STUDIES ON BIOMETRICS AND GONADAL ACTIVITY OF SNOWTROUT (*Schizothorax esocinus*)

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THESIS

Submitted to

The Faculty of Postgraduate Studies
Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir in partial fulfilment of requirement for the award of the degree of

MASTER OF FISHERIES SCIENCE

2008

Dedicated

to my Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Fisheries, Rangil, Ganderbal C/o Shuhama Campus
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Certificate – I

This is to certify that the thesis entitled **"Studies on biometrics and gonadal activity of snowtrout (*Schizothorax esocinus*)"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science**, to the Faculty of Postgraduate Studies, Sher-e-Kahsmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Mr. Shabir Ahmad Dar (Regd. No. 2006-F-06-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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Certificate – II

We, the members of the Advisory committee of **Mr. Shabir Ahmad Dar (Regd. No. 2006-F-06-M)**, a candidate for the degree of **Master of Fisheries Science**, have gone through the manuscript of the thesis entitled, **"Studies on biometrics and gonadal activity of snowtrout (*Schizothorax esocinus*)"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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This is to certify that the thesis, **"Studies on biometrics and gonadal activity of snowtrout (*Schizothorax esocinus*)"** submitted by **Mr. Shabir Ahmad Dar (Regd. No. 2006-F-06-M)** to the Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science,** was examined and approved by his Advisory Committee and external examiner on ………………..

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ABSTRACT

Studies on the biology of *Schizothorax esocinus* were carried out with regard to morphometry, length-weight relationship, condition factor, histology of gonads and spawning season. On the basis of histology, oocyte development and gonadosomatic index (GSI), ovarian cycle has been divided into five stages viz. Immature Phase, Preparatory Phase, Maturing phase, Ripe/Spawning Phase and Depletion/Spent Phase. Similarly testicular cycle has been divided into Relaxation/Preparatory Phase, Slow Spermatogenesis Phase, Rapid Spermatogenesis Phase, Spawning/Partly Spent Phase and Depletion Phase. Maximum GSI values were recorded around spawning season during April, May and June with peak values of 20.11 for female and 18.21 for male in May. It has been observed that *S. esocinus* is an annual breeder i.e. spawns once in a year. High degree of positive correlations were recorded amongst various morphometric characters being highest (r = 0.96) between body weight and maximum depth and least (r = 0.81) between total length and head length. The length-weight relationship established for the fish suggested fairly isometric growth closely following cube law. The regression equation obtained was Log w = - 4.7441 + 2.8973 Log L (r = 0.94). Coefficient of condition factor (K) was found to vary from 0.57 - 0.85 with a mean value of 7.28.

Key words: *Schizothorax esocinus*, morphometric characters, length-weight relationship, condition factor, histology of gonads, GSI

Signature of Student
Dated : ___________

Signature of Major Advisor
Dated: ________________

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(Shabir Ahmad Dar)

Place : Shalimar, Srinagar
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CHAPTER – 1

INTRODUCTION

The Valley of Kashmir is famous for its peerless scenic beauty. It has been rightly called the “Paradise on the earth”. Spread over an area of about 39921.8 hectares (Anonymous, 1998), it has been bestowed with enormous and cleanest resources of water in the shape of lakes, streams, rivers, sars, low lying areas etc. These water bodies due to their peculiar natural conditions may become prime centres of cold water fisheries which have a major contribution to make the blue revolution a success. The Kashmir Valley water bodies support a wide variety of indigenous and exotic fish species. In all 40 species of fish have been reported (Balkhi, 2002). Of these, *Schizothoracids* (snow trouts) are considered more important. Among *Schizothoracids*, *Schizothorax esocinus* (Heckel) is the most important food fish from the commercial point of view.

Schizothorax esocinus locally known as Churoo is found in Indus and Jhelum rivers in good numbers and scarcely in Chenab river. It also occurs in Dal, Wular, and Mansbal lakes of Kashmir Valley. While as *Schizothorax* species form the major catch in the rivers of Kashmir, more than 50 per cent of the fish catch comprises of common carp in the lakes of Kashmir (Kamal, 2000). *Schizothorax esocinus* fetches more economic returns to the fisherman community (Das, 1965). Moreover, it could also be included in the polyculture programmes along with common carp, grass carp, silver carp etc. However, during the past a few years, *Schizothorax esocinus* has shown tremendous decline in its population as well as size (Yousuf, 1996 and Bhat, 2003). The decline is attributed to a number of factors such as over exploitation of fish, pollution of aquatic resources, destruction of breeding and rearing grounds etc. Although, studies have been carried out on the nutritional aspect of the fish (Balkhi, 1998), there has been hardly any detailed study on gonadal development and other aspects of breeding biology and conservation aspects of *Schizothorax esocinus*.

The study of morphometric characters in fishes is very important from taxonomic point of view (Balli *et al.*, 2007). Studies on length-weight relationship are of considerable importance in fishery because it shows relevance to fish population dynamics and patterns of growth on fish stocks. The length-weight relationship changes with various biological processes of metamorphosis, growth and maturity (Beverton and Holt, 1957). As such it is a necessary tool for assessment of the stock (Sparre and Venema, 1989). Nevertheless such studies are usually undertaken to know whether variation from expected weight for the known length groups are indicative for fatness, wellbeing, gonadal development in relationship with the environment (Lecren, 1951 and Bagenal, 1978) and fluviatile condition of the habitat. A voluminous data is available on this aspect on a global scale. However, in Kashmir valley, where the fish fauna is very distinct, only a few reports are available on the length-weight relationship in fishes inhabiting different aquatic habitats (Yousuf *et al.*, 1992).

The knowledge of gonadal cycle and their functional mechanism is of prime importance for successful fish seed production and breeding practices. Determination of spawning period is highly essential for devising a breeding programme of a fish. The reproductive potential of a population is a function of the selection of brooders for production purposes. Sexual maturation (development of gonads) is an important prerequisite for continuation of the race in living being of higher order. During the development of functional gametes in fishes there occurs meiotic arrest which has to be resumed at a specific stage for successful reproduction. The resumption of meiosis is called oocyte maturation (Najar and Qadri, 1999; Najar *et al.*, 2000).

Sexual maturation depends on many factors including availability of feed. The amount of feed eaten largely determines the availability of the year classes, as well as the growth rate and sexual maturity. Besides above temperature, photoperiod, internal rhythm and endocrine system also influence the maturation of gonads in fishes. Sexual maturation, among fishes includes seasonal cycle of gonads, fecundity and spawning etc.

Studies on the reproduction biology of fishes of Indian sub-continent have been made by a number of researchers. However, scanty information is available on the reproductive aspects of fishes of hill streams and cold waters (Shrestha and Khanna, 1976; Pathani, 1980). Hence, the present study was undertaken to observe the process of morphology, gametogenesis, seasonal cyclicity, breading behaviour and other reproductive aspects of *Schizothorax esocinus*. This will be a tool for the development and rational management of *Schizothorax* fishery.

The study will provide a deeper insight into the biology of spermatogenesis, oogenesis, environmental influence on spawning activity and morphology. This may help to explore the possibilities of improving fertility and in developing the *Schizothorax* fishery of snow-fed rivers which are undergoing serious changes due to various natural and human activities like silitation, flash floods, land floods, pollution, road construction, dams and hydroelectric projects, etc. As such present investigation on *Schizothorax esocinus* was intended with following objectives:

1. To study morphometric characters of *Schizothorax esocinus*.
2. To study the gonadal development of *Schizothorax esocinus*.

CHAPTER – 2

REVIEW OF LITERATURE

Review of literature pertinent to the present investigation has been broadly discussed under following headings :

2.1 Histology

2.2 Spawning

2.3 Gonadosomatic index

2.4 Length-weight relation and condition factor

2.1 Histology

Seasonal histological changes in the ovary and oogenesis have been described by Khanna and Pant (1967) in *Glyptorthorax pectinopterum*; Malhotra (1971) in *Schizothorax richardsoni* and Rita Kumari and Nair (1979) in the hill stream Loach, *Nemacheilus Triangularis*. However, the seasonal and developmental changes in the oocytes and oogenesis in *Schizothorax esocinus* have not been described so far. Similarly, the use of classical and subsequent process of spermatogenesis is restricted to few individual cases and very little information is available (Upadhyay and Guraya, 1973). Therefore, morphological and histological studies on the gonads of *S. esocinus*, an important representative of *Schizothoracid* species have been undertaken.

Singh and Das (1990) studied the seasonal changes in the morphology of the ovaries of *Crossochilus lateus punjabensis* (Heckel) Cypriniformes and concluded that the ovary of *crossochilus lateus punjabensis* after spawning from May upto the end of July, undergoes an ovarian vitellogenesis phenomenon from August to December. In the months of January, February and March the ovary undergoes period of winter rest (diapause). With the return of good climatic conditions, the vitellogenesis starts again. The gonadosomatic index shows a gradual change with change in the weight and maturity of the ovary which is affected by the external environment condition.

Najar (1994, 2002), Najar and Qadri (1999) and Najar *et al.* (2000, 2001) studied progesterone and estradiol-17β levels in ovarian tissue of *Schizothorax niger* in relationship with various development stages of its ovary. Maximum levels of these ovarian hormones were observed during September to November which forms the peak season for vitellogenesis. Although oocytes were fully deposited with yolk by the end of November, the spawning does not occur which was restricted by low winter temperature, thus there occur winter diapause. Spawning occurred only when temperature of holding water increased during ending February and March.

Mazzoni and Caramaschi (1997) observed that the spawning season of *Hypostamus affinis* extended from September to February coincidental with the warm, rainy season. Histological and oocyte size frequency analyses indicated a serial spawning and an asynchronus mode of ovarian development. Five phases of oocyte development were described. Garcia *et al.* (1997) studied the gonadal development of *Serranus cerbrilla* and confirmed that this species is synchronously hermaphrodite. Ovarian tissue development is asynchronous and testicular tissue depicts continuous spermatogenesis. Six stages of oogenesis and five stages of spermatogenesis are described based on differences in staining, in size, on differences in the nucleus and cytoplasm structure. The spawning season of *S. cerbrilla* is from February to July with peak in May. Yonada *et al.* (1998) examined the reproductive cycle and sexual maturity of the angler fish, *Liphiomus setigerus* and observed that spermatids were released from the germinal cysts into the lumin of the seminal lobules. Both spermatids and spermatozoa were present in the lumin of the seminal lobules and sperm ducts. Spermatogenesis and vitelogenesis occurred throughout most of the year with spawning from May to November. There were clear seasonal cycles in the gonadosomatic index.

The reproductive cycle of *Creagrutus bolivari* females in a tropical stream in northern Venezuela was divided in three stages as function of volume of ovary which are ‘rest’, ‘immature’ and ‘mature’ and it was reported that body weight has a higher correlation with ovarian weight than with standard length. The slope of ovarian weight versus body weight increased with the stage of ovary development. The main period for gonadal maturation and spawning was the dry season with a secondary period in the wet season. The condition factor decreases as the gonadal maturation process reaches the end of this cycle (Ortaz, 1997).

