ANNUAL REPRODUCTIVE CYCLE OF SEA CUCUMBER *HOLOTHURIA (HALODEIMA) ATRA* JAEGER (HOLOTHUROIDEA : ASPIDOCHIROTA) AT TUTICORIN, SOUTHEAST COAST OF INDIA

Thesis submitted in
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JANUARY, 2001

Dedicated to
The Animals which sacrificed
Their Lives
for this Study

DECLARATION

I hereby declare that this thesis titled "**Annual reproductive cycle of sea cucumber, *Holothuria (Halodeima) atra* Jaeger (Holothuroidea : Aspidochirota) at Tuticorin, Southeast coast of India**", has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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29 - 1 - 2001.

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CERTIFICATE

Certified that the thesis titled "**Annual reproductive cycle of sea cucumber, *Holothuria (Halodeima) atra* Jaeger (Holothuroidea : Aspidochirota) at Tuticorin, Southeast coast of India**" is a bonafide record of the work carried out by **Mr. RAM MOHAN M.K.** under my guidance and supervision and that no part thereof has been presented for the award of any Degree, Diploma or any other similar titles.

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PREFACE

The processed holothurians or *Beche-de-mer* commands high price in the International market. The value of *Beche-de-mer* is determined by the species value and the thickness of the body wall. Besides, edible value, the toxins present in the body of many holothurians are of bio-medical importance. These Saponin-based toxins are used in several allergic and anti-tumor treatments.

The species studied, *Holothuria atra* is one of the commonest species of sea cucumbers in the Indo-West Pacific region. Although it offers a low yield and price after processing, due to its thin body wall, this species is one of the most toxic among the sea cucumbers. The entire animal, including body wall and viscera, contains toxic material, which is used in bio-medical research and eradication of weed organisms from fish ponds. Although common, the biology of this species is not intensively studied. A knowledge of reproduction is a pre-requisite for the production of young ones and culture, which can enhance the economic viability of the species. It may in turn help the poor fisher folk in the coastal area by offering a potential resource for fishing and improve their economic status. With this in view, the candidate took up the study on the annual reproductive cycle of *H. atra* at Tuticorin, Southeast coast of India.

The thesis consists of four chapters. The first chapter is an Introduction, followed by a review of related literature in the second chapter. Chapter-3 deals with material used and methods followed in this study. Chapter 4 deals with Results and Discussion, which is again divided into two parts: first part dealing with the reproductive cycle of *H. atra* and the second on the asexual reproduction of the same species at Tuticorin. These chapters are followed by a Conclusion, Summary and References.

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सारांश

समुद्री कक्कड़ी होलोथूरिया अट्रा के वार्षिक जनन-चक्र पर टूटिकोरिन में नवंबर, 1997 से लेकर दिसंबर, 1999 तक 18 महीनों का अध्ययन किया गया. लिंग की अनिर्धारित स्थिति में जीव के पूरे वज़न में जनन-ग्रंथि सूक्क (gonad index) 0.037±0.06 था. पुरुष कक्कड़ियों में दिखाया पड़ा अधिकतम सूक्क 2.995±0.02 और मादा कक्कड़ियों में सूक्क 4.486±3.7 था. जनन-ग्रंथि सूक्क और अंडजनन के निरीक्षण के आधार पर यह देखा गया कि टूटिकोरिन में होलोथूरिया अट्रा का द्विबहुलक प्रजननकाल (bimodal breeding season) होता है, जूलाई - अगस्त और फरवरी - मार्च. अगस्त 1998 में औसत जनन-ग्रंथि सूक्क उच्चतम (2.21±2.57) और दिसंबर, 1997 में न्यूनतम (0.119±0.14) था. प्रजननकाल में पुरुष और मादा नमूनों की प्रतिशतता अधिकतम देखी गयी. करीब 90 ग्रा वज़न के होने पर यह जाति प्रथम परिपक्वता प्राप्त करती है. माध्य जनन क्षमता 3,175,938 अंडे थी. टूटिकोरिन के पारिस्थितिक प्राचलों में इस जाति की प्रजनन अवधि से संबंध जोड़ने वाला एकमात्र घटक लवणता था.

