REPRODUCTION
3. REPRODUCTION

3.1. Introduction
The global population is expected to rise to over 9 billion by the year 2050, placing tremendous pressure on food production systems. Currently, the majority of human nutritional needs are met by terrestrial plant agriculture and this source of food will remain dominant in the future. Additional food supplies are acquired through animal productions (primarily mammalian and avian species) that are produced by agriculture rather than by harvesting wild populations. Recent FAO studies indicate that global production of meat from livestock exceeds 188 million metric tonnes and is rising approximately 2.3% per annum (Tacon et al., 1996).

In contrast to terrestrial food production, supplies from aquatic resources have traditionally depended upon hunting and gathering of wild populations of plants, crustaceans, molluscs and vertebrates (Primarily fish and marine mammals). Since the middle of the twentieth century, increased fishing effort yielded augmented harvests from oceanic and fresh water ecosystems (Grainger, 1998) a trend that was sustained until the mid 1980’s. Available data suggests that the oceans retained a vast capacity for production that human activity did not significantly impact other than in specific local fisheries. However since the late 1980’s and early 1990’s, further increases in global wild fish supplies have not been achieved from capture fisheries, indicating that
limits do exist: harvest levels have plateaued at approximately 90 mmt and are not expected to increase significantly under current global climate regimes.

There exist two major areas that show potential for increasing global food supplies. The first involves the application of new and improved technologies to current production systems. A second approach to increasing world food supplies will be the utilization of aquatic environments in a manner similar to that practiced for terrestrial systems, by increasing production through the application of aquaculture. Both these approaches required a detailed and thorough understanding of the biology and reproductive behaviour of commercially important aquatic organisms, especially fishes. Sexual dimorphism is the rule in fishes and the breeding season varies in different species. Hermaphrodite gonads have also been reported in some perches, darters and black basses. Fishes in general are oviparous. Ovaries and testes are paired organs. They are elongated structures lying close to the kidneys. In the breeding season, the gonads are much enlarged. Mature ova are released by oviduct to the exterior or they are shed into the body cavity and the eggs pass out through temporary genital funnels to the exterior. Fertilization is external.

Fishes attain sexual maturity at different ages. Some fishes are ready to reproduce at the age of one year, others when two or three years old. The longest period of sexual maturity among fishes is observed in Eels and they attain sexual maturity at the age of ten or eleven. The time and periodicity of spawning vary from region to region depending on the climatic conditions.
In Europe several food fishes breed in the first half of the year. The spawning season of the Plaice (Pleuronectes) is January to April and that of the cod (Gadus) in the North Sea is from February to May. The spawning season of soles falls in the month of April and it continues up to July. Many fish species in the north Pacific have a long reproductively active life span which increases the likelihood of producing offspring during periods of favourable environmental conditions. This kind of reproductive strategy reduces the impact of environmental variations on reproductive success (Goodman, 1984; Leaman and Beamish, 1984; Shutly, 1989). In species with age-structured spawning schedules, a broad age distribution will maximize the length of the spawning season. The more protracted the reproductive period, greater the likelihood that some spawning will occur during conditions favourable to larval survival (Lambert, 1990). Age related differences in the timing of spawning have been observed in many fishes, usually larger, older fish spawn earlier (Shepherd and Grimes, 1984; Lambert, 1987) but in some cases younger fish spawn earlier in the season (Hutchings and Myers 1993). Factors that might affect individual reproductive success include the number of eggs produced, the quality of eggs (e.g. yolk or oil globule volume) and the size or health of eggs and larvae.

In fishery biology studies, the knowledge of reproduction is important for elucidating both short-term and long-term variations in the production of fish broods which are finally recruited in the population as exploitable stocks. Most teleost fishes spawn more than once during their lives. In fishes, two basic types
of spawning have been observed. Synchronous where all the oocytes develop at the same time inside the ovary and spawn at once, serial or batch spawning, ovaries contain batches of oocytes at different stages of development leading to repeated or multiple spawning, (Yamamoto and Yamasaki, 1961). Coastal and estuarine teleosts in the sub tropics and tropics are characterized with a long spawning season (Pauly, 1987). In Indian waters most teleosts are prolonged breeders as evidenced by their ovaries containing several batches of ova destined to mature and to be released periodically on maturation. Methods used for identifying spawning season of fishes are reviewed by West (1990) who suggested that the assessment of the stage of gonad development of individual fish is an important component in reproductive biology of fish.

The complete development of the oocyte has been divided into several stages, according to the most relevant morphological characteristics used to identify them (Yamamoto, 1956). That is, as each oogonium matures, its microscopic characteristics gradually change until it becomes a mature cell that present the main element known as yolk. At the beginning stages were recorded in the development of the oocytes or vitellogenesis (Yamamoto and Yamazaki, 1961).

Male gonads were classified microscopically into two stages: sexually mature or immature (White. G., 2003). In Female microscopic gonad stages were assigned based on the most advanced type of oocytes present, regardless of their abundance (Wallace et al., 1987; West 1990 and Hunter et al., 1992).
Oocyte development and maturation is a continuous process, which has been subdivided into various stages to simplify histological classification of ovaries. The presence of post ovulatory follicles (POF), migratory nucleus oocytes or hydrated oocytes in ovaries were used to identify individuals that had begun to spawn (Hunter and Macewicz, 1985b) or were capable of spawning. After spawning, residual oocytes and unwanted materials are reabsorbed in a process known as atresia (Hunter and Macewicz, 1985a) Atretic oocytes were recognized by their irregular shape, breakdown in fine structure (disintegration of the nucleus and liquefaction of yolk granules) and hypertrophy of granulosa cells (Davis, 1977).

Ovaries containing yolked oocytes were classified as active, i.e., capable of spawning in the current spawning season. Active females were separated into spawning and non-spawning groups. Spawning females contained ovaries with stage VI oocytes. Imminent spawning ovaries contained hydrated oocytes or migratory nucleus stage oocytes. The non-spawning females that displayed no such characteristics were assumed to be capable of spawning in the near future. Females with 50% or more oocytes were in $\alpha$-stage atresia, had no yolked oocytes but only post ovulatory or atretic follicles. The active and the post spawning females were both considered mature. Females that showed no histological evidence of imminent or future reproductive activity were classified as immature. The developmental stages of oocytes can be determined simply by microscopic examination of whole oocytes, according to Hilge
(1977). However, as with all attempts at dividing a continuum of development into discrete stages, there are problems in categorizing some of the transitional stages. Based on average diameter of large ovarian eggs, Mako – Hiroshi & Tagawa - Masaru, (1958) classified ovaries of sole *Cynoglossus robustus* into five stages. Gopalan (1968) observed seven stages in the ovaries of silver pomfret.

In *Sprattus sprattus*, De Silva, (1973) observed seven stages of ovarian maturation. His observation also revealed a long-spawning season and high proportion of females in the population of this species. The studies on maturation of ovaries, development of ova, length of reproductive season of American cyprinid determined by examination of ovaries, showed ovarian classification into four stages of maturity such as immature, maturing, mature and partially spent (Heins and Clemmer, 1976). Naumov (1956) described six stages of gonad ripeness based on histological aspects. Based on natural developmental phases of the ovary and oocytes, four stages of gonad ripeness in female bony fishes have been reported. Four stages are, I. Juvenile; II. Ripening; III. Running ripe and IV. Spent (Hilge, 1977). He described spent ovaries as slack, shrinking and containing residual eggs. The characters used to define spent ovaries are: flaccid, empty, often bloodshot in appearance and containing small number of ripe eggs. Recent spawning may be indicated by the presence of empty follicles but in many species these may persist only few days after spawning (Yamamoto and Yoshioka, 1964; Hunter and Goldberg, 1980).
Ovarian maturation of bisexual and unisexual fishes of Genus Poecilia was analyzed. Five stages of oocyte development based on morphological features and cytochemical staining properties of chromatin, nucleoli and the ooplasm occurred (Monaco et al., 1978). In anchovy, studies exist on spawning frequency, fecundity, sexual maturity of females and atretic state of the ovary using histological criteria was done. For estimation of spawning frequency, females were examined histologically and classified into five classes as hydrated, age 0 day, age 1 day, mature and immature stages (Hunter and Macewicz, 1980).

Histological examinations were made to determine the relationship between the morphological differences and the reproductive state of the gonads (Ratty et al., 1990). Maturity and spawning of Nibea albida was studied. The spawning season was determined from the percentage occurrence of different maturity stages of gonads during different months of the year and confirmed by the gonado-somatic indices. Five maturity stages were determined based on the colour, shape, size and microscopic structure of gonads (Kurup and Samuel, 1991).

Breeding biology of a gobid fish have been studied. Maturity stages were classified arbitrarily into seven stages based on appearance of fresh gonads, sizes, changes in GSI, extension of the gonads into body cavity and frequency distribution of ova diameters (Hoda, 1995).
Biological aspects of maturity, spawning time, sex ratio, fecundity and ova diameter of *Euryglossa orientalis* were examined. Seven stages of maturity such as virgin immature, developing virgin, developing, maturing, mature, ripe and spent were observed. (Khan and Hoda, 1998). Histological studies in mackerel revealed the presence of early perinuclear oocytes even in partially spawned ovaries suggesting a continuous oogenesis and production of more than 6 batches of ova (Yohannan and Abdurahiman, 1998).

