CHAPTER V

DISCUSSION
General Morphology and Histology of fish Ovaries

The ovaries in the fishes studied are 'Cystoarian' type and are enclosed in a peritoneal sac; as against the 'Gymnoarian' condition described for elasmobranchs, Dipnoi, Chondrostei, Amia and some teleostei such as Salmonidae, Hyodontidae, Notopteridae etc. (Goodrich 1930). This condition of cystoarian is secondary as in other teleosts with similar ovaries (Brock 1957). It is possible that in these cases the peritoneum, arising from the dorsal body wall, encloses the ovarian primordia lying in the form of bands of cells, the germinal epithelium. A similar the mesovarium extends from the dorsal body wall to continue with the ovarian tunic. Thus the present work confirms that the ovarian origin is completely free from the Müllerian complex, a view put forth for some teleosts by Goodrich (1930). Thus it is established that the Müllerian ducts and their primitive openings have disappeared altogether and are replaced by new formations. This condition is supported by Dodds (1960) for oviducts of other teleosts, in sharp contrast to the majority of other cold blooded vertebrates.
In all the fishes studied, it has been observed that the gradual increase of the ovaries weight is from July to end of December, attaining maximum by the end of March. The active growth does not continue during the winter months. But it is possible that vitellogenesis continues at a low rate within some immature and maturing oocytes even during this winter period (winter diapause). Thus the author's observations reveal that the ovary weight in the fishes studied is maximum during the prespawning and spawning phase, and it is minimum not in the period immediately following the completion of spawning act, but during the recovery phase when discharged ova are resorbed and removed from the ovaries. Mathews (1930) made similar observations on Fundulus. But according to Stern and Corb (1939) the fall in weight is not sudden because spawning is a long drawn-out process.

It has been observed by the author that the oocytes in the adults of the five fishes studied are derived from the germinal epithelium of the ovigerous lamellae projecting inwards from the outer peritoneal epithelium of the ovary. The germinal epithelium is thus the living potential of the ovary by the migration into it of the primordial germ cells. This view is in line with that of Turner (1937) who stated that the germ cells are embedded in the stroma beneath the germinal epithelium in the ovary of Cymastogaster.
Mendoza (1941) observed that the germinal tissue is confined to the lobulated ovigerous folds in the ovary of Naotoea bilineats, but he did not trace the origin of these ovigerous folds. According to the observations of the author, the general activity of the germinal epithelium becomes very much restricted in the summer months (when no oocytes are formed) till spawning. After that it again regains activity when some fresh oocytes are formed for the new cycle, which immediately enters into the dormant phase of winter in Kashmir. These fresh oocytes have been observed to be formed from ovigerous lamellae arising from the germinal epithelium, which in turn arises from the peritoneal epithelium.

The mature oocyte, in all the five fishes studied by the author, is surrounded by the following concentric structures from inside to outside: i) a striated vitelline membrane; ii) a single layer of granulosa; iii) a basement membrane of granulosa; iv) an outer epithelium layer, which in mature oocyte consists of two layers, theca externa and theca interna, the cells being of the squamous type; v) a basement membrane associated with the theca.
These observations are in line with those of Moser (1967), who however showed six layers in Sebastodes. Jollie and Jollie (1964) described in Lebistes, that the vitelline membrane consists of an inner amorphous layer perforated by pore canals, through which microvilli project and develop an intimate association with the theca interna of the follicle. He further stated that it is on account of these microprojections that the impact of bilamellar membrane to the vitelline membrane, which was also recorded in Sebastodes by Moser (1967). It may be pointed out that bilamellar vitelline membrane appears to be characteristic of viviparous or ovoviviparous fishes. It may be also pointed out that vitelline membrane has been designated variously as Zona radiata, Zona pellucida, Oolemma or Choroin.

Stroma is a dense tissue occupying the space between the follicles of the ovary. This stroma is present or better developed in the stage I oocytes in the ovary and in the later part of quiescent phase with indefinite disintegrating cells in the inter-oocytic spaces. It has been observed by the author that the stroma is a product of these disintegrating tissues of the follicular and
corpora lutea cells.

Brambell (1956) has stated that this is derived mainly from the mesenchymatous core of the germinal ridge. Turner (1937) suggested that germ cells are embedded in stroma; while Odum (1936) had suggested that the formation of germ cells is by the transformation of stroma cells.

The function of the stroma is usually protection, this protective function is limited only in the I stage oocytes as in this stage these oocytes are without any protective membrane like granulosa or vitelline membrane. In the advance stages when the protective membranes are formed around the oocytes, the stroma is seems to disappear. The other function of the stroma is as a carriers of nutrition, this too in the early and in I stage oocytes in the ovary. In the later stages this function is carried out by the membrana granulosa of the ova.

Thus it is concluded by the author that the presence of the stroma in the inter-oocytic spaces in the ovary of the I stage oocytes is a temporary feature; while since it has no function in the advance stages, the stroma disappears.

Hoar (1969) states that "the fish ovary does not contain interstitial tissue comparable to in development and histochemistry to the Leydig cells of the testes; the
theca of the ovarian follicle, which in some mammals participates in the formation of corpus luteum (Brambell 1956; Hann 1965) and probably secretes progesterone, never shows a sudanophilia in fishes, where it is evidently a simple fibroelastic connective tissue. Where then are the ovarian hormones synthesized in the teleosts? According to Hoar (1969) they must be synthesized by the ovum (which appear improbable to author) or the glandular granulosa or the corpus luteum, including corpus atreticum, which develops from the granulosa. It appears that the conclusion of Hoar (1965), after discussing this controversy, is correct; and "that a estrogen synthesis was one of the responsibilities of granulosa from the earliest vertebrate phylogeny". However, Gottfried (1964) showed high estrogen contents of yolk in the ova of some fishes and stated that this may represent their entire reserve of estrogen.

**Ovarian Cycles of five Kashmir fishes**

A study of the ovarian cycle in the five Kashmir fishes reveals, that the each stages of maturity (I to V) can have a periodicity of one to three months.

Although the international stages I-V were elucidated long back by the international council for exploration of
seas, a recent criteria for staging ovaries is given by Barlow and Ylaming (1972) who delineate the five stages as follows:

- **Stage I.** Regressing or regressed ovary; ova being resorbed;
- **Stage II.** Early phase of ovarian development;
- **Stage III.** Intermediate phase of ova development;
- **Stage IV.** Ripe or prespawning phase;
- **Stage V.** Spent or post-spawning condition.

It is possible to separate stage II, III & IV by merely stating early phase, intermediate phase and ripe phase respectively. The present author therefore has given the following detailed criteria for identification of the five ovarian stages:

- **Stage I** (Regressed with smallest oocytes stage)

  This stage corresponds to the Stage I according to the international council for the exploration of the seas, to Stage I of James (1946) in Blue gill, Nair (1958) in *Hilisa ilisa* and Gokhale (1958) in *Gadus*. But this stage is not equivalent to Stage I of Sathynaesan (1958, 60), because he includes discharged follicles of Stage V also in Stage I.

  The author's observations reveal that the diagnostic
features of Stage I oocytes are as follows:

1) the oocytes have a clear cytoplasm;

2) a distinct central nucleus is present;

3) the most advanced oocytes of Stage I have a single layer of follicular epithelium;

4) some regressing or regressed ova are being reabsorbed;

5) the oocytes are not visible to naked eye on the surface of the ovaries.

In *S. esocinus* and *C. c. communis* stage I is entered upon as early as August and persists till the end of October; in *C. c. specularis* it persists from September to end of November; while in *Barbus* and *Crossocheilus* Stage I lasts from September late to ending November.

**Stage II** (Early phase of Oocyte development)

In Stage II ovary increases slightly in volume, and the general condition of the ovary appears turgid. A few oocytes become visible to the naked eye on the surface of the ovaries.

The authors observations reveal the diagnostic features of Stage II are as follows:
i) a single ring of nucleoli on the periphery of nuclear membrane;

ii) a single ring of vacuoles;

iii) in advanced stage II a second vacuolar ring is formed interior to the first vacuolar ring;

iv) the appearance of Nucleolar extrusions in many fishes;

v) Appearance of yolk plates;

vi) Appearance of very thin vitelline membrane.

This stage II coincides with the Stage II of James (1946) in Blue gill, Nair (1958) in Hilisa ilisa; Gokhale's II & III stage for Gadus, and to the international stage II. The maturing stage of Nayak (1959) should also fall in Stage II.

Stage III (Mature phase with formed ova)

In Stage III stage the ovaries are much enlarged with pale ova distinctly visible through the ovarian tunic. The ovary increases in weight and volume considerably. This stage corresponds to Stage III of international council; of Nair (1958) in Hilisa ilisa; James (1946) in Blue gill and of Gokhale's Stage III in Gadus.

