CHAPTER I

STUDIES ON THE REPRODUCTIVE CYCLE OF FEMALE AND MALE SEA-CUCUMBER

HOLOTHURIA ATRA.
Introduction:

Giese (1959) in his review article defined the reproductive cycle as "the series of events from the time of activation, growth and maturation in the gonad to spawning of the gametes and recession of gonadal activity to a relatively sustained resting level and including the duration of the rest period". He has also referred the reproductive cycle as the total course of events, regardless of the time period over which these occur annually, monthly, weekly or daily.

Since, the past several years, the reproductive cycles of many holothurian species, are being studied from various marine habitats all over the world, the species studied are, Cucumaria frondosa (Runnstrom*, 1921); C. pseudocurata (Rutherford, 1973); Psolum bullatum (Mc Euen and Chia, 1991); P. chitonoides (Mc Euen and Chia, 1991); P. fabricii (Hamel, et al., 1993); Thyone (Turner and Boolootian*, 1964); Aslia lefevrei (Costelloe, 1988); Parastichopus californicus (Courtney*, 1927); Stichopus japonicus (Tanaka and Choe*, 1962); S. variegatus (Conand, 1993); S. mollis

* Cited in Boolootian (1966)
(Sewell and Bergguist, 1990); Actionopyga echinites (Chen, et al., 1991); Thelenota ananas (Conand, 1981); Leptosynapta tenuis (Green, 1978); L. inharen L. roseola (Costelloe et al., 1957); Synapta hydiformis (Clark, 1898); Bathyploites natory (Lonning, 1976); Cherbonniera utriculus (Tyler et al., 1987); Benthogone rosea and Laetomogone violacea (Tyler et al., 1985a and 1985b); Peniagone azorica (Tyler et al., 1985b); genus Paroriza (Tyler et al., 1992). From the genus holothuria the species studied are, Holothuria tubulosa (Selenka*, 1876 and Bulteel et al., 1992); H. spinifera (Mortenson*, 1938); H. arenicola (Mortenson*, 1938); H. mormorata (Mortenson*, 1938); H. scabra (Krishnaswamy and Krishnan, 1966); H. nobilis and H. fuscosgliva (Conand 1981); H. impatiens (Pearse, 1968a); H. edulis (Pearse, 1968a); H. leucospilota (Jayashree, 1988); H. forskali (Tuwo and Conand, 1992) and H.atra (Pearse, 1968a; Harriott, 1985; Conand and Ridder 1990).

Though, many holothurian species are stable gonochoric, a few are hermaphrodites. Such species are Cucumaria crocea (Ludwig**, 1898); C Lamperti (Ohshima**, 1915); C. Laevigata (Ackerman**, 1902); Pachythyone rubra (Chaffe**, 1982);

* Cited in Boolootian (1966)
** Cited in Smiley et al., (1991)
Pseudopsolus macguariensis (Simpson*, 1982); Synapta vivipara (Clark*, 1898); Leptosynapta minata (Becher*, 1906); L. inharens (Runnstrom*, 1927 and Kegami*, et al., 1976); L. clarki (Everingham*, 1961); Labidoplax buskii (Nyholm*, 1951); Rhabdomolgus ruber (Menker*, 1976); Ophialobatis bidenta (Tyler and Gage 1982); Mesothuria intestinalis (Theel*, 1901);

In the populations of Oneirophanta mutabilis and Deima validum, oogenesis occurred in about half the members but in the other half gametogenesis was absent. These were the males which were reproductively inactive (Tyler and Billett, 1987).

Reproduction by fission is quite common in a number of holothurian species, viz. Cucumaria lactea (Dalyell*, 1851), C. plancii (Dalyell*, 1851, Chadwick*, 1891; Monticelli*, 1896); Stichopus chloronotus (Harriott*, 1980); S. horrens (Harriott*, 1980). Among the members of the genus Holothuria, fission is quite frequent in the following species. Holothuria difficilis (Deichman*, 1922);

* Cited in Smiley et al., (1991)
H. parvula (Crozier*, 1917, Deichman*, 1922; Emson and Mladenov, 1987) H. surinamensis (Crozier*, 1917) and H. edulis (Harriott, 1985); H. atra (Bonham and Held, 1963; Pearse, 1968a; Harriott, 1985) from Heron Reef Island, H. atra (Chao, et al., 1993) from Guam Heron Ananjii Island and H. atra (Conand and Ridder, 1990) from New Caledonia and Papua New Guinea. All the above mentioned species of various genera adopt fission as a means of reproduction besides the conventional sexual method.

During our studies for four years on the reproductive pattern in H. atra of Karwar coast, we have carefully attended to this aspect too.

Though many holothurian species show an annual and well defined breeding pattern, some are continue breeders. Such species are,

Synapta hydiformis (Clark*, 1898); Chirodota rotifera (Clark*, 1898); Ypsilothuria talismani (Tyler and Gage 1983); Benthogone rosea, Deima validum, Laetomogone violacea; Peniagone azorica and P. diaphana (Tyler, et al., 1985a and 1985b); Eumolpadia violacea (Feral and Magniez, 1985);

* Cited in Smiley et al., (1991)
Benthothuria funebris, Mesothuria intestinalis, M. lactea, M. verrilli, Paelopatides grisea, Benthodytes sordida, Psychropotes longicauda (Walker et al., 1987), Cherbonniera utriculus (Tyler, et al., 1987); Holothuria edulis (Harriott 1985) and H. atra (Pearse, 1968a)

While the species like Leptosynapta tenuis (Green, 1978) Holothuria scabra (Krishnaswamy and Krishnan, 1966); H. atra (Harriott, 1985) breed twice a year as H. leucospilota of Goa breeds thrice a year (Jayashree, 1988).

Spawning event in holothurians yet another interesting aspect to study. Information on spawning event pattern is fairly well documented in the following species. The species are,

Cucumaria lubrica (Engstrom*, 1974, 1982); C. miniata (Mc Euen*, 1987); Psolus chitonoides (Young and Chia*, 1982); Leptosynapta clarki (Everingham*, 1961); Opheodesoma spectabilis (Berrill*, 1966); Stichopus Spp (Holland and Holland*, 1969); Orphnurgus Spp (Pawson*, 1976); Thelenota ananas (Shelley*, 1982); Actinopyga echinites

* Cited in Mc Euen (1988)
** Cited in Smiley et al., (1991)
(Shelley*, 1982); A. agassizi (Hammond*, 1982) and among the genus holothuria, Holothuria leucospilota (Toma*, 1980); H. mexicana (Mosher*, 1982); H. scabra, Holothuria marmorata (Shelley*, 1982) and H. atra (Olson*, 1988).