Histological examination of ovaries in cobia revealed all classes of maturity, from early developing to regressed stages. Ovarian tissue in all classes of maturity showed atresia throughout the reproductive season (Brown-Peterson *et al*, 2001). Seven stages of maturity were recognized based on both macroscopic and microscopic observations in blue spot mullet. Linear relationships were found between fish length, gonad weight and fecundity; and between fish length, fish weight and ovary weight (Venkatesha Moorthy *et al*, 2002).

The spawning and maturity determination by gonado-somatic index and mean ova diameter in two local teleosts (Andra Pradesh) were reported by Shashi and Gupta, (2002). Variability in body weight was found correlated with the variation in the developmental stages of ovary from microscopic and macroscopic stages.

Reproductive biology of tautog was investigated. Ovarian development was described by eight microscopic gonad stages. Immature ovaries were characterized by the presence of oogonia and primary growth oocytes.
Developing stage ovaries were characterized by the presence of cortical alveoli and partially yolked oocytes. The fully developed ovaries were characterized by the presence of primary growth to advanced yolked oocytes and absence of oocytes in final maturation classes. Hydrated ovaries were distinguished by prominence of hydrated oocytes. Running ripe stages were identified by the presence of ovarian lumen. Partially spent or redeveloping ovaries are classified by lack of ovarian lumen. Spent stages were characterized by resorption of yolked oocytes and resting stage ovaries by a thickened ovarian membrane (White et al., 2003). An analysis was made of sexual pattern, spawning season, sizes at sexual maturation and sex change in black grouper. Sexually active males and females were observed year round, although ripening females with stage-III, IV and V vitellogenic oocytes in the ovaries dominated in December and March (Brule et al., 2003). Analysis of life history data shows that, both the size-specific fecundities and the age specific spawning frequencies differ for two halfbeak species, the ballyoo and the balao. Histological data was used to describe oocyte development and estimate spawning frequency (McBridge, 2003). The reproductive activity and recruitment of white mullet was determined by observations of gonad development and coastal juvenile abundance. Gonadal stages were classified as Stage-I, Stage-II, Stage-III and Stage IV (Marin et al., 2003).

The reproductive biology of gold-lined seabream has been examined focusing on duration, timing and frequency of spawning and on determining
potential annual fecundity. Histological sections of numerous ovaries were used to determine the timing of the formation and degeneration of postovulatory follicles, the relative abundance of different stages of atresia in ovaries at different times during the spawning period. (Hesp et al, 2004). Sex specific demography and reproductive biology of stripey bass were examined. Macropscopic features of histologically processed samples were compared within and between reproductive stages to determine whether any macroscopic characteristics could be used to accurately stage ovaries. Five female reproductive stages were identified through histological analysis (Kritzer, 2004). Histology and histochemistry on gonads in Glytocidaris crenularis and Strongylocentrotus intermedius was observed. The results showed that their gonads consisted of follicles containing germ cells and nutrient granules. Number of nutrient granules gradually decreased with the growth and development of the germ cells and increased after the germ cells were released (Li,-Xia et al., 2004).

Reproductive and growth parameters of Rex Sole were studied. Through histological analysis, ovaries that contained a sufficient number of advanced yolked oocytes or oocytes with migratory nucleus or unovulated hydrated oocytes for one spawning were classified as active. Ovaries without advanced yolked oocytes (AY) or major atresia of AY oocytes were classified as inactive (Abookire, 2006). Annual potential fecundity, batch fecundity and oocyte atresia was estimated for Atka mackerel. Histological examination of the
ovaries indicated that oocytes in the vitellogenic stage and higher had been spawned in the current spawning season (McDermott et al., 2007).

The mode of fertilization of Leiognathids is dioecism that means these fishes exhibit external fertilization. Maturation refers to cyclic morphological changes which the male and the female gonads undergo to attain full growth and ripeness.”Spawning” means the emission of male and female gametes from the body of the fish to the exterior, where fertilization occurs.”Breeding season” signifies the time of peak maturity and the period during which spawning occurs in the population. Thus the specialization in reproduction, which the teleosts have undergone as a group, is almost unique in the entire animal kingdom (Qasim, 1973). Even though there is some information on reproductive biology of *Leiognathus dussumieri* from Gulf of Mannar (James and Badrudeen, 1981) and *Secutor insidiator* in Porto Novo Coast (Jayabalan and Ramamoorthy, 1984), no detailed information in histological aspect is available on the reproductive biology of these two species. Therefore the present study was undertaken on *Leiognathus dussumieri* and *Secutor insidiator* to find out spawning frequency, length at first maturity based on histological sections of gonad tissue, spawning frequency, gonado somatic index, peak spawning period and synchronous or asynchronous spawning.

Histological studies while expensive and time consuming, yield the most reliable objective information on spawning cycle. Visual staging based on the external appearance of the gonad is possibly the least certain but the most rapid.
While histological studies provided very precise information on oocyte developmental stage, their interpretation is sometimes confused because different authors use different terms for the same structures. Some attempt at standardization is desirable (Forberg, 1982).

Histological examination is considered essential for detecting details within the maturation cycle as maturity fish, spawned fish, post ovulatory follicles and atretic oocytes. (Hunter and Maceviez, 1985a,b; West, 1990; Davis and West, 1993).

In all the oviparous animals yolk is deposited in the oocyte during oogenesis and serves as a reserve food material for the developing young. In many oviparous animals alterations occur in the composition of serum during vitellogenesis. These alterations involve accumulation of lipids, lipoproteins and complex phosphoproteins molecules. The liver has been implicated as the site of synthesis of these multi component proteins. During the process of growth of the oocyte the yolk is stored and accumulated in the form of granules.

Fecundity has been considered as the number of ripening eggs in the female prior to spawning. It varies from species to species depending on their age, length, weight, environmental conditions etc. Fecundity estimation is combined with estimates of the abundance of eggs in the sea and they can be used to estimate the biomass of a stock (Hunter et al., 1992).
3.2. Materials and Methods

Random samples of *Leiognathus dussumieri* and *Secutor insidiator* were collected from Puducherry landing centres during January 2004 to March 2006. We report only on results of studies of fish collected from commercial samples unless otherwise stated. Fishes were randomly collected from the catch. Fish were held on ice and transported to the laboratory. Females and males of each species for each month were dried of surface moisture, weighed (± 0.001g) and some of them were stored in 10% formalin for future observations; others were cut open to expose the gonads for the identification of sex, as sex of these fishes cannot be differentiated externally. Both ovaries from the females and testes from the males were weighed to the nearest mg. After gross examination of the gonads, the fishes were classified into maturity stages. Macroscopic characteristics for classifying gonads as mature correspond to Bagenal (1968), Nielson and Johnson (1983). Female characteristics included the presence of eggs visible to the naked eye and light yellow to reddish appearance from increased vascularisation of the ovary. Characteristics for mature males included white appearance and relative enlargement of testes within the body cavity.

In gonads randomly chosen by coin toss for histological processing the medial portion of about 5 to 10 mm of gonad was removed (Forberg, 1982) to avoid possible variation in the developmental stage of oocyte due to their position in the ovary. Monthly random samples of gonads of *Leiognathus*
*dussumieri* and *Secutor insidiator* were fixed in 10% neutral buffered formalin (NBF); tissue were dehydrated and embedded in paraffin, sectioned with a rotary microtome at 6 µm thickness and stained with Hariss hematoxylin and counter stained with eosin. This procedure gave good results and specific steps can be found in Luna (1968), Preece (1965) or any histotechnology handbook. Bouins fixative gave better results than 10% NBF, but it required transferring ovaries into 70% ethanol within 1 to 2 days or the ovaries become hard and brittle. During processing, hardening of ovaries may occur if time spent in xylene or toluene is not kept at a minimum or if temperature of paraffin is not kept below 60°C. It is necessary to have 10 – 20 good serial sections per slide for histological analysis; cold blocks (4°C) and knives usually improve sectioning. If shattering occurs, it is possible to soak blocks (prefaced-off) in ice water for a short time (1 – 15 min) to improve sectioning with little or no shattering.

**Mounting:** Wiping slides with a very fine coat of Mayers Albumen, sections were spread on the slides by floating the serial sections on warm water and attaching the sections to the slide by draining the water. By mounting as many sections as possible on a slide, it may be possible later to microscopically trace questionable structure. Gonad maturation stages were defined following Cyrus and Blaber (1984) and Hunter and Goldberg (1980) and were similar to those of Moussac and Poupon (1986). Young *et al.*, (1987) classified gonads
according to the relative number of cells at each developmental stage and the presence of any post ovulatory follicles.

The ovarian sections were also examined for the presence of $\alpha, \beta, \gamma$ and $\delta$ stages of atresia. In addition to relative frequency of developmental stages; we classified each histological section for the presence of post ovulatory follicles and atretic oocytes to aid in determination of spawning frequency. If no atresia of yolked oocytes were observed, we denoted the ovary as atretic state 0. Tissue sections exhibiting yolked oocytes undergoing atresia at $<50\%$, $>50\%$ and $100\%$ were classed as atretic states 1, 2 & 3 respectively. Our definition of an atretic follicle, which characterizes an oocyte undergoing atresia in $\beta$ or subsequent atretic stages were adopted from Hunter and Macewiez (1985b).