दिसंबर 1998 के दौरान विखंडन (fission) अधिकतम और अप्रैल 1998 और अप्रैल 1999 में न्यूनतम देखा गया. विखंडन केलिए कोई विशेष प्रतिमान नहीं दिखाया पड़ा. माहिक नमूने के 9.00±5.69 विखंडन करने वाले थे. विखंडन और पुनर्जनन में किसी भी पारिस्थितिक प्राचलों का संबंध नहीं देखा गया.

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CHAPTER 1

INTRODUCTION

Knowledge on population parameters is a pre-requisite for the rational management of holothurians. Information on the various species is scarce despite their abundance and size, which qualify them as a significant component of the benthic macrofauna of lagoon and coral environments. The reproductive biology studies of sea cucumbers are of great importance in understanding the annual breeding season, recruitment patterns and stock enhancement in the natural environment. Likewise, it also helps in establishing a hatchery system to produce seeds. Induced spawning could be achieved through thermal stimulation for seed production. This enhances importance of a species for mariculture activities, where adequate seed supply is an important criterion.

Seasonal reproduction is a mode associated with the storage of gametes, followed by subsequent release in a mass spawn-out. It implies a non-random allocation of resources to reproductive activities in relation to environmental and biological inputs.

Holothuria atra is one of the commonest sea cucumbers in the tropical waters. It is well distributed in the Indo-West Pacific, Oriental and Australian regions. In India, it is reported from the Gulf of Kutchh, Bombay (Mumbai), Ratnagiri, Goa, Karwar, Quilon, Vizhinjam, Kanyakumari, Gulf of Mannar, Palk Bay, Madras (Chennai), Visakhapatnam, the Lakshadweep islands and the Andaman and Nicobar islands. This species occurs in shallow waters in good densities. In Tuticorin port region, about 5-8 specimens could be found in 10 sq. m. area.

Many holothurians possess saponin-based toxin in their body. The degree of toxicity varies with each species. This toxin is found to inhibit bacterial and fungal action. When *H. atra* is handled in live condition, a red toxin known as holothurin, stains hand. Rao *et al.* (1985a and b;1991) states that the toxins of *H. atra* are of biomedical importance, since they exhibit anti-tumoural, anti-fertility and anti-cancerous properties. The toxins proved lethal for unwanted organisms in culture ponds during the experiments conducted by James (1986a). The significance of the toxins of *H. atra* in biomedical research enhances the potential commercial importance of the species. Many holothurian toxins are found to inhibit bacterial and fungal action.

This species is processed only very rarely because of its thin bodywall. Still, large specimens are processed in some parts of Gulf of Mannar, including Tuticorin. This species is extensively processed in Philippines and commands fairly good price.

Besides the sexual mode of reproduction, *H. atra* propagates asexually too by way of binary fission. So both sexual and asexual methods of reproduction help in the propagation and stock enhancement of this species, which is quite distinct and interesting in nature. Despite its abundance in the Gulf of Mannar region, only meager information is available on the reproductive biology. The works by Hiremath and Desai (1994) at Karwar and that of James *et al.* (1995) at Tuticorin are of preliminary nature.

Judicious exploitation is a must for the management of the fishery of these defenceless animals. Unless the complete biology of a particular species is known, such management measures cannot be taken. Research on the reproductive biology of holothurians allow recommendations to be made on the size regulations for fresh or processed product based on the size at first maturity. A study on the reproductive cycles of *H. atra* could bring all these facts to light. With this in view, and also the potential biomedical importance of the species, the candidate selected the study on the annual reproductive cycle of *H. atra* at Tuticorin on the southeast coast of India. The study is intended to fulfill the following objectives :

i) To study the annual reproductive cycle of *Holothuria atra* at Tuticorin, southeast coast of India.

ii) To classify different maturity stages of the gonad.

iii) To study monthly gonad index variations.

iv) To study the variations in gonad index along with the progression of maturity stages.

v) To study the variations in gonad morphology in different maturity stages.

vi) To study the histological characteristics of gonad in different maturity stages.

vii) To study the asexual reproduction characteristics of *H. atra*

CHAPTER 2

REVIEW OF LITERATURE

Reproduction in Echinoderms as such is of critical importance by way of their characteristic nature and larval stages, with respect to each class. Various researchers have worked on the reproductive biology and related physiological events of the Echinoderms. Giese (1959) in his review, defined reproductive cycle as "the series of events from the time of activation, growth and gametogenesis 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". Reproductive cycle refers to the total course of events, regardless of the time period over which these occur daily, weekly, monthly or annually.