Though not exhaustive, some information on this aspect of H. atra has been presented in this study also.

Fecundity forms one of the integral aspects of studies on the reproductive cycle of animals. However, information on this aspect is rather limited to a few species of holothurians up till now. Species so far studied from this aspect are, Cucumaria pseudocurata (Rutherford, 1973); Microthele nobilis and Thelenota ananas (Conand, 1981); Ypsilothuria talismani (Tyler and Gage, 1983); Microthele fuscogliva (Conand, 1981); Cherbonniera utriculus and Molpadia blakei (Tyler et al., 1987). We have gathered some information on the fecundity of H. atra and the same has been reported in the present work. Fecundity of animals is always correlated with their egg size. Smaller the egg size (< 500 μ in diameter) larger is the fecundity.

* Cited in Mc Euen (1988)
Further, the egg size also provides a clue to the mode of the egg retention or release by the parent (brooding or broadcasting) and also the mode of development (direct or indirect). Useful information on this aspect is available for the species: Cucumaria pseudocurata (Rutherford, 1973); C. frondosa (Lonning 1976); C. lubrica (Mc Euen*, 1987); C. hyndmani (Lonning, 1976); C. miniata* (Mc Euen, 1987); Psolus chitonoides (Mc Euen*, 1987); P. fabricii (Hamel et al., 1993); Aslia lefevrei (Costello, 1988); Eupentacta quinquesemita (Mc Euen, 1987); Ypsilothuria talismani (Tyler and Gage, 1983); Microthele nobilis (Conand, 1981); Parastichopus californicus (Cameron and Fankboner, 1989); Stichopus tremulus (Lonning, 1976); Leptosynapta clarki (Mc Euen*, 1987); L. tenuis (Green, 1987); Mesothuria intestinalis (Lonning, 1976); Cherbonniera utriculus, Molpadia blakei (Tyler, et al., 1987); Lissothuria antillensis (Miller, 1984); Peniagone azorica, P. diaphana (Tyler et al., 1985b); B. sordida, Psychropotes depressa, Oneirophanta mutabilis and Deima validum (Tyler and Billett, 1987); Benthogone rosea and Laetomogone violacea (Tyler et al., 1985a).

* Cited in Mc Euen (1988)
Among the species of Holothuria such information is available for, H. edulis, H. impatiens and H. atra (Harriott, 1985)

Materials and methods:

Adults individuals of H. atra were collected on monthly basis from Binaga beach of Karwar coast. Each time 25 to 30 individuals were collected during the period June 1990 to May 1993. The specimens so collected were used for, (i) morphometrical and (ii) histological studies of the gonadal cycle. For the morphometric study the body weight of each specimen was taken irrespective of its sex. Then an incision was made along the mid dorsal line of the body to expose the gonads and ascertain the sex of the specimen. The position, the colour, the size and the weights of the gonads were recorded. To ascertain the sexes during the post-spawning phase and the regeneration phase, smears were taken on slides and observed under the microscope for the relict oocytes or sperms. Subsequently the body weights and the gonad weights were sorted out sexwise. Finally, the gonad index (G.I) of each specimen was calculated by applying the formula,
Wt. of the gonad \( \times 100 \)

Wt. of the body

The gonad index is expressed as the ratio of the wet gonad weight to the total wet animal weight \( \times 100 \) (Giese*, 1958). The mean value of gonad index was calculated each time separately for the males and females. These were recorded in a tabular form, statistically processed and the graphs were plotted.

From the data collected the average weights of the entire body of five females and the gonads were sorted out in early growth phase (June), first maturation phase (December), second maturation phase (April) and at the post-spawning phase (May), in order to understand the extent to which the weight of the entire body and of the gonads increase during the annual cycle.

For histologic studies tubules of the ovaries and testes of five specimens each were fixed in Bouins fluid for 24 hrs. They were washed in ethyl alcohol (70%) several times until they were almost devoid of the fixative, and treated with alcohol grades 90% and absolute to bring about a complete dehydration. Then the tissues were cleared in

* Cited in Boolootian (1966)
benzene, embedded in paraffin wax (m.p. 58°C to 60°C) for 3 hours with three changes of an hourly duration each and finally blocks were prepared. Sections were cut at 5 μm. thickness. After deparaffinizing the sections were stained with alcoholic haematoxylin and counter stained with eosin. The permanent preparations were made as usual.

The histological features of the gonads in different stages of the gonadal cycle with reference to the gonadal wall, genital-hemal sinus, germinal epithelium and the developing oogonia or spermatogonia, etc. were observed. The germinal epithelium in the testis was carefully examined to detect the presence/absence of Sertoli cell-like cells. Microphotographs of important-features of different stages of the gonads have been taken on Binocular Research Microscope and are presented in the thesis in support of our findings. Histological sections of ovarian tubules in different months of one calendar year 1991-1992, were also used to study the oocyte frequency during the different phases of oogenesis. The results are presented in the form of a graph.

In our present work fecundity in H. atra has also been studied, with the help of the data on the wt(gm) of the
entire animal, wt(mg) of a piece of the gonad tubule at maturity phase and the number of oocytes (n) there in, and by applying the formula.

\[ P = \frac{n \times G}{P} \]

where \( G \) = Wt. of the body, \( P \) = Wt. of piece of a gonad and \( n \) = Number of oocytes

The results are presented in the form of a graph.

Observations:

*Holothuria atra* is an important member of the invertebrate fauna found on Binaga beach of Karwar coast. Eventhough the cucumber is dioecious, there is no sexual dimorphism. Sex can be determined only by examining the grown up gonads.

Morphological features of the gonads:

A single gonad located in the anterior part of the coelom. The mature gonad is held in position by the anterior mid-dorsal mesentry. The gonad consists of numerous tubules sprouting from a tubular chamber - the gonad basis-which opens anteriorly to the outside by a gonopore situated in the mid-dorsal line between the
tentacles. The ovaries appeared dark pink and the testes ivory or cream in colour at maturity (Figs. 8a and 8b). After spawning the gonads shrunk in size to minute hair like tufts, and at this stage the ovaries and testes were identified by examining the smears under the microscope.