It has been observed by the author that the Stage III is characterised by :-
i) the presence of a thick vitelline membrane;

ii) the appearance of a membrana granulosa;

iii) the formation of a theca outside the membrana granulosa;

iv) and the nucleus becomes slightly ecentric in position.

This stage III persists from late February to March in *S.esocinus* and *C.c.communis*; while in *C.c.specularis*, *Barbus* and *Crossocheilus* Stage III is from March to end of April.

Stage IV (Spawning phase)

In Stage IV, which is the spawning phase, the ovary is greatly enlarged with maximum weight and length. It is only in this stage of the ovary that the fish exhibits the sexual dimorphism. The eggs during this stage lie loose in the ovary and can be stripped with a slight pressure.

This stage IV corresponds to stage IV of James (1946) in Blue gill; Nair (1958) in *Hilisa ilisa*; the spawning stage described by Nayak (1958), (without numbering the stage), corresponds to the present stage IV and Stage IV of international council.

The author's observations reveal the following criteria for identifying stage IV:
i) the nucleus in Stage IV ova becomes excentric;

ii) the peripheral rings of vacuoles in the cytoplasm become obliterated;

iii) a thick band of yolk at the peripheral region;

iv) differentiation of surrounding theca interna and theca externa of the oocytes.

The stage IV ovary persists from March to ending June in *S.esocinus* and *C.c.communis*; while in *C.c.specularis*, *Barbus* and *Crossocheilus*, it persists from April to July.

**Stage V**  
(Post-spawning phase)

In Stage V, which is the spent stage, the ovaries are shrunken, flaccid, cord like structures, occupying very little space in the body cavity. Almost all the ripe ova have been shed, but there are present some ripe ova left unspawned and even these undergo resorption at a latter stages.

It has been observed by the author that the tunica albuginea of Stage V oocytes is thicker than that of Stage IV; while the epithelium of ovigerous lamellae of Stage IV become disintegrate in Stage V. Besides these there are empty follicles, showing the formation of corpora lutea.

This stage corresponds to Stage V of international council; of James (1956) in Blue gill and of Nair (1958) in *Hilisa*. But it covers the Stage V and VI of Sathynanesan (1958).
This stage persists from July to August in case of *S. esocinus* and *C. c. communis*; while in *C. c. specularis*, *Barbus*, and *Crossochilus* it persists from late July to September.

The observations on *S. niger* Malhotra (1965, 74) appear contradictory to the observations of the present author of *S. esocinus*. In *S. niger* Malhotra states, "from the study of histology it has been observed that the vitellogenesis is completed by the end of December, but ovulation does not take place till May ... all these factors confirm that completely mature oocytes are carried from November to March without any ovulation taking place. This winter dormancy observed in mature oocytes of *S. niger*". He has not identified the stage of the oocytes in which winter dormancy takes place. In *S. esocinus* the present author finds that the winter dormancy, Diapause is not in mature oocytes (Stage III) but the oocytes remain in Stage II during December, January and February (three months) only. Ovulation of *S. esocinus* takes place in May but may continue up to early June. It is not understandable how "winter diapause" in *S. niger* is from November to March when the spawning period of the two fishes is about the same; and how the vitellogenesis is completed from November to March during the winter dormancy period. Therefore the statement by Malhotra (1965), "It is also
during the period (November to March) the diameter of the oocytes also attain the maximum; appears exactly the period in which *Schizothorax* exhibits winter dormancy of the oocytes in Stage II, but their diameters can never attain maximum size.

**Atretic follicles of five species**

The phenomenon of degeneration and reabsorption of maturing and mature oocytes, after and even during spawning has been named "ovular atresia". The degenerating follicles are called atretic follicles or 'Pre-ovulatory corporus lutea' and the degenerating oocytes as atretic oocytes. These have been variously reported in many fishes by workers like Brook (1878); Canningham (1897); Sathynesan (1941); Mendoza (1943); Bulloch (1951); Hoar (1955); Ball (1960); Dodd (1960); Moser (1967).

The author in the present investigations has observed the atretic follicles in all fishes studied and the diagnóstic characters are as below :-

i) The cytoplasm takes on a granular or lumpy appearance and the Zona pellucida becomes irregular and finally ruptures;

ii) The granulosa cells hypertrophy, invade the interior of the ovum, and phagocytize the yolk;
The area formerly occupied by the ovum is now inhabited by a body of cells and bounded by a thin theca.

This is in line with that of Wiebe (1963) working on Seaperch, who suggested that in this phenomenon the entire interior of the follicles becomes filled with granulosa cells; however sometimes groups of tiny cells are present which are dark and have crenate nuclei; which are similar in appearance to those of theca cells.

Wallace (1903), reported the hypertrophy of granulosa cells in pre-ovulatory degenerating follicles in Zoarces. But he did not clearly show that these follicles resemble the corpora lutea of mammals. While Bretschneider and Duyvene de wit (1949) have reported pre-ovulatory corpora lutea in Zoarces, which according to them is a source of progesterone and their function mainly is in the production of nutrition.

Atretic follicles have been observed in the ovaries in stages III & IV of the ovaries of all the fishes studied.

In Schizothorax esocinus and Cyprinus carpio communis have been observed by the author in late February and March (early stage II, III & IV).

In C.c. specularis, Crossocheilus and Barbus the atretic follicles have been observed from early April to June. They make their appearance in early April and atrestia
continues up to end of June, in stages late II, III and early IV. The author is of the view that atresia of some oocytes help vitello genesis and rapid growth in other oocytes.

In all these cases of pre-ovulatory atresia it have been observed that the cytoplasm of the oocyte is drawn out into irregular processes and the nucleus loses its original shape. Theca interna and externa are separated from each other at a distance. In some cases it may be even thrown into folds. Usually the follicular cells multiply and replaced the ovular contents to form the atretic follicles. However, in some cases (Barbus and Crossocheilus), no follicle cells are found inside the degenerating oocytes. In Crossocheilus this structure resembles very much in appearance to pre-ovulatory corpora luteum of Bretschneider and Duyvene de wit (1949). The presence of Follicle cells within the degenerating ovum and also outside the vitelline membrane shows that these cells have the function of removal of degenerating tissue and also blood cells.

Sathyanesan (1961) has also recorded ovular atresia in Heteropneustes. The mechanism of atresia in the same ovary shows variations which depend on the growth stage of the oocyte at which atresia sets in.
Hisaw (1959) is of the opinion that atresia is concerned with yolk phagocytosis in pre-ovulatory follicles or the removal of tissue fragments and blood cells in the post-ovulatory follicles. Chieffi (1961, 67) attributes an endocrine function to the atretic follicles. Hoar (1969) has suggested "that a steroidogenesis was one of responsibility of the granulosa - from the earliest vertebrate phylogeny that this capacity developed in association with the synthesis of lipid material in the yolk". Moser (1967) reported pre-ovulatory corpora lutea in Sebastodes and according to him it may be a source of pregestational hormones.

It seems entirely possible that some granulosa cells may have responsibility in hormone production at the time when corpora atretica becomes active.

Nucleolar Extrusion & Vitellogenesis

Nucleolar Extrusions

It is seen that in the I stage oocytes during the period of growth, the centrally placed nucleus also increases in size, which becomes maximum in the II stage oocytes. In the completely mature prespawning stage (III and IV) the nucleus becomes indistinct and becoming eccentric.
The nucleolar extrusions have been observed by the author in the I & II nuclear growth stage. Among the earlier workers on the nucleolar extrusions, the first detailed account was given by Scharff (1937) in some marine teleosts; Wallace (1903), said that the nuclear membrane does not allow any transmission of solid material through it. This view was supported by Nath and Nangia (1931), as a result of their work on Hita and Ophicephalus, where in they observed the appearance of a large number of nucleoli inside the nucleus during the oogenesis but they did not observed nucleolar extrusions.

Loewy and Sickeritz (1953) observed in some marine fishes, that the nucleoli as seen under electron microscope are rich in ribosomes, the nucleoli eventually being extruded through the nuclear pores into the cytoplasmic matrix.