Amin (1997) in his study on gonad differentiation and early gonadal development of the European eel, *Anguilla anguilla* in Egyptian waters reported that primordial germ cells first appear in 6.4-10.0 cm elvers and the oogonia in 15.0 cm ones. He further reported that Elver’s destined to become females differentiate sexually at a length beyond 19.0 cm and those destined to become males beyond 27.0 cm but some adult fish 38.0 cm in length still have undifferentiated gonads.

Maddock and Burton (1998) studied the largest increase in gonadasomatic index of American plaice, *Hippoglassoides platessoides* occurred between January and February and reported that gonad development advanced from December to April. Ovaries from spawning females showed hydration patterns and histological detail consistent with batch or serial spawning strategy. Evidences of spawning activity including the presence of post-ovulatory follicles were found in ovaries which also contained oocytes undergoing exogenous vitellogenesis during this period. Oocyte size frequency distributions show a gap in size between cortical alveoli and vitellogenesis from a previtellogenic condition during the spawning period.

Kumar *et al.* (2003) studied the annual reproduction cycle of male rohu, *Labeo rohita* (Ham.) and observed that the testicular maturity and spermiogenesis during spawning phase seem to be correlated with the lowering of water temperature, attributable to rainfall. The primary spermatogonial cells and spermatocytes, a few lobules with spermatozoa and spermatids were observed in preparatory phase. The peak of spermatogenesis having active interstitial leydig cells was recorded during pre-spawning phase. During spawning phase, most of the testicular lobules were packed with sperm masses alongwith a few secondary spermatocytes and spermatids. Decreased interlobular space with some inactive interstitial cells was noticed during post-spawning phase.

Danley *et al.* (2005) studied the reproductive cycle of the Thorny skate (*Amblyraja radiata*). Gonadosomatic index (GSI), follicles size and egg case formation were observed. There was significant increase in GSI during certain months. The females had large preovulatory follicles present in their ovary with the exception of June and September. He observed monthly fluctuation in spermatogenesis and GSI.

2.2 Spawning

The spawning is one of the most significant phases of the reproductive cycle. It comprises ovulation and oviposition in the female, spermiation and sperm release in the male. The success or failure of spawning has direct impact on the population size, continuation and survival of the species. Hence, determination of the spawning season and spawning frequency during a season or during the life span of the fish are of utmost importance and essential prerequisites for assessment of the reproductive potential of species. In addition, the study of locality and nature of spawning ground, has greater relevance to several applied aspects of fishery.

The term spawning season is referred to the time of peak maturity and the period of ovulation or spermiation in a population (Quasim, 1973). It differs from species to species. According to Jhingran (1982), majority of fishes all over the world are seasonal spawners and in the Indian Sub-continent, a vast majority of the freshwater fishes spawn at the time of heavy rainfall. According to Nikolskii (1963), the spawning timings in fishes are so precise that the chances of survival of their eggs and larvae are maximal. This is possible through the optimal co-action of a number of factors. The chief among them are the internal-endocrinology and external-environment or ecological factors (Amoroso and Marshall, 1960). Of the many possible environmental factors, the temperature, photoperiod and rainfall are the important ones in regulating the reproductive cycles in teleosts. Besides, the social factors (e.g. sex ratio in the population courtship behaviour, pheromone, crowding etc.) may be important in synchronizing spawning (spermiation and/or ovulation) in some teleosts (Chew, 1972; Chien, 1973; Soloman, 1977; Liley, 1982).

Vass *et al.* (1980) studied the reproductive biology of *Schizothorax niger* and observed that *S. niger* breeds in shallow parts of Dal Lake. Breeding extended over a period of 2 months from the first week of March till end of April. Qadri *et al.* (1983) reported that *Schizothorax plagiostomus* spawns during April-June and chiefly in May. Extrinsic factors such as warmer water and increased photoperiod were necessary for successful breeding. Sunder (1984) studied the spawning biology of *Schizothorax curviforns.* Ova were ripe in January, but spawning only occurred from May till July and chiefly in June. However, Koul (1988) observed that *Schizothorax* brooders move from Lakes to nearby streams and from the main river Jhelum to tributaries with gravel beds. Micale *et al.* (1987) reported that white bream *Diplodus sargus* spawning occurred in winter. Environmental factors are likely to have influenced the reproductive activity of the captive fish. Shah *et al.* (2006) observed that maximum body weight for both male and female recorded during March to May coincided with pack spawning period of *Schizothorax esocinus*.

Yoon (1981) observed the maturity of the spawners gradually advanced to the yolk vesicle stage with a low level of GSI value from spring to summer thereafter active accumulation of yolk occurred and GSI value increased steadily through summer, though the time of yolk formation was different among individuals in walleye pollock, *Theragra chalcogramma*.

Fowler *et al.* (1999) studied the reproductive mode in King George whiting and revealed that King George whiting is a multiple batch spawner with asynchronous development. Spawning occurred from March-May with peak in April.

2.3 Gonadosomatic index (GSI)

GSI values increase with the maturation of the fish become maximum during peak of maturity and decrease abruptly and sharply when the fish becomes spent (Khan, 1945; Ganpatti and Chako, 1954; Pathak and

Jhingran, 1977; Piska and Devi, 1993). Prasad *et al.* (2005) observed peak values of GSI in the month of May were to be 19.1148 in female (*Puntius sophore*) which fell sharply in June which coincided with the mature stage and spawning stage respectively. Phukon and Biswas (2002) observed the seasonal fluctuation in GSI for males and females in *Erethistes pussilus*. The GSI showed increasing trend from October onwards in both males and females and the peak value was found during June and July indicating the beginning of spawning. Thereafter, the value dropped abruptly and remained low till September showing termination of breading season. According Bouain and Siam (1983), Biswas *et al.* (1984), Sinovcic (2000), GSI is an indicator of Fish Spawning in temperate and tropical region.

Narejo *et al.* (2003) studied the reproductive biology of air breathing freshwater mud eel, *Monopterus cuchia* (Hamilton) and observed high GSI values during April-June for female (7.52 ± 1.15) and male (5.50 ± 1.25) indicated that the fish has only one breeding season during summer. However, Agarwal (2007) calculated gonadosomatic indices for male and females fishes separately. In male fish, it increased gradually from 0.4165 (January) to 9.7405 (September) attaining a peak during October (13.231) corresponding to the GSI values observed in females 'i.e' 0.625 (January) to 14.841 (October (3.243 to 9.745) in male and 6.667 to 13.363 in female) was indicative of onset of spawning activity. In number the GSI values decreased from 13.231 to 8.626 in male fishes. Furthermore, an abrupt fall in the GSI values in December from 10.666 to 4.656 in females and from 8.626 to 2.549 in the males was observed and thus showing the cessation of the spawning act in *S. plagiostomus*.

2.4 Length-weight relationship and condition factor

Length-weight relationship and the condition factor are useful tools for understanding the biological factors in fish stocks (Lecren, 1951). Sarker *et al.* (2004) studied the morphometry and length-weight relationship of *Megalaspis cordyla*. The morphometric characters revealed positive allometric growth and high interdepence ('r' = 0.85-0.98). The length-weight relationship was described log W = -4.74440573 + 2.87664383 log L. The condition factor of the population varied from 0.7 to 1.2.

Kumar *et al.* (1979) studied the length-weight relationship and ponderal index of brown trout catches from five Kashmir streams. The value of exponents in the length-weight equations estimated for males and females indicated that females depart more from the cube law. The general length-weight for the fish was calculated as log W = -5.2844+3.14862 log L. Condition factor ranged from 1.19 to 1.31.

Kosygin and Vishwanath (1999) studied the biology of *Semiplotus manipurensis* and found that the length-weight equation for the fish was calculated as Log W = -5.1984+3.1508. The average value of relative condition factor ‘Kn’ was found to be one indicating a general good condition.

Singh and Singh (2005) studied the length-weight and condition factor of *Puntius sophore* and showed the applicability of the cube law to *P. sophore* (a =2.675, r = 0.98). C0-efficient of condition factor (K) for both sexes combined was found to vary from 1.13 to 1.60 with a mean value of 1.38. Jhingran (1972) recorded the condition factor in gangetic anchovy, *Septipinna phasa* (Hamilton) and found that the point of inflexion on the ponderal index curves plotted at various lengths for male and female gave the size at first maturity. In April-May, decline in the condition factor coincided with peak spawning and low feeding intensity of the fish. According to Hart (1946) the condition factor value can be taken as fair indication of the spawning months of the fish. However, according to Papageorgiu (1979) the fall in the K value of a fish in the non-breeding months may be due to low food intake, adverse environmental conditions and high degree of parasitism. Doddamani *et al.* (2001) studied the length-weight relationship and condition factor of *Stolephorus bataviensis*. The relationship for male was expressed by log w = -1.036946+1.809955 log L and for female, log w = -1.836013+2.63931 log L. The mean values of relative condition factor (Kn) varied between 1.00172-1.01433, respectively. Kumar (2005) studied the maturation and spawning of *Decapterus russelli* (Ruppell). The spawning period of the *Decapterus russelli* is prolonged extending from March to December with peak in April-May and September. Fishes with mature, ripe and spent gonads were noticed from March to December. The relative factor (Kn) and gonadosomatic index (GSI) values ranged between 0.93-0.98 and 0.75-3.85 respectively with peaks in March-May. Vinci *et al.* (2005) studied the Biology of *Gudusia chapra* (Hamilton) and observed the regression equation which was log w= -4.756 log L 2.8576 (r = 0.9176). The fish has a prolonged breeding season, extending from March to October, during which it releases eggs in batches. The relative condition factor shows well being of the fish. Kumar (2004) studied the some aspects of the biology of *Nemipterus japonicus*. The length-weight relationship of *Nemipterus japonicus* was log w =-4.7338 + 2.9902 log L (r =0.9786). Spawning occurs mainly from November to December and in February the fecundity ranged from 14212 to 46387.

Olurin and Aderihighe (2006) studied the length-weight relationship and condition factor of pond reared *Oreochromis niloticus* and observed the value of regression coefficient obtained from the length-weight relationship which was found to be 3.10. This suggests an isometric growth form in all the specimens sampled. The condition factor computed was 1.11 which suggested that the specimens were healthy.

CHAPTER – 3

MATERIALS AND METHODS

The present investigations on *Schizothorax esocinus* were carried in Fishery Biology Laboratory, Faculty of Fisheries. However, histology of gonads was conducted in Veterinary Pathology Laboratory Faculty of Veterinary and Animal Husbandry, Shuhama.

3.1 Fish

Fresh specimens of *Schizothorax esocinus* (Plate-1) with different length-weight groups were procured from fish landing centre on monthly basis.