Gonadal Tubule Types:


The ovary of _H. atra_ of early June samples was in resting phase after total spawning and was showing a large tubular gonad basis. The posterior-most portion of the gonad basis showed numerous elongated minute translucent tubes which were almost devoid of oocytes. Rest of the gonad basis was completely devoid of tubules of any kind. On the contrary, the ovary of September (late) collection showed three types of tubules arising from the ventro-lateral regions of the gonad basis. The first type (a) (Fig. 6) were a few and extremely small confined to the anterior most region, the second type (b) (Fig. 7) were immediately behind the type a, they were also a few in number but compared to the former, they were very large in
size and branching dichotomously. Both the types 'a' and 'b' were found in the anterior third of the gonad basis. In the rest of it were numerous and still larger tubules packed with oocytes. These formed the third type of tubules the 'c' type (Fig. 8). Yet another picture emerged from the females of late November samples. Tubules of type 'a' so clearly seen in the ovary of the previous sample were conspicuously missing. Tubules of type 'b' had increased in number and size. Ovarian tubules of type 'c' had further grown and finally a few from this lot had become slender, shrunken in girth and were empty, suggesting that they were the spent tubules due to the first spawning event (Fig. 8).

Finally, the gonads (testes as well as ovaries) of late March samples showed a complete gravid condition with the tubules largest in size and packed with gametes. However in the anterior portion one finds the tubules type 'a' and 'b' also (Figs. 8a and 8b). This clearly shows an asynchronous growth of the tubules of the gonads of both the sexes.

Histology of the gonads:

In H. atra the ovarian tubules and testis tubules are composed of an outer peritoneum layer, then an epithelial
layer with squamous to columnar cells depending on the reproductive state of the tubule. Then an adjacent circular and a subjacent longitudinal muscle cell layer. Underlying this is the connective tissue compartment composed of connective tissue ground substance, connective tissue fibres, fibroblasts and genital hemal sinus. The genital hemal sinus is lodged on the basement membrane lined by the germinal epithelium. The germinal epithelium composed of primordial germ cells (Fig. 9).

OVARY:

Following are the histological data of the ovary during the gonadal cycle.

Regeneration Phase:

During this phase (June-Sept) oogonial cells proliferate from the germinal epithelium. The oocytes are characterized by their small size (less than 10μm). Their cytoplasm is finely granular. The nucleus is large and conspicuous. The nuclear membrane was eosinophilic. Interestingly enough, many oogonial cells are found to grow within the genital hemal sinus (Fig. 9).
Active-growth-phase:

During this phase (Sept-Nov) oocytes have grown in size, and are surrounded by numerous small accessory or follicle cells (Fig. 10). The oocytes measure on an average 60µm. The cytoplasm becomes more and more coarsely granular, indicating the commencement of vitellogenesis. The nucleus is round, enlarged and located in the central position. A large spherical nucleolus with a vacuole is clearly seen within it. The nucleolus has started to migrate to the periphery (Fig. 11).

Maturation phase:

During this phase (Nov-Dec) the oocytes attain the maximum size and measure 150µm in diameter. The cytoplasm becomes more coarsely granular. The large nucleus grown along with the oocyte. The cytoplasm and nucleus are more eosinophilic. Nucleolus which is less eosinophilic has moved to the periphery (Fig. 12). A prominent conical protuberance has appeared on one side of the oocyte, suggesting the animal pole of the future embryo (Fig. 13).
Spawning phase:

Spawning in *H. atra* is asynchronous. While majority of the tubules were still gravid, some had already spawned out to various degrees.

Partial spawning phase:

This event begins from December and is completed by February (Fig 14).

Second Maturation phase:

From January to April, the ovary again shows some increase in weight indicating the second maturation phase. The ovarian wall is fully distended and the genital hemal sinus is highly compressed in between the visceral side of the ovarian wall and masses of fully grown up oocytes. (Fig. 15)

Total spawning phase:

The animals start spawning for the second time from April and is completed in May (Fig. 30).

Post-spawning phase:

During the early part of post-spawning phase the
ovarian tubules are still in a distended condition and a few unspawned oocytes of various sizes are left in the lumen with a lot of cytoplasmic debris (Fig. 16).

Atretic and phagocytosed oocytes

During maturation stage some oocytes in various tubules are found highly vacuolated (Fig. 17). Probably the oocytes are undergoing atresia. In the tubules of post-spawning phase cytoplasmic debris as well as the unspawned oocytes are found to be invaded by numerous phagocytes (Figs. 18 and 19). Probably such oocytes undergo disintegration and their nutrient materials are engulfed by the phagocytes only to furnish the same to the oocytes of next generation.

Testes:

Regeneration phase:

In this phase (June-Sept) the spermatogonial cells are seen proliferating from the germinal layer. The germinal epithelium forms deep infoldings projecting into the lumen (Fig 20). The spermatogonial cells are large with conspicuous nuclei. Clusters of spermatogonial cells are also found within the genital hemal sinus. (Fig. 21).
Active growth phase:

During this phase (Sept-Nov) the lumen is filled with spermatogonia and spermatocytes. (Figs. 22 and 23). Primary spermatocytes are arranged in the form of colonettes. The primary spermatocytes situated at the distal portion of the colonettes are being transformed into secondary spermatocytes and are being filled in the lumen.

Maturation phase:

During this phase (Nov-Dec) the accelerated rate of gametogenic activity is observed. The secondary spermatocytes and spermatids are cutting off at increasing rate from the distal portions of the colonettes. The spermatozoa are also observed in sections of some tubules (Fig. 24).

Spawning phase:

Spawning is asynchronous. While majority of the tubules were still gravid, some had already spawned out to various extent.
Partial spawning phase:

This event begins from December and is completed by February (Fig. 25). Owing to partial spawning, the lumen of some spawning tubules are never totally empty. Other tubules are not yet ready for spawning.

Second Maturation phase:

From February to April almost all the testis tubules show their gravid nature indicating the second maturation phase. The lumen is completely filled with spermatids and spermatozoa. (Fig. 26)

Total spawning phase:

The animals starts spawning for the second time from April and is completed in May. The spawning now is total. The tubules are almost empty (Fig. 29).

Post-spawning phase:

During this stage a few upspawned relict sperms are left in the lumen. Some tubules in such tubules the unspawned sperms are found to be invaded by numerous phagocytes (Figs. 27 and 28). Probably the sperms are
engulfed by the phagocytes in which they undergo disintegration.