Eggert (1929, 1931) observed in Salarinus, a number of pockets projecting out of the nuclear membrane, each pocket according to him containing one nucleolus. Each pocket later elongates and its nucleolus is discharged into the extranuclear cytoplasm. Chaudhary (1951) in some teleosts, observed a smooth nuclear membrane in such of the oocytes where nucleolar extrusions have taken place.
In the present investigations the author observed that in *Schizothorax, Crossocheilus* and *C. c. communis*, the nucleolar extrusions are clearly marked. In stage I the nucleoli are seen in the centre of the nucleoplasm and in stage II these multiply and a large number of nucleoli and come to lie at the periphery of the nucleoplasm, which can be differentiately stained from the rest of the nucleoplasm by Mallory's triple stain. Some of these are seen to pass out into the extranuclear cytoplasmic matrix and finally disintegrate (Plate No. II, Fig. 3 and Plate No. XXIX). It has been observed that the passage of a nucleoli through nuclear membrane is not by pockets like formations, contrary to what Eggert (1929) observed in his work on some marine fishes. In the present studies on *Schizothorax* and *Cyprinus* the nuclear membrane has been found to present a wavy appearance, without the formation of definite pockets at the points of the nucleolar extrusions.

Nath et al. (1944) remarked that so-called pockets formed at the time of nucleolar extrusions is due to selectivity of the nuclear membrane at this stage to different fixatives. He observed that the pockets are most prominent in *Colisa*; while in *Mystus* the nuclear membrane appears perfectly even. The view, that the pockets are artefacts was supported by his
observations that the pockets may or may not contain nucleoli in the fishes he studied. The present author's view is, that the nucleoli are extruded by pressure through the very delicate areas in the nuclear membrane, is supported by the findings of Nath et al. (1944) himself, that the nuclear membrane of the fish oocyte is extremely delicate since it breaks down by *in vacuo* centrifugation, whereas that of *Lumbricus* egg similarly treated remained perfectly intact (Singh and Boyle (1938), Norminton (1937).

Guraya (1963, 64, 65) in his comparative study on the yolk nucleus in *Channa* and *Heteropneustes* reports the migration of a few nucleoli into the Boplasm. These according to him consist of R.N.A. and Protein. Droller and Roth (1966) in the young Oocytes of *Lebistes* have described accumulations of coarse granules on the surface of the nuclear membrane, which bear a striking morphological resemblance to the granules that comprise the nucleoli of the egg.

In the present investigations the peripheral nucleolar layer of nucleoplasm appears to hold the rest of the nucleoplasm firmly. It is possible that the granules of Droller and Roth (1966) appertain to the granules in the peripheral layer of nucleoplasm, which is distinct only at the time of nucleolar extrusions, specially in *Schizothorax* (Plate No. XXIX)
The mode of nucleolar emission by osmosis Aieyer (1935) is out of question. The formation of pockets Eggert (1931); Chaudhar (1951) is not also universal as it was not reported by Nangia (1931); Nath et. al. (1944); Cheffi (1951); Malhotra (1963) in S. niger and E. birdii. The author is of the opinion that the process of extrusion is by active pressure of the peripheral nucleoplasm on the nucleoli, which are extruded through the delicate nuclear membrane at certain pre-weakened zones in the nuclear membrane. It has also been seen that the points at which one nucleolus passes out, another may follow suit. It can be stated hence that these perforation are entirely temporary as evidenced in the present work.

Singh and Boyle (1938) recorded a unique type of nucleolar extrusions not so far found either in fishes or in eggs of any animal. They resembled nucleolar extrusions in Gasterosteus in form of fine threads consisting of granules emerging from the nucleoli lying on the inner surface of the nuclear membrane. This is possibly an artefact caused by extrusion of several nucleoli (and then disintegrating) in a chain.

The importance of nucleolar extrusion and their
possible function is elaborated in the discussion on vitellogenesis. The fact of nucleolar emissions and the method of extrusion have been clearly established. Yamamoto (1955, 57, 58) has also demonstrated the occurrence of the nucleolar extrusion of mouse Melanoma cells by microsmatography. Another factual observation by the author is that there is a decrease in the number of intranuclear nucleoli previously at the time when the second stage oocytes are vigorously increasing in size due to vitellogenesis and cytoplasmic synthesis. This is in agreement with the observations of Hisaoka and Firlit (1962) in Zebra fish.

**Vitellogenesis**

It is the phenomenon of yolk formation in the growing oocytes.

In the past some workers assigned vitellogenesis and stated that the extranuclear yolk nucleus to (the yolk nucleus of Balbiani, 1893), due to its golgi bodies, mitochondria and other cytoplasmic inclusions, takes part in the formation of yolk. (Canningham, 1897) stated that the presence of a yolk nucleus in the vicinity of the yolk layer. He stated that the yolk is formed from the yolk nucleus. Battachrya (1925) in tortoise; Linis and Doorme (1908) in mammals; Brambell (1925); Das (1941) in birds, also reported the yolk nucleus of Balbiani and its role in vitellogenesis. Narain (1930), assigned yolk
formation to the golgi bodies of the yolk nucleus in Ophicephalus, Heteropneustes, Clarias and Anabas.

Nath and Nangia (1931) did not report yolk nucleus in Eita rita and Channa, but stated that the formation of albuminous yolk is from mitochondria and fatty yolk from golgi bodies. According to them these cytoplasmic inclusions begin to disperse, till finally the vacuoles and the mitochondria get uniformly distributed in the cytoplasm. While the golgi elements migrate to the periphery in the course of oogenesis but become slightly fatty.

Wheeler (1924) working on the egg of the dab was of the opinion, that the golgi bodies are not themselves transformed into yolk but help in its formation. Chaudhry (1949) reported that the golgi and mitochondria of the yolk nucleus gets transformed into fatty and protein yolk.

Guraya (1963,64,65) worked out the histochemistry of yolk nucleus and clearly showed that the yolk nucleus substance in Channa consists of ribonucleo-proteins. Closely associated with it there are the mitochondria of lipoprotein composition and lipid inclusion composed of unsaturated phospholipids. Yolk nucleus has also been recorded in Channa and Heteropneustes by Nayyer (1964). Butt (1964) also records the yolk nucleus appearing in early oocytes near the nucleus in Anabas.
The present author has failed to identify any yolk nucleus in the five fishes studied. But extruded extranuclear nucleoli appear to migrate towards the region of peripheral cytoplasmic vacuoles of the oocyte; and disintegrating nucleoli can be seen between the vacuolar layer and the nucleus (Plate No. xx Fig. 4).

It has already been established (Brambell 1925; Dutt, 1964; Nayyer, 1964) that quite a large amount of R.N.A. and proteins is passed into the extranuclear cytoplasm by the extruded nucleoli. This view is also supported by Hisaoka and Firlit (1902) who showed that R.N.A is an important factor of yolk formation. It is therefore reasonable to state that the nucleolar extrusions take part in the formation of vitellogenesis.

Narian (1937) stated that nucleolar extrusions are responsible for the production of yolk in the oocytes of Heteropneustes. Subramanian and Aiyar (1935) suggested that the nucleoli before extrusions through the nuclear membrane become fatty. Nath and Nangia (1931), while disagreeing with the occurrence of the phenomenon of nucleolar extrusion, suggested that some undetectable material passes out of the nucleus into the cytoplasm to take part in the formation of yolk. This is a possibility which cannot be
ruled out, since in the present investigations three of the five fishes exhibit nucleolar extrusions; and in most animal cells R.N.A. is passed out of the nucleus (without nucleolar extrusions) which act as messenger for cytoplasmic synthesis.

It is apparent that nucleolar extrusions is not a universal phenomenon, amongst fishes. Not only are the records few and far between, but Nath (1958) categorically denies this phenomenon and explains away all the past observations on Nucleolar extrusions as artefacts. The author therefore concludes the so-called yolk nucleus is nothing but collection of disintegrating nucleolar extrusions in fishes, which are the active centres of the vitellogenesis. But yolk nucleus does not appear to be essential for yolk synthesis in fishes, due to its widespread absence in a large number of fishes. In the present fishes studied it is seen that the nucleolar activity starts at a very early stage and the nucleoli pass out and disintegrate in the cytoplasm even before any visible signs of yolk have appeared in the oocytes. It is, therefore, suggested that the view of Hisaoka and Firlit, that the extruded bodies by their disintegration and subsequent action with the cytoplasm help in the process of yolk formation, is correct. This is probably because of the nucleic
acid contents including R.N.A. act as messangers, and
seats of feed back principle to stimulate the release
of F.S.H. from the pituitary, with which is associated
the onset of vitellogenesis.

It has been observed by the author that while the
extruded nucleoli lying internal to the ring vacuoles
disintegrate, this probably increases the nucleic acid,
content of cytoplasm and subsequent action with the
cytoplasm to create internal condition for the synthesis
of yolk.

It can be, however, safely be concluded that
whether agglomerated as yolk nucleus are lying separately
as extruded nucleoli, and then disintegrate, the sythesis
of yolk by R.N.A. messanger takes place in all cases. It
has been shown that at the places of disintegration of
extruded nucleoli (yolk nucleus of Balbiani), and the
synthetic areas in the cytoplasm, where ever they be, are
composed of nucleic acid, Lipoproteins, Sulphydral groups,
Carbohydrates and certain amino-acids (Dutt 1964). It
is also be observed that these are associated with both
mitochondria and golgi bodies, which are growth inducers.
It is not surprising, therefore, that Nucleolar extrusions
have been seen by the authors before the formation of yolk
plates, yolk vesicles, or vacuoles. The question what
starts the vitellogenesis is certainly a complex one,
which is still to be answered fully.