3.2 Morphometry

The weight of the fish (in grams) and other morphometric data including total length, standard length, head length, prepelvic length, predorsal length, preanal length, prepectoral length were recorded in millimetre (mm). The length-weight relationship was calculated using the formula :

$$W = aL^b$$

Where,

L = Total length in mm

W = Total weight in grams

a and b are constants

Plate-1 : Collection of *Schizothorax esocinus* samples

The condition factor (K) was determined by the formula :

$$\text{Condition factor } K = \frac{W \times 10^5}{L}$$

Where,

W = Weight in grams

L = Total length in cm

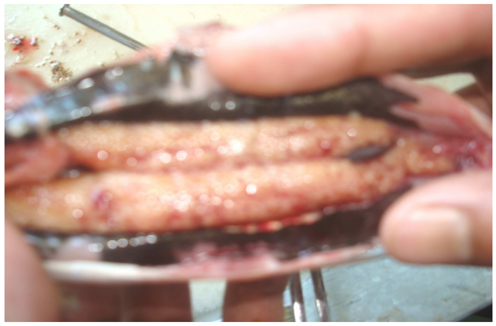
10^5 = is a factor used to bring of K near unity

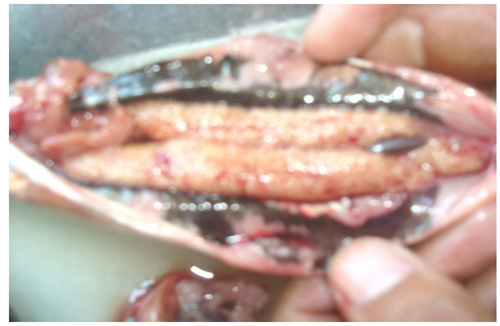
3.4 Morpho-histological study of gonads

Fish specimen were dissected open, gonads were collected (Plate-2) and their length and weight recorded. GSI was determined using the formula :

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

After recording morphometric characters gonads were immediately fixed in aqueous Bouin’s fluid for 24 hours. After fixing in aqueous Bouin’s fluid, tissues were washed in 70 per cent alcohol in various changes till the yellow colour of picric acid was removed. Further gonads were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax (58-60^{o}C congealing point) [Plate-3]. Sections, 6-8 µ thickness were cut and stretched on albuminised slides and fixed at 60^{o}C for overnight.





Dissection of *S.esocinus* for collection

Mature Testis of *S. esocinus*

Ripe Ovary of *S. esocinus*

Plate 2 : Collection of gonads of *S. esocinus*

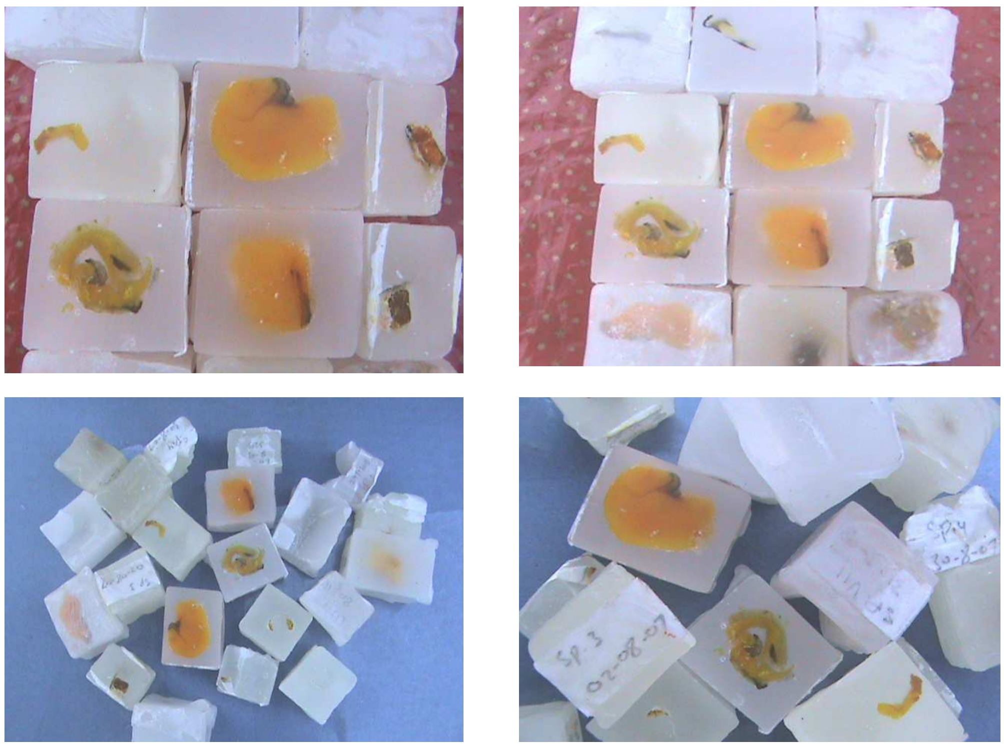


Plate 3 : Wax blocks containing gonads prepared after embedding with wax

Sections were deparaffinised in three changes of xylene and rehydrated in descending grades of alcohol to distilled water. Sections were stained in haematoxylin for 20 minutes, differentiated in 1% of alcohol and blued in ammonia water. After washing sections were stained with eosin (working) for 10 minutes. Dehydrated and cleaned sections were then mounted in DPX (Luna, 1968). Various events of gonadal development were recorded through microphotography.

CAPTER – 4

EXPERIMENTAL FINDINGS

The results of the present study on biometrics and gonadal activity of snowtrout *Schizothorax esocinus* are as under :

4.1 Morphometry

During the present investigation on the morphometry of *Schizothorax esocinus*, the coefficient of correlation (r) for various characters i.e. total length v/s body weight, total length v/s head length, standard length v/s body weight and body weight v/s maximum depth ranged from 0.819-0.968 indicating very high degree of relationship among the characters compared (Table-1; Fig. 1). The values of correlation coefficient amongst various morphometric characters have been depicted Table-2; in Figs. 2-5. The correlation coefficient (r) value was noted highest between body weight and maximum depth and least between total length and head length (Figs. 2-3). Regression analysis observed was as follows :

$$\text{Log } W = -4.7441 + 2.8973 \text{ Log } L$$

4.2 Condition factor

The condition factor of the *Schizothorax esocinus* varied from 0.57 to 0.85 with an average value of 0.75 close to 1 indicating that the fish in the population are in good health condition (Tables 3-4; Fig. 6). This shows the sufficient availability of food for the species.

Table-1 : Morphometric characters of *Schizothorax esocinus*

Measurement	**N**	**Min.**	**Max.**	**Mean**	**SEM±**
Body weight (g)	72	60.00	575.00	206.1	13.1
Total length (mm)	72	180.00	387.00	289.90	4.89
Standard length (mm)	72	155.00	335.00	250.46	4.12
Head length (mm)	72	42.00	85.00	65.06	1.01
Max. body depth (mm)	72	13.00	37.00	23.02	0.505
Predorsal length (mm)	72	81.00	173.00	128.72	1.98
Prepectoral length (mm)	72	39.00	82.00	61.21	1.02
Prepelvic length (mm)	72	82.00	183.00	133.39	2.44
Preanal length (mm)	72	116.00	256.00	188.50	2.81

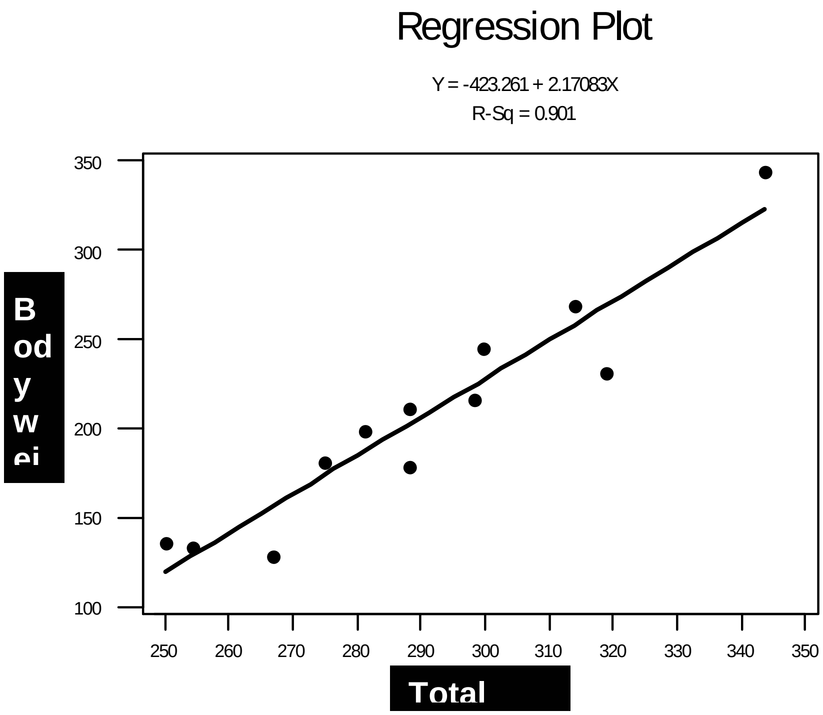


Fig. 1 Length-weight relationship of *Schizothorex esocinus*

Table-2 : Correlation coefficient (r-values) for various morphometric characters of *Schizothorax esocinus*

Morphometric characters	**r-value**
Total length and body weight	0.94
Total length and head length	0.81
Standard length and head length	0.85
Standard length and body weight	0.90
Total length and maximum body depth	0.89
Body weight and maximum depth	0.96

