull. Inst. Zool., Academia Sinica* **25**(1): 57-66. Correlative changes in the hypothalamic nuclei, hypophysis and gonads of a fresh water perch *Colisa fasciata* has been undertaken to assess the endocrine role of hypothalamus and pipuitary in controlling the reproductive cycle of this fish. During the prespawning phase the attainment of large size and heavy accumulation of Neurosecretory material (NSM) around the cell body points towards an increased activity of the cells of NPO & NLT. Their activity, however, declines during the successive phases of early spawning and spawning and finally decreases maximally in the post Spawning phase when these cells become very small with scanty NSM. Changes parallel to this have also been recorded in the cyanophils (gonadotrops) of the adenohypophysis related with the gonadal activity. This phenomenon, thus, suggests that the secretion of NPO & NLT triggers the secretory activity of the gonadotrops which in turn influences the gonadal maturation and spawning in *C. fasciata*.

It was shown that the hypothalamus controls the secretion of pituitary gonadotropins which in turn regulates gonadal function. Depletion in the amount of neurosecretory material in the nucleus preopticus and the neurohypophysis during the spawning phase, and its accumulation during the pre-spawning phase has been examined in various fish species (Sokol, 1961; Rai, 1973; Pantic and Lovren, 1975; Saxena, 1976 and Moitra and Medya, 1980) has led to a renewed interest in the study of the hypothalamo-adenohypophysial gonadal interrelationship. These authors have demonstrated that if neurosecretory material (NSM) does not reach up to the cells of anterior pituitary through the hypophysial vessels, the gonadotropic function of the adenohypophysis is markedly interfered with.

Like majority of the teleosts, the *Colisa fasciata* is also an annual breeder and spawns during the monsoon season (June to August). It was, therefore, of interest to examine whether the nucleus pre-opticus (NPO) and the nucleus lateralis tuberis (NLT) are the hypothalamic sites for the synthesis of neurosecretory materials and exhibit concomitant cyclical activities during the gonadal cycle of this species.

MATERIAL AND METHODS

Specimens of *Colisa fasciata* were regularly procured every month for two consecutive years from local Ramgarh lake Gorakhpur. The brains, together with pituitaries and gonads (testes and ovaries) were isolated and fixed in Bouin's solution. Sections about 6 μm were subjected to following histological and the pituitary cytology was studied using Aldehyde fuchsin (AF), Herlants tetra chrome, chrome-alum-haematoxylin Phloxine (CAHP), Periodic acid-schiff's (PAS), azan's and Lead haematoxylin techniques while gonads weле subjected to haematoxylin-eosin and Heidenhain's-iron haematoxylin methods.

Cytometric studies of these section was made and parameters were measured using occular micrometer. The mean and standard error (M+SE) were calculated for various neurosecretory cells (Nucleus preopticus, nucleus lateralis tuberis), cyanophils (gonadotrops of the proximal pars distalis and testicular and ovarian parameters. Student's 't' test (Campbell, 1974) was used to test significant monthwise size differences of these cells.

RESULTS

Histological examinations of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT) and parenchyma cells particularly the cyanophils of adenohypophysis reveal seasonal fluctuations in the secretory activity corresponding to the sexual cycle of *Colisa fasciata*. It has been recorded that this fish attains gonadal maturity only once during a calender year and spawns from June to August with peak in July. Aforesaid secretory activities are described in the following five phases of the reproductive cycle of this fish.

Resting phase (November–December)

During this phase the cells of preoptic nuclei and nucleus lateralis tuberis do not show any marked change and appear to be inactive. They are small in size with depleted neurosecretory material. The nuclei of preoptic cells measure 6.67 ± 0.017 to 6.93 ± 0.048 μm and of nucleus lateralis tuberis measure 5.25 ± 0.038 to 5.48 ± 0.034 μm in diameter. The cyanophils of the proximal pars distalis also appear to be highly inactive. The cytoplasm of these cells is highly sparse and exhibit scanty granulation. These nuclei measure 3.67 ± 0.024 to 3.98 ± 0.049 μm in diameter during this phase.

The testes appear to be small with thick walled lobules. The lobules mainly contain sperm mother cells and spermatogonia. The ovary shows prominent ovigerous folds enclosing nests oogonia and numerous immature oocytes. The oogonia are found in chromatin nucleolus stage while the oocytes in early perinucleolus stage.