A number of works have been carried out on the control of abiotic and biotic factors over echinoderm reproduction. Hyman (1955) published an account of echinoderms and their biology. Annual spawning cycles of benthic invertebrates from Passamaquoddy Bay was studied by Lacalli (1981). The spawning behaviour and its correlation to temperature in seastar *Marthasterias glacialis* was studied by Minchin (1987). The effect of seawater temperature and rainfall was correlated to seasonal spawning pattern of the sea urchin *Lytechinus variegatus* by Moore and Lopez (1972). Keats *et al.* (1984) conducted a study on the depth-dependent reproductive output of sea urchin, *Strongylocentrotus droebachiensis* and observed that it was highest at depths where preferred microalgae were abundant. Tyler and Young (1992) consider the availability of food as a criterion for the initiation of reproduction process. A similar view was also expressed by Tyler *et al.* (1982) after their study in five species of deep-sea echinoderms. The photoperiod control over gametogenesis was studied in sea star *Pisaster orchraceus* (Pearse and Eernisse,1982), sea urchin

Strongylocentrotus purpuratus (Pearse *et al.*, 1986; Bay-Schmith and Pearse, 1987) and *Eucidaris tribuloides* (Mc Clintock and Watts, 1990). A relation of spawning with full moon was given by Pearse *et al.* (1988) for six echinoderm species from the British Columbia. Starr *et al.* (1990) correlated spawning of green sea urchin *S. droebachiensis* with various phytoplankton blooms. Out of season gametogenesis was induced in the same species through artificial diet and photoperiod control by Walker and Lesser (1998).

The reproductive period or the breeding season is the time during which mature fertilizable gametes are present. Holland *et al.* (1975) studied the gonad development in the crinoid *Comanthus japonica* during the annual reproductive cycle in Koaziro Bay, Japan. Vail (1987) studied the reproduction of five species of crinoids at Lizard island, Great Barrier Reef. In asteroids too, a few works have been conducted on reproductive biology. Morphology and histology of the gonoduct of *Asterias vulgaris* was studied by Walker (1975). The histological changes of gonad of *A. rubens* was studied by Jangoux and Vloebergh (1973). Vevers (1949 and 1952) studies the biology of the same species. The sexual and asexual reproduction in geographically separated populations of fissiparous seastar, *Coscinasterias calamaria* were also studied (Crump and Barker, 1985). Hamel and Mercier (1995a) studied the pre-spawning behaviour, spawning and development of brooding starfish *Leptasterias polaris*. The reproductive cycle of *Astropecten brasiliensis* in Cabo Frio, Brazil was studied by Ventura *et al.* (1997). Spawning of two Indo-West Pacific asteroids was studied by Marsh (1988). The gonad and pyloric caeca production in the nine-armed starfish *Lucidia senegalensis*

during its annual reproductive cycle at Southeast Florida Gulf coast was studied by Miller and Lawrence (1999). The bimodality of echinoderm egg size distributions were tested by Sewell and Young (1997).

Similarly, a number of works have been conducted in sea urchins in this line and other related aspects. The annual reproductive and nutritional cycles of *Strongylocentrotus pupuratus* and *S. franciscanus* was studied by Bennet and Giese (1955) and that of echinoids from Gulf of Suez was studied by Pearse (1969). Boolootian *et al.* (1959) was studied the reproductive aspects of a deep sea echinoid *Allocentrotus fragilis*. The gametogenic events in purple sea urchin *S. purpuratus* were determined autoradiographically by Holland and Giese (1965). Whereas, Pearse and Phillips (1968) studied the continuous reproduction in sea urchin *Echinometra mathei* at Rottnest island, western Australia. They detected very little seasonal change in the reproductive activity for this species. Annual reproductive cycle and gametogenic studies of cidaroid sea urchin *Stylocidaris affinis* was studied by Holland (1976). An annual cycle of reproduction with spring spawning was observed in *Strongylocentrotus droebachiensis* from Atlantic and Pacific coasts of Canada (Himmelman,1978). Munk (1992) also studied reproduction of the same species. Sukarno *et al.* (1979) has studied the reproductive cycle of *Psammechinus miliaris* and observed gamete release in the months of June and July. Gonadal growth and gametogenesis in Sand dollar *Mellita quinquiesperforata* was studied by Lane and Lawrence (1978). They observed a maximum gonad index during January and February. Annual reproductive cycle of *Paracentrotus lividus* from contrasting habitats on the west coast of Ireland was studied by Byrne (1990).