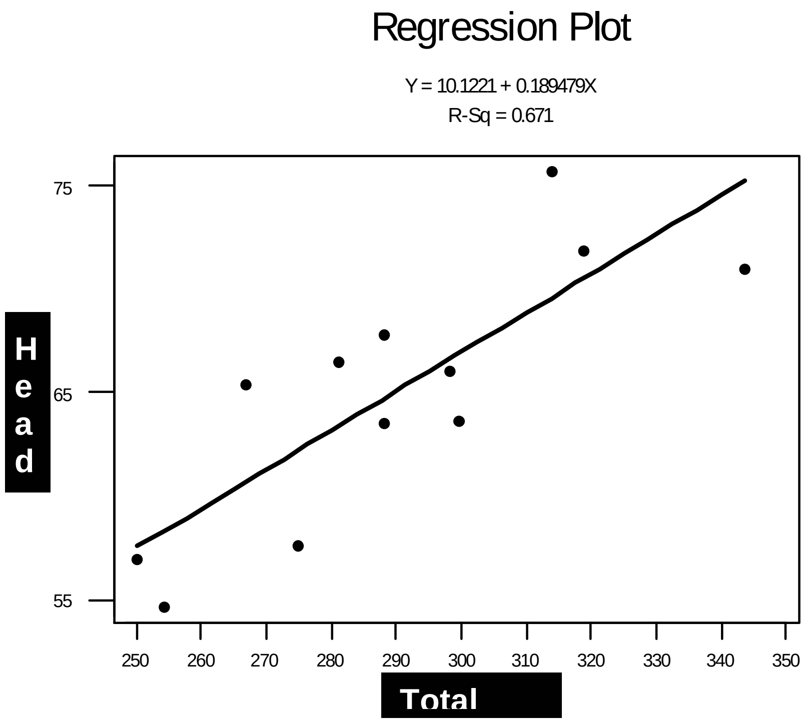


Fig. 2 : Relationship of head length with total length

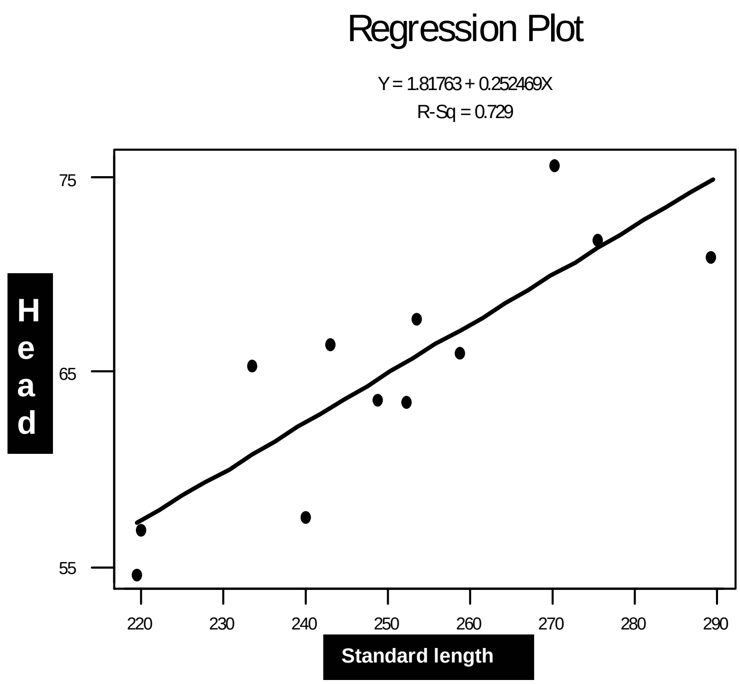


Fig. 3 Relationship of head length with standard length

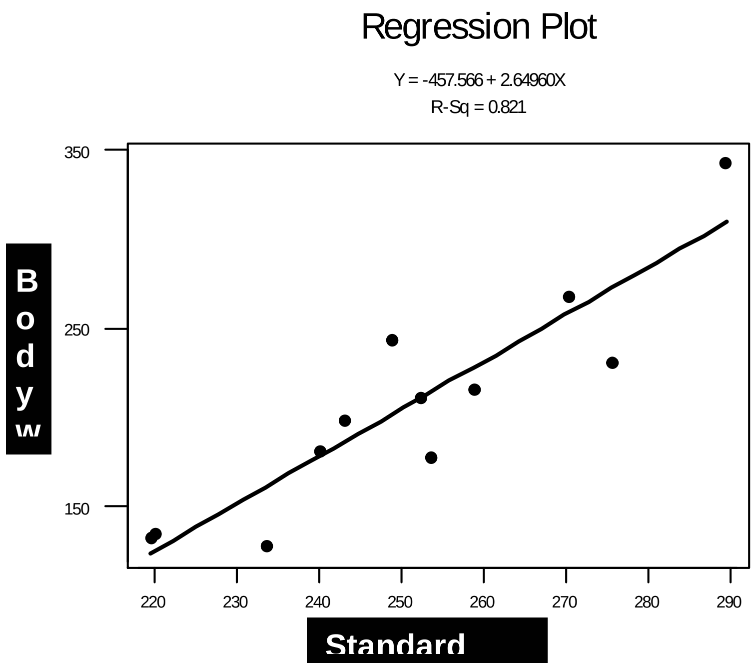


Fig. 4 : Relationship of body weight with standard length

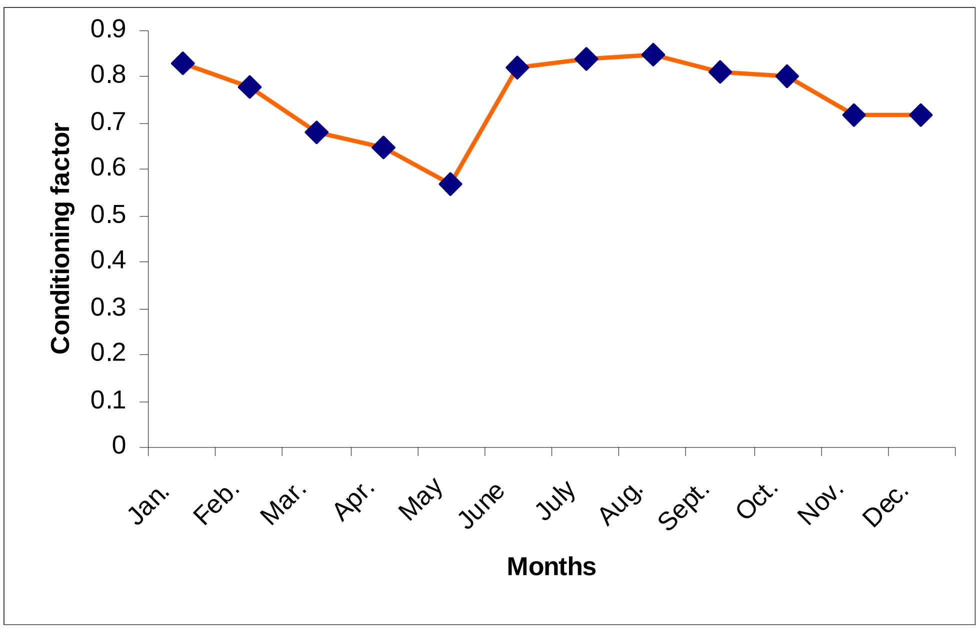


Fig. 5 : Monthly variation in conditioning factor (K) in *S. esocinus*

Table-3 : **Gonadosomatic index (GSI) and conditioning factor (K) of *Schizothorax esocinus***

Measurement	**Min.**	**Max.**	**Mean**	**SEM±**
GSI (female)	0.94	20.11	8.65	1.65
GSI (male)	0.74	18.21	7.28	1.53
Condition factor (K)	0.57	0.85	0.75	0.02

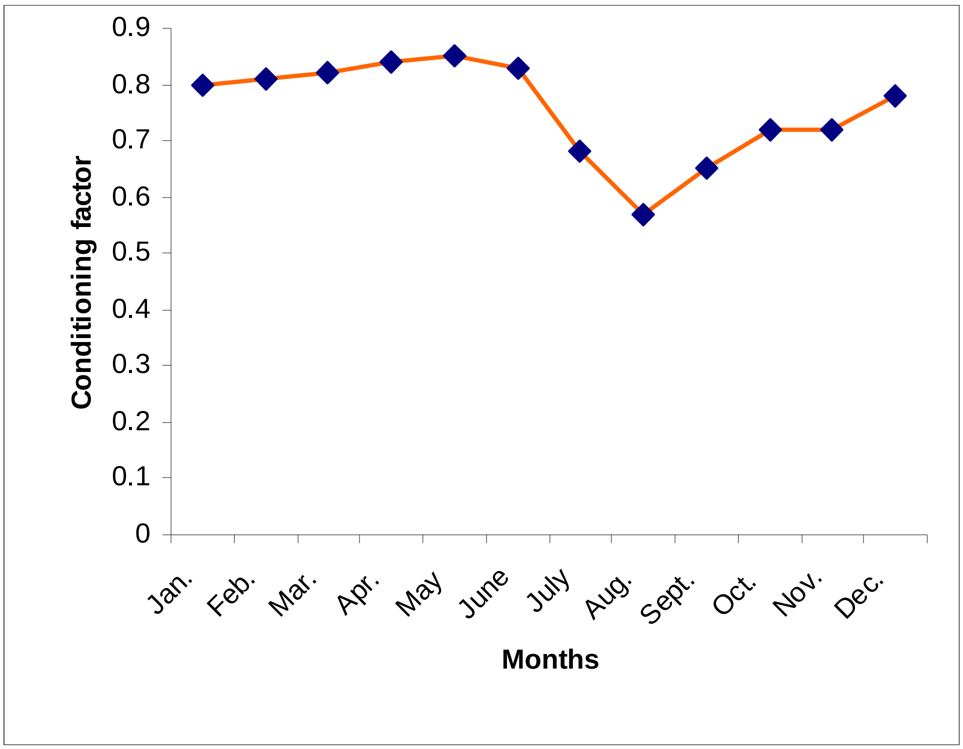


Fig. 6 : Monthly variation in conditioning factor (K) of *Schizothorax esocinus*

Table-4 : **Monthly variation in conditioning factor (K) of *Schizothorax esocinus***

Month	**Min.**	**Max.**	**Mean**
January	0.66	0.90	0.80
February	0.55	1.11	0.81
March	0.77	0.86	0.82
April	0.70	1.05	0.84
May	0.66	0.95	0.85
June	0.76	1.02	0.83
July	0.30	0.88	0.68
August	0.09	0.86	0.57
September	0.50	0.81	0.65
October	0.58	0.90	0.72
November	0.58	0.90	0.72
December	0.68	0.86	0.78
Pooled mean	**-**	**-**	**0.75±0.02**

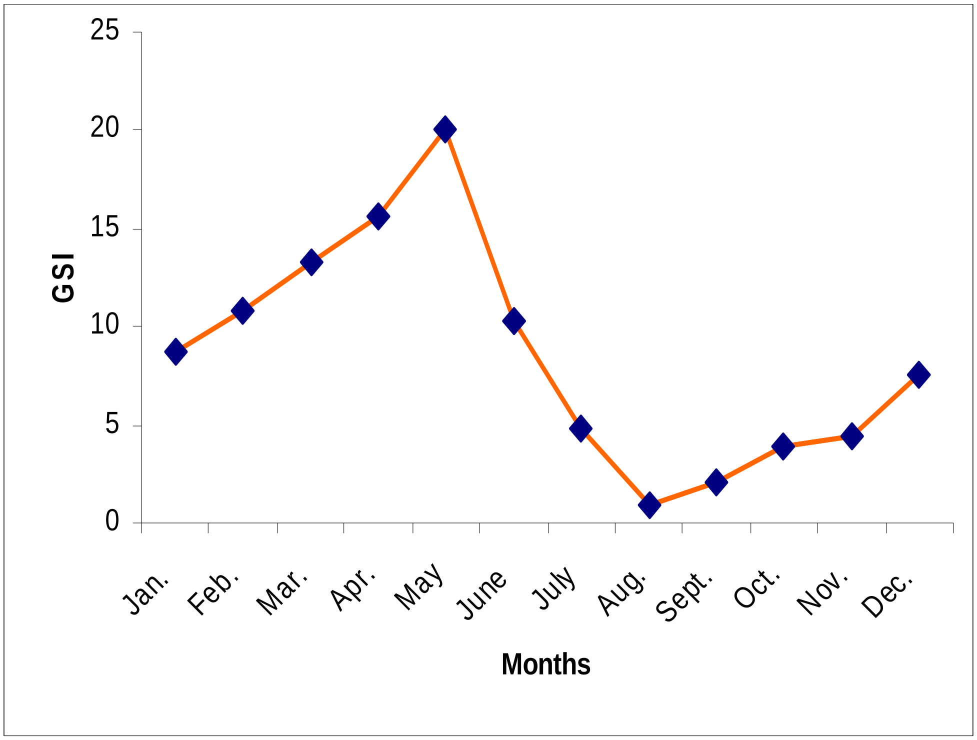


Fig. 7 : Monthly variation in gonadosomatic index (GSI) of female *Schizothorax esocinus*