I has been observed that vitellogenesis starts in
February in *S. esocinus* and *C. c. communis* and in *C. c. specularis*, *Barbus* and *Grossocheilus* it starts from March. The progress of vitellogonesis, once it starts, can be seen from the figures of *C. c. specularis*. While the average oocyte diameter is 510 micra in March, it becomes 816 micra in April, 935 micra in May and 1106 micra in June, when the ova are ripe. It is also apparent that in the same fish the average diameter of oocyte is from 289 to 340 micra during December, January and February, i.e. there is not much growth and no vitellogenesis during this period occurs in *C. c. specularis*.

The first sign of vitellogenesis in stage II oocyte of *Schizothorax*, *C. c. specularis* and *Grossocheilus*, is the extrusion of Nucleoli into the Cytoplasm, although the few nucleoli of stage I have already multiplied and arranged in large numbers at the periphery of the nucleoplasm during the long winter months of ovarian diapause. In the other two fishes viz *C. c. communis* and *Barbus*, the first sign of vitellogenesis is the formation of a peripheral ring of vacuoles in the Cytoplasm, which later becomes double in nature. At the time of vitellogenesis the follicular cells surround the oocytes appear enlarged. This is due to their well-known function of passing on the food material into the oocytes activity (Beddard, 1856; Hatt, 1924). If the food input is stopped by starvation due to cold wave or nonavailability of food vitellogenesis is at a stands still, although all its pre-requisites are present in ooplasm.
Fecundity Studies

Assessment of the fecundity (egg production) of fishes is of special importance in fishery biology studies, particularly in the management of heavily fished commercial species in confined waters (lakes, ponds and tank), as in Kashmir. Fecundity studies help in rational exploitation of the fish stocks, since we can estimate the starting point of production in each species.

Das (1964) initiated this work on fresh water fishes of Kashmir after working out the fecundity of several Indian species. The value of the fecundity studies lies in determining the population pressure of the one species on the other species. Actually it is the number of eggs spawned by each species that determine the year-class strength.

Three types of fecundity have been recorded in the past, (i) total fecundity and (ii) Relative fecundity by Vladykov (1956); while the third type comparative fecundity has been reported by Das (1964).

The total fecundity of the fishes studied are as follows: - Cyprinus capio communis 125,400; Schizothorax esocinus 92,627; Cyprinus corpio specularis 105,200; Labeo dero 91,210; Barbus conchonis 28,440; Crossocheilus latius 21,432 and Nemachilus kashmirensis 8,290. (as shown in the fecundity tables).
When compared with sea fishes these fecundity figures appear rather meagre. The Cod has a total fecundity of above 6 million; the conger 15 million; the Ling 160 million. But when compared to figures for fresh water fishes of Europe, the Kashmir figures are comparable. The Pike 136,000-293,000; the Roch 25 thousand; the Carp 1,310,600; and Minnow only 500 (Pincher 1947).

McFadden et al. (1965) working on brown trout stated that the use of the ovary weight as a measure of fecundity, in contrast to egg number, is desirable in the same species, because weight overcomes the influence of difference of egg size. In a sense, ovary size provides an estimate of the ability of the female to mobilize material for reproduction.

It was observed by the present author that egg number agreed more closely with body weight than body length of the fish in a particular species. As in case of C. c. specularis a fish measuring 14.4 cms has the fecundity 16,104 while another fish with a length of 15.00 cms. shows less fecundity (9,286). But taking the fish weight in consideration the fecundity goes on increasing with the increase in the weight of the fish. The same results have also been noted in other fishes as shown in fecundity graphs. Similar results were obtained by Bulkey (1967) in Steelhead trout (Salmo gairdneri), he also compared the egg size and body length with fecundity to determine if differences in egg size could account for the fecundity
difference to the fish size. He further stated that if egg size is influenced by nutrition, as mentioned by Nikolskii (1962), then it is possible that a fish species producing fewer eggs could produce larger eggs within limits than if it were producing numerous eggs. Thompson (1962) has concluded that fecundity length relationship for Pacific Cod is constant.

Hodder (1963) states 'nowhere in the literature by the author has any attention being given to the possible relationship between the fecundity of individual fish and number of times that the fish had spawned'. The Kashmir fishes studied spawned once in a year, as the first spawning is in second year, the number of times that the fish has spawned can be ascertained from its body length and body weight. While Hodder (1963), working on Grand Bank haddock, proposed that fecundity increased at a rate proportional to about the fifth power of the body length and to the square of the age, the correlations between fecundity and length and between fecundity and weight being better than that between fecundity and age. He further stated that the number of eggs produced by an individual fish in any one spawning season is the result of environmental conditions during the several months immediately preceding spawning. Vladykov (1956) for Salvelinus said that an abundant food supply for adults, during several months preceding spawning resulted in higher fecundity at spawning time. The author's results also support these views.
Comparative fecundity as explained in the chapter of observation, has been worked out in all the studied species on the lines of the comparative fecundity data given by Das (1964) and it shows that (i) a fish with high total fecundity may have low comparative fecundity (as in case of *Wallago attu*); (ii) the real indicator of productivity in interspecific competition is not the total but comparative fecundity; (iii) total fecundity increases with the increasing length, weight and age of the fish, but not in the case of comparative fecundity.

The comparative fecundity of five fishes studied along with two more species for comparison are as follows:

i) *Barbus conchonius* 4569
ii) *Nemachilus kashmirensis* 1238
iii) *Crossocheilus laterus* diplochilus 856
iv) *Cyprinus carpio communis* 545
v) *Cyprinus carpio specularis* 483
vi) *Schizothorax esocinus* 427
vii) *Labeo dero* 422

This is a significant picture for species population competition in the lakes of Kashmir. Since *Barbus conchonius* has the highest comparative fecundity, although in total fecundity it stands sixth among the seven fishes, the production of this fish is increasing in the lake due to its comparative fecundity in competition with other species. While the population of *Labeo dero* and *Schizothorax esocinus* is decreasing due to its low
comparative fecundity, 422 & 427 respectively. The population of *Cyprinus carpio* is also going up due to its higher comparative fecundity when compared with the endemic fishes.

The present investigations have been compared with the comparative fecundity data given by Das (1964); 703 for *Nystus bleekiri*; 245 for *Gonialosa mambinna*; 110 for *C. c. specularis*; 35 for *Chiccephalus*; 37 for *Rita rita* and 33 for *Wallogonia attu*.

The high fecundity of Carp has been of help in elucidating the problem of its rapid increase, which is a very good breeder, each female producing as many as about 100,000 eggs in comparison to the other fishes found in Dal lake in Kashmir Valley. In the last ten years it has spread in almost all the flat-land waters of Kashmir Valley. It is observed that in a fish catch in Dal lake the fishermen obtain 75% of Carps and about 25% of all the other species put together by weight. This can be only explained on the basis of the comparative fecundity, which is comparatively higher than the other good food fishes of Kashmir. As pointed out by Das (1960) the impact of one species upon the other in a mixed population as in a lake can be adjudged and anticipated by studying the comparative fecundity of the fishes concerned.

*Crossocheilus* and *Barbus conchonius* have no fear of extinction due to the propagation of *Cyprinus carpio*.
because the comparative fecundity ratio of these two fishes (856 and 4569 respectively) is higher than that of *Cyprinus* (545). While the convergence of feeding habits, and the fecundity ratio points towards the impact of the prolific *Cyprinus* on the endemic species like *Schizothorax*, *Labeo*. The comparative fecundity ratio being low (427 and 422 respectively); and this is of a great consequence to the future of endemic fishes of Kashmir.

**Gonado-Somatic Index**

It can be stated at the outset that fresh water fishes of Kashmir have only one breeding season during the year. The sexual state of a fish may be measured by the Gonado-somatic Index (GSI). (Where GSI is the ratio between the weight of gonad to the weight of body divided by 100). The range in the number of eggs produced by an individual fish in successive spawning makes for differences in the weight of gonad in similar stages of ripenes.

It has been observed by the author in all the fishes studied that the post spawning ovaries become fractional in weight, actual regression takes place a month after spawning. During this time any unshed contents are resorbed and the ovary then represents the characteristic spent condition.

After this period GSI value rises slowly and as the gonadal active condition is re-established by initial vitellogenesis. The GSI value in *Schizothorax*
is maximum from March to June (6-7) in spawning period, 1-2 in the post-spawning (from July to September) and 2-3 in the preparatory phase from September to February; while in the two winter months it usually remain unchanged (Plate No. XXXVIII).