Morphometric Analysis:

Ovary

The morphometric analysis of the ovary of *H. atra* during the gonadal cycle yielded the following data. During the year 1990-91 the gonad index (G.I.) showed a gradual increase from June to September (0.822 ± 0.04 to 1.670 ± 0.22) and steep increase from September to December (1.670 ± 0.22 to 6.250 ± 0.50). It fell slightly in December and January (6.250 ± 0.50 to 5.680 ± 0.15) indicating a partial spawning. Again it began to rise to reach the peak in April (6.620 ± 0.13). It reached the lowest value in May (0.370 ± 0.60) owing to the total spawning. Variations in the gonad indices during the years 1991-92 and 1992-93 were consistent with these (Tables 1-3; and Graph 1 and 2)

Testes

The morphometric analysis of testes of *H. atra* during the reproductive cycle (1990-91) showed the following features. There was a gradual rise in the values of gonad index (G.I.) from the regeneration phase to maturation phase
(June 3.565 ± 0.60 to 6.985 ± 0.30 December). Then fell slightly in December and January (6.985 ± 0.30 to 6.235 ± 0.40) indicating a partial spawning. Again it began to rise to reach the peak in April (6.650 ± 0.40). It reached the lowest value in May (2.680 ± 0.48) owing to the total spawning. Variations in the gonad indices during the years 1991-92 and 1992-93 were consistent with these (Tables 1 -3 and Graphs 1 and 2).

During the annual reproductive cycle, the body of H. atra, the body weight in females was nearly doubled from the early gonadal growth phase to the second maturation phase, whereas the increase in the gonadal weight was about 14 fold as observed during our three years study on the morphometric analysis (Tables 1-3).

Sertoli cell-like cells:

In course of our study we have observed at frequent intervals along the germinal epithelium some non-germinal cells. These cells are triangular in shape with prominent nucleus. They are totally different from the germinal cells in every aspect. They are comparable to the Sertoli cells.
of vertebrate testes. Hence we have designated them as Sertoli cell-like cells (Fig. 20).

Oocyte size frequency:

During regeneration phase sections of the ovarian tubules showed 100% of oocytes < 30 μm in diameter. During the growth phase oocytes measuring 50 - 70 μm in diameter were 66.23%. During the late growth and maturation phases the oocytes reached their maximum size (140 - 150 μm in diameter) and they were 51.05% in number. Besides these, oocytes of different size groups were also present but in smaller proportions. The distribution of mean oocyte size during the reproductive cycle of _H. atra_ suggests the reproduction is a highly seasonal act in this cucumber (Graph 3).

Body size and Fecundity:

The number of eggs per female is mainly the function of body size, the larger the female, the greater the number of eggs produced.

The number of eggs versus total body weight shows linear regression. A regression of egg number on body
weight (total weight minus gonad weight) when calculated gave a high correlation (intercept = 216030, slope = 1818.8, and $r = 0.7901$) (Table 5 Graph 4).

Discussion:

Holothurians are well known not only for their variety in sea patterns, but also for the modes of propagations. While majority of holothrians are dioecious, there are examples of hermaphrodites and also examples of populations where only females are concerned with reproduction and those populations lack the functional male members.

Similarly, as regards the modes of propagation, the sexual mode of propagation is the commonest mode adopted by the majority, some have adopted propagation by fission as an additional device. Of course this depends largely upon the local conditions. A review of the literature reveals the following details.

The majority of holothurians are dioecious. They are, Cucumaria planci and C.kriclebergii (Selenka*, 1876); C.frondosa (Runnstroms*, 1921); C.pseudocurata (Rutherford, 1973); Thyone (Turner and Boolootian*, 1964); Aslia lefeveri (Costello, 1988); Psolus chitonoides (Mc Euen and Chia, * Cited in Boolootian (1966)
1991); P. fabricii (Hamel et al., 1993), *Parastichopus californicus* (Courtney*, 1927 and Cameron and Fankboner, 1989); *Stichopus japonicus* (Tanaka and Choe*, 1962); S. mollis (Sewell and Bergquist, 1990); S. variegatus (Conand, 1993); S. californicus (Smiley 1988); *Synapta hydiformis* (Clark*, 1898); *Leptosynapta inharens, Leptosynapta roseola* (Costello*, et al., 1957); L. tenuis (Green, 1978); Bathyploites natans (Lonning, 1976); Chirodota rotifera (Hyman, 1955); Actinopyga echinites (Chen, et al., 1991); genus Paroriza (Tyler, et al., 1992); Thelenota ananas (Conand, 1981); Peniagone azorica, P. diaphana, Benthogone rosea, and Laetomogone violacea (Tyler, et al., 1985a and b); Cherbonniera utriculus and Molpadia blakei (Tyler, et al., 1987).

The dioecious species amongst the genus Holothuria are Holothuria tubulosa (Selenka*, 1876, Bulteel, et al., 1992); H. spinifera, H. arenicola, and H. mormorata (Mortenson*, 1938); H. scabra (Krishnaswamy and Krishnan, 1966); H. nobilis, and H. fuscogilva (Conand, 1981); H. impatiens and H. edulis (Harriott, 1985); H. leucospilota (Jayashree,

* Cited in Boolootian (1966)
Finally, the members of the population of H. atra of Karwar coast are also strictly dioecious as we have observed during our studies.

There are a few hermaphrodites among the dendrochirotans, such as Cucumaria crocea (Ludwing**, 1890); C. laevigata (Ackermann*, 1902); C. lamperti (Oshima**, 1975); Pachythone rubra (Chaffee**, 1982); Pseudopsolus macquariensis (Simpson**, 1982); among the aspidochirodans, Mesothuria intestinalis (Theel**, 1901); and among the apodids, Labidoplax buskii (Nyholm**, 1951); Leptosynapta clarki (Everingham**, 1961); L. inhaerens (Ikegumi**, et al., 1976); L. miniata (Becher**, 1906); Rhabdomolgus ruber (Menker**, 1970); Synapta vivipara (Clark**, 1898); Opheodesoma glabra (Diechman**, 1930 and Green, 1978).

Among the hermaphroditic synaptids each tubule produces both the eggs and sperms, generally at the same time (Clark***, 1907). In Leptosynapta inhaerens the animal is

* Cited in Boolootian (1966)
** Cited in Smiley et al., (1991)
*** Cited in Hyman (1955)
male when young and becomes female only when it is of a few years of age (Runnstrom*, 1927). In Cucumaria laevigata, the developing tubules at the gonadal base are sexually indifferent at first, but functions as ovarian tubules and release eggs as they lengthen, with still further elongation the female elements are destroyed by phagocytic coelomocytes and the same tubules then produce sperms (Ackerman*, 1902), in Mesothuria intestinalis the gonadal tubules branch distally forming tufts and some of these tufts develop as testes and the others as ovaries (Thell*, 1901).