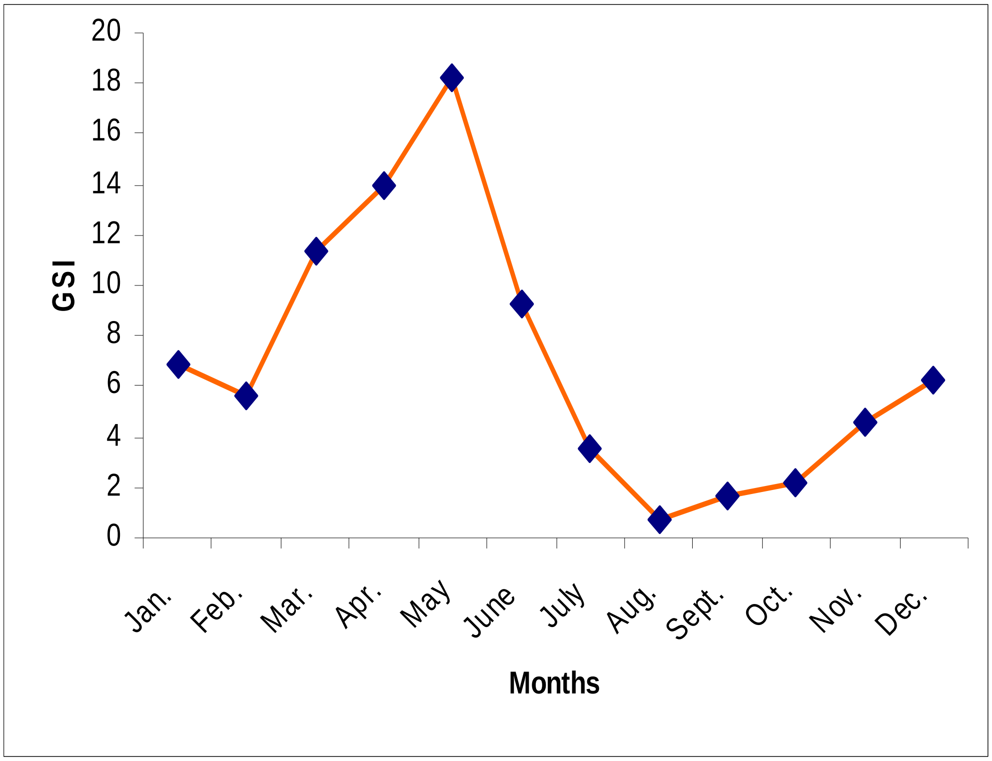


Fig. 8 : Monthly variation in gonadosomatic index (GSI) of male *Schizothorax esocinus*

4.3 Gonadosomatic index (GSI)

The gonadosomatic index is a suitable indicator of the gonadal development that can be useful for determination of fish reproductive period because increasing GSI values are associated with maturation and decreasing values with gamete extrusion or resorption (Lecren, 1951).

GSI values showed increasing trend from March onwards forming peak in May (GSI = 20.11 in female, 18.21 in male) in both sexes (Table-5; Fig. 7-8). During July GSI values declined sharply in both the sexes (GSI = 4.82 in female, 3.56 in male). GSI values declined further in August and thereafter improved with some fluctuation. The peak in the month of April-May indicated the mature (ripe) stage of the fish whereas sharp fall in the month of June-July are indicative of its spawning season (Table-5).

4.4 Histology

4.4.1 Ovaries

Ovarian reproductive cycle of *S. esocinus* exhibits the phases of multiplication, growth, differentiation, maturity, depletion. On the basis of gross morph-histological changes occurring in the ovary, percentage and duration of different stages of developing oocytes and changes in gonosomatic index, the ovarian cyclicity of *S. esocinus* has been divided into :

i) Immature phase

ii) Preparatory phase

iii) Maturing phase

iv) Ripe/Spawning phase

v) Depletion/Spent phase

Phase-I : Immature phase

This phase extends from September to October. During this phase the inactive ovaries are long, slender, thread-like, translucent, and slightly fleshy in texture and running along almost $3/4^{th}$ length of the body cavity. GSI values recorded are 2.09 and 3.91 for September and October, respectively (Table-5; Fig. 7). The ova are not visible by the naked eyes. The ovaries show prominent alligerous folds, budded off from the germinal epithelium of variable size as revealed by light microscopy oogonia and oocytes nests are clearly visible. This phase corresponds mainly to the oocytes of chromatin nucleolus and early perinucleous stage. Some oocytes of late perinucleolus stage are also visible. The blood supply in the ovary is relatively poor at this stage and is mostly confined to the ovarian wall (Plate-4a).

Table-5 : **Monthly variation in gonadosomatic index (GSI) of *Schizothorax esocinus***

Month	**GSI (F)**	**GSI (M)**
January	8.78	6.89
February	10.84	8.59
March	13.28	11.36
April	15.65	13.96
May	20.14	18.21
June	10.32	9.29
July	4.82	3.56
August	0.94	0.74
September	2.09	1.68
October	3.91	2.21
November	5.44	4.59
December	7.58	6.23
Pooled mean	**8.65±1.65**	**7.28±1.53**

Phase-II : Preparatory phase

During this period, the ovaries increase in weight and volume and become light yellow in colour. The blood supply is still inconspicuous. This is the beginning of the maturation phase, which is characterised by the formation of yolk vesicles in the peripheral ooplasm (Plate-4b, c). This phase spans from November to January with GSI values of 5.44 for November, 7.58 for December and 8.78 for January (Table-5; Fig. 7).

Phase-III : Maturing phase

This phase extends from February to March. The ovary becomes yellowish in colour, increases in volume and weight and covers nearly the entire length of the body cavity. There occurs increase in GSI with values of 10.84 in February and 13.28 in March (Table-5; Fig. 7). The tunica albuginea becomes thin. The colour of the ovary becomes yellow with reddish tinge due to the increased supply of blood. Some atretic previttelogenic eggs in different stages of resorption also make their appearance (Plate 4d and Plate-5a).

Phase-IV : Ripe/Spawning phase

This phase extends from April to June with peak spawning in May. The ovaries are greatly enlarged attaining their maximum weight and volume filling up the entire body cavity. Maximum GSI values recorded during this period are 15.65, 20.14 and 10.32 for April, May and June, respectively (Table-5; Fig. 7). The oocytes are ripe and heavily loaded with yolk and may extrude out if slight pressure is applied on the abdomen. There are also few atretic vitellogenic oocytes in different stages of resorption. The discharged postovulatory follicles make their appearance first in April/May, suggesting the beginning of spawning (Plate-5b, c, d, Plate-6a).

Phase-V : Depletion/Spent phase

This phase extends from July to August. During this phase, the ovary is very much shrunken, flaccid, dirty yellowish in colour and of much reduced weight. GSI values observed are 4.82 for July and 0.94 for August (Table-5; Fig. 7). The tunica albuginea becomes thicker. It shows unovulated mature yolk eggs as well as postovulatory follicles in different stages of resorption. As a result of artretic process, corpora atretica may be observed abundantly in the vascular stroma (Plate-6b).

4.4.2 Testes

On the basis of morpho-histological observations, the seasonal testicular cycle of *S. esocinus* may be divided into following phases :

Phase-I	:	Phase of Relaxation/Rehabilitation
Phase-II	:	Phase of Slow Spermatogenesis
Phase-III	:	Phase of Rapid Spermatogenesis
Phase-IV	:	Phase of Spawning/Partly spent
Phase-V	:	Phase of Depletion

Phase-I : Phase of Relaxation and Rehabilitation

The phase spans from September to October during which testes are thin slender, translucent and dull coloured. The GSI values found are 1.68 in September and 2.21 in October (Table-5; Fig. 8). During this period the testicular lobules are small and the interlobular spaces are packed with dense stroma consisting of loose connective tissue and blood vessels. Towards the end of this phase, lobules increase in size and they possess a good number of primary and secondary spermatogonia (Plate-6c).

Phase-II : Phase of Slow Spermatogenesis

It extends from November to January. The testes seem to be slightly enlarged but opaque. The GSI exhibits its increasing tendency having a value of 4.59 in November and 6.89 in January (Table-5; Fig. 8). The spermatogenetic activity shows progressive enhancement. The size of the lobules increases gradually with corresponding decrease in the interlobular space with prominent blood vessels. The lobule mass depicts spermatogenic activity as revealed by the presence of primary spermatogonia, secondary spermatogonia and primary spermatocytes (Plate-6d; Plate-7a).

Phase-III : Phase of Rapid Spermatogenesis

This phase extends from February to March with a considerable increase in the weight and volume of the testes during this period. The colour of the testis becomes pinkish due to rich blood supply.

The GSI increased from 8.59 in February to 11.36 in March during this phase (Table-5; Fig. 8). Milt extrudes out if the belly of the fish is pressed slightly.

The histological observation of testes reveal peak spermatogenic and spermiogenesis (transformation of spermatids into spermatozoa) processes during this period. Testes have rich blood supply during this phase (Plate-7b, c).

Phase-IV : Phase of Spawning and Partly spent

The spawning occurs from the month of April to June with peak spawning in May with GSI value of 18.21 (Table-5; Fig. 8). The weight of the testis starts reducing and freely oozing of spermatozoa are observed. The thin testicular and lobular walls and the presence of spermatozoa are the characteristic features of this phase. However, some empty lobules are also present (Plate-7d, 8a, b).

Phase-V : Phase of Depletion

The testes reduce considerably in weight and volume. They appear flaccid, thin and slender due to extrusion of spermatozoa. The colour changes from pinkish red to light brown because of reduced blood supply. The GSI values are 3.56 in July and 0.74 in August (Table-5; Fig. 8). The tunica albuginea becomes thick and interlobular space increases. Some interstitial cells also make their appearance. The sertoli cells are observed in close proximity of lobule wall. The phagocytosis is quite active during this phase and plays an important role in removing the residual spermatozoa. This phase extends from July to August (Plate-8c).