Histochemically, due to the lack of glycoproteinaceous materials, the cyanophils show very feeble reaction to PAS, AF, and aniline blue.

Preparatory phase (January–March)

These formentioned cells appear to increase in size along with concentation of the neurosecretory material during this phase. The nuclei diameter of pre-optic cells, nucleus lateralis tnberis, and cyanophils of the proximal pars distalis measure 7.14 ± 0.067 μm, 5.70 ± 0.040 to 6.54 ± 0.027 μm and 4.16 ± 0.035 to 4.82 ± 0.033 μm respectively. The cytoplasm appears gaining granulation.

The spermatogenic activity is also in progress and most of the lobules exhibit cysts with enhancing number of spermatogonia, primary and secondary spermatocytes. The oocytes of primary and secondary yolk stage are fairly common, while a very few oocytes in early yolk vesicle stage are also observed. The yolk nucleus and nucleolar extrusion are frequently visible.

Pre-spawning phase (April to early June)

During these months of reproductive cycle the cells of nucleus preopticus and nucleus lateralis tuberis show maximum size with maximumum accumulation of the neurosecrecretory material in their cell bodies (Fig. 1, 2). This is probably because of the presence of large amount of the intranuclear granules and neurosecretory material around the nuclei which appear darker and brighter. The nuclei measure $11.38 \pm 0.057\ \mu m$ and $7.35 \pm 0.032\ \mu m$ in diameter respectively. The neurosecretory material located mainly at the peripheral part of the cell body can be clearly seen.

Concomitantly, the cyanophils of the proximal pars distalis increase in volume getting tightly packed and became strongly PAS, AF and aniline blue positive. In the beginning of this phase these cells show a definite and specific contour (Fig. 3). The cytoplasm of these cells exhibit intense granulation. The cyanophilic cells constitute the major component of this region, chiefly because of their number, granulation of cytoplasm, well differentiated nuclei, chromatin net work and cell diameter. Occasionally certain degranulated and vacuolized cells are also encountered. Blood capillaries are gorged and turbid with blood cells within the branches of the neurohypophysis.

Spermatids and sperms are the dominant germ cells though secondary spermatocytes are also present in fair number. The interlobular septa became more thin.

The oocytes with heavy yolk globules and abundant yolk vesicles are dominant in this phase.

Due to the increase in secretary material of cyanophils the averagea rea of the proximal pars distalis appears enlarged.

Spawning phase (June-August)

During the spawning phase (June, July, August) the cells of NPO and NLT due to the release of the nurosecretory materials from their cell body show a general decrease in their size. The nuclei of these cells measure 10.13 ± 0.039 to $6.39 \pm 0.027\ \mu m$ and 6.97 ± 0.037 to $4.26 \pm 0.033\ \mu m$ in diameter respectively. The neurosecretory material along with the axons could well be seen. Sign of degranulation was noticeable just prior to June and it further deplets during July, August and September.

In the month of June and July there is greater degranulation in the cytoplasm of a large number of cyanophils resulting small vacuoles in the cytoplasm of these cells. In July and August the cyanophils are highly degranulated with increased number of the cytoplasmic vacuoles rending the cell boundaries more or less indistinct. The nuclei measure during this phase ranges 6.06 ± 0.042 to $4.27 \pm 0.087\ \mu m$ in diameter.

On account of the release of secretory material from their cytoplasm the degranulation of the cyanophils results. Blood vessels became more conspicuous. This is recorded maximum in the month of July indicating higher rate of hormone release in this month. The granulation and degranulation of cyanophils show a definite correlation with the reproductive cycle and spawning of fish.

Majority of the testes lobules are almost filled with spermatids and sperms. The sperm production seems to be at its peak and practically fill all available space of the lobules.

The ovaries are fully gravid due to the presence of heavily yolk laden ova. However, few discharged follicles are also encountered indicating the beginning of spawning. The tunica albuginea become extremely thin.

Histochemically, during early part of the spawning the glycoproteinous material of the PAS positive cells does not appear to be decreasing but soon after the act of spawning a general decrease of glycoproteinaceous material is observed.

The release of the secretion of these cells during this phase thus seems to be concerned with final maturation process of the gonads as well as an overall control on the spawning of the fish.