3.3. Result

3.3.1. Sampling

A total of 1445 specimens of *Leiognathus dussumieri* (761 Males and 684 Females) and 1453 specimens of *Secutor insidiator* (709 Males and 744 Females) were collected from medium seized gillnet and beach seine from Puducherry landing centre the size (Total Length) of *Leiognathus dussumieri* ranged from 72mm to 155mm TL. The size of *Secutor insidiator* ranged from 70mm to 112mm TL.
3.3.2. Histological Analysis.

i. Oocyte Development

Ovaries were collected every month, weighed and processed for histology. Maturation stages were defined following Cyrus and Blabber (1984), Hunter and Goldberg (1988) and Moussac and Poupon (1986). Ovaries were staged according to the relative number of cells at each development stage.

Changes in various cellular organelles of the oocyte during oogenesis have been described in a number of teleost species (Wallace & Selman, 1981; De Vlaming 1983). The first stage of the development of female gamete is similar to that found in spermatogenesis. Oogenesis undergo proliferation by mitotic divisions and become primary oocytes when the chromosomes become arrested at the diplotene stage of the first meiotic prophase. Oocytes unlike male gametes, then enter a period of growth which varies from species to species. Enlargement of oocytes is caused mainly by the accumulation of yolk.

Several criteria have been employed for staging the process of oogenesis; they are size, amount and distribution of various cell inclusions, especially yolk granules and morphology of the chromosomes. Yamamoto et al., (1965) divided the development of the oocytes of the rainbow trout into 8 stages; each stage is defined cytologically by size, appearance of nucleus and nucleolus and the type and localization of cytoplasmic inclusions. They are chromatin – nucleolus stage, perinucleolus stage (sub divided into early and late stages), oil drop stage, primary yolk stage, secondary yolk stage, tertiary yolk stage and
maturation stage. The chromatin nucleolus stage is characterized by a conspicuous nucleolus associated with chromatin threads. Concomitant with oocyte growth, the nucleus increases in size and multiple nucleoli become located around the periphery of the nucleus (early perinucleolus stage). The late perinucleolus stage can be distinguished from the previous stage by the enlargement of oocytes. During this period (Diplotene stage of Meiosis) lampbrush chromosomes are formed which disappear immediately prior to the breakdown of germinal vesicles during oocytes maturation. In the perinucleolus stage, most teleost oocytes, like those of other animals, accumulate a small juxtanuclear mass which is basophilic in histological sections. They are termed yolk nucleus. Recent electron microscopical studies have revealed that the yolk nucleus is not a homogeneous structure and that it is composed of various cellular organelles such as mitochondria, Golgi bodies, smooth endoplasmic reticulum, multivesicular bodies and lipid granules. Although its role is as yet not clear, it has long been considered that the yolk nucleus functions as a centre for the formation of organelles within the oocytes (Guraya, 1979).

**Vitellogenesis**

The enlargement of teleost oocyte is attributable mainly to the accumulations of yolk. There are three distinct types of yolk material in teleosts: Oil droplets, yolk vesicles and yolk globules. In general the oil droplets first appear in the perinuclear area and then migrate to the periphery in later stages. The sequence of the appearance of this yolk material varies with
species. Yolk vesicles appear during the secondary growth of oocytes. It appears in the outer and midcortical zones of oocytes. Electron microscopic studies by various authors suggest that yolk vesicles are synthesized within the oocyte (autosynthetic). As the oocyte grows, the yolk vesicles increase in both size and number and at maturity they move to the periphery of the oocyte, where they become known as cortical alveoli (Wallace and Selman, 1981). Cortical alveoli function in the cortical reaction at fertilization, the components of cortical alveoli being released into the perivitelline space when the egg is inseminated.

Yolk globules are formed by the fusion of small, coated vesicles. They first appear peripherally later they fuse with each other to form a single mass of yolk. In teleosts as in other non-mammalian vertebrates, it has been demonstrated that a female specific protein (vitellogenin) which is synthesized by the liver in response to $17\beta$ estradiol, is released into the blood and then transported to the ovary.

**Atretic oocytes**

Follicular atresia, which involves the hypertrophy of the granulosa cells and possibly the thecal calls may occur in follicles at any stage of oocyte development. Most investigators have divided teleost follicular atresia into four consecutive stages. Khoo (1975) provided a detailed description of histological changes in follicular atresia in the goldfish after hypophysectomy and classified five consecutive stages.
The following are the stages identified histologically in the ovarian development of *Leiognathus dussumieri* and *Secutor insidiator*.

**Stage – I**

Right and left ovaries are more or less equal in length and size. Ovaries are colourless to whitish; small and thin; slight vascularisation observed. Eggs very minute; distinct only under microscope. The eggs were 16 - 32 \( \mu \text{m} \) in diameter and were found in clumps or “nests” along the lamellar branches (Plate 3 (A) & 4(A)). Oocytes are surrounded by follicular epithelium. Large oogonia possessed a deeply stained nucleus with chromatin network attached to a single large basophilic nucleolus. The cytoplasm is seen as a narrow weakly basophilic zone around the more basophilic nucleus. The diameter of the nucleus is about 75% of the total oocyte diameter. One large and several smaller threads like basophilic nucleoli are found in the nucleus. The largest nucleolus is typically half moon shaped and lies close to the cytoplasm during the synaptic phase. Later the big nucleolus occupies a more central position, with smaller nucleoli dispersed around it. The oocytes are by now commonly found in small clusters. The oocytes of this stage have been described in several teleosts by various authors and they are observed to pass through the leptotene to the zygotene stage of chromosome development, giving the so called post synaptic stage. The nucleoli are arranged around the periphery of the nucleus with the chromatin distributed towards the centre.
Stage - II

The oocytes were 112 - 128 µm in cell diameter. Oocytes growing into the next stage move away from oogonial nests. Chromatin threads are visible; this stage marks the initiation of the period of major growth of the oocytes. A large, round and strongly basophilic nucleolus is seen to lie centrally in the nucleus with a number of smaller nucleoli situated peripherally. The cytoplasm becomes strongly basophilic and the nucleus is weakly basophilic. Numerous nucleoli of different sizes are situated in the periphery. The nucleus is still round in shape with a smooth surface. Oocytes of this nature are seen year round in ovaries of mature females of both *Leiognathus dussumieri* and *Secutor insidiator*. A ring of discrete vacuoles begin to appear around the periphery of the oocyte. The follicle cells are distinguishable into two layers.

Stage - III

The oocytes were 128 - 208 µm in cell diameter. Depending upon bodily maturation and environmental stimuli, 10 - 30% of the stage II oocytes (First growth phase) enter into the next phase of development. The remaining oocytes do not develop beyond the stage of the resting oocytes until the spawning season. This stage is marked by accumulation of yolk in the oocytes. Yolk accumulation begins with the appearance of yolk vesicles in the cytoplasm. Multiple vacuoles appear between yolk vesicles and the nucleus. These vesicles increase in number and volume. (Plate.4(F) & 8(F)). The vacuoles tend to coalesce and become less discrete. The nuclear membrane becomes irregular in
appearance. The nucleoli are in close contact with the cytoplasm. According to Balinsky (1970) the irregular nature of nuclear membrane reflects the RNA transportation from the nucleoli into the cytoplasm where it is used in the building of yolk.

**Stage – IV**

A further increase in oocyte size was observed and the mean diameter was in the range of 192 - 288 µm. Oocytes are more eosinophilic. Multiple vacuoles appear between yolk vesicle and the nucleus (Plate.4(H) & 8(H)). These vacuoles increase in number and volume. Yolk granules are identifiable in the cytoplasm. The nucleoli are less distinct than in the previous stages. The ovary also contains batches of vacuolated oocytes some of which contain developing yolk granules. The general size distribution in the ovary is not uniform. All stages of oocytes could be seen in the ovary, though stage IV oocytes are predominant. This suggests that the development is a continuous process in the ovaries of *Leiognathus dussumieri* and *Secutor insidiator*.

**Stage – V**

Oocytes are eosinophilic. Oocytes are in various stages of yolk accumulation. Most of the oocytes at this stage show cytoplasm entirely filled with yolk. The oocyte diameter at this stage measured 208 – 384 µm. The vacuoles which were distributed throughout the cytoplasm begin to coalesce forming larger vacuoles. The follicle layer is thin and appears stretched due to
the rapid growth of the oocyte and is often ruptured during histological processing (Plate.5(I) & 9(I)).

**Stage – VI**

(Plate.5 & 9) Ripe oocytes undergo hydration resulting in transparency and considerable increase in volume. The content of the oocyte is similar to that in preceding stage. Hydration leads to the rupture of follicles and the eggs lie in the lumen of ovary before passing out. The spent ovary is characterized by thick tunica and empty follicles. Large number of germ cells and resting oocytes are present forming the reserve for the next season. Some mature oocytes and vacuolated oocytes with discrete yolk granules are also present. The unovulated mature oocytes undergo atresia. Granulosa layer increase in thickness in these oocytes. It is suggested that they are phagocytic in function. Most of the empty follicles also collapse and are eventually resorbed. Once resorption is complete only those resting oocytes and germ cells comprising the reserve stock for the following season are left.