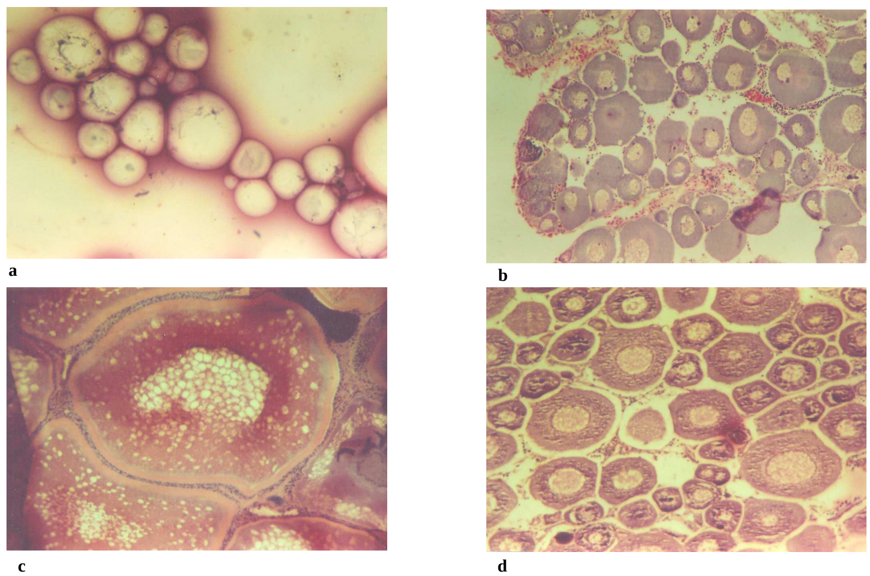
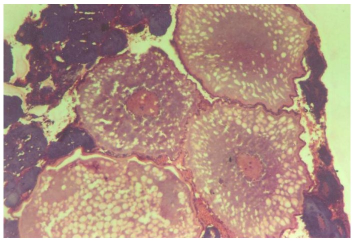


Plate 4 :

- Photomicrograph of T.S. of Ovary of *S. esocinus* (September) depicting proliferation of oogonia and oocyte nests (Immature Phase) X 40
- Photomicrograph of T.S. of Ovary (November) depicting immature oocytes (Preparatory Phase) X 100
- Photomicrograph of T.S. of Ovary (December) depicting immature ovary showing lipid bodies in the germinal vesicle (Preparatory Phase) X 400
- Photomicrograph of T.S. of Ovary (February) depicting maturing oocytes with many nucleoli near the periphery of nuclear membrane with



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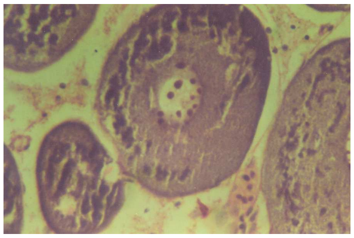

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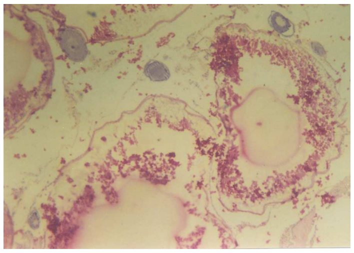

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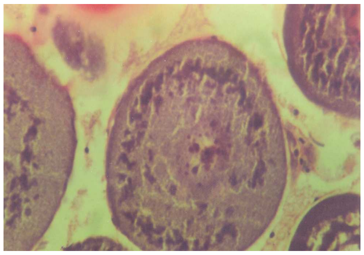

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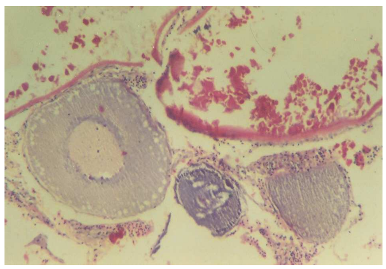
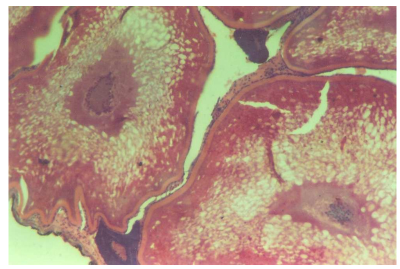
Plate 5 :

- **Photomicrograph of T.S. of Ovary (January) illustrating a large number of cortical alveoli (yolk vesicle) and prominent follicular envelope (Maturing Phase) X 400**
- **Photomicrograph of T.S. of Ovary (April) depicting oocytes with vitellogenesis in progress and some extruded nucleoli in the cytoplasm alongwith atretic oocyte (Ripe/Spawning Phase) X 400**
- **Photomicrograph of T.S. of Ovary (May) illustrating ripe oocytes with cortical alveoli with indistinct nucleus likely to disappear before spawning with new crop of oocytes (Ripe/Spawning Phase) X 400**
- **Photomicrograph of T.S. of Ovary (May) illustrating ripe egg ready for ovulation (Ripe/Spawning Phase) X 400**





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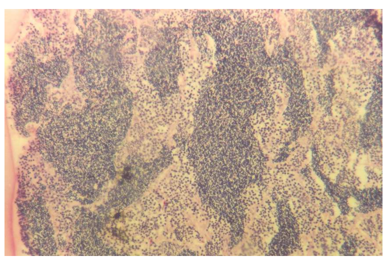

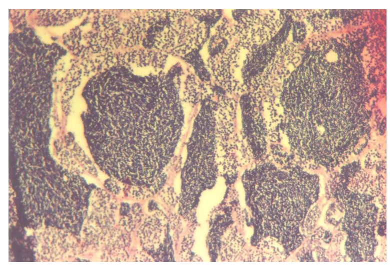

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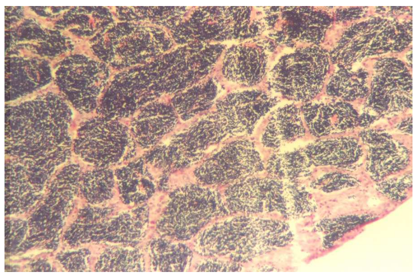
Plate 6 :

- **Photomicrograph of T.S. of Ovary (June) showing ripe oocyte alongwith cortical alveoli and atretic oocytes. Also showing theca and follicular membrane (Ripe/Spawning Phase) X 400**
- **Photomicrograph of T.S. of Ovary (August) depicting unovulated and atretic follicles undergoing shrinkage and distortion (Depletion/Spent Phase) X 400**
- **Photomicrograph of T.S. of Testis (September) showing testicular wall, primary and secondary spermatogonia (Phase of Relaxation/Rehabilitation) X 400**
- **Photomicrograph of T.S. of Testis (November) showing thick lobules of varying sizes containing secondary spermatogonia (Phase of Slow Spermatogenesis) X 400**



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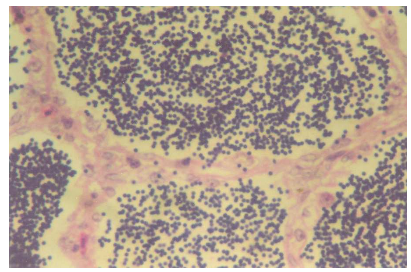

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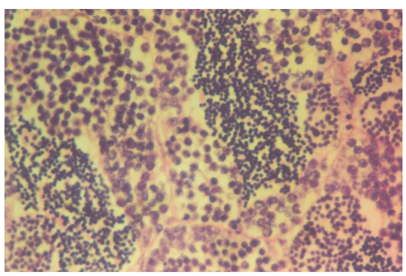

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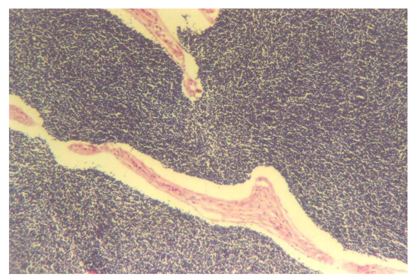

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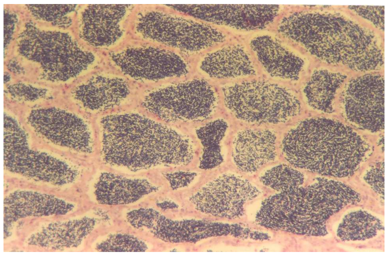
Plate 7 :

- **Photomicrograph of T.S. of Testis (December) showing lobules of primary spermatogenesis (Phase of Slow Spermatogenesis) X 400**
- **Photomicrograph of T.S. of Testis (February) showing secondary spermatocytes, prominent lobule wall and rich blood supply during peak maturity (phase of Rapid Spermatogenesis) X 400**
- **Photomicrograph of T.S. of Testis (March) showing secondary spermatocytes and spermatids (Phase of Rapid Spermatogenesis) X 400**
- **Photomicrograph of T.S. of Testis (April) showing thin walled lobules fully of spermatozoa during spawning period (Phase of Spawning) X 400**



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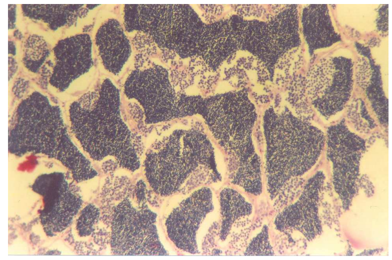

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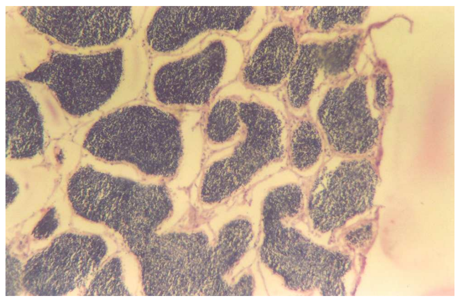

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Plate 8 :

- Photomicrograph of T.S. of Testis (May) showing lamallae full of spermatozoa (Phase of Spawning) X 400
- Photomicrograph of T.S. of Testis (June) showing partly spent lobules (Phase of Spawning/Partly Spent) X 400
- Photomicrograph of T.S. of Testis (August) showing sertoli cells, lobule wall, empty lumen, interlobular space and decreased blood supply (Phase of Depletion) X 400

CHAPTER – 5

DISCUSSION

Some fishes exhibit a distinct sexual dimorphism (Lehri, 1967) while others do not (Rai, 1965; Khanna and Pant, 1966; Bisht, 1974; Shrestha and Khanna, 1976). Owing to the absence of sexual dimorphism, the sexes in *S. esocinus* can be identified by opening the abdomen. However, during the breeding period, milt oozes out by applying slight pressure on the abdomen, and that the female fish have a bulging belly due to enormously enlarged ovaries fully packed with ripe ova. The appearance of melanophores (pigmentation) on the surface of the testes during the breeding season has been taken as a sign of ripeness in *Gasterosteus aculeatus* (Swarup, 1958). Such type of pigmentation has not been observed in *S. esocinus* though the body colour becomes darker and colour of the testes appears pinkish due to the increased vascularization as also reported in *Barbus tor* (Rai, 1965), *Glypotothorax pectinopterum* (Khanna and Pant, 1966), *Channa gachua* (Sanwal and Khanna, 1972) and *S. richardsonii* (Bisht, 1974).

In teleosts the paired testes are varied in their morphology. They are either fused along the entire length (Khanna and Pant, 1966) or completely separate (Dixit and Agarwal, 1974) or fused at the posterior region only (Rai, 1965; Sanwal and Khanna, 1972; Bisht, 1974; Pandey and Misra, 1981). The testes in *S. esocinus* are fused posteriorly to form common spermatic duct and remain smooth during non-breeding period. When the maturity advances the testes look somewhat flattened and exhibit extensive labulation as in *Mystus seenghala* (Sathyanesan, 1959), *Barbus tor* (Rai, 1965) and *Schizothorax richardsonii* (Bisht, 1974). Both left and right testes are generally equal in length as observed during the present study. However, the length of the testes does not show any correlation with their periodic activity whereas their volume and weight vary accordingly.