This is in contrast to the results of Malhotra (1970) in S. niger, who states that GSI value is maximum (3-10) from November to March, when the diameters of oocytes have attain their maximum. Again he observes that "the oocytes of S. niger show a period of growth from May to December; although the growth is steady in May, June & July, as the oocytes during these months are in primary growth phase". He also states that "this growth does not result in maximum increase in diameter of oocytes or in GSI of the ovary" (without giving the figures of the GSI). According to the present author's observations the maximum GSI (6-7) is in the period from March to June, being the spawning period of Schizothorax esocinus. Therefore the maximum GSI (3-10) recorded by Malhotra (1970), from November to March is theoretically and practically impossible.

The observations are in line with that of Moser (1967) who stated that GSI value of Sebastodes during the post-extrusion period, from March to June, was low. A slightly elevated median gonad index for July was caused by initial vitellogenesis, and gonad indices increased steadily until October when pregnant
females appeared in the collections. The gonad indices is very low in spent females in January and February in *Sebastodes*, confirming the findings in Kashmir fishes.

**Condition factor**

The condition factor, which is the ratio between the observed weight and the length cube of a fish, forms an important part of fishery research science. The $K$ value in the fishes studied depends on the amount of good in the gut. The state of gonads affects the fatness, and the $K$ value decreases when mature fish spawn. Le Cren (1951) founded that nearly the seasonal value of $K$ of the mature fish was due to cyclical changes in gonad weight. Differences in $K$ reflect differences in nutritive level or the effects of environmental factor in a media of the fish.

Hart (1946) pointed out that, since the adolescent fishes have higher $K$ values than the older fishes, the increase and decrease in the $K$ values related to the increasing length can be employed to determine the size at first maturity. Fluctuation in the gonad weight is the main factor which seems to regulate the condition factor (Le Cren 1951; Morrow 1951). The other factor which seems to govern the rise and fall of $K$ value is the feeding rate of fish.

Alexander (1967) stated that the factors which tend to limit the number of eggs produced by a female of
given size. Within a teleost species the number of eggs produced tends to be proportional to the weight of the body. Big females produce more eggs rather than bigger ovaries. The average growth rate of fish in a population depends on the amount of food they eat and the amount of energy they use in metabolism.

The seasonal variations in the condition factor has been observed by the author in all the five fishes studied and it is maximum in both sexes and coincides with the time when gonads reach peak maturity. The time of the poorest condition factor is probably due to complete loss of reserve, for both sexes remain busy in brood until July. The cycle of the condition factor seems entirely connected with the maturation of the gonads and spawning in both sexes. (Plate No. XLIII). These results are in line with those of Qayyum and Qasim (1965), on Bloch.

General morphology and Histology of fish testes.

The structure and location of teleostean testes have been described by variously authors from abroad; Sedgwick (1905); Turner (1919); Geiser (1922); Van oodt (1924); Goodrich (1930); Bullough (1939); Parker (1943); Wieble (1943); Lofts and Marshall (1952); Moser (1967); Hoar (1969). Among the few Indian workers Kamala veni (1961); Ahsan (1966); Jones (1940); Gokhale (1959) have significant contributions to our knowledge of the male gonad in Indian fishes. The general condition of the
teleostean fishes according to these authors are compact-lobular structures suspended by mesorchia in the body cavity. The substance of the testes consist of lobules which are short and tabular and divided by partitions. This condition has been observed in present work in all the five fishes. Two types of testes have been differentiated by Hoar (1969); the first type which is the most common, is the compact testes with a complex network of elongated and much divided lobules and enveloped in a common capsule of connective tissue (Epigonal tissue) and peritonium. The second type (Glyptothorax; Hoar 1969); and Glyptothorax, Koul (1965) is represented by free lobules projecting from the vas deferens and without any covering of coelomic epithelium. The five fishes studied by the author fall in the first category viz compact testes. Again Hoar (1969) has classified two type of testes (i) Acinar type in which Vas deferens inside testes are short; and (ii) lobular type in which Vas deferens forms an extension duct system up to periphery of testes. According to this classification Schizothorax and Crossocheilus have testes of Acinar type; while the testes of Cyprinus and Barbus are of the tubular type. Koul (1965) represented a third type in which Vas deferens totally absent in Glypto-thorax and Weisel (1949) in Gillichthys.

Spermatogenesis occur within the unit of the testes which in teleostes, take the form of sacs,
ampullae, tubules or lobules. Dodds (1930); Walvig (1963); found spermatogenesis to occur in small ampullae which are separated by a delicate connective tissues. A number of such units may be grouped together and bounded by somewhat thicker connective tissues called lobules.

Each cyst consists of nests of cells (somewhat like primary ovarian follicle), which proliferate to form small tubules or ampullae or cysts in which spermatogenesis occurs. Stanley (1962) and Mellinger (1965) stated that the serotini cells surrounded the ampullae at this stage and are clearly associated with sperm production. These tubules when extremely short form the acinar type testes and when complex of extensive form the tubular form.

It may be pointed out that the seminiferous tubules of the fishes studied lack a permanent germinal epithelium as also pointed out by Hoar (1969) in other fishes. In the present case of tubular testes the germ cells (resting stage) are pact together at the blind ends of lobules near the periphery, but as already mentioned many of them are displaced along the walls of the tubules. In spermatogenesis the nests of spermatogonia multiply from the ends of tubules and also from the resting germ cells along their walls, the latter being the usual method and in the five fishes studied.

The lobule boundary cells referred to earlier were also noted by Marshall and Lofts (1956) in
Esox lucius, who noted them as hormone producing cells located in the walls of the seminiferous tubules. While other interlobular cells aggregation in the teleost testes have described as interstitial cells by Courrier (1921); Craig & Bennet (1931) and Potter and Hoar (1954). Hoar (1965) showed that these cells produce testicular androgen.

The present author have observed these cell masses to occur in between the lobules more at the completion of spermatogenesis and in post-spawning stage I of the testes. Craig & Bennet showed that in Gasterosteus, the interstitial tissue bears a clear histo-chemical resemblance to that of same tissue in mammals; and concluded that it has a characteristic of a gland of internal secretion. Again Courrier (1921) had found that these tissues are prominent in individual with marked secondary sexual characters. In the opinion of the present author the interstitial cells can hardly be differentiated from lobule-boundary cells, the difference being larger one of the distribution. The two types of cells are similar histochemically as confirmed by Marshall (1960); Dodds (1960); Hoar (1965), but the fact that in addition to interstitial cells, Urodels also possess lobule boundary cells, confirms the author's view that even in fishes the interstitial cells and lobule boundary cells must be separate in character and function.

The Sertoli cells have been found prominent in the testes in all groups of fishes, (Mathews, 1950;
The spermatogenetic units - whether cysts, ampullae or lobules are bounded by a cellular layer containing two types of cells: one of these is the gonocyte which give rise to generation of spermatogenetic cells; while the other is the Sertoli or the supporting cells, believed to play a nutritive role during spermatogenesis. This conclusion is supported by the present investigation in the studied fishes. In higher vertebrates, (Nelson, 1953; Patten, 1953; Ham, 1965) reported that the spermatids become embedded in the centripetal ends of Sertoli cells, which confirms the nutritive hypothesis.

In the fishes studied the spermatids appear intimately associated with Sertoli cells and apparently draw nourishment from them till they transform into spermatozoa. Sertoli cells have also been assigned the role of phagocytosis of unshed sperms (Vaupel, 1929; Nilson, 1953). The author feels this view to be correct since even in the ovaries the same follicle cells supply nutrition as well as participate in phagocytosis. It is surprising that Collenot and Ozon (1964) and Simpson and Wardle (1967) have demonstrated hydroxysteroid dehydrogenases in the Sertoli cells in dog fish and surfperch. What then is the difference between Sertoli cells and interstitial cells? The author can only conclude that interstitial cells suggest two functions: Buffer and space-filter and production of male hormones;
while sertoli cells suggest three functions; i) nutritive; 
ii) phagocytic; & iii) hormonal function.

The source of the seasonal supply of the germ 
cells in the fishes studied appear similar to that of 
Mathews (1938) in *Fundulus*; Bullough (1939) in *Phoxinus*. 
The process is that the germ cells take the form of small 
cysts filling the lobules. The present investigations do 
not show any indication that the spermatogonia present 
had migrated from a card of germ cells outside the testes 
to their position along the lobules walls as described by 
Turner (1919), and the migration of germ cells into the 
lobules of the testes from certain extratesticular cells— 
cords in the yellow perch (*Perca flavescens*). Turner 
further stated that the germ cells are found along the 
septa of the lobules from the center to the periphery of 
the testis during the time in which clusters of germ cells 
are formed and increase at the periphery of the testes.