In the course of our study over four years we have not come across any cases of hermaphroditism in the population of H. atra of Karwar coast.

Tropical species have been found to exhibit a range of seasonalities including continuous, annual, biannual reproduction (Stephenson**, 1934, and Giese and Pearse, 1974).

Echinoderm species having 1-3 months of breeding period are considered to be the short breeders, those having 4-8

*Cited in Hyman (1955)
**Cited in Harriott (1985)
months considered as the long/extended breeders and rest are considered as continuous breeders (Boolootion, 1966; Bellary, 1989).

Accordingly the holothurian species having a short breeding periods are,

Cucumaria doliolum (Selenka*, 1876) March-April,
C. syracusana (Lo.Bianco*, 1899) June-July,
C. grubii (Lo.Bianco*, 1899) June-July,
C. frondosa (Clark*, 1902) Aug,
C. saxicola (Orton*, 1914) March-May,
C. frondosa (Runnstrom and Runnstrom*, 1919) Feb-March,
C. echinata (Ohshima*, 1921) June-Aug,
C. frondosa (Jordan*, 1979) July,
C. fallax, (Mc Euen*, 1987) March-May,
C. miniata (Mc Euen*, 1987) March-May,
Thyone intermis (Lo.Bianco*, 1899) May-June,
T. briareus (Colwin and Colwin*, 1956) June,
Psolus chitonodies (Jones*, 1960) Mar-April,
Pentamera chiloensis (Deichmann*, 1941) Jan,

* Cited in Smiley et al., (1991)
<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Appearance</th>
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<tbody>
<tr>
<td>Phyllophorus conchilegum (Deichmann*, 1930)</td>
<td>May</td>
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<tr>
<td>Aslia lefeveri (Costello, 1985)</td>
<td>Feb-April</td>
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<tr>
<td>Stichopus regalis (Lo.Bianco*, 1899)</td>
<td>April-May</td>
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<td>S. variegatus (Mortenson*, 1937)</td>
<td>June</td>
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<td>S. tremulus (Holland*, 1981)</td>
<td>July</td>
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<tr>
<td>S. californicus (Smiley et al., 1991)</td>
<td>May-June</td>
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<tr>
<td>Actinopyga mauritiana (Mortenson*, 1937)</td>
<td>May</td>
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<tr>
<td>A. serratidens (Mortenson*, 1937)</td>
<td>May</td>
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<td>A. echinates (Conand, 1982)</td>
<td>Jan-Feb</td>
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<td>Microthele fuscogliva (Conand, 1981)</td>
<td>Jan-Feb</td>
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<td>M. nobilis (Conand, 1981)</td>
<td>May-June</td>
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<tr>
<td>Thelenota ananas (Conand, 1981)</td>
<td>Feb-April</td>
</tr>
<tr>
<td>Bohadschia marmorata (Hendlerand Mever*, 1982)</td>
<td>July-Aug</td>
</tr>
<tr>
<td>Leptosynapta girardii (Mead*, 1898)</td>
<td>April</td>
</tr>
<tr>
<td>L. inhaerens (Runnstrom*, 1927)</td>
<td>Aug-Sept</td>
</tr>
<tr>
<td>Labidoplax digitata (Bury*, 1985)</td>
<td>Mar-May</td>
</tr>
<tr>
<td>Labidoplax buskii (Nyholm*, 1951)</td>
<td>Oct-Dec</td>
</tr>
<tr>
<td>Caudina arenta (Gerould*, 1896)</td>
<td>Feb-April</td>
</tr>
<tr>
<td>C. chilensis (Inaba*, 1930)</td>
<td>May-June</td>
</tr>
<tr>
<td>Molpadida roretzii (Hatanaka*, 1939)</td>
<td>Nov-Dec</td>
</tr>
<tr>
<td>M. intermedia (Mc Euen and Chia*, 1985)</td>
<td>Nov-Jan</td>
</tr>
<tr>
<td>Synapta reciprocans (Mortenson* 1939)</td>
<td>May</td>
</tr>
</tbody>
</table>

* Cited in Smiley et al., (1991)
Opheodesoma grisea (Mortenson*, 1939) 
Rhabdomolgus ruber (Menker*, 1870) 
Eupyrgus scaber (Heding*, 1935)

The following species are the extended breeders.

Cucumaria piperata (Mc Euen*, 1987) 
Psolus phantapus (Runnstrom*and Runnstrom*, 1919) 
Pentamera populifera (Mc Euen*, 1987) 
Eupentacta quinquesmita (Byrne*, 1983) 
Stichopus japonicus (Choe*, 1963) 
Leptosynapta galliennei (Chebronnier*, 1953) 
L. tenuis (Green, 1978) 
Opheodesoma spectabilis (Berrill*, 1966) 
Epitomapta roseola (Clark*, 1809)

The continuous breeders are,

Mesothuria intesinalis; M. verrilli; M. lactea B. funebris; 
and Paelopatides grisea (Walker*, et al., 1987); Synapta 
hydriformis (Clark*, 1898); Eumolpadia violacea (Feral and 
Magniez, 1985); Cherbonniera utriculus (Tyler et al., 1987); 
Ypsilothuria talismani (Tyler and Gage, 1983); Benthogone

* Cited in Smiley et al., (1991)
rosea; and Laetomogone violacea (Tyler et al., 1985a); Peniagone azorica (Tyler et al., 1985b); Benthodytes sordida (Walker et al., 1987a); Deima validum ((Tyler and Billett 1987); Psychropotes longicauda (Walker*, et al., 1987), and Holothuria edulis (Harriott, 1985).

Among the genus Holothuria following are the short breeders.

Holothuria kefersteini (Mortenson*, 1921) Oct-Nov,
H. poli (Horsudus*, 1925) July-Aug,
H. arenicola (Mortenson*, 1937) May,
H. spinifera (Mortenson*, 1937) June,
H. difficilis (Mortenson*, 1937) Aug,
H. paradalis (Mortenson*, 1938) Sept,
H. glaberrima (Lewis*, 1960) June-Aug,
H. moebi (Noumara and Kantanni*, 1962) July-Aug,
H. scabra (Krishnaswamy and Krishnan, 1966),
H. tubulosa (Pladellorens and Sabirana*, 1975) Aug,
H. leucospilota (Maruyama*, 1980) July-Aug,
H. floridana (Engstrom, 1980) Sept-Oct,
H. mexicana (Mosher*, 1982) July-Sept,
H. impatiens (Harriott, 1985) Dec-Feb,

* Cited in Smiley et al., (1991)


The populations at Heron Reef (Harriott, 1985), at New Caledonia (Conand and Ridder, 1990) and also of Karwar coast as we have observed are the short breeders, while the population at Eniwetok Atoll is a continuous breeder (Pearse, 1968).