Within the testes of fish undergoing reproductive activity, six spermatogenic elements namely – primary spermatogonia, secondary spermatogenia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are observed. Primary spermatogonia after multiplication by mitotic division are called secondary spermatogonia and later give rise to primary spermatocytes which undergo mitotic division to form secondary spermatocytes. The life span of secondary spermatocytes is relatively short and they rapidly divide to form spermatids. Thus starting from one primary spermatocyte after one complete meiotic mature spermatozoa by reorganisation of nucleus, golgi bodies, mitochondria, centriole and other cytoplasmic components and acquire flagellum for mobility (Nagahama, 1983).

All the spermatogenic elements have some common cytological and histochemical features such as diffused sudanophilic lipids, granular mitochondria phospholipid bodies and nuclear DNA the histological and histochemical studies on the testes of *S. esocinus* reveal that all the spermatogenic elements posses sudanophillic lipid and phospholipids bodies relatively get reduced in number and size. However, some lipid bodies are also seen during the stage of spermiogenesis. Hence, it may be concluded that phospholipid bodies are the endogenous source of energy, similar observation were also made by Upadhyay and Guraya (1973). However, Vasisht (1954), Stanley (1969) and Mattei (1970) did not report the presence of lipid bodies in the fish spermatogenic cells. Besides, being the endogenous source of energy, phospholipids are the important constituents of membranes of cell organelles. During the spermatogenesis, few phospholipid bodies are retained in the middle piece of the spermatozoa. These phospholipid bodies serve the purpose of endogenous source of energy by their aerobic oxidation needed for the motility of spermatozoa as the sperms are shed into the aqueous environment which lacks metabolic nutrients. This is in accordance with the observations of Minassian and Turner (1966) and Upadhyay and Guraya (1973). Biochemical studies also indicated that the endogenous respiration of spermatozoa depends mainly on the oxidation of intracellular phospholipids present in the middle piece (Nelson, 1967). Upadhyay and Guraya (1973) further stated that phospholipids in the middle piece of spermatozoa might also serve as a phosphorous donor for the reconstitution of adenosine triphosphate (ATP) from adenosine diphosphate (ADP).

The existence, origin, structure and formation of interstitial cells in teleost have been described variously by a number of workers (Hyder, 1970; Lofts and Bern, 1972; Gresik *et al.*, 1973; Guraya, 1976). In *S. esocinus* interstitial cells occur tissue cells distributed singly or in small groups in the interstices between the lobules. But in *Poecilia laptipinna* interstitial leydig cells are distributed around the efferent duct periphery of the testes (Vanden, 1973). Oota and Yamamoto (1966) and Nagahama *et al.* (1978) confirmed their presence in immature testes of rainbow trout and pink salmon. The evidence that interstitial leydig cells originate from fibre blast-like connective tissue elements of the interstitium comes from the ultrastructural studies of testes of *Cichlasoma nigrofasciatum* (Nicholls and Graham, 1972). The cells of fibroblast like tissue exhibit elongated nuclei, small mitochondria and isolated vesicles of smooth membrane (Nicholls and Graham, 1972).

The interstitial cells exhibit well established features of sterioid-secretion in the testes of *Labeo rohita*, *Labeo calabu*, *Labeo goenius* and

Ompok bimaculates (Upadhyay and Guraya, 1973) where abundant diffuse *Sudanophillic lipoprotein* and deeply sundanophiic lipid droplets have been observed. These cells also resemble in respect of some cytological and cytochemical features with the steroid-secreting cells of mammalian gonads (Hyder, 1970; Nicholls and Graham, 1972). Such features include abundant lipoprotein, agranular endoplasmic reticulum, mitochondria with a complex structure (with tabulovesicle cristae) enzyme activity (Hydroxy steroid dehydragenese, HSDH) indicative of steriodogenesis and accumulation of cholesterol containing lipid droplets (Guraya, 1976, 1979, Upadhyay, 1977).

The interstitial cells of the testes of *S. esocinus* exhibit marked changes in their morphological and cytochemical characteristics and also conspicuous seasonal cyclicity in their number and size, being maximum from July-October (phase of depletion, relaxation and rehabilitation) diminishing gradually in number as well as size by the mobilization of their lipoidal content such as – lipid, phospholipids, neutral lipid and cholesterol during maturity following by complete disappearance during April to June (phase of spawning/partly spent). This lipid mobilization may be related to active secretion of steroid hormone which controls the development of spermatogenic elements and reproductive behavioural activity (Guraya, 1976).

Guraya (1976) considered that beside the interstitial and lobule boundary cells testes of some teleost species also have sertoli cell-sites of steriodogenesis. According to him the cholesterol containing lipid accumulation in the lobules of spent phase in *Ompak bimaculatus* is due to the activity of sertoli cells. This accumulated lipid rapidly disappears as the spermatogenetic activity advances, suggesting the possible conversion of cholesterol positive lipid into steroiod hormone. Some investigators are of the opinion that the lobule-boundary cells are homologous with interlobular space by a thin but distinct basal lamina and their basal lamina and their close proximity to spermatids and spermatozoa (Upadhyay, 1977). Functionally, these cells appear to be actively engaged in phogocytosis of residual bodies, degenerating germ cells and in the transport of metabolities (Billard *et al.*, 1972). According to de Valaming (1974), these may perform nutritive, conductive, supportive and steroid producing functions. In *S. esocinus*, sertoli cells were observed in the ripe as well as spent testes intimately associated with lobule wall and mature spermatozoa. Their precise function in the fish under study seems to be obscure. They may play some role in resorption of residual spermatozoa or they may be nutritive in function.

The testicular annual cyclicity of *S. esocinus* is concerned with successive stages of relaxation and rehabilitation, spermatogenesis, activation and depletion. In different species, the spermatogenic activity starts at different times of the year (Rai, 1965; Nair, 1966; Shrestha and Khanna, 1976; Nautiyal, 1983). Such variations are probably due to the local physiochemical and environmental factors. Accordingly to Swarup (1958), though the testes of *Gasterosteus aculeatus* may be sexually mature is attained only during breeding period (April-May). Some investigators reported the period of quiescence or inactivity during the reproductive cycle of teleost species studied by them (Ruby and McMillian, 1970). But there is no resting period in some fish species (Sanwal and Khanna, 1972). The testicular cyclicity is closely related to the changing environmental factors (Ahsan, 1966).

In the present fish testes also undergo cyclic changes. The spermatogenic activity starts in November increasing gradually till June and reaching its peak in the month of May when the lobules are full of sperms. Now the activity slows down to almost cessation by July and August. The testes after passing over a period of relaxation and rehabilitation upto

September and October enter the new annual cycle.

Among teleost fishes the origin of new crop of oocytes presents a controversial picture and the earlier observations are variable and much disputed regarding the formation of germ cells. The new crop of oogonia in liopsetta (Yamamoto, 1956) develop from the follicle cells of postovulatory follicle, while on other hand, the origin of new germ cells have been described from the germinal epithelium in certain teleosts (Rai, 1967; Khanna and Pant, 1967; Nauriyal, 1983) and pre-existing residual oogonia of the previous cycle (Khanna and Sanwal, 1974). But according to Gopal and Govindan (1969) the germ cell in *Anabas scandens* have dual origin i.e. from germinal epithelium as well as from the germinal epithelium which increases in number by repeated division inside the oogonial nest and most of them form the eggs of the current cycle, while some of them are left behind and serve as a part of the parent stock for the succeeding cycle which is also contributed by the cells from the ovarian germinal epithelium.

The oogonia undergo proliferation by mitotic division and are known as oocytes, which enter a period of growth because the egg contributes greater part of substance used in development, the growth appears to be of great significance in oogenesis. It may be divided into two phases, previtellogenesis and vitellogenesis.

There are several views regarding the origin, extrusion and function of the nucleoli in teleost fishes. According to Raven (1961), Khanna and Pant (1967), Bisht and Joshi (1975) and Shrestha (1980), there may be one or two nucleoli initially present in the germinal vesicle which produce a number of nucleoli by their division or fragmentation. However, McGregor (1972), Guraya *et al.* (1975) and Rita Kumari and Nari (1979) believed that nucleoli originate from certain heterochromatic regions of chromosomes known as nucleolar organiser. Chouinard (1963) opined that non-heterochromatic region distinct from nucleolar organising region is also instrumental in the formation of extra nucleoli. In *S. esocinus* initially 2-3 nucleoli are seen in the nucleoplasm. As the growth advances, nucleoli increase in number but decrease in size which clearly suggests that extra nucleoli are formed by division and fragmentation of initial nucleoli.

Many divergent views have been expressed regarding the nucleolar extrusion in fish oogenesis. Lal (1963) observed complete absence of nuclear extrusion in fish oocyte. According to him nuclear envelope does not allow any transmission of nucleoli through it and so called nucleorlar extrusion is to be mere artifacts due to mechanical disturbance during sectioning of the material. On the other hand extrusion of nucleoli are observed in some growing previtellogenic oocytes of teleost (Khanna and

Sanwall, 1974; Bisht and Joshi, 1975; Guraya *et al.*, 1975, 1977; Ramadan *et al.*, 1978; Shrestha, 1980; Guraya, 1986). Kapoor (1977) observed the presence of nucleoli in contact with the nuclear envelope and suggested that they are passed into surrounding cytoplasm of oocytes in *Puntius ticto* and finally disappear near the periphery of oocyte.

In *S. esocinus* nucleolar extrusion has been observed in some growing previttelogenic and early vitellogenic oocytes. It suggests the actual extrusion of nucleoli from nucleoplasm to ooplasm through the nuclear envelope, as observed by some earlier workers (Yamamoto, 1956; Hoar, 1965; Braeckevelt and McMillan, 1967; Guraya, 1986). The morphological and cytochemical changes in extruded nucleoli also provide strong evidence to their extrusion. Nucleoli similar to those of nucleoplasm are seen in the ooplasm of previtellogenic and early vitellogenic oocytes. After the extrusion of nucleoli into the ooplasm they finally break up into pieces and simultaneously disappears as also observed by Guraya (1965) in *Channa marulius*.

The occurrence of large cortical alveoli is the most characteristic feature of the teleost egg (Donato *et al.*, 1980; Takahashi, 1981). In the oocyte of dog fish (Guraya, 1982) and other cartilaginous fishes (Ginshburg, 1968). These are absent throughout the oogenesis. Various investigators have termed the cortical alveoli as cortical vacuoles, intra vacuolar yolk, intravesicular yolk, carbohydrate yolk, yolk vesicle etc.

The vitellogenic oocyte of *S. esocinus* in early phase develops a population of bodies (Cortical alveoli) of variable size, randomly distributed in the outer region of ooplasm from where they spread towards the inner cytoplasm. Later on with the maturity of oocyte they aggregate in the cortical ooplasm of mature eggs by forming a conspicuous zone. This is in agreement with the observations made by Guraya (1965) and Guraya *et al.* (1975, 1977).