According to Jones (1940) in *Salmo salar*, stated 
that no cells other than these resting and migrating germ 
cells are present in the stage I testis; while the resting 
germ cells with their lightly staining homogeneous cytoplasm 
completely fill the region which will later form the 
spermatogenetic crypts.

Gokhale (1957) on the *Herring* and the *Norway Pout* reported that the spermatogenesis begins from these 
migrating germ cells. About the origin of the germ cells
he stated that the nuclei of the mesothelium are much smaller than the nuclei of the germ cells; and do not show any mitotic activity and are therefore not a likely source of germ cells. On the contrary, the fact that the migrating germ cells are present in all stages of gonad development would suggest that they have arisen from the primordial germ cells which become segregated early in life, as also suggested by Dodds (1960) which are similar to the observations of the present author. On the other hand Moser (1967) described germ cells inward from the fibrous envelope of the testis in *Sebastodes*.

The germ cells in the fishes investigated are the dominant element of the testes during post spawning months of the reproductive cycle. Although some germ cells are present at all times of the year, but are most obvious at the end of the reproductive cycles.

**Spermatie cycles of the five species.**

Seasonal spermatie changes in testes of bony fishes have been studied, Craig Bennet (1931); Bullough (1939); James (1946); Copper (1952); Harrington (1957); Hoar (1965); Ahson (1966); Dadzie (1969). The testicular cycle in five fresh water fishes studied in Kashmir, undergo a regular cyclical changes during the year and these are divided into five phases of maturations.

**Stage I** *(Immature phase)*

The author's observations reveal the diagnostic features of Stage I testes as i) they are small tiny
transparent cord like structure; ii) each testes consist of primary spermatogonia and germ cells; iii) there are a few dividing primary spermatogonia and some secondary spermatogonia; iv) each spermatogonium was spherical and contained a small round dark staining centrally placed nucleous; v) pycnotic degeneration has been also observed with the sperms nests; vi) interstititial cells are also prominent in this stage I testes.

This stage occurs during late August to October in case of *S. esocinus* and *C. c. communis*; while it persists from September to November in other fishes studied.

This stage I corresponds to the Stage I of international council; stage I of Dadzie (1969) in *Tilapia*; (early resting testes), Stage I of Ahsan (1966) in *Couesius* and to the immature testes stage A of James (1946) in *Blue gill* and stage I & II of Gokhale (1957) in *Gadus*.

**Stage II** (Maturing phase)

The characters of the maturing phase as observed by the author are as follows: - i) testes macroscopically are similar to that of the Stage I of maturation, except an increase in the weight; ii) the testes at this stage is filled with germ cells, spermatogonia and primary spermatocytes; iii) Secondary spermatocytes and sometimes even spermatids have also been observed; iv) the inter-lobular walls of the lobule are thin containing fibroblasts and sometimes they do possess migrating germ cells; v) no
pycnotic degeneration is observed.

This stage II testes corresponds to stage II of international council; stage II of Dadzie (1969) in *Tilapia* (late immature stage); Ahsans (1966) in *Couesius*, II stage; and Stage B of James (1956) in Blue gill.

The Stage II persists from October to middle of the January in *S.esocinus* and *C.c.communis*; while in *Cyprinus carpio specularis*, *Crossocheilus* and *Barbus* it is from ending November to February.

**Stage III** (Mature phase)

The observations of the author reveals that Stage III shows i) rapid spermatogenetic activity; ii) secondary spermatogonia and spermatocytes are transformed into secondary spermatocytes and spermatids and also even spermatozoa; iii) secondary spermatogonia are stain more deeply than primary spermatogonia and nucleolus disappears iv) each spermatogonium forms the centre of one cyst, the descending generation being found in that same cyste which in Stage III may contain 16 - 20 secondary spermatagonia.

This stage III corresponds to international council stage III; stage C (enlarged testes) of James (1946) in Blue gill; stage III of Dadzie (1969) in *Tilapia*; Stage III of Ahsan (1966) in *Couesius* and Stage III of Gokhale (1957) in *Gadus*.

This stage III persists from late February to March in *S.esocinus* and *C.c.communis*; while in other fishes studied it is from March to end of April.
Stage IV  

( Spawning phase )

The stage IV as observed by the author has the following chief characters:- i) testes attain a whitish colour and shows a positive stripping condition; ii) testes have the labules containing ripe spermatozoa; iii) the labule boundary cells and interstitial cells are well observed in this stage in the interlabular species; iv) the spermatozoa have a round head containing the nucleus, and a long tail, in some cases spermatogonial cells can be seen around the periphery of the labules; v) hypertrophy of the interlobular connective tissue has been also observed; vi) the appearance of the nucleus in spermatids is elliptical and in structure is rounded; vii) the cysts wall disappear and the spermatozoa lie in the entire labule.

The stage IV testes persists from March to ending June in S. esocinus, C. c. communis; while in other fishes studied it persists from late March to July.

This stage IV, corresponds to stage IV of Dadie (1969) in Tilapia; of Ahsan (1966) stage IV (maturing stage) in Couesius; of stage D (spawning stage) of James (1946) in Blue gill and stage IV of international council of maturity.

Stage V  

( Spent phase )

The stage V shows the following diagnostic features as observed by the author :- i) shows completion of spermation; ii) germ cells and primary spermatogonia are predominate in this stage; iii) unexpelled spermatozoa have also been observed in the empty labules, these soon
phagocytized; iv) the testes are regressed and much reduced in size.

This stage persists from July to August in case of *S. esocinus* and *C. c. communis*; while it persist from August to September in *C. c. specularis*, *Crossocheilus* and *Barbus*.

This stage corresponds to stage V of Dadzie (1969) in *Tilapia*; of Stage V of Ahsan (1966) in *Couseus*; of stage E & F (poorly and complete sponic stage) of James (1946) in Blue gill and Gokhale's (1957) spent stage in *Gadus*.

During winter, the testes remain in primary spermatocyte stage and later development begin only in spring. Scott (1952) reported that in *Mylocheilus*, the quiescent winter condition is passed in the secondary spermatocyte stage. It is agreed that the secondary spermatocyte is a comparatively transient phase in the spermatogenetic cycle of fishes (Folley, 1952); Weisel (1943). It appears that Scott's secondary spermatocytes were probably the primary stage. The author is of the view, based on his observations, that the months of December to middle of February is the period of quiescent (winter diapause). While it is only in the late February or early in March the testes show some spermatogenetic activities in the fishes studied.

Mathews (1938) observed the presence of spermatids in the testes during Autumn and Winter in *Fundulus*, maturity ahead of breeding season was also observed by
Turner (1919) in *Berea*; Geiser (1924) in *Gambusia* and Craij-Bennet (1931) in *Gasterosteus*. The author is of the opinion that these differences in maturity are due to some fishes being spring breeders (*S. esocinus*), other summer breeders (*Cyprinus* and *Crossocheilus*) and still other Autumn breeders (*the trout*). Hoar (1955) pointed out that in spring breeder a short period of rest after spawning is followed by testicular activity during the later summer, Autumn and sometimes in winter. However no winter activity was observed in the testes of five fishes studied. This is possible because Kashmir is extremely cold (-6 to -10°C) in winter.

**Spawning seasons**

The time of the spawning seasons and its duration in Bony fishes depend upon both the exteroceptive and interoceptive factors governing the intragonadial stages in the species during the year. In the majority of fishes there is always a fixed spawning season during the year, which is even more marked in fresh water fishes.

The spawning season in different fishes have been described by various authors from abroad: Harrington (1959) *Fundulus*; Strawn (1958) *Hippocampus*; Barlow (1963) Largjaw gobies; Walker et al. (1961) Longjaw gobies; Barlow and Vlaming (1972) *Gillicithys*. The spawning season of fresh water fishes of India have been given by Khan (1943) *Carps*; Ahmad (1944) *Lissochilus*; Karandikar and Palekar (1956); *Thrissocoles* and *Polynemus*; Prabhu (1956).
in some Indian fishes; and Ahsan (1966) *Cousieus*.

The spawning season in the fishes studied have been determined by a study of gonadal structure as well as observation of actual spawning in nature. The stage of the gonad of ripe is a true indication of spawning, which can be determined by the following features:

1) Histological maturation and ripe condition of the Gonads;
2) The occurrence of spent fishes in the catches;
3) Sudden fall in the GSI value;
4) The running state of eggs and spermatozoa from the gonads;
5) Absence or scanty presence of Atretic follicles;
6) Presence of collapsed ovarian follicles and spermatic lobules.