Spirlet *et al.* (1998) studied the reproductive cycles of this species in France and observed three main phases for it. Spawning period of *Stomopneustes variolaris* from east coast of South Africa was from December to mid-February (Drummond, 1991). Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* in New South Wales was studied by Laegdsgaard *et al.* (1991). Seasonal breeding aggregations of *Stylocidaris lineata* was studied by Young *et al.* (1992). In Taiwan, the miniature Sand dollar *Sinaechinocyamus mai* spawned during October and November (Chen and Chen,1993). Vernon *et al.* (1995) observed spawning of *Clypeaster raveneli* in late February in the northern Gulf of Mexico. An increase in the tempo of gametogenesis in May and onset of spawning in June was reported for *Centrostephanus rodgersii* in contrasting habitats along New South Wales coast, Australia (Byrne *et al.*, 1998). In India too, sexual maturity of *Stomopneustes variolaris* has been worked out by Sastry (1997). Hamel and Mercier (1994) reported inter-specific cross fertilization among molluscs and echinoderms.

The reproductive biology of sea cucumbers were dealt by many researchers from temperate and tropical waters. Studies on different families of Holothuroids are reviewed below.

Lawson (1966) published an account on the ecology of holothurians with comments on reproduction. Sewell *et al.* (1997) postulated a reassessment of the "tubule recruitment model" in the ovarian development of holothurians. Bakus (1973) and Conand (1990) gave detailed accounts on the studies in biology of tropical holothurians. Green (1978) conducted a study on the breeding season of the apodid sea cucumber *Leptosynapta tenuis* in North Carolina. The reproductive

seasons, brooding and protandric characters of *L. clarki* was studied by Sewell (1994 and 1996) and Sewell and Chia (1994). Minchin (1992) presented an account of the observations of invertebrate reproductive behaviour and recruitment at Lough Hyne marine reserve, Ireland, where the spawning of *Leptosynapta* has been portrayed. A study on the aspects of reproduction and population biology of two elasipodid holothurians, *Peniagone azorica* and *P. diaphana* from northeast Atlantic ocean, revealed the similarity existing between these two species (Tyler *et al.*, 1985). Reproductive biology of *Cherbonniera utriculus* and *Molpadia blakei* was noted by Tyler *et al.* (1987). Heavy spawning of *Psolus chitinoides* and *Psolidium bullatum* was observed both in the laboratory and San Juan Archipelago of Washington by Mc Euen and Chia (1991). The reproductive cycle of the sea cucumber *Psolus fabricii* was studied by Hamel *et al.* (1993) from the St. Lawrence estuary, eastern Canada. The spawning behaviours of twelve species of northeast Pacific sea cucumbers were described and compared from the waters of San Juan Archipelago, Washington by Mc Euen (1988). Spawning of *Thyone briareus* was observed from Massachusetts by Colwin (1948). Tyler *et al.* (1994) also studied reproduction of *Bathyplotens natans*.

The reproductive biology of *Ypsilothuria talismani* from north-east Atlantic was studied by Tyler and Gage (1983) and that of the dendrochirote holothurian *Aslia lefevrei* in the West Ireland by Costelloe (1985). Costelloe (1988) also found that the reproductive season varied with geographic separations.