The stages described above are common to both *Leiognathus dussumieri* and *Secutor insidiator* and we could not identify any histological difference between the two species in the developmental pattern of the oocytes and the ovary in general. The features that help to distinguish different stages of oocyte development are presented in Table. 3.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic Criteria</th>
<th>Microscopic/ Histological Criteria</th>
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<tr>
<td>II – Maturing</td>
<td>Pale yellow, granular ova visible to naked eye. Medium sized ova, spherical, opaque.</td>
<td>Size increases with dark blue stained cytoplasm. Primary growth; cortical alveoli and some partially yolked oocytes present. A large darkly stained nucleolus with many peripheral nucleoli appears.</td>
</tr>
<tr>
<td>III – Matured/ Ripe</td>
<td>Yellow, blood vessels prominent, occupying about full body cavity. Slightly grainy. Oocytes visible clearly.</td>
<td>Beginning of accumulation of yolk; Multiple vacuoles appear and coalesce; nuclear membrane irregular.</td>
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<tr>
<td>IV – Hydrated</td>
<td>Ovaries very large, yellow to orange in colour, yolked oocytes interspersed with large transparent (hydrated) Oocytes.</td>
<td>Oocytes more eosinophilic; vacuoles increase in number and volume; nucleoli less distinct; yolk granules identifiable in the cytoplasm.</td>
</tr>
<tr>
<td>V – Running Ripe</td>
<td>Ovaries large to very large almost totally transparent. Occupying the full length of the body cavity.</td>
<td>Cytoplasm filled with yolk granules. Vacuoles become large as they coalesce; follicle layers appear thin and often ruptured.</td>
</tr>
<tr>
<td>VI – Spent and Resting Stage</td>
<td>Ovaries flaccid, loose, ovaries wall thickened and wrinkled. Some residual Oocytes visible within translucent material.</td>
<td>Post Ovulatory follicles are clearly visible. In the mature oocyte the nucleus is not visible. Some vitelline vesicles occur and yolk globules are completely fused. Primary growth and cortical alveoli present, occasional atretic oocytes.</td>
</tr>
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Plate 3. Histological stages of *Leiognathus duumlieri* ovaries.
(Scale bar, 120 μm) (A), Immature (Stage I). (B), 1/4th Maturing (Stage II).
(C), 1/2 Maturing (Stage II). (D), 3/4th Maturing (Stage II).
PG = Primary growth oocyte. CA = Cortical alveoli. PY = Partially yolked oocyte.
AY = Advanced yolked oocyte. OG = Oil globule. YG = Yolk globule.
Plate 4. Histological stages of *Leiognathus dussumieri* ovaries.

(Scale bar, 120 μm) (Enlarged) (E). 3/4<sup>th</sup> Maturing ovum (Stage II).
(F). Matured (Stage III). (G). Matured ovum (Stage III).
(H). Hydrated (Stage IV). PG = Primary growth oocyte.
CA = Cortical alveoli. AY = Advanced yolked oocyte.
OG = Oil globule. YG = Yolk globule. N = Nucleus. NU = Nucleoli.
HO = Hydrated oocyte. POF = Postovulatory follicle.
Plate: 5. Histological stages of *Leiognathus dussumieri* ovaries.
(Scale bar, 120 μm) (I). Running ripe (Stage V). Enlarged
(J). Partially spent (Stage VI). (K). Spent (Stage VI). (L). Resting (Stage VI).
PG = Primary growth oocyte. CA = Cortical alveoli. AY = Advanced yolked oocyte.
NU = Nucleoli. GVM = Germinal vesicle migration oocyte.
GVBD = Germinal vesicle breakdown oocyte. POF = Postovulatory follicle.
AO = Atretic oocyte.
Plate: 7. Histological stages of *Secutor insidiator* ovaries.
(Scale bar, 120 μm) (A). Immature (Stage I).
(B). 1/4th Maturing (Stage II), (C). 1/2 Maturing (Stage II).
(D). 3/4th Maturing (Stage II). PG = Primary growth oocyte.
CA = Cortical alveoli. PY = Partially yolked oocyte.
AY = Advanced yolked oocyte. OG = Oil globule. YG = Yolk globule.
Plate: 8. Histological stages of *Secutor insidiator* ovaries.
(Scale bar, 120 μm) (Enlarged) (E). 3/4th Maturing ovum (Stage II).
(F). Matured (Stage III). (G). Matured ovum (Stage III).
(H). Hydrated (Stage IV). PG = Primary growth oocyte.
CA = Cortical alveoli. AY = Advanced yolked oocyte.
OG = Oil globule. YG = Yolk globule. N = Nucleus. NU = Nucleoli.
HO = Hydrated oocyte. POF = Postovulatory follicle.
Plate: 9  Histological stages of *Secutor insidiator* ovaries.
(Scale bar, 120 μm) (I). Running ripe (Stage V) (Enlarged)
(J). Partially spent (Stage VI). (K). Spent (Stage VI). (L). Resting (Stage VI).
PG = Primary growth oocyte. CA = Cortical alveoli. AY = Advanced yolked oocyte.
NI = Nucleoli. GVM = Germinal vesicle migration oocyte. 
GVBD = Germinal vesicle breakdown oocyte. POF = Postovulatory follicle. 
AO = Atretic oocyte.
We have not attempted to classify atretic oocytes. Atretic oocytes were found in samples throughout the year except in months during which the ovary was in the matured/ripe stage. Frequency of occurrence of atretic follicles was highest in the post ovulatory stages (spent) and in recovering stages. Oocytes that ovulated but remained in the ovigenous folds and were resorbed later were treated as atretic oocytes because it was difficult to distinguish between them and atretic oocytes, if they were somewhat absorbed. Atretic oocytes did not always correspond to the most advanced oocytes in the ovaries.

ii. Testis Development

The testes of *Leiognathus dussumieri* and *Secutor insidiator* are lobed organs that appear attached to the dorsal body wall by a mesentery. The mesothelium of the peritoneum surrounding the testis is a very thin and delicate membrane resting upon the fibrous connective tissue of the stroma. The stroma consists mostly of loose white fibrous connective tissue. Among the fibrous connective tissue cells are found with darkly staining nucleoli. The testis is made up of a system of lobules. The lobules converge on the main sperm duct. The lobular units are tubular with their apices at the centre of the organ and their broader ends directed towards the periphery.

Two distinct zones can be identified in cross section of testis. Outer proliferative region of the testis and the inner region which includes the duct system. At the outer region, the seminiferous lobules have a thick wall formed
<table>
<thead>
<tr>
<th>Stages</th>
<th>Macroscopic Criteria</th>
<th>Histology Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Immature</td>
<td>Small, transparent, pale in colour. Occupying very small portion of body cavity.</td>
<td>Testis contains spermatogonia isolated pockets of spermatocrypts. These mainly contain spermatocytes.</td>
</tr>
<tr>
<td>II Early developing</td>
<td>Flat, translucent. Slightly larger</td>
<td>Cytoplasm of the spermatogonium stains very faintly. Some spermatogonia become degenerate. The primary spermatogonia have prominent basophilic nucleoli.</td>
</tr>
<tr>
<td>III Developing</td>
<td>Creamy white, round occupying about half of body cavity.</td>
<td>Secondary spermatogonium appears and stained deeply. Few spermatocytes and even some spermatozoa are seen. Germ cells present in all stages of spermatogenesis.</td>
</tr>
<tr>
<td>IV Late developing</td>
<td>Creamy white, soft, occupying about 3/4th of the body cavity.</td>
<td>Secondary spermatocytes are round and much smaller than primary spermatocytes. The nucleus of spermatocytes stains deeply. Bundles of spermatozoa appear. Spermatocytes dominate in the sperm tissue.</td>
</tr>
<tr>
<td>V Ripe</td>
<td>Creamy white, soft, lobed, occupying 3/4th of the body cavity.</td>
<td>Testis contains abundance of spermatozoa and spermatid in the outer portion of gonad.</td>
</tr>
<tr>
<td>VI Running ripe</td>
<td>White, sometimes blood shot, translucent.</td>
<td>Testis large in size and dominated by large peripheral and central sperm sinuses filled with spermatozoa.</td>
</tr>
</tbody>
</table>
Plate: 6 Histological Stages of *Leiognathus dussumieri* testis. (Scale bar, 120 μm)

(A). Transverse section showing the outer proliferative region (PR) of the testis. (Immature – Stage I) (B). Peripheral zone of the testis where the distal ends of some tubules terminate below the tunica albuginea (Maturing – Stage II) (C). Outer region of testis contain developing germinal cysts and the inner regions contain spermatids and mature spermatooza (Matured – Stage III) (D). Main sperm duct filled with a compact mass of spermatooza (Spent - IV) PR = Proliferative region SCP = Spermatocyte present in pockets of spermatocysts. BV = Blood vessel IC = Interstitial cell. SG = Spermatogonia. SC = Spermatocyte. ST = Spermatid. SZ = Spermatooza. TMW = Thick muscular wall CSS = Central sperm sinus. SD = Sperm duct filled with spermatooza. PZT = Peripheral zones of testis
Plate: 10 Histological Stages of *Secutor insidiator* testis. (Scale bar, 120 μm)

(A). Transverse section showing the outer proliferative region (PR) of the testis. (Immature – Stage I) (B). Peripheral zone of the testis where the distal ends of some tubules terminate below the tunica albuginea (Maturing – Stage II) (C). Outer region of testis contain developing germinal cysts and the inner regions contain spermatids and mature spermatozoa (Matured – Stage III) (D). Main sperm duct filled with a compact mass of spermatozoa (Spent - IV) PR = Proliferative region SCP = Spermatocyte present in pockets of spermatocysts. BV = Blood vessel IC = Interstitial cell. SG = Spermatogonia. SC = Spermatocyte. ST = Spermatid. SZ = Spermatozoa. TMW = Thick muscular wall. CSS = Central sperm sinus. SD = Sperm duct filled with spermatozoa, PZT = Peripheral zones of testis.
by the germinal epithelium, where germ cells develop in association with sertoli cells; spermatozoa are found in the cavities of the lobules which are released at the end of spermatogenesis. Plate 6 & 10 shows the spermatozoa accumulated in the lobules prior to their release. As a result of the release of mature sperm from spermatocysts into the lobule lumina, the germinal epithelium becomes discontinuous.