Regarding the origin of cortical alveoli there are several views. These are supposed to originate from palial substance identical to yolk nucleus substance (Malone and Hisaoka, 1963), from pre-cortex in the egg of Medaka (Akata, 1954) and from vacuolar or ground substances of ooplasm (Yamamato, 1964). Guraya (1965) noted their origin near the plasma membrane by means of pinocytotic activity in *Channa marulius*. The Golgi complex have been described as a source of cortical alveoli (Danato *et al.*, 1980).

The cortical alveoli are well known to play significant role during fertilization, the cortical breakdown and their colloidal substance and spherical bodies release into the previtelline of the egg, which widens rapidly preventing polyspermy in fish (Guraya, 1982). Keeping in view their location in *S. esocinus*, cortical alveoli help in some way to check the polyspermy.

According to Rajalakshmi (1966) in Gobius, the atretic oocytes are noticed only during the post-spawning period. However, Brackevett and McMillan (1967) observed atresia throughout the year but more abundant during and after spawning. There are several factors responsible for follicular atresia. These are mainly hormonal of intra-ovarian and extra-ovarian sources (Saidapur, 1978) and unfavourable environmental conditions i.e. photoperiod (Saxena and Anand, 1977), over crowding, temperature and inadequate feed supply (Lam, 1983). In addition, increasing water pollution by insecticides, pesticides and industrial effluents also affect the metabolism of fish ovary resulting in the increased incidence of follicular atresia (Saxana and Bhatia, 1983; Mani and Saxena, 1985).

In *S. esocinus*, atretic oocytes are seen during pre-spawning, spawning and post-spawning period. Small atretic previttogenic oocytes are relatively of rare occurrence. Atresia are accompanied by the shrinkage of follicles, resulting in disorganisation of nuclear and ooplasmic component. Hypertrophied granulosa cells and blood cells (Macrophages) invade the nucleo-ooplasmic components, digest it and finally leave a residual minute nodule of stromal element. Such type of invasions have been reported by Rastogi (1968) and Guraya *et al.* (1975, 1977). However, Rajalakshmi (1966) observed atresia without the invasion of oocytes by granulosa cells.

Teleosts exhibit different spawning periodicity and are seasonal breeders. In Indian subcontinent most of the freshwater fishes are monsoon breedrs (Jhingran, 1982). According to Badola and Singh (1984), most of the Garhwal Himalayan hillstream fishes spawn during summer and monsoon months as *Tor tor* and *Tor putitora* (April to July), *Labeo dyocheilus* and *L. dero* (March to June), *Barilius* spp. (April-June), *Glyptothorax pectionpterus* and *Pseudecheis sulcutaus* (April to August) and *Noemacheilus* spp (July to August). In schizothoracids, diversity in spawning season and periodicity exists because of varied ecological environments. According to Jhingran (1982), *S. richardsonii* in Himachal Pradesh spawns from March to June, in Kumaon waters. It spawns from July to December (Bisht, 1974) and in Garhwal Himalaya from July to September (Misra, 1982). However, Kashmir snowtrout, *S. niger* exhibits spawning from mid April to May end (Malhotra, 1966). Bhatnagar (1964) reported that *S. plagiostomus* of Bhakra reservoir breeds twice in a year i.e. from July to August and from December to January. Similarly, two breeding seasons (from September to October and February to March) in *S.*

plagiostomus of Nepal waters have also been reported by Shrestha and Khanna (1976). A single GSI peak value throughout the year, histology of gonads and occurrence of sexually mature fishes from April to June as observed during the present study reveals that the spawning season of *S. esocinus* stretches from April to June with peak in May. Similar observations have been reported by Raina (1977).

The length-weight relationship in fish is generally determined so that if at any other time only one of these two parameters is known, the other can be computed easily by substituting the values of co-efficient ‘a’ and ‘b’ in the formula as mentioned in materials and methods. Further, variations from the expected weight or length of individual fish are an indication of the general well-being of the animal or the state of gonadal development (Lecren, 1951; Sunder, 1985).

Allen (1938) suggested that the value of ‘b’ remains constant at ‘3’ in an ideal fish. However, significant deviations have been reported by later workers. Hile (1936) and Martin (1949) found the value of experiment ‘b’ to be fluctuating between 2.5 and 4.0. Antoney Raja (1967) recorded 35 values of regression coefficient for different fish species ranging from 2.0 to 5.4. Out of these 12 were found to depart significantly from the isometric growth value of ‘3’.

A perusal of length-weight relationship worked out for mirror carp from Mansbal Lake exhibits the exponential value of ‘b’ equal to 2.89 (Yousuf *et al.*, 1992). Sunder *et al.* (1984) have reported the mirror carp from Dal Lake to possess an exponential value for ‘b’ equal to 2.98 in river Jhelum. Soni and Kathal (1979) have reported the value for *Cyprinus carpio* as 3.75 in the tropical lake Sagar. Choudry *et al.* (1982) have reported the value of regression co-efficient to be 2.3477 in case of *Labeo rohita*.

Yousuf *et al.* (1992) have reported the ‘b’ value for *S. niger* of Mansbal Lake as 3.014. Sunder *et al.* (1979) have reported the value of regression coefficient to be 2.347 in case of *S. niger* in Dal Lake of Kashmir. However, Pandit (1987) reported the regression coefficient to 2.977 for the same fish in Dal Lake in Kashmir. Sunder (1985) recorded the value of regression coefficient ‘b’ of *S. curviforns* in river Jhelum (Kashmir) to be 2.888. Qadri and Mir (1979) calculated the value of ‘b’ to be equal to 2.448 in *S. richardsonii* of Sindh Nallah. Bhagat and Sunder (1984) reported the value of ‘b’ in *S. esocinus* of Dal Lake (Kashmir) was to be 3.191. However, in the present study the value of exponent ‘b’ in *S. esocinus* was recorded to be 2.897. Thus the length-weight relationship established for the *S. esocinus* suggested fairly isometric growth closely following cube law.

From the above comparative account, it is quite clear that the regression coefficient not only varies from species to species but also between the populations of the same species occurring in different habitats. However, the limnological features of these habitats do show appreciable differences (Qadri and Yousuf, 1978; and Balkhi *et al.*, 1987) which points to the fact that intra-specific fluctuation in the length-weight relationship in fish are influenced by environmental factors and when the environmental conditions are conducive to the growth, the fish shows value of exponent ‘b’ very nearer to ‘3’.

Condition factor

Hart (1946) stated that the ponderal indices may give a very good idea of the broad outline of the seasonal cycle for the species concerned. According to him seasonal variations in the ponderal index occur with increase in age. He also observed a lower level of condition throughout the seasonal cycle, on account of the increased metabolic strain of spawning. The point of inflexion on the curve was found by him to be a good indication of the length at which the fish attains sexual maturity. During the present study it was observed that highest values of K during April to June (spawning months) were mainly due to maturity of gonads while the lowest values observed during July-August was due to spent condition. The values of condition factor showed fluctuation in all size groups of *S. esocinus* observed during present study. Similar observations were reported by Qaddus (1993) for *Gudusia chapra*, Azadi and Naser (1996) for labeo bata, Shafi and Quddus (1974) for *Cirrhina mrigala*. From the present study it becomes evident that the breeding cycle has a significant effect on the condition factor of the fish. Thus, it may be concluded that *S. esocinus* spawns once in a year during the months of April, May and June with peak spawning in May.

CHAPTER – 6

SUMMARY AND CONCLUSION

Studies on the reproductive aspects of the snowtrout *Schizothorax esocinus* inhabiting the snowfed waters of Kashmir have been carried out so as to generate information regarding the morphology, condition factor, histology and cyclicity of testes and ovaries. The other equally important aspects viz. GSI, spawning periodicity of *S. esocinus* have also been considered during the study. The data generated will provide a basis for the development and rational management of *Schizothorax* fishery of Kashmir valley and for better understanding of biology of spermatogenesis and oogenesis. This will further encourage the biological and experimental approaches utilizing the recent techniques for cryopreservation of gamates, induced breeding and genetic manipulations etc.

Under the study, the morphology and histological aspects of gonads, highlighting development, differentiation, structure and function of various components of the testes and ovaries have been taken care of. As a result of the present study, it has been observed that the testes of *S. esocinus* are paired and elongated structures lying on both the sides of the air bladder. Both the testes, left and right are generally of equal length and free from each other throughout their length except posteriorly where both are united to form a common spermatic duct communicating exteriorly through the urinogenital aperture.

The testes undergoing reproductive activity exhibit six spermatogenic elements namely primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The new crop of spermatogonia arises by the division of resting sperm mother cells. The annual testicular cycle is concerned with the successive stages of relaxation/rehabilitation, spermatogenesis (slow and rapid), activation and depletion. The spermatogenic activity starts in the month of March and reaching its peak in the months of May when lobules are full of sperms and active, spawning takes place.

The ovaries are paired elongated structure lying posterior-dorsally occupying the entire length of the abdominal cavity, Anteriorly, both the ovaries are free while posterior portions join each other to form oviduct, which opens exteriorly through the urinogenital aperture.

Unlike the testis, the whole of the ovary is fertile. The oogonia pass through five maturation stages to form the ripe ova. The germinal epithelium is responsible for the new crop of oogonia. However, the residual oogonia also serve as a part of the parent stock for the succeeding cycle.

The ovarian cycle has been divided into five phases on the basis of histology, duration of various developmental stages of oocytes, increase and decrease in gonadosomatic index. These are (i) Immature Phase (ii) Preparatory Phase (iii) Maturing Phase (iv) Ripe/Spawning Phase (v) Spent/Depletion Phase. The gonadosomatic index exhibits the maximum values during April, May and June. This period forms the spawning phase of the fish.

The length-weight relationship established for the fish suggested fairly isometric growth, closely following cube law. The regression equation obtained was log W = – 4.744 + 2.897 Log L. The condition factor of the present study varied from 0.57-0.85 near to 1 indicating that the majority of fish in the population are in good health condition.

CONCLUSION

On the basis of present investigation, following conclusions could be drawn :

- *Schizothorax esocinus* is an annual breeder and spawns from April to June with peak spawning in May.

- Testicular cycle consists of five phases viz. (i) phase of relaxation/ rehabilitation, (ii) phase of slow spermatogenesis, (iii) phase of rapid spermatogenesis, (iv) phase of spawning/partly spent and (v) phase of depletion.

- Ovarian cycle consists of five stages viz., (i) Immature Phase (ii) Preparatory Phase (iii) Maturing Phase (iv) Ripe/Spawning Phase (v) Spent/Depletion Phase.

- Gonadosomatic index (GSI) values were maximum during beginning of spawning period peak with the value of 20.14 in female and 18.21 in male in May.

- Length-weight reveals that *S. esocinus* has fairly isometric growth closely following cube law.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner during Viva-Voce examination held on 22.12.2008 have been incorporated in the manuscript entitled **"Studies on biometrics and gonadal activity of snowtrout (*Schizothorax esocinus*)"** submitted by **Mr. Shabir Ahmad Dar (Regd. No. 2006-F-06-M)**.

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