Annual reproductive cycles of three inter-tidal holothurians, *Roweia stephensoni, Pseudocnella sykion* and *Neostichopus grammatus* from Eastern Cape Province of South Africa was studied by Foster and Hodgson (1995). Fish (1967) observed no breeding activity in *Cucumaria elongata* off Northumberland coast. Rutherford (1973) observed spawning in *C. pseudocurata* at Shell Beach, California. This sea cucumber is a brooder which lays large eggs. The recruitment and biology of *C. frondosa* also have been studied at Northeast America and in the Gulf of Maine (Hamel and Mercier, 1996b; Dorothy and Miles, 1997). Early development and settlement of the same species was also studied by Hamel and Mercier (1996a). The same authors (1995 b and 1996 c), carried out a study on the spawning, gametic dispersion and fertilization of *C. frondosa* at St. Lawrence estuary, Canada. Active spermatogenesis resulted in deep invaginations of the germinal epithelium of the testis in *Cucumaria japonica* (Reunov, 1994). Gaschen *et al.* (1993), studied the reproduction of *Cucumaria ferrari* at Antartica. A study by Sewell and Levitan (1992) provides valuable information on natural spawning fertilization rate of *Cucumaria miniata* in British Columbia. Hadel *et al.* (1997) studied reproduction of *Chiridota rotifera*.

Cameron and Fankboner (1986 and 1989) studied the reproductive periodicity, spawning behaviour and recruitment of *Parastichopus californicus* in British Columbia, Canada. Martinez *et al.* (1997) studied the population and reproductive biology of *Isostichopus fuscus* in the Galapagos Islands. Hopper *et al.* (1998) carried out a study on the sexual reproduction of *Actinopyga mauritiana* in

Guam. The biology of *Parastichopus parvimensis* was studied by Perez-Plascencia (1997) at Mexico.

The reproductive biology of the aspidochirote holothurian, *Holothuria forskali* of Atlantic and Mediterranean was studied at Penfret Island by Tuwo and Conand (1992). Eckelbarger and Young (1992) investigated the ovarian ultrastructure and vitellogenesis in ten species of sea cucumbers. Bulteel *et al.* (1992) observed and analyzed the biometry, distribution and reproductive cycle of *Holothuria tubulosa* from the Mediterranean.

Conand (1981) investigated the reproductive biology of three commercially important species of holothurians from the New Caledonia lagoon. The species studied were *Thelenota ananas*, *Microthele nobilis* and *M. fuscogilva*. The same author carried out a study on the reproductive biology of *Actinopyga echinites* (1982) and also on *Stichopus variegatus* (1993b). Reproductive biology of some holothurians including *H. atra* from the New Caledonia lagoon was studied by Conand (1993a and 1994). Biology of juvenile *A. echinites* was studied by Wiedemeyer (1994).

The fine structure of ovarian tubules and ovulation characteristics of *Stichopus californicus* was studied through microscopical observations by Smiley and Cloney (1985) and Smiley (1988). Reproductive season of *Stichopus mollis* on northeast coast of New Zealand was studied by Sewell and Bergquist (1990). Reproductive cycle of temperate aspidochirote *S. mollis* was studied by Sewell (1992) in New Zealand. Seed production of *H. scabra* was achieved by Battaglene *et al.* (1998) and Battaglene (1999) at Solomon Islands. Induced spawning by thermal

stimulation and early larval rearing of *H. atra* has been carried out at Solomon Islands by Ramofafia *et al.* (1995). A few works have been cited on the reproductive biology of *H. atra* from tropical waters. Pearse (1968) studied the reproductive periodicities of four species of Indo-Pacific echinoderms including *H. atra*. Harriot (1985) studied the reproductive biology of *H. atra*, along with *H. impatiens* and *H.edulis* Heron reef, Great Barrier Reef.

Oocyte maturation inducement and development patterns in *Holothuria leucospilota* and *H. pardalis* was studied by Maruyama (1980 and 1990) in Japan. Arakawa (1990) describes the maximum development of gonads for *Stichopus japonicus* during January and February. Tanaka (1958) studied the seasonal changes of gonad in *S. japonicus*. Induced oocyte maturation using radial nerve extracts in *S. japonicus* was studied by Maruyama (1985) and Drozdov *et al.* (1991). Catalan and Yamamoto (1994) studied the annual reproductive cycle of *Eupentactachro chronhjelmi*. Ito and Kitamura (1997 and 1998) achieved the larval development and seed production of *S. japonicus* in Japan. Kubota and Tomari (1998) studied the semi-lunar spawning rhythm and sex change in apodid sea cucumber *Polycheira rufescens* at Iso marine station, Kyushu, Japan. Chao *et al.* (1993b) observed a short discrete breeding period for the dendrochirote holothurian *Phyrella fragilis* in southern Taiwan.