While studying the general biology of the Leiognathids, we found different stages of gonad development (Table 4.) by a macroscopic examination but it was not always possible to say whether the gonad was developing to maturity for the first time (virgin developing) or whether it had spent and was recovering again (Spent-recovering). It was thought that a histological examination might reveal some difference between virgin and spent recovering testis. We also wanted to find out a correlation, if there are any, between the macroscopic appearance of gonads and their histological structure.

In a virgin testis many cysts appear. The cysts are not permanent structures like stroma and the lobules. The gametes develop in groups of isogenic cells called germinal cysts or spermatocysts. Primary spermatogonia are large single cells that are distributed all along the germinal epithelium. Successive mitosis of spermatogonia A, results in the formation of spermatogonia B. They are found in small groups. At the outer region, the seminiferous lobules have a thick wall formed by the germinal epithelium, where germ cells develop in association with sertoli cells; spermatocysts and spermatids are grouped within
large spermatocysts. Late spermatids and spermatozoa, prior to spermiation orient themselves towards the lobule wall and the flagella are directed towards the seminiferous lobule lumen.

Active spermatogenesis was observed to occur in both species of *L. dussumieri* and *S.insidiator*. In both species all stages of germ cell lines were present in the gonads. In addition, the large amounts of spermatozoa had accumulated in the central system of ducts. After completion of spermatogenic process spermatozoa are released into the lumina of the lobules. As a result of the release of mature sperm from spermatocysts the germinal epithelium becomes discontinuous. With the release of spermatozoa the structures of the lobules change. Their function changes from sperm production to sperm storage.

**Stage I and II**

Stage I & II are observed in virgin developing as well as spent recovering testis. Migrating germ cells are observed and they appear elongated. Nucleus is large; it occupies a large portion of the cytoplasm. The migrating germ cells appear to migrate from the peripheral stroma towards the interior of the testis along the interlobular walls. Once the germ cells become firmly lodged in a cyst it undergoes transformation. It becomes rounded, increase in size and its nucleolus becomes more prominent. These cells were called primary spermatogonia by Wilson, (1925). Some authors call them resting germ cells. The primary spermatogonia have prominent basophilic nucleoli and are found
in cysts with varying numbers of individual cells. These cells can be seen dividing mitotically and giving rise to similar cells. Such mitotic divisions are only seen in stages I & II and never later. The cytoplasm of the spermatogonium stains very faintly: the size of the nucleus increases. Some of the spermatogonia become degenerate as the number of spermatogonia increase. Some scattered blood cells and a few connective tissue cells are found in the interlobular septa. Interstitial cells are observed in these stages in the space between lobules and the interlobular walls.

**Stage III**

The testis appears creamy white in colour. Occupying the body cavity. The secondary spermatogonia appear in stage II testis but are more common in stage III from December to January in *S. insidiator* and January, April – May in *L. dussumieri*.

They are stained more deeply than the primary spermatogonia, but less so than the primary spermatocytes. The nucleus of the secondary spermatogonium is smaller than that of the primary spermatogonium. In a stage III testis there are fewer migrating germ cells. Some lobules in stage III testis show advanced spermatogenesis and may contain quite a few spermatocytes and even some spermatozoa. This is true particularly during December - January in *S. insidiator* and January, April – May in *L. dussumieri*. 
Stage IV

Spermatocytes appear in this stage. Two types of spermatocytes can be distinguished from the appearance of chromatin material and the shape of the nucleus. Secondary spermatocytes are round and much smaller than the primary spermatocytes. The nucleus of spermatocytes stain deeply and they are smaller than the spermatogonium. Bundles of spermatozoa appear in a stage IV testis in some of the sections observed. The interlobular walls of stage III and IV testis appear thin. Germ cell populations are not very prominent in the walls of stage IV testis.

Stage V and VI

Sections observed at this stage showed more number of spermatozoa. The cyst walls have almost disappeared. Some of the sperm bundles are seen grouped at the centre of the lumen of the lobule whereas others are seen pressed against the wall of the lobule. The interlobular walls are thin and appear similar to their structure in stage IV testis. A few migrating germ cells and resting spermatogonia are seen in the stroma. Possibly these cells act as stem cells for proliferation in the next spawning season. Number of spermatozoans are more in stage VI compared to stage V.

Stage VII Spent Stage

The size and weight of the testis is reduced due to release of spermatozoa. Stage VII testis hardly occupies 1/2 of the body cavity whereas stage V occupied almost 3/4 th of the body cavity. Histological sections showed loss of
sperm bundles and in some sections only few number of spermatozoans could be identified. The wall of the stroma and interlobular walls again started increasing in thickness. It may also be due to shrinkage of testis that these lobular walls appear thicker. Blood cells are seen scattered. Blood vessels which appeared dilated in cross sections of stage V & VI testis seem to have returned to their normal size.

**Spent Recovering Stage**

Such testes are found in fishes captured during the months of Sep. – Dec. in *L. dussumieri* and during the months of Oct. – Dec. and May – June in *Secutor insidiator*. The lobule walls contain many migrating germ cells and a few resting spermatogonia can also be seen. Few spermatozoa that appeared in stage VII testis have started disintegrating and new cysts are formed. The entire testis seems to be in a process of reorganization. The number of spermatogonia increases progressively during the months of January and May in *L. dussumieri* and during the months of January and April in *S. insidiator*. The cycle of growth of the testis along with its morphological changes takes place in the testis of these two species till the next spawning season is reached. At the initial stages of this cycle only resting spermatogonia are observed in the testes.
Fig. 4a(i). Month-wise occurrence of males and females of *Leiognathus dussumieri* in various stages of maturity for the year 2004
Fig. 4a(ii). Month-wise occurrence of males and females of *Leiognathus dussumieri* in various stages of maturity for the year 2005 – 2006.
Fig. 4b(i). Month-wise occurrence of males and females of *Secutor insidiator* in various stages of maturity for the year 2004
Maturity Stages

Fig. 4b(ii). Month-wise occurrence of males and females of *Secutor insidiator* in various stages of maturity for the year 2005 - 2006
Fig. 5a. Ova–diameter frequency distribution of *L. dussumieri*
Fig. 5b. Ova–diameter frequency distribution of *Secutor insidiator*
3.3.3. Monthly Distribution of Maturity Stages of Gonads

Monthly distribution of maturity stages of gonads *Leiognathus dussumieri* and *Secutor insidiator* are illustrated Fig. 4a(i & ii), 4b(i & ii). Stage I was recorded less in April 2004 and 2006 than April 2005. High percentage of spawning was recorded in May 2004. Mature stage was observed from January to July. It shows that *L. dussumieri* has a prolonged spawning period. In *S. insidiator* the mature fishes were seen in Feb to May and again Aug and sep. It indicates that *S. insidiator* spawns twice in a season.

3.3.4. Spawning Frequency

The percentage occurrence of various maturity stages of ovaries in different months was computed for both fishes by pooling the data for two years, and it was represented graphically. Maturity stages recognized macroscopically were categorized into 4 stages.

*Leiognathus dussumieri*

Monthly percentage occurrence of gonads in different stages of maturity in males and females are presented in Fig. 4a(i & ii). The advanced stage of ovary of a fish contained an intermediate group of ova which had undergone half the maturation process apart from mature group of eggs; it was expected to spawn twice a season. So the presence of maturing group of ova in the ovaries of *L. dussumieri* of Puducherry coast indicates that these fishes have a prolonged spawning period.
Throughout the period of the present investigation, mature gonads were noticed and spent individuals were also found during most part of the year. However it was evident from the frequency distribution of mature gonads that spawning activity was higher during Jan. to June, Jul.

*Secutor insidiator*

Monthly percentage occurrence of gonads in different stages of maturity in both males and females are presented in Fig. 4b (i & ii). The advanced group of oocytes were seen in stage III Ovaries. These advanced groups of oocytes were well separated from the immature and mature group of ova. These types of ovaries indicate that the individual spawning of these fishes are restricted to a short period. The occurrence of different stages of maturity of gonads of male and female in different months indicate that *S. insidiator* has two spawning seasons in a year.