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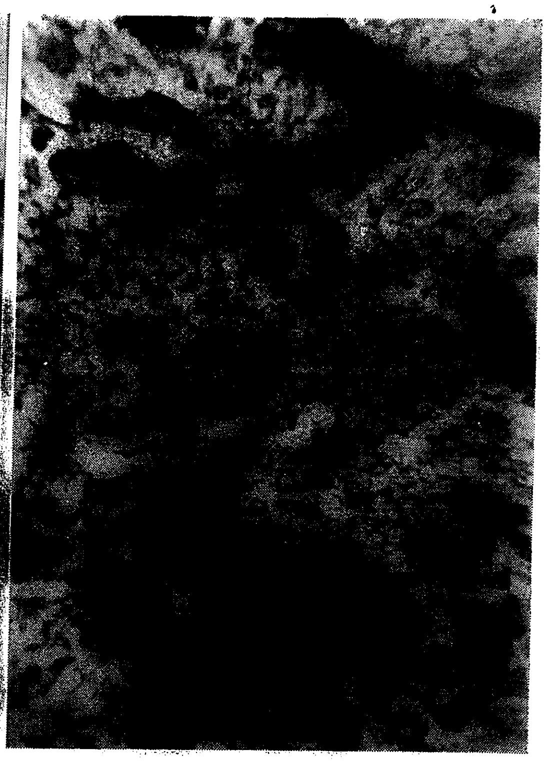

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Fig. 1. Nucleus preopticus cells (NPO) in May showing accumulation of neurosecretory material. AF ×900.
Fig. 2. Nucleus lateralis tuberis cells (NLT) in May showing accumulation of neurosecretory material. HA ×900.
Fig. 3. A portion of proximal pars distalis (PPD) of pituitary gland in May showing granulated cyanophils (C). HA ×405.
Fig. 4. Nucleus preopticus cells (NPO) in August showing degranulated cells. AF ×900.
Fig. 5. Nucleus lateralis tuberis (NLT) cells in August showing degranulated cells. HA ×900.
Fig. 6. A portion of proximal pars distalis in August showing degranulated and vacuolized cyanophils (C). HA ×405.

Post-spawning phase (September–October)

The size of the NPO cells as well as of the NLT in this phase are greatly reduced and attain their minimum size due to total depletion of the neurosecretory material from their cell body (Figs. 4, 5). The nuclei of these cells measure during this phase $6.39 \pm 0.027\ \mu m$ and $4.08 \pm 0.039\ \mu m$ in diameter respectively. Their staining affinity is considerably reduced and show the sign of inactivity.

The cyanophils of the proximal pars distalis get degranulated and vacuolated with reduced staining affinity. Their size is greatly reduced and exhibits signs of inactivity (Fig. 6). The nuclei of these cells ranges from 3.80 ± 0.031 to $3.59 \pm 0.028\ \mu m$ in diameter.

After spermiation, the lobules become more compact and show a considerable decrease in their size. Few residual sperms are seen in some of the lobules. The sperm mother cells are in active stage of division along the inner wall of lobules. In October most of the lobules are packed with cysts of sperm mother cells.