Most of the holothruian species including the population of H. atra at New Caledonia (Conand and Ridder, 1990), which are already mentioned are annual breeders, breeding once in a year. However, Holothuria scabra (Krishnaswamy and Krishnan, 1966), Leptosynapta tenuis (Green, 1978) and H. atra from Heron Reef (Harriott, 1985) breed twice in a year. While Holothuria leucospilota (Jayashree, 1985) breeds thrice a year. Finally H. atra of Aniwetok Atoll, breeds throughout the year. (Pearse, 1968)

There are many theories explaining the significance of asexual reproduction in organisms, in terms of its genetic advantage (Williams* 1966; Maynard and Smith* 1971), and those which account for its effect on individual energetics.

* Cited in Smiley et al., (1991)
prey capture or predator avoidance (Sebens* 1979; Emson and Wilkie*, 1980). And fission, a method of population maintenance and increase. Asexual reproduction is maintained high densities of small individuals (Ebert, 1978). The asexual reproduction by fission probably is triggered by emersion, during low tides through desiccation and thermal stress or solar radiation at unusually low water level (Chao et al., 1993).

In holothurians three different types of fission are observed. In the first the animal attaches the anterior and posterior ends to the substrate by its tube feet. The ends then slowly walk away from each other in opposite directions stretching and thinning in the middle of the body until the body wall eventually ruptures leaving two separate parts connected only by the remnant gut, which soon breaks (Chadwick*, 1891; Monticelli*, 1896). The second type involves dividing the body into three parts, the animal twists and writles constricting its body at both ends, while the middle portion inflates. One end twists back and forth vigorously until a only thin narrow strand of bodywall remains at the constriction. After a brief rest, the

* Cited in Smiley et al., (1991)
process is repeated at the other end of the animal (Monticelli*, 1896). In the third type the animal narrows sharply about its middle. This constriction deepens until the two halves are linked by only a thin strand of body wall. The animal completes the division by twisting and contracting the bodywall around the region of constriction. This type is the least common (Monticelli*, 1896).

In Cucumaria planci the circular muscles in the bodywall contract strongly while the tube feet are attached to the substratum and the anterior and posterior ends pull in opposite directions (Crozier*, 1917). In the same species fission was observed shortly after spawning. (Chadwick*, 1891).

In genus Holothuria also the asexual reproduction by fission has been noticed. In Holothuria surinannensis the circular muscles in the body wall contract strongly, while the tube-feet are attached to the substratum and the anterior and posterior ends pull in opposite directions (Crozier*, 1917). Studies on the freshly collected the above said species showed only 11% were regenerating. Another species v.i.z. Holothuria edulis from the same

* Cited in Smiley et al., (1991)
locality also showed evidence of a low rate of asexual reproduction (Harriott*, 1982). 50% of Holothuria difficile and 65% of Holothuria parvula were regenerating following fission indicated more tendency for fission (Deichmann*, 1922, Emson and Mladenov, 1987). In both the species, the division occurred before the animals were fully grown. Holothuria parvula showed the evidence of multiple fission and it also appeared that the fission was likely to be an annual event (Emson and Mladenov, 1987).

Populations H. atra throughout the tropical region except the one in Nanwan region (21°57'N and 120°45'E) invariably show evidences of fission. Evidences of fission throughout the year are abundant in the population at Rongelap Atoll, of Marshall Islands (Bonham and Held, 1963). In Holothuria atra from Heron Reef showed 6 to 7% of individuals examined were regenerating following fission (Harriott*, 1980, 1982), and in Holothuria atra from Guam, Heron Island, and Ananiji Island, the yearly rates of fission were, 0.35, 1.04 and 1.78% respectively (Ebert, 1978). Fission has been observed in the field in all sizes of individuals at a mean rate of 1% of the population

* Cited in Smiley et al.. (1991)
sampled in New Caledonia (Conand and Ridder, 1990). Then _H. atra_ at Wanlitung, small individuals with evidences of fission and regeneration were abundant in shallow tide pools on the wide reef flats and fission occurred here throughout the year with an average monthly fission frequency of 4.5% with a peak at 18% in September. In this species fission was accomplished by revolving, twisting and stretching of the body resulting in two unequal fragments. When the small individuals were transferred from Wanlitung to Nanwan and large individuals from Nanwan to Wanlitung, no evidence of fission could be observed. Fission occurs in small individuals living in shallow tide pools, suggesting that fission probably is triggered by stressful environment resulting from solar radiation at the unusually low water level of spring tide (Chao, et al., 1993).

During our studies on _H. atra_ of Karwar coast for four years, we never obtained specimens undergoing fission nor the products of fission. As far as we are aware of the literature perhaps this is the only population of _H. atra_ besides the one in Nanwan that does not show fission.
In the absence of fission as a mode of reproduction individuals of *H. atra* of Karwar have to rely on sexual type of reproduction alone. Naturally they are expected to allocate more energy for their gonadal growth, even at the cost of somatic growth to some extent. In the recent years two studies have been carried out to estimate the amount of energy allocated by the adult individuals for the somatic growth and the gonadal growth. In *S. japonicus* there is a 12 to 15 fold volume increase in the gonads during their annual cycle (Tanaka* 1958), and in *Cucumaria pseudocurata* the increase in the gonadal weight is 9 fold (Rutherford 1973). In both these cases the authors interpret that much of the energy is allocated to the gonadal growth and this reproductive effort may account of decreased growth rate as reproductive age is reached. Our data reveals that in *H. atra* of Karwar also the increase in the gonadal weight during the gravid phase is around 14 fold (Table 4), and that the individuals allocate a considerable amount of energy for their gonadal growth.

The amount of energy allocated for the reproductive aspect is manifested by the animals in the form of fecundity.

* Cited in Rutherford (1973)
Fecundity of animals in turn is correlated with their egg size. Smaller the egg size, (< 500 µ in diameter) larger is the fecundity. Our knowledge on the fecundity of holothuroids is confined to the following species only.