3.3.5. Month wise Sex Ratio

Sex of the fish was assessed externally from several dimorphic characters. Leiognathids have a well developed internal luminescent system with light organ. For example in *Leiognathus bindus* the outer surface of the light organ in male is densely pigmented with chromatophores and is visible to the exterior as a black spot close to the pectoral fin base where the muscle is transparent. In females, there is no transparent region under the pectoral fin and hence the light organ is not visible. This helps to distinguish the sexes.
In *Leiognathus dussumieri* and *Sector insidiator* the sex cannot be differentiated externally, the fishes were brought to the laboratory and the abdomen of the fishes was cut open to expose the gonads for identification of the sexes.

This study is based on random samples of fish collected at Puducherry by trawl nets operated from mechanized boats and bag nets. Sex ratio was determined from the number of specimens of each sex sampled every month. To test the significant deviations from an expected 1:1 sex ratio for all male and female fishes, the sex ratio values obtained every month were subjected to chi-square (Sokal and Rohlf, 1981) employing the formula,

\[
X^2 = \sum \frac{(o-e)^2}{e}
\]

Where

- \(o\) = observed number, \(e\) = expected number

*Leiognathus dussumieri*

During 2004-2006, a total of 761 males and 684 females were recorded. Chi-square values calculated month wise showed that the sex ratio conformed to the expected 1:1 in all the months \((P<0.05)\) except February-2004, June-2004, September-2005, March-2006 & overall sex ratio, slightly varying significantly from an expected 1:1 ratio, with slightly less number of females than males \((1.113:1, X^2=0.284, P<0.05)\). The percentage of females in monthly samples ranged between 35.714% and 60.714% while males ranged between 39.286% and 64.286% (Fig. 6a).
### Table 5a. Month wise Sex - ratio of *Leiognathus dussumieri*

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of Females</th>
<th>No. of Males</th>
<th>Total no. of fish</th>
<th>% Females</th>
<th>% Males</th>
<th>Sex Ratio (F:M)</th>
<th>Chi ^2 value</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan'04</td>
<td>12</td>
<td>11</td>
<td>23</td>
<td>52.174</td>
<td>47.826</td>
<td>1:0.917</td>
<td>0.189</td>
<td>&gt; 0.05</td>
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<tr>
<td>Feb'04</td>
<td>10</td>
<td>18</td>
<td>28</td>
<td>35.714</td>
<td>64.286</td>
<td>1:1.800</td>
<td>8.163</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Mar'04</td>
<td>50</td>
<td>47</td>
<td>97</td>
<td>51.546</td>
<td>48.454</td>
<td>1:0.940</td>
<td>0.096</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Apr'04</td>
<td>18</td>
<td>23</td>
<td>41</td>
<td>43.902</td>
<td>56.098</td>
<td>1:1.278</td>
<td>1.487</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>May'04</td>
<td>15</td>
<td>11</td>
<td>26</td>
<td>57.692</td>
<td>42.308</td>
<td>1:0.733</td>
<td>2.367</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>June'04</td>
<td>19</td>
<td>34</td>
<td>53</td>
<td>35.849</td>
<td>64.151</td>
<td>1:1.789</td>
<td>8.010</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>July'04</td>
<td>33</td>
<td>35</td>
<td>68</td>
<td>48.529</td>
<td>51.471</td>
<td>1:1.061</td>
<td>0.087</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Aug'04</td>
<td>38</td>
<td>35</td>
<td>73</td>
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<td>47.945</td>
<td>1:0.921</td>
<td>0.169</td>
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<tr>
<td>Sep'04</td>
<td>25</td>
<td>28</td>
<td>53</td>
<td>47.170</td>
<td>52.830</td>
<td>1:1.120</td>
<td>0.320</td>
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<td>Oct'04</td>
<td>27</td>
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<td>62</td>
<td>43.548</td>
<td>56.452</td>
<td>1:1.296</td>
<td>1.487</td>
<td>&gt; 0.05</td>
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<tr>
<td>Nov'04</td>
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<td>26</td>
<td>44</td>
<td>40.909</td>
<td>59.091</td>
<td>1:1.444</td>
<td>3.306</td>
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<tr>
<td>Dec'04</td>
<td>16</td>
<td>15</td>
<td>31</td>
<td>51.613</td>
<td>48.387</td>
<td>1:0.938</td>
<td>0.104</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mar'05</td>
<td>24</td>
<td>30</td>
<td>54</td>
<td>44.444</td>
<td>55.556</td>
<td>1:1.250</td>
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<tr>
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<td>67</td>
<td>117</td>
<td>42.735</td>
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<td>1:1.340</td>
<td>2.111</td>
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<tr>
<td>May'05</td>
<td>35</td>
<td>50</td>
<td>85</td>
<td>41.176</td>
<td>58.824</td>
<td>1:1.429</td>
<td>3.114</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>June'05</td>
<td>16</td>
<td>15</td>
<td>31</td>
<td>51.613</td>
<td>48.387</td>
<td>1:0.938</td>
<td>0.104</td>
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<td>July'05</td>
<td>18</td>
<td>25</td>
<td>43</td>
<td>41.860</td>
<td>58.140</td>
<td>1:1.389</td>
<td>2.650</td>
<td>&gt; 0.05</td>
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<tr>
<td>Aug'05</td>
<td>40</td>
<td>43</td>
<td>83</td>
<td>48.193</td>
<td>51.807</td>
<td>1:1.075</td>
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<td>25</td>
<td>41</td>
<td>39.024</td>
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<td>1:1.563</td>
<td>4.819</td>
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<td>Oct'05</td>
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<td>19</td>
<td>37</td>
<td>48.649</td>
<td>51.351</td>
<td>1:1.056</td>
<td>0.073</td>
<td>&gt; 0.05</td>
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<tr>
<td>Nov'05</td>
<td>28</td>
<td>30</td>
<td>58</td>
<td>48.276</td>
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<td>57</td>
<td>107</td>
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<td>1:1.140</td>
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</tr>
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<td>Jan'06</td>
<td>36</td>
<td>26</td>
<td>62</td>
<td>58.065</td>
<td>41.935</td>
<td>1:0.722</td>
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<tr>
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<td>23</td>
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<td>47.727</td>
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<td>84</td>
<td>60.714</td>
<td>39.286</td>
<td>1:0.647</td>
<td>4.592</td>
<td>&lt; 0.05</td>
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<tr>
<td>TOTAL</td>
<td>684</td>
<td>761</td>
<td>1445</td>
<td>47.336</td>
<td>52.664</td>
<td>1:1.113</td>
<td>0.284</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

![Fig. 6a. Month wise sex ratio of *Leiognathus dussumieri*](image-url)
### Table 5b. Month wise Sex - ratio of *Secutor insidiator*

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of Females</th>
<th>No. of Males</th>
<th>Total no. of fish</th>
<th>% Females</th>
<th>% Males</th>
<th>Sex Ratio(F:M)</th>
<th>Chi² value</th>
<th>'p' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan' 04</td>
<td>12</td>
<td>10</td>
<td>22</td>
<td>54.545</td>
<td>45.455</td>
<td>1:0.833</td>
<td>0.826</td>
<td>&gt; 0.05</td>
</tr>
<tr>
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<td>11</td>
<td>13</td>
<td>24</td>
<td>45.833</td>
<td>54.167</td>
<td>1:1.182</td>
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<td>&gt; 0.05</td>
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<tr>
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<td>35</td>
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<td>52.703</td>
<td>47.297</td>
<td>1:0.897</td>
<td>0.296</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Apr' 04</td>
<td>30</td>
<td>26</td>
<td>56</td>
<td>53.571</td>
<td>46.429</td>
<td>1:0.867</td>
<td>0.510</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>May' 04</td>
<td>27</td>
<td>30</td>
<td>57</td>
<td>47.368</td>
<td>52.632</td>
<td>1:1.111</td>
<td>0.277</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>June' 04</td>
<td>25</td>
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<td>56</td>
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<td>55.357</td>
<td>1:1.240</td>
<td>1.148</td>
<td>&gt; 0.05</td>
</tr>
<tr>
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<td>57</td>
<td>42.105</td>
<td>57.895</td>
<td>1:1.375</td>
<td>2.493</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Aug' 04</td>
<td>37</td>
<td>34</td>
<td>71</td>
<td>52.113</td>
<td>47.887</td>
<td>1:1.091</td>
<td>0.179</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sep' 04</td>
<td>30</td>
<td>43</td>
<td>73</td>
<td>41.906</td>
<td>58.094</td>
<td>1:1.433</td>
<td>3.171</td>
<td>&gt; 0.05</td>
</tr>
<tr>
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<td>40</td>
<td>78</td>
<td>48.718</td>
<td>51.282</td>
<td>1:1.053</td>
<td>0.066</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Nov' 04</td>
<td>21</td>
<td>17</td>
<td>38</td>
<td>55.263</td>
<td>44.737</td>
<td>1:0.810</td>
<td>1.108</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dec' 04</td>
<td>17</td>
<td>25</td>
<td>42</td>
<td>40.476</td>
<td>59.524</td>
<td>1:1.471</td>
<td>3.628</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mar' 05</td>
<td>40</td>
<td>22</td>
<td>62</td>
<td>64.516</td>
<td>35.484</td>
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</tr>
<tr>
<td>Apr' 05</td>
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<td>32</td>
<td>69</td>
<td>53.623</td>
<td>46.377</td>
<td>1:0.865</td>
<td>0.525</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>May' 05</td>
<td>21</td>
<td>17</td>
<td>38</td>
<td>55.263</td>
<td>44.737</td>
<td>1:0.810</td>
<td>1.108</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>June' 05</td>
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<td>45</td>
<td>85</td>
<td>47.059</td>
<td>52.941</td>
<td>1:1.125</td>
<td>0.346</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>July' 05</td>
<td>66</td>
<td>53</td>
<td>119</td>
<td>55.462</td>
<td>44.538</td>
<td>1:0.803</td>
<td>1.193</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Aug' 05</td>
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<td>37</td>
<td>83</td>
<td>55.422</td>
<td>44.578</td>
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<td>18</td>
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<td>43.750</td>
<td>56.250</td>
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<td>1.563</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Oct' 05</td>
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<td>19</td>
<td>41</td>
<td>53.659</td>
<td>46.341</td>
<td>1:0.864</td>
<td>0.535</td>
<td>&gt; 0.05</td>
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<tr>
<td>Nov' 05</td>
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<td>19</td>
<td>34</td>
<td>44.118</td>
<td>55.882</td>
<td>1:1.267</td>
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<td>&gt; 0.05</td>
</tr>
<tr>
<td>Dec' 05</td>
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<td>23</td>
<td>58</td>
<td>60.345</td>
<td>39.655</td>
<td>1:0.657</td>
<td>4.281</td>
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</tr>
<tr>
<td>Jan' 06</td>
<td>9</td>
<td>12</td>
<td>21</td>
<td>42.857</td>
<td>57.143</td>
<td>1:1.333</td>
<td>2.041</td>
<td>&lt; 0.05</td>
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<tr>
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<td>30</td>
<td>52</td>
<td>42.308</td>
<td>57.692</td>
<td>1:1.364</td>
<td>2.367</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mar' 06</td>
<td>66</td>
<td>45</td>
<td>111</td>
<td>59.459</td>
<td>40.541</td>
<td>1:0.682</td>
<td>3.579</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>TOTAL</td>
<td>744</td>
<td>709</td>
<td>1453</td>
<td>51.204</td>
<td>48.796</td>
<td>1:0.953</td>
<td>0.058</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