Applying the above criteria to determine the spawning season in *S.esocinus*, it has been observed that histologically the gonads are in stage IV (ripe condition) in the period from late March to June, during these months the diameter of oocytes are maximum and the sperms are fully developed. The GSI is high prespawning and shows a decline after the spawning season. From late June in females the collapsed follicles have been observed and after June the spent specimens of fish are obtained.

According to above mention criteria, the spawning of the other fishes is as: - *C.c.communis* is from late
spring to early summer (April - June); while in C.C. speculiris, Barbus and Crossocheilus the spawning season is summer (April to July).

Nair (1958) reported two spawning seasons in Hilsa, although the ova mature in spring "March", the exact spawning takes place in September. Barlow and Vlaming (1972) in Gillichthys stated that spawning apparently continued through May and June, and after middle of June many spent individuals were collected in which reabsorption of yolky oocytes was in progress.

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Hickling and Rutenberg (1956) in Lake herring, Lepidogaster; Karandikar and Palekar (1950) in Polynomus; Prabhu (1956) in nine species of fishes; Gokhale (1958) in Gadus were the workers who had studied the spawning season only by measuring the diameter of ova and nothing its frequency. According to the present author's opinion, this method can be a way of determining the spawning season in fishes, but it is not the exact positive proof for recording the spawning season in fishes. While discussing this view Nair (1958) stated that "although the ovaries may macroscopically show an enlarged condition and microscopically show the majority of oocytes in stage IV, still it is possible that the majority of them, if not all, may turn atretic subsequently as has been found in the case of March "brood" of Hilsa."

The spawning seasons of Kashmir fishes studied are different from the spawning season of fishes in the rest of India. The fishes of India have their spawning season coinciding with the summer monsoon which is usually from
middle of June to middle of September, only a few species
spawn during March, April and May. It is also on record
that the fishes in India are sexually mature when
they are one year old. It is apparent that this is not
on account of the low temperature of Kashmir waters but
also due to less availability of food as observed by
the author in the fishes studied.

As quoted by Pincher (1948), however, when compared
to fresh water fishes of Europe with those of Kashmir, the
spawning periods appear similar e.g. Barbel (British
rivers) May and June; Carp (*Cyprinus carpio* of European
lakes). May and June; Loach (*Misgurnus fossilis* of
continental rivers) March and April; Minnow (*Phoxinus
of British rivers*) May to July; Perch (*Barca fluclolus laeris*)
March to May; Pike (*Esox lucius*, European fresh waters);
Stickle back (*Gasteroneus*) May and June; Trout (*Salmo
tutta*, European rivers) September and January.

In Kashmir spring and summer climatic seasons are
ideal conditions for fishes to spawn, as the spawning
season is correlated with the maximum availability of its
food for their young ones, which is at its peak in these
months. The water temperature during this period also
rises, and is quite suitable for the optimum growth of the
young ones.

The age at which the first sexual maturity occurs
is different in the different species. *S. esocinus* and
*Cyprinus* species mature in the second year after hatching,
their approximately length at first spawning being 37-50
cms in *Schizothorax* and 30-40 cms in *Cyprinus* species.
Whereas *Crossocheilus* and *Barbus* spawn after attaining two
years age with a size 6.12 cms.

**Reproductive Ecology**

Successful reproduction is an essential factor for
species survival, and the breeding periods of animals are
adjusted in time to that particular phase of the seasonal
cycle which is suitable for rearing of the offspring.
However, gonadal development is a complicated physiological
process of long duration.

In Kashmir fresh water environment provides great
diversity in Physical, Chemical and biological condition
from season to season whether it is lotic or lentic environ­
ment. The fishes, whether in ponds, lakes or rivers undergo
progressive and predictable changes. Breeding is adopted
to an annual rhythm. The cycles of maturation and depletion
of the gonad being fairly regular and being repeated almost
at the same time of the year.

The environmental factors and the life processes
of a fish are closely interconnected. Changes in the environ­
ment (waters) affect similar changes in the fishes
inhibiting the water. The spawning are also governed by these
factors.

(1) **Physical Factors**

**Temperature**

The water temperature play a very important part in
the maturation of the gonads of the fishes. Low water
temperature adversely effects the maturation of gonads.

A study of GSI proves that the highest values are obtained when the temperature rises after the cold winter, from ending March to June. It has also been recorded in present findings that in all the fishes there is a long period of winter dormancy or quiscent (December to February), during which the gonads remain static. Some food is always available in the water; and therefore the dormancy is a direct effect of temperature in all the fishes studied. The reproductive activity is at a stand still during this period.

Mathews (1939) for Fundulus heteroditus found that for the complete activation of the testes, besides the high temperature, the low temperature exerts a retarding influence of the maturation of the sperms. Implying thereby the warmer conditions of water should prove favourable for reproduction. Burger (1939) experimenting with the same fish corroborates the view of Mathews. However, it is worth mentioning here that a temperature of about 30°C in July and August has an inhibiting effect on the gonadial activity as revealed by the histology of the gonads.

Nair (1958) has shown in Hilisa ilisa that temperature exerts some influence upon the ripening of the gonads. According to him the average temperature is lowest in November, December and January, although a slight increase in temperature is in January. The reproductive activity is almost nil during these months. On the other
hand, when the temperature has risen to 25°C in March, the oogenetic and spermatic activities are in full swing.

Barlow and Vlambing (1972) also reported in gobies that temperature is the primary environmental factor controlling sexual cycling. The goby spawns in May and June when temperatures are high.

It may be therefore stated that histologically and morphologically the gonads of the Kashmir fishes studied are active from early spring to summer. In the summer late months (July to August) when water temperature is maximum (above 27°C), the fish has already spawned and the gonads show the least active phase of the seasonal activity. And after this up to late autumn recovering of gonads takes place gradually due to plenty of available food. But even then the development of gonads stops in international stage II and remains as such throughout the winter period.

In the present thesis a comparison of temperature of the waters of the lake (Dal) and its correlation with the principal histological changes, in the gonad throughout the year, has shown that temperature centrally exerts some influence upon the ripening of the gonads. It may be concluded therefore that temperature stimulus normal impulse for inducing sexual activity of gonad of fishes; and that temperature is a central factor in reproductive ecology.

Light

Light being correlated with temperature is also
an important factor in reproductive ecology of fishes. Exposure to artificial light for long periods has been found to increase the fertility and advance the spawning of the female brook trout (*Salvelinus*). Vladkoy (1956), when the brook trout was subjected in spring to light conditions similar to those of summer and in summer, to autumn light condition, it spawned in summer instead of autumn. Similarly the oocytes in the ovaries of *Gasterosteus* ripe extra rapidly if the fish is exposed to extra light (Craig - Bennet (1919). In the present studies although no artificial light experiments were performed, it has been observed that the intensity of light increases suddenly in March in Kashmir and in highest in summer when all the fishes has spawned.

Light has a direct effect on the hormone secreting activity of the neuro-secretting cells in the hypothalamus, the chief centres of activation being the MFC and NLT. The release of these hormones induces the release of gonadotrophine, L.S.H. and F.S.H. from fish pituitary, which activates the gonads. If the fishes are blinded or kept in total darkness, the ovaries develop very slowly and spawning is postponed.

We also know that pituitary hormone treatment induces breeding (Pickford and Atz, 1957), and also with pituitary hormone tynopheine exerelates maturity in fish in non-maturation condition (Ahsan and Hoar 1963). The author has also observed that the stimulus for spawning in an entire shoal of fish can be provided by the spawning
of a few individuals in the shoal, when all the other ripe fishes start spawning. Similarly, Das and Hussain (1968), injected mammalian gonadotrophines into Schizothorax, Cyprinus and Salmo trutta, and observed riped oogenesis and spermatogenesis, the spawning taking place even one month earlier than the untreated fishes.

The interplay of temperature and light on gametogenesis and reproductive behaviour in Cymatogastar was investigated by Wiebe (1968), he found that increasing a long photo period from late winter to early summer induces spermatogenesis and reproductive behaviour at warm temperature. Low temperature and short photoperiod retards these processes. Similarly Marke et al. (1967) reported that the Carp (Cyprinus carpio) could be induced to spawning after every five hours by pituitary injection when they were maintain in warm running water aquaria.

(2) Chemical factors

The pH value table shows that the maximum value is 8.8 in March and in August and minimum in the months of November and December. It is clear from the graph that there is a tendency for pH value to increase from January, February and reaching its maximum in the months of March and April, after this it remains somewhat uniform (Av. 8.0) till August, when it again attains maximum value.

Carbon dioxide (which affects the value of pH) is chiefly obtained as a result of decomposition of organic matter by the bacteria, through respiration of living
organisms within the waters.