*Microthele nobilis* which produces smaller oocytes in large numbers since the ovary weight is high, whereas *Thelenota ananas* and *Microthele fuscogilva* have more voluminous ovaries and oocytes (Stearns*, 1976 and Harriott*, 1980). A coefficient of fecundity equals to 4,750 for *Thelenota ananas*, 22,800 for *Microthele nobilis* and 7,350 for M. *fuscogilva*. The inverse relationship between ovum size and number is confirmed for these species. The fecundity related to body weight in the above species revealed the highest values *M. nobilis* 3.5 times greater than for M. *fuscogilva* and 6.0 times greater than for *Thelenota ananas* (Harriott, 1985). *Cucumaria pseudocurata* is able to brood all the eggs produced. The number of eggs produced and brooded ranges from 1 to 340 depending on the size of the individual. (intercept = -32.5, slope = 3.37, r = 0.863) (Rutherford, 1973). In *Cherbonniera utriculus* examination of the whole mounts of ovaries, suggests that

* Cited in Harriott (1985)
the fecundity of oocytes (> 20 μm) is less than 100 oocytes per individual (Tyler et al., 1987).

In Holothuria edulis and H. impatiens the fecundity index is 2,800 and 800 respectively (Harriott, 1985). In H. scabra the fecundity is estimated at ten lakhs (James et al., 1994). In Holothuria atra the fecundity index is 6,600 (Harriott, 1985).

During our study on H. atra there was a highly +ve correlation between the body weight and the number of oocytes (intercept = -2160, slope = 1818.8, r = 0.7901).

We have also some information on the actual fecundity of some asteroids. The fecundity of Japanese seastars is as follows, Distolasterias nipon, 34 million eggs, Asterias rubens, 19 million eggs, Pateria pectinifera, 15 million eggs, whereas in Henricia layashi 100-1000 eggs only (Kasynov, 1988). In Pteraster militaris also the relative fecundity is shown to increase with increase in body size (Mc Clary and Mladenov, 1988).

As already mentioned fecundity is closely associated with the egg size and numbers. The egg size also provides a
clue to their retention (brooding) or release (broadcasting) by the parent and also the mode of development (direct or indirect).

In *Pisaster militaris* a sub-arctic-arctic species having a wide circumpolar distribution as far south as Japan sea is the only echinoderm species that is known for both brooding and broadcasting. Small sized adults are the brooders that change to broadcasting as they increase in size. The same pattern ontogenic shifting has been noticed in *Henricia leviascula*, whereas in *Echinaster spp.* reverse is the phenomenon (Mc Clary and Mladenov, 1988). In one species of synaptid (external brooding) and two species of dendrochirote (lecithotrophy) and seven species of dendrochirotides and one molpadid species (planktotrophy) extremes in fecundity and egg size range from 149 eggs (1015 \( \mu m \) of diameter) of brooding species to nine million eggs of (206 \( \mu m \) diameter) produced by the planktotrophic species (Mc Euen*, 1985).

* Cited in Mc Euen (1988)
The lecithotropic eggs (> 500 μm in diameter) with brooding and direct form of development are produced by,

Cucumaria pseudocurata (Rutherford, 1973), 1 μm,
C. lubrica (Mc Euen *, 1987) 973 μm,
C. miniata (Mc Euen *, 1987) 520 μm,
C. frondosa (Lonning, 1976) 650 μm,
Aasia lefevrei (Costello, 1988) 650 μm,
Benthogone rosea (Tyler, et al., 1985) 750 μm,
B. sordida (Tyler and Billett, 1987) 1,200 μm,
Phychropotes depressa (Tyler and Billett, 1987) 1,800μm,
Oneirophanta mutabilis (Tyler and Billett, 1987) 950μm,
Deima validum (Tyler and Billett, 1987) 700μm,

On the other hand, the below mentioned species produce smaller eggs, which are broadcast. As to be expected the development is indirect incorporating a larval phase. Following is the list of such species along with their egg size.

Microthele nobilis (Conand, 1981), 140-160 μm
Eupentacta quinquesmita (Mc Euen *, 1987), 397 μm
Ypsilothuria talismani (Tyler and Gage, 1983), 350 μm

* Cited in Mc Euen (1988)
Leptosynapta clarki (Mc Euen*, 1987), 345 µm
Leptosynapta tenuis (Green, 1987), 200 µm
Cherbonniera utriculus (Tyler, Billett and Gage, 1985)

Molpadia blakei (Tyler, Billett, and Gage, 1985), 200 µm
Lissothuria antillensis (Miller*, 1984), 400 µm
Laetomogone violacea (Tyler, et al., 1985), 350-400 µm
Peniagone azorica (Tyler and Gage, 1985), 300 µm
P. diaphana (Tyler and Gage, 1985), 300 µm
Bathyplotes natans (Lonning, 1976), 400 µm
Cucumaria hyndmani (Lonning, 1976), 300 µm
Stichopus tremulus (Lonning, 1976), 150 µm

In the genus Holothuria, following is the information on the egg-size in a few species,

Holothuria scabra (James et al., 1994), 180-200 µm
H. edulis (Harriott, 1985), 103 µm
H. impatiens (Harriott, 1985), 184 µm

In synaptid species

H. atra (Harriott, 1985), 88 µm
H. atra of Karwar coast (our finding) 150 µm

* Cited in Mc Euen (1988)
The culmination of gametogenesis is spawning or the release of gametes. At the time of spawning, the gravid brooders display some patterns of behaviours peculiar to different species, although in majority of the species some behavioural patterns are common. Following are some of the details on this subject. A typical spawning behaviour of many species of holothurian, particularly among the aspidochirotes is the lifting or stretching of the anterior end of the body into the water away from the substratum before releasing gametes (Mc Euen, 1988). A back and forth swaying or swinging motion described as cobra like is observed in aspidochirotes (Mortenson*, 1937). Among dendrochirotes a tentacle waving is another type of spawning behaviour. In *Cucumaria lubrica* and *C. miniata* male spawning proceeds the female. In this case the females are called the 'opportunist' spawners. The male arches oral end away from substratum with tentacles crumpled, while the female detaches two ventral rows of tube feet and arches the anterior end to deposit the eggs between the ventral surface and substratum (Mc Euen* 1987), *Psolus chitonoides* releases the eggs in cylindrical mucoid strands released.

* Cited in Mc Euen (1988)
The females of this species also are the "opportunistic" spawnners. They extend the tentacles widely apart before spawning (Young and Chia* 1982). In Actinopyga agassizi a stationary, raised posture is observed (Hammond*, 1982).