**Fig. 6b. Month wise sex ratio of *Secutor insidiator***
**Secutor insidiator**

A total of 709 males and 744 females were recorded. Chi-square value calculated month wise showed that the sex ratio conformed to the expected 1:1 in all months (P< 0.05) except March-2005 and December-2005. Overall, sex ratio did not vary significantly from an expected 1:1 ratio, with slightly more number of females than males (0.953:1, X^2=0.058, P< 0.05). The percentage of females in monthly samples ranged between 40.48 & 64.52% while males ranged b/w 35.48% and 59.52% (Fig. 6b).

3.3.6. **Month wise GSI**

Monthly gonado-somatic index was calculated by using total weight (TW) for each male and female fishes. Applying the method of June (1953) the GSI of *Leiognathus dussumieri* and *Secutor insidiator* was calculated by using the following equation.

\[
\text{Gonado – Somatic Index} = \frac{\text{Weight of Gonad}}{\text{Total weight of the fish}} \times 100.
\]

A more precise estimate of spawning season was determined from microscopic examination of gonad stages. The monthly average GSI values were computed by dividing the total value of each month by the number of fishes examined in that month.

**Leiognathus dussumieri**

The male and female fish showed weight changes in the gonads corresponding to the three gametogenic stages (pre spawning, spawning and post spawning). In the pre spawning period there was a gradual increase in the
gonado somatic index (GSI) from January and reached a peak in June, July. A gradual decrease in the post spawning period in the weight of the gonads is recorded from August and again it started increasing through December.

High GSI were found in males than females in all the months (Fig. 7a.). In general, higher values observed during the spawning periods are due to the occurrence of higher percentages of mature gonads. Highest GSI values (3.88 for females and 4.23 for males) were recorded in July. The higher values in females during February, March and June, July showed the availability of large number of mature individuals also in these months. The low values during October, November, December and January might be due to the occurrence of more number of immature and spent gonads.

Fig. 7a. Month wise GSI of *Leiognathus dussumieri*

The patterns of seasonal changes in the mean GSI values of males were relatively consistent during three years of sampling. The seasonal patterns of mean GSI values of females were also similar from 2004 – 2006. In all three
years mean GSI increased from May – July and subsequently decreased in August – October.

**Secutor insidiator**

High gonado-somatic index were found in females than males in all months (Fig. 7b.). In general, higher values observed during the spawning periods are due to the occurrence of higher percentages of mature gonads. Highest GSI values (3.14 for females and 2.58 for males) were recorded in February. The higher values in both males and females during Feb – May and again in August and September showed the availability of large number of mature individuals. The low values during October – January and June may be due to occurrence of more number of immature and spent gonads. Thus mean GSI values appeared to be bimodal in *Secutor insidiator* with one period of peak values during February – May and another in August – September. Comparatively GSI values of males of *Leiognathus dussumieri* seems to be
high compared to the males of *Secutor insidiator*. GSI values of females *Secutor insidiator* seems to be higher consistently throughout the year compared to *Leiognathus dussumieri* which showed higher values only during February – July.

3.3.7. Fecundity

The capacity of fish in terms of egg production is called fecundity. All fecundity estimates were based on fish that had undamaged ovaries and showed no sign of previous spawning in that season. The number of ova in the most advanced modal group (mature group) was calculated by multiplying the total weight of the sub sample. Fecundity estimation was made by the gravimetric method (Hunter and Macewicz, 1985 b). The fecundity was estimated from the counts of oocyte samples of the ovarian tissue.

i. Materials and Methods

The ovaries were removed, weighed and preserved in neutral buffered 10% formalin. From the whole ovary, a sub sample of known weight was used for counting. The ovaries were treated with modified Gilson’s fluid. Thus the ovaries with modified Gilson’s fluid were individually stored in small glass vials. Prior to this, each ovary was split longitudinally to assist penetration of the preservative. The preserved ovaries were stored for 2 – 3 weeks and periodically shaken to ensure the separation of the eggs from ovarian tissue.

After the eggs get separated, the eggs were taken with the help of a pipette and a single streak of eggs were placed on a microscopic slide. Then this slide
was viewed under a microscope and the number of eggs counted in the sub samples. The total number of mature eggs in the whole sample i.e., the fecundity of a fish, could be calculated by using the following formula.

\[ \text{Fecundity} = \left( \frac{\text{No. of ova in sub samples}}{\text{Weight of the sub sample}} \right) \times \text{Total ovary weight}. \]

Then, the relationship between fecundity and total length, fecundity and weight and the ovary weight and fecundity were worked out by the least square method. The regression was fitted using the formula,

\[ \log F = a + b \log x \]

Where, \( F = \) Fecundity, ‘a’ and ‘b’ are two constants, \( x \) – length or weight of the fish or ovary weight. The co-efficient of correlation ‘r’ was calculated.

**ii. Results**

**iii. Fecundity of *Leiognathus dussumieri***

The relationship between total length and fecundity, total weight and fecundity and ovary weight and fecundity of *Leiognathus dussumieri* were estimated by least square method. The fecundity varied from 6197 to 33825 based on 22 ovaries (Only mature and ripe stages) of fishes ranging in total length from 103 to 144mm and weight from 17 to 49 gm. The number of ova increased generally with increase of fish length . However, at the same time fecundity of fish of same length or same weight or same ovary weight showed variations.
a. Relationship between Total length and fecundity is logarithmically expressed by the equation.

\[
\text{Log } F = -5.56714 + 4.66158 \text{ Log } L
\]

Based on this formula, the expected fecundity value was calculated for different lengths and linear relationship is evident as shown in Fig. 8a.

The correlation coefficient \((r = 0.81444)\) between total length and fecundity was found to be significant at \(p < 0.001\) level. Hence, the present study suggests that the fecundity increase with increasing length of the fish.

![Fig. 8a. Relationship between log annual fecundity and log Total length of *Leiognathus dussumieri*](image)

b. Relationship between total weight \((W)\) and fecundity \((F)\)

A linear relationship was found as represented in Fig. 9a. Logarithmically the relationship between the two variables was calculated by the method of least square as expressed by the regression equation.

\[
\text{Log } F = 1.81293 + 1.6006 \text{ log } W
\]
The correlation coefficient \( r = 0.913852 \) between total weight and fecundity was found to be significant at \( P < 0.001 \) level. Hence it can be concluded from the present observation that the fecundity appears to increase with increasing body weight of the fish. At the same time the data also shows that fecundity is variable in individuals of the same weight.