This dissolved $\text{CO}_2$ is used up by photoplankton and microphytes of the lakes for photosynthesis; and thus an increase of phytoplankton and macrophytes causes increase in $\text{pH}$ in March and August. Das (1961) observes "that $\text{pH}$ value of water coincides with low value of $\text{CO}_2$ and with phytoplankton peaks; while low $\text{pH}$ values coincide with high value of $\text{CO}_2$ in water during the Zooplankton, necton peak periods". He concludes "high $\text{pH}$ values means high phytoplankton and low zooplankton whereas low $\text{pH}$ means low phytoplankton and high Zooplankton under ordinary circumstances".

The high $\text{pH}$ value in March and August in Kashmir water indicates high phytoplankton, which is the first food of the fish larvae, even in early spawners like Schizothorax, therefore, the larvae have plenty of food at hatching. Later, in April, May and June, which are spawning periods of the other fishes studied, the most of hatched fish larvae reach the fry stage, which feed mostly on Zooplankton. And this is the peak period of Zooplankton associated with low $\text{pH}$ values.

The correlation of phenomena with reproduction is therefore, apparent, not only that it has been recorded by Das (1961) that more fishes are present in Natural lakes with $\text{pH}$ range of 7.2 to 8.0; but declined rapidly in highly alkaline waters which remain above $\text{pH}$ 9.0. And, if the $\text{pH}$ rises above 9.0 many fishes remain unproductive.
or die. It may be concluded therefore that pH of the waters is an important factor in the reproductive ecology of the fishes.

**Oxygen**

The gases which are found dissolved in any water body generally are oxygen, carbon dioxide, nitrogen, hydrogen, sulphides etc. Lakes receive the supply of oxygen from (i) atmosphere; (ii) photosynthetic activities of Chlorophyll bearing photoplanktons and microphytes.

In the lake the upper layers get oxygen from the atmosphere, by mechanical admixture of air through wind and wave action, and when the water is in complete circulation entire lake becomes impregnated with oxygen. Dal lake being shallow has complete oxygen circulation almost throughout the year.

In the present investigation, oxygen content was found high in spring and early summer (11.2 ppm) and lowest in autumn period (9.2 ppm). It has been observed that high oxygen contents in the lake coincides with the high peak of phytoplankton. During this period (March to June) is suitable for the supply of food (Phytoplankton) for newly hatched fish larvae.

The high oxygen period (March to June) in Dal lake is highly advantageous for the vitality of the fish larvae and fry, which obtained waters at that time. With even some depletion of oxygen *Schizothorax* larvae and
and fry have been observed to die; while the larvae of all the other fishes studied cannot withstand much depletion of oxygen. It is only Cyprinus Carpio fry and fingerlings are thrown even in semi-oxygenated lake arms of Dal.

Some fishes can be and reproduced in high oxygen waters. Blaska (1958) and Mathur (1967) have reported extensive survival of fish under completely anaerobic conditions. Kutty (1968) demonstrated that Gold fish can live for months with a low oxygen contents, although it is not stated, if its larvae or fry can do so. It can safely be concluded that fish larvae and fry can hardly stay alive in deoxygenated or poorly oxygenated waters. Similarly, oxygen is essential for energy supply in reproductive activities of any fish, and the high oxygen period in lakes coincide with the spawning seasons of the fishes studied.

Carbon dioxide

In natural waters the most important oxide is the carbon dioxide and its study is of great importance in understanding the H-ion concentration of waters (Hutchingson, 1957). This is usually obtained as a result of decomposition of organic matter and plant organisms in the lake.

As estimated by the author the value of CO₂ in Dal lake is highest in autumn and early winter and minimum in spring and early summer.
Different species of fish display different sensibility to Co₂. The larvae and fry of Carpcan withstand Co₂ concentration above 6.0 ppm; while those of Schizothorax can hardly live at such Co₂ concentrations.

It is apparent therefore that low Co₂ contents of the water coincides with the reproductive activity of all the fishes studied. High Co₂ is hardly limiting factor for fish reproduction, since (i) acclimation for Co₂ is attained in a short time (Saunder, 1962). This is also supported by the data of Lloyd & White (1967), who suggested that the change in blood carbohydrates in Rainbow trout, in response to receive the Co₂ is largely completed in 24 hours. It is also on record Basu (1959) that the effect of given concentration of Co₂ is least at highest temperature for a given specie. And this hold true for the Dal lake fishes as well.

The gonadial development is a complicated physiological process of long duration. This internal physiological process makes the animal ready for actual breeding behaviour to occur at the most appropriate time.

Since, in the present investigations, it has been observed that the snow melted water coming into the lake by small streams stimulate the fish Schizothorax to migrate and spawn in those water adjoining the falling streams and spawning is only once a year.
While John (1963) described two spawning periods within one year for the Speckled dace in Arizona. The major peak of this bimodal rhythm in April - May is correlated with flooding from melting snow. A lesser peak in spawning in late July - August, coincides with flooding by rainwater. If the later summer freshets occurred before the July, no spawning was observed.

Several reports indicates that spawning activity in many species of Carp coincides with seasonal flooding by periodial monsoon rains. The observation by Khanna (1958), extending over three out of the eight years was obviously of related to insufficient flooding. Other studies confirming the coincidence of flooding and spawning in Carp is by David (1959). A study of Lake (1967) suggests the presence of soil substance which when leached out by flooding could incitiate spawning in fresh water fishes, although he did not safely mentioned what those substances are.

Reproductive activity in Schizothorax often coincides with extensive flooding during the rainy season. Since spawning is frequently reported to occur almost immediately after rains, but this factor cannot be held responsible for long term anticipatory timing of the sexual cycle.

The migrations to special breeding grounds occur preparatory to spawning, one must assume that it is not
the sudden incidence of directly acting stimuli of favourable environmental conditions which trigger reproductive behaviour but the entire annual sexual cycle is subject to synchronization by external factors.

The effect of day length in seasonal reproduction and its role in the timing of reproductive rhythms in animals was shown by Rowan (1926), that seasonal changes in temperature, light intensity, rainfall were responsible for causing the appropriate adjustment in reproductive cycles. Craig Bennett (1931) working with Gasterosteus found that the male reaches potential maturity before the female and that spermatogenesis was completed several months in advance of the next breeding season. His conclusion that day length was not affecting gonadial development was based on the appearance of secondary sex characteristics and has been contradicted by several workers (Baggerman, 1957; Schneider, 1969).

Van de Eeckhoudt (1948) employed gradual increases in photoperiod, reaching 10 hours of light per day from February to April, and found spermatogenesis to be continuing normally but observed no nuptial colour development. And those males which were exposed to consistently short days under otherwise identical conditions, seemed to revert to earlier stages of spermatogenesis. Increasing day length caused progressing oogenesis which became arrested in the short-day group.
Temperature has also been shown to affect the rhythm of gonadial maturation and spawning aside from directly influencing physiological processes, there seems to be some interaction with day length, in some instances, the breeding cycle was found to proceed when day length and temperature were kept uniform.

In Kashmir water temperature is not a limiting factor, as it only helps the gonads to come out of the winter dormancy quiescent phase of two cold months. While taking oxygen into consideration it seems that it is an essential for energy supply in reproductive activities of any fish, and the high oxygen period in lakes coincide with the spawning seasons of the fishes studied and it is further observed by the author that the high oxygen period is suitable for the supply of food (Phytoplankton) for newly hatched fish larvae. Thus making the oxygen as a limiting factor.

It is obvious in Kashmir which is a temperate region, to that the changing photoperiod effects timing of breeding rhythms in fishes, where the annual cycle of changing day length is of substantial magnitude. This is in contrast to tropicals zone where these conditions do not vary much. It is possible that day length plays only a synchronizing role with reproduction and that, in their natural habitat in the temperate zone, days of less than 12 hours light prevent renewed breeding in fall and still high temperature;
whereas, spawning activity during the following year cannot begin before sufficiently high temperatures are present in spring or summer.

Baggerman (1957) on the three spined stickleback, *Gasterosteus aculeatus*, a reasonably clear account of the complex interactions between an internal rhythm and day length-temperature combinations as factors seems to result. Nest building in males and oviposition in females. While Merriman and Schedl (1941) on the four spine stickleback *Apeltes* showed no difference in spermatogenesis and ovogenesis for groups of fish exposed from October to November-December either to a gradually increasing length of day. Hoover (1937) on *Salvelinus*, showed that by first increasing and later decreasing the light duration much in advance of the natural photofraction change, the spawning season could be advanced by about four months to August.

Thus it may be concluded that the internal physiological rhythm of gonadal maturation remains arrested at a prespawning phase for a considerable length of time and that the consummatory breeding behaviour is triggered by the same environmental situation to which the reproductive rhythm is ultimately timed.