Seeto (1994) studied the reproduction of *H.atra* in Fiji, while Chao *et al.* (1994) did a similar work in southern Taiwan. Reproductive cycles of nine species of sea cucumbers of Dendrochirotida, Aspidochirotida and Apodida were studied by

Chao *et al.* (1995) in Southern Taiwan. The reproductive cycle of *H. leucospilota* was investigated at the Nha Trang Bay in Viet Nam by Viet-Nam and Britaev (1993). *H. scabra* was studied for its reproductive cycles in southwest Sulawesi, Indonesia by Tuwo (1999).

Indian researchers also have contributed much to study the reproductive biology of sea cucumbers. Of a dozen commercially important species, only a few have already been studied in India. The reproductive biology of *Holothuria leucospilota* was studied by Jayasree and Bhavanarayana (1994) from Anjuna, Goa. Hiremath and Desai (1994) carried out a small study on the reproductive biology of *H. atra* at Karwar, South West Cost India. Kandan (1994) studied the reproductive biology of *H. nobilis* and *A. mauritiana* from Minicoy Island, Lakshadweep.

James (1969) brought out a catalogue on echinoderms of India. James (1995d) studied the animal associations in echinoderms. Again the same author (1988a, 1989a and 1991a) detailed the echinoderm resources of the Indian region. Management and conservation of *Beche-de-mer* resources in India was studied by James (1991b, 1994b and 1996b) and by James and James (1994a and b). A good deal of work has been done on the taxonomy, resources, fishery, utilization and processing of sea cucumbers in India (James,1973,1983a, 1986c, 1986e, 1987, 1988b, 1989b, 1989c, 1989d, 1994e, 1995a and b; James and Baskar,1994; James and Ali Manikfan,1994). Ecology of echinoderms including holothurians was also studied from the Indian region (Gopalakrishnan, 1969; Rane and Chhapgar,1962; Parulaker, 1981; James,1982a, 1986d, 1994c,1994d and 1998a). James (1994a) and James and Lalmohan (1969)

published bibliography of sea cucumbers and echinoderms respectively from the Indian seas. The quantum of work done in this region signifies the importance of holothuria in the Indian seas. James (1965) observed light purple gonad tubules occupying the posterior two thirds of the coelom in *Phyllophorus parvipedes* from Vedalai, Gulf of Mannar in south east coast of India. The biology and reproduction of the most valuable sea cucumber, *H. scabra* has been studied by Krsihnaswamy and Krishnan (1967) and Baskar (1993 and1994) from the south east coast of India. Krishnan (1968) brought out the research on the reproductive and nutritional cycles of *H. scabra*. James *et al.* (1988 and 1994) perfected the technique of induced spawning and larval rearing of *H. scabra*, to raise seeds in hatchery to improve its culture prospects (1994). The hatchery and culture prospects of *H. scabra* was also studied in depth James and James (1993) and James (1993,1994f,1996c and 1998b). James *et al.* (1999) tried broodstock development of *H. scabra* in prawn farms. James (1983b) reviewed the research on Indian echinoderms. James and James (1994) published a handbook on the sea cucumbers of India. James (1999) brought out a review of hatchery and culture techniques of *H. scabra* in India. James (1995c and 1996a) attained seed production of *H. scabra* and also could rear *H. atra* seeds upto doliolaria larva at Tuticorin, south east cost of India. James *et al.* (1995) at Tuticorin commented on the reproductive biology of *H. atra* .