**Fig. 9a. Relationship between log annual fecundity and log total weight of *Leiognathus dussumieri***

**c. Relationship between ovary weight (OW) and fecundity (F)**

This relationship was linear as represented in Fig. 10a. Logarithmically the relationship between the two variables was calculated by the method of least square and can be expressed by the regression equation as given below.

\[
\text{Log } F = 0.36697 + 1.25311 \text{ log OW}
\]

The correlation coefficient \( r = 0.95785 \) between the ovary weight and fecundity was found to be significant at \( p < 0.001 \) level. It indicates a high degree of relationship between these two variables.
iv. Fecundity of *Secutor insidiator*

The fecundity was estimated from 22 specimens of size ranging between 97mm and 109 mm (Total length). The minimum weight of the fish was 12 gm and the maximum weight was 20 gm. Fecundity varied from 5878 to 13868 ova. The number of ova increased generally with increase of fish length. However at the same time fecundity of fish of same length showed variation.

The relationship between Total length and fecundity, Total weight and fecundity and Ovary weight Total weight and fecundity of *Secutor insidiator* were estimated by least square method.

a. Relationship between Total length (L) and Fecundity (F).

The relationship between total length and fecundity is logarithmically expressed by the equation.
Log \( F = a + b \log L \)

Where,

\( F = \text{Fecundity} \)

\( L = \text{Total length and 'a' and 'b' are two constants examined by the method of least square and expressed by the following equation.} \)

\( \log F = -2.9222 + 3.4225 \log L. \)

Based on this formula, the expected fecundity values were calculated for different length and linear relationship is evident. The correlation coefficient \( (r = 0.590513) \) between total length and fecundity was found to be significant at \( p < 0.005 \) level (Fig. 8b.).

Hence the present study suggests that the fecundity increase with increasing length of the fish.

**Fig. 8b. Relationship between log annual fecundity and log Total length of *Secutor insidiator*
b. **Relationship between total Weight (W) and Fecundity (F).**

The relationship between total weight (W) and fecundity (F) showed a linear relationship as represented. Logarithmically the relationship between the two variables was calculated by the regression equation (Fig. 9b.).

\[
\log F = 2.9518 + 0.8302 \log W.
\]

The correlation coefficient \( r = 0.5921 \) between total weight and fecundity was found to be significant at \( p < 0.005 \) level. Hence, it can be concluded from the present observation that the fecundity appears to increase with increasing body weight of the fish.

![Fig. 9b. Relationship between log annual fecundity and log total weight of Secutor insidiator](image)

c. **Relationship between ovary weight (OW) and Fecundity (F).**

The relationship between ovary weight and fecundity showed a linear relationship. Logarithmically the relationship between these two variables was calculated by the method of least square and can be expressed by the regression equation (Fig. 10b.).
Log F = 1.9689 + 0.7306 Log OW.

The correlation coefficient $r = 0.8435$ between ovary weight and fecundity was found to be significant at $p < 0.001$.

![Fig. 10b. Relationship between log annual fecundity and log ovary weight of Secutor insidiator](image)

3.4. Discussion

Macroscopic and Microscopic gonad staging

Macroscopic ovarian staging of multiple spawning fishes can be difficult because subtle difference at the cellular level may not be detectable macroscopically. However, macroscopic analysis does provide a rapid estimate of maturity and result in a general description of spawning season at a reduced cost compared to time consuming histological methods. West (1990) noted that there have been few attempts to assess the accuracy of macroscopic gonad staging with histological analysis. In the present study we have made an attempt to correlate the microscopic staging of gonad development with microscopic histological observations. Balan (1963) has classified the gonads of Leiognathus bindus into seven stages macroscopically. Many of the schemes
devised for the study of ovarian development were designed for individual species. There are arguments for and against developing staging schemes for individual species as the variation in teleost gonads are small. Similarly there are differing views on the number of stages identified macroscopically. Pollard (1972) feels that most species can usually be fitted into the basic framework of the seven stage scale. Qasim (1973) proposed that in tropical and subtropical species the number of stages should be limited to about five. In the present observation we have classified the ovary into six stages macroscopically and histologically excluding the atretic stage. As both the species studied show prolonged spawning season, it is difficult to obtain a specimen with completely spent ovaries. The different stages of maturity varied both within the spawning season and between years in both *L. dussumieri* and *S. insidiator*. From the Fig. 4a (i & ii) and 4b(i & ii), that represent month-wise occurrence of males and females of *L. dussumieri* and *S. insidiator* it is clear that variations occur in the number of individuals that attain different stages of maturity. For example percentage of female *L. dussumieri* showing stage IV ovary was higher in May 2004, where as this stage is represented less during May 2005. Stage I is represented less in April 2004 and 2006 while its representation is fair in April 2005. But at the end of the spawning season from Aug 2004 onwards till Nov 2004 number of reproductively active females decreased when compared to the main spawning peak (May 2004). Satyanarayana Rao (1967) observed that in both sex of *L. splendens* mature or ripe stages occur in all the months excepting July, August and November. According to him, the occurrence of advanced
stages of sexual maturity in most of the months suggests prolonged breeding in *L. splendens*. From the recorded values in the present study it is understandable that *L. dussumieri* also has a prolonged breeding period from January to June, July. Available data presented shows that *S. insidiator* is bimodal in spawning and the two peaks are observed during Feb to May and again in Aug and Sep. According to Jayabalan (1985), who observed the maturation and spawning of *S. insidiator* in the Porto Novo waters, two spawning seasons could be recorded. According to him percentage occurrence of different stages of maturity of gonads of males and females in different months indicates that spawning in *S. insidiator* takes place between Jul and Nov and again during Mar and Apr. He has also studied the presence of juveniles in the Porto Novo waters during these months thus confirming the fact that *S. insidiator* has two spawning seasons in a year in the Porto Nova waters. Our present observation that the peak gonadal activity in *S. insidiator* occurs during the months of Feb and May and again in Aug to Sep in Puducherry waters is almost similar to the observations by Jayabalan (1985).

Gonad indices have traditionally been used as an objective support for field staging and they provide a useful general indication of seasonal trends. They are not however, a good predictor of developmental stage and are not independent of fish size. In general, larger fish have proportionately larger ovaries and the effect increases with increasing gonad development. To overcome this size dependence for statistical comparisons, the best method
appears to be to standardize the size composition of the samples. This may be done either when the animals are collected or afterwards by taking subset of data of a restricted range of body sizes. This approach may not be possible with small sample sizes in which case the magnitude of the bias should be assessed in case it could affect the interpretation of the results. Apart from indicating the state of development of the ovary, gonad indices may reflect other aspects of the reproductive cycle. High gonad index values for ripe fish may indicate that maturation of more than one complement of oocytes is unlikely, while the shape of the annual gonad index curve may also indicate the spawning strategy of the species. The value of gonad indices is that they reflect the status of all oocytes in ovary and provide a measure of development additional to that provided by staging methods or oocyte measurements, which are directed only at the upper limit of the oocyte size range. It is pointed out that ovarian maturation is a complex process and that attempting to describe it by a single parameter (eg. Oocyte size), however, precise could be misleading. GSI calculated for *S. insidiator* and *L. dussumieri* support the assessment by other methods that *S. insidiator* is bimodal and *L. dussumieri* has a prolonged breeding season from Feb to May, June.
Sex Ratio

Sex ratios vary greatly among published studies on pony fish’s life history. This variability may be due to true differences in the composition of local populations or it may be artifacts of sampling strategies rooted in collection season or gear biases. In our study, sex ratios were slightly skewed towards females in both *L. dussumieri* and *S. insidiator* and did not differ significantly from a 1:1 ratio. Collections were mostly made from commercial catches by trawl nets and bag nets. Jayabalan (1986) in his study on *L. splendens* 1976-1978 found that the males and the females were not homogeneously distributed between the years. Balan (1963) had observed that there was no prevalence of either sex of *L. bindus* in boat – seine catches; whereas James and Badrudeen (1975) had recorded more females than males in *L. brevirostris* in the trawl catches. Observation by Jayabalan (1986) shows that *L. splendens* differ from *L. bindus* and *L. brevirostris* in that males were consistently more in number than females in the trawl catches.

Fecundity

The fecundity of 22 females of *L. dussumieri* ranging in total length from 103 to 144 mm and weight between 17 to 49 grams was estimated. The numbers of ova varied from 6197 to 33825. The number of ova increased generally with increase of fish length. Regression analysis between the total length (x) of fish and number of ova (y) and total weight of the fish (x) and the total number of ova (y) for *L. dussumieri* are given in fig. 8a and 9a. Similarly
relationship between total length and number of ova and total weight of the fish and number of ova for *S. insidiator* are given in fig 8b and 9b. For *L. splendens* Arora (1951) had given an average fecundity of 7,566 ova and a maximum of about 11,000 in larger specimens of Thangachimadam region. Jayabalan (1986) reported a maximum of 21,507 ova for *L. splendens* from Porto Novo waters. Hence the individuals of the *L. Splendens* of Porto Novo waters appear to be more fecund than that of Thangachimadam region. Jayabalan (1985) estimated the fecundity of *S. insidiator* from Porto Novo waters. Fecundity was estimated from 24 specimens of the size ranging from 92mm – 109 mm (TL). The minimum weight of the fish was 10.4 gm and the maximum weight was 19.6 gm. Fecundity varied from 5085-12584. In the present observation of fecundity of *S. insidiator*, fecundity was estimated from 22 specimens and the weight range was 12-20 gms. Fecundity varied from 5878-13868 and it showed no much variation from the estimates by Jaybalan (1985) from Porto Novo waters.