Thelenota ananas and Bohadschia marmorata the anterior end of the body is raised vertically and slowly waved around. (Shelly*, 1982) In Leptosynapta inharens males spawns first followed by females which erect themselves part way out of burrows and swing about while emitting the sex cells (Costello*, et al., 1957). The Caudina chilensis spawns during high tide, with the body and tentacles of both sexes kept entirely still (Inaba*, 1930).

Spawning activity in the following species of Holothuria has also been observed in the past by different workers. In H. mexicana, one third to two thirds of the oral end is elevated perpendicularly with pursed mouth, undulating bows. Males precedes the females (Mosher*, 1982). In H. scabra anterior end raised to vertical position and slowly waves around (Shelley*, 1982). In H. thomasi a stationary raised posture is exhibited (Hammond*, 1982). In H. tubulosa males spawns first, emit sperms at

* Cited in Mc Euen (1988)
intervals of 2-20 minutes. (Thorson *, 1946). *H.atra* also lifts its anterior end up and spews 30-38 cm long stream of red eggs (Lawrence and Olson * 1988). As we have observed in *H. atra* too the males spawn first and this is followed by the opportunistic females. The males first lift the anterior end and exhibit swaying movements, after sometime they start slowly releasing the sperms, for one or two hours; in contrast the eggs are released in powerful jets. Similar observations have also been made by James and James (1994).

The following are some details of spawning periods in echinoderms in different parts of the world.

In the species of Asteroidea *Patiria miniata* of Pacific Grove U.S.A. (36°38'N 121° 56'W) (Farmanfarmaian, et al., 1958) spawns from Jan to Dec and the same species in Monterey Bay U.S.A. (36° 37' N, 121° 55'W) spawns only during April-May months (Newman**, 1921).

In Echinoidea *Strongylocentrotus franciscanus* of Vancouver Islands B.C., Canada (49° 16' N 123° 07'W) (Mortensen*, 1921), Puget Sound, U.S.A. (47° 50' N and 122°

*Cited in Mc Euen (1988)*

**Cited in Boolootian (1966)
30°W) (Johnson, 1930) and Pacific Grove USA (36° 38' N and 121° 56'W) spawn (Newman, 1923) during May-Jun, Mar-Apr, and Feb-July respectively.

Then among the species of Holothuroidea

*Stichopus tremulus* of North-sea (55° 20' N, 0.03° 0'E) spawns during March-April (Runnstrom and Runnstrom, 1921).

*S. californicus* of Puget Sound U.S.A. (47° 50' N, 122° 30'W) breeds in Mar-Jun (Johnson and Johnson, 1950).

*S. japonicus* of Japan (36° 0' N, 138° 0' E) spawns during Apr-Aug (Tanaka, 1958).

*S. variegatus* of Red-sea (20° 0' N, 38° 0' E) breeds during Jun-Aug (Mortensen, 1937).

*Cucumaria crocea* of Antarctic (69° 30'S and 65° 0'W) breeds in Jan-Feb (Thomson, 1878).

*C. frondosa* of North sea and arctic sea (55° 20' N, 03° 0' E) breeds in Feb-Mar (Runnstrom and Runnstrom, 1921).

*C. miniata* and *C. brica* of Puget Sound, U.S.A. (47° 50' N, 122° 30' W) breeds during March-June (Johnson and Johnson, 1950).

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* Cited in Boolootian (1966)
C. planci of Naples, Italy (40° 51' N, 14°, 17' E) breeds in Mar-April (Selenka*, 1876).

Holothuria atra of North-west Red-Sea (27° 15' N and 33° 49' E) spawns during July-Sept (Pearse, 1968).

Holothuria atra of Suri Ilot Maitre, New Caledonia (22° 20' S and 166° 24' E) breeds once from Dec-Apr and again July-Sept (Conand and Ridder 1990).

Holothuria atra of Karwar Coast (14° 18' N and 74° 97' E) breeds during May, Dec and Jan (our finding).

H. atra Papua Island, New Guinea (4° 10' S and 144° 42' E) the spawning period from Dec-April (Conand and Ridder, 1990).

From the above data it becomes clear that breeding (spawning) season becomes more and more wide-spread in populations distributed more and more close to the equator.

Further, regarding the gonadal tubules type Conand (1981) and Smiley et al., (1991) have observed similar types of ovarian tubules in Microthele nobilis and Stichopus californicus respectively. They have named these tubules as the primary, secondary, fecund and spent tubules. Following

* Cited in Boolootian (1966)
this, Smiley et al., (1991) have described the events of oogenesis in details. According to them the primordial oocytes from the dorsal mesentry connecting the gonad basis to the body wall flow to the gonad basis from where they are lodged in the minute hollow buds - the primary tubules. The authors have further described the details of oogenesis concurrent with propagation of the subsequent types of the ovarian tubules harbouring oocytes in different stages of development upto phagocytosis of the relict oocytes in the spent tubules. Based on their findings in *S. californicus* they opine that the model of oogenesis in *S. californicus* may serve as a model common to all other species of holothuroidea. Views of Conand (1981) and of Smiley et al., (1991) appear to be one and the same, while Tyler and also Byrne (personal communications) are cautious and have reservation on the applicability of *Stichopus* model to holothurians at large. Though our observations on the morphology of the ovary in different stages of reproductive cycles do resemble those of Conand (1981) and Smiley et al., (1991), we do not have information as yet on the structural details of oocytes in different types of tubules. Hence, we just mention that the structure of ovary and the programmed events in oogenesis in *H. atra* may have close resemblances
with those in *S. californicus*. Examination of the gonad and gametogenic events in many species of different genera and families of holothuroids is essential before treating Smiley et al., (1991) model as having a limited or wide range applicability. Further, the development of the primary and secondary tubules in the gonads of mid-growth (late September) and maturation phases (March) and also the spent tubules in the November sample are clear indications of asynchronous development of the gonads to some extent.
Summary

1. The reproductive cycle of female and male Sea-cucumber *Holothuria atra* has been studied for a period of three years histologically and by morphometric analysis.

2. Though, the Sea-cucumber *H. atra* is a gonochoric species, the sexes are indistinguishable externally. The gonadal tubule morphology has also been studied during the different stages of the reproductive cycle of the Sea-cucumber.

3. *H. atra* of Karwar coast is a short breeder broadcasting its eggs.

4. In matured ova a protuberance has been observed on one side which is supposed to form the animal pole of the embryo.

5. In the testes *Sertoli* cell-like cells have been observed.