A large number of discharged follicles

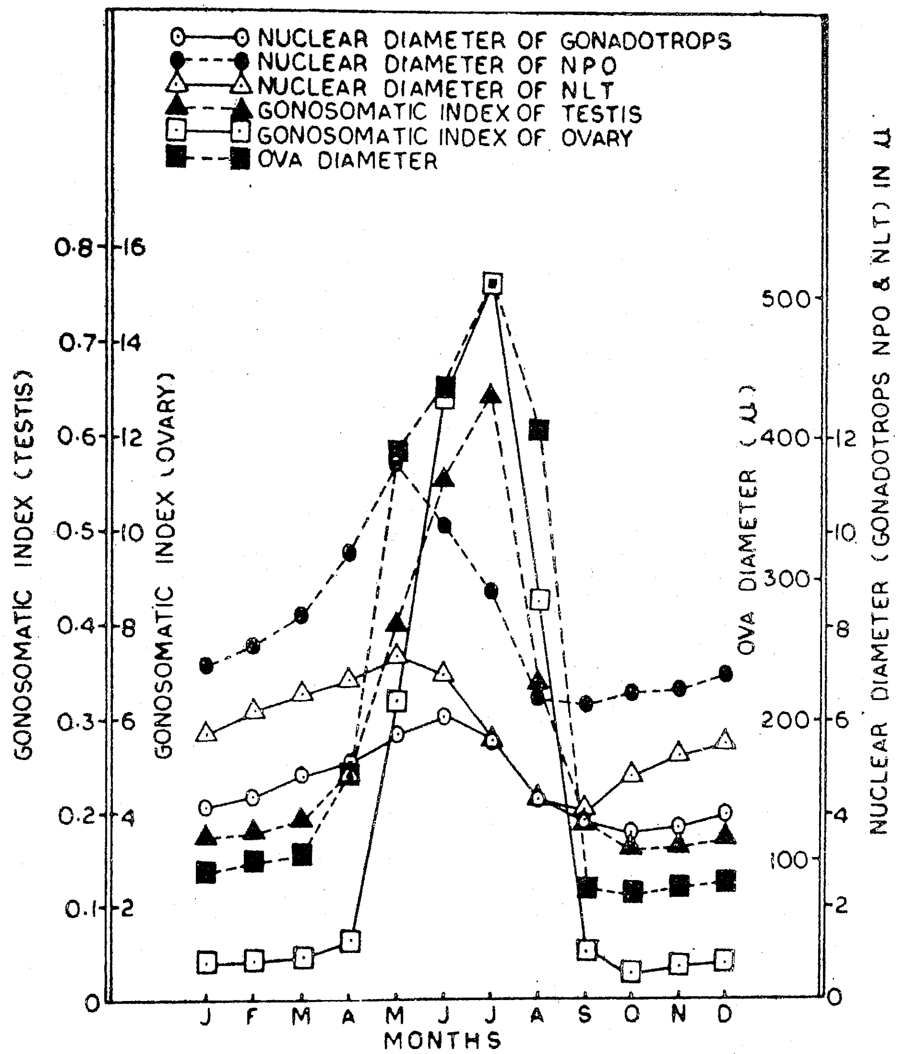

Fig. 7. Showing correlation in the cells of NPO, NLT, Cyanophils (gonadotrops), ova diameter and gonosomatic index (Testis and Ovary) in *Colisa fasciata*.

and few unovulated yolky eggs are seen in various stages of resorption. The oogonial nests and young oocytes are seen abundantly along with the increased somatic tissues. The formation of large number of early spermatogenetic and oogenetic stages, during this phase are, therefore, associated with the scanty secretion of these cells.

Histochemically also the cyanophil cells show a gradual decrease in glycoproteinous material. It is only the pycnotic nuclei which are stained deeply with nuclear dyes.

The activity of the cells of the NPO, NLT and cyanophils of proximal pars distalis have been shown in Fig. 7. They also show correlative changes with the gonosomatic indices (Testes and Ovaries) and Ova diameter supporting the above findings.

DISCUSSION

Various workers have correlated the neurosectory activity of the NLT with the reproductive activity in a number of teleost species (Stahl, 1953, 1954, 1957; Zaitsev, 1955; Brehm, 1958; Billenstein, 1962, Stahl and

Leray, 1962; Honma and Tamura, 1965; Honma and Suzuki, 1968; Peter, 1970; Pantic and Lovren, 1975; Moitra and Medya, 1980), while contradictory statement regarding the secretory activity in the cells of the nucleus lateralis tuberis and maturation of gonads are also not uncommon (Gabe, 1966). Oztan (1963) has described active preoptic nuclei and inactive nucleus lateralis tuberis in the sterile hybrid of *Xiphophorus maculatus* and *Xiphophorus hellerii.* Lesions of the NLT significantly decreased the ganadal activity in goldfish *Carassius auratus* (Peter, 1970). Dodd *et al.* (1978) observed that in male salmon parr *Salmo salar* lesions of the NLT blocked spermatogenesis, reduced testis size and lowered pituitary gonadotropin content. In *Colisa fasciata* the examination of the neurosecretory cells of the NLT during annual gonadal cycle showed their higher activity and attainment of large size in prespawning phase and depletion or degranulation in the neurosecretory material during spawning phase. Scanty accumulation of NSM and smaller nuclear size during post-spawning and resting phases indicate that these cells are possibly involved in the regulation of gonadal maturation and reproduction of this fish. Further, the concomitant changes in the activity of the nucleus lateralis tuberis, the cyanophils (gonadotrops) and maturation of gonads are also known in a number of teleost species (Stahl and Leray, 1962; Honma and Tamura, 1965; Rai, 1973, Viswanathan and Sundararaj, 1974, Pavlovic and Pantic, 1974). In the present study, degranulation process as an evidence of hormone release appears in the NLT parallel to those of cyanophils approaching prespawning phase. Concomitantly the degranulation is maximum at the peak of spawning period indicating their partioipation in maturation of gonad as well as spawning in *Colisa fasciata.*

According to Peter and Crim (1978), the role of the nucleus preopticus in the gonadotropin control can not be overlooked in the light of the recent report that the lesions of the gold fish NPO caused ovarian regression and blocked ovarian recrudescence. Furthermore, according to Peter and Paulencu (1980), a factor that inhibits ovulation (Gonadotropin inhibiting factor or GnIF) may originate in the anterior preoptic region of gold fish. Additionally, certain changes in the AF-positive nucleus preopticus and in neurosecretory material in the neurohypophysis are related to reproduction (Stahl and Leray, 1962; Honma and Tamura, 1965; Honma and Suzuki, 1968; Ball and Baker, 1969; Pantic and Lovren, 1975); and while some of these changes could reflect the involvement of neurohypophysial octapeptides in oviposition and spawning behaviour (Peter, 1973; Holmes and Ball, 1974). On the other hand correlative seasonal activity in the nucleus preopticus and gonads was neither observed in certain teleosts (Schiebler and Brehm, 1958; Schiebler and Hartmann, 1963; Oztan, 1963), nor there exists any direct evidence, indicating the role of nucleus prepoticus in the control of gonadal activity (Peter, 1970; Dodd *et al.*, 1978).

Depletion in the amount of neurosecretory material during the spawning and postspawning phases while its accumulation during prespawning was observed in the neurohypophysis of *C. fasciata.* This secretion of the neurosecretory material probably from nucleus preopticus, suggests its implication in spawning of this species. Earliier observations in this regards of Olivereau, 1960; Olivereau and Lovren, 1975 are in conformity to the present results.

In *Colisa fasciata* the hypothalamohypophysial tract conducts the neurosecretory material and becomes more prominent during early spawning and spawning period due to the rapid conduction of the neurosecretory material during this phase. Similar observation has also been made by Tishchenko *et al.* (1976) in *Corejonus autumnalis migratorius.* Polenov (1960) observed that the main preoptico-hypophysial tract in the carp and sazan is often rich in neurosecretory granules in its more caudal regions. Leatherland *et al.* (1966)

have observed that the tracts of the eel, *Anguilla anguilla*, shows a small caudal accumulation of neurosecretion, which is followed by short empty area. This area, which was termed the "subterminal area" lies above the anterior margin of the adenohypophysis and it may represent the terminations of certain neurosecretory axons. Pantic and Lovren (1975); Sathyanesan and Jose (1975) and Moitra and Medya (1980) reported similar observations in *Serranus scriba; Channa punctatus* and *Cirrhinus mrigala*, respectively.

The chemical nature of the secretions of the nucleus preopticus and nncleus lateralis tuberis tuberis may or may not be the same. Various workers have demonstrated the presence of bioamines in the hypothalamus (Schally *et al*., 1973; Kambery, 1973). According to Kambery (1973) and Pantic and Lovren (1975), the NLT cells probably have a role in the biosynthesis of bioamines and also the role of bioamines in the regulation of the activity of the pituitary cells, specially the gonadotropic one.

It is now unanimously accedted that pituitary gland controls the activity of gonads (Pickford and Atz, 1957; Sathyanesan, 1958; Robertson and Wexler, 1962; Ball and Baker, 1969). Most of the workers who have studied the cyclical changes in the gonads (testes and ovaries) have recorded that a parallelism exists between the spermatogenic and oogenic activity of gonads and gonadotropic cells of the hypophysis. This suggests that testes and ovaries are under the hormonal control of pituitary gland which in turn is directly under the control of hypothalamus (Gorbman and Bern, 1962; Barrington and Jorgensen, 1968). Histocytological changes in the pituitary gland and hypothalamus in relation to the gonadal changes in the present study have been found to be highly correlative with each other. This indicates clear possibility of hypothalamo-hypophysial gonadal relationship in *Colisa fasciata*.

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熱帶鱸魚 *Colisa fasciata* 下視丘核、腦垂腺細胞及性腺之季節性相關變化

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本研究探討熱帶鱸魚 *Colisa Fasciata* 之下視丘與腦垂腺對生殖週期之內分泌控制所扮演之角色。排精或排卵前，視前區核及側梗核細胞體出現大型神經分泌顆粒物質。該分泌物質在排精與排卵早期或整個期間漸次減少；而在排精與排卵後，該二核區細胞變小，且細胞內所含神經分泌顆粒極少。