It is well known that echinoderms in general, and holothurians in particular, have the capacity to regenerate lost parts and resort to asexual reproduction. Asexual reproduction by way of fission is common in some holothurians. "Fission is an asexual process in which an animal divides into two parts and each part is then

capable of regenerating the whole animal." In *Holothuria* spp. the body undergoes transverse binary fission, dividing the body into an anterior and posterior parts. The studies on asexual reproduction and regeneration had been confined to a few species of holothurians. The studies covered the areas of regeneration, binary fission *etc.*, mainly carried out in tropical waters. Agametic reproduction is possible only in a taxa with good regenerative capacity. This is engendered by a ready supply of undifferentiated cells, scattered about the soma, but impeded to a great extent by sequestering the germline or by cell consistency. The autotomy in *Leptosynapta inharens* and *Thyone briareus* was studied by Pearse (1908 and 1909). The regeneration capability of the holothurian *T. briareus* after induced autotomy, was studied in depth by Kille (1936). The regeneration of the gonad tubules following extirpation was also studied in this species by the same author (1939). Again Kille (1942), studied the regeneration of reproductive system following binary fission in *H. parvula*. It is observed that there exists no correlation between the stage of development of gonad and the occurrence of fission in *H. parvula*. Fission may be repeated under natural conditions without the intervention of sexual reproduction. Bakus (1973) observed formation of buds on the body of *H. atra*. Fission studies in *H. parvula* was also carried out by Emson and Mladenov (1987) in a Bermudan population.

Observations were carried out to study fission of *Stichopus chloronotus* and *H. leucospilota* populations in Reunion Island by Conand *et al.* (1997 and 1998). Asexual reproduction of Pacific and Indian ocean populations of *S. chloronotus* was compared by Conand and Uthicke (1999). The consequences of asexual

reproduction on population structure was studied by Conand (1999). The Reunion Island population of *H. atra* was studied for its fission characteristics by Boyer *et al.* (1995), Conand (1996) and Jaquemet *et al.* (1999). Asexual reproduction of *H. atra*, *H. edulis* and *S. chloronotus* figured the study by Uthicke (1997a and b) at Great Barrier Reef. Uthicke *et al.* (1998) also studied the genetic structure of fissiparous *H. atra* in Great Barrier Reef. The fission in Indo-Pacific tropical holothurian populations was studied from fringing reefs of La Reunion and Great Barrier Reef (Conand. 1999). Populations of the holothurian *H. atra* at Taiwan was subjected to fission studies and its effect on the population by Chao *et al.* (1993a). Reichenbach and Holloway (1995) studied the asexual propagation potential of six species of holothurians at Laamu atoll in the Maldives and fission in *H. parvula* was studied by Emson and Mladenov (1987).

Kandan (1994) studied the asexual propagation of *A. mauritiana* at Minicoy of Lakshadweep Islands. James (1986b) reported contraction and subsequent evisceration by *Phyrella fragilis* from the Andaman Islands immediately after collection. *H. scabra* was induced for evisceration and subsequent regeneration was studied in detail by Mary Bai (1971). She also studied the anatomy and histology of the same species (1978). James (1982b) reported constriction followed by breaking of body into small bits by *Protankyra tuticorinensis* when kept in state water, from the Gulf of Mannar coast.

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLING

A shallow water bay near the South Break Water region inside the Tuticorin port was selected as sampling site, because of the abundance of the species there.

Live specimens of *Holothuria atra* for the study were collected from a shallow water bay near the South Break Water region inside the Tuticorin harbour. The specimens were collected by hand picking every fortnight for a period of 18 months from November 1997 to April 1999. The collections were made at random, preferably during low tide, when the water depths ranged from 0.2 to 0.5m. Usually sampling was done during morning hours.

During sampling, the atmospheric as well as water temperatures were noted. Water samples for estimating salinity and pH were also taken. The salinity was primarily checked using an ATAGO salinity refractometer and then analysed in the laboratory following `Mohr - Kundson method" as given by Strickland and Parsons (1968) for confirmation. In this method, 10ml of seawater sample was pipetted out into a 250ml conical flask. Four drops of potassium chromate were added as indicator and the sample was titrated against silver nitrate solution, till the colour turns brick-red.

The pH of the water sample was checked using a digital laboratory pH meter. The pH meter was standardised by using buffer solutions of acidic (4.0) and alkaline (9.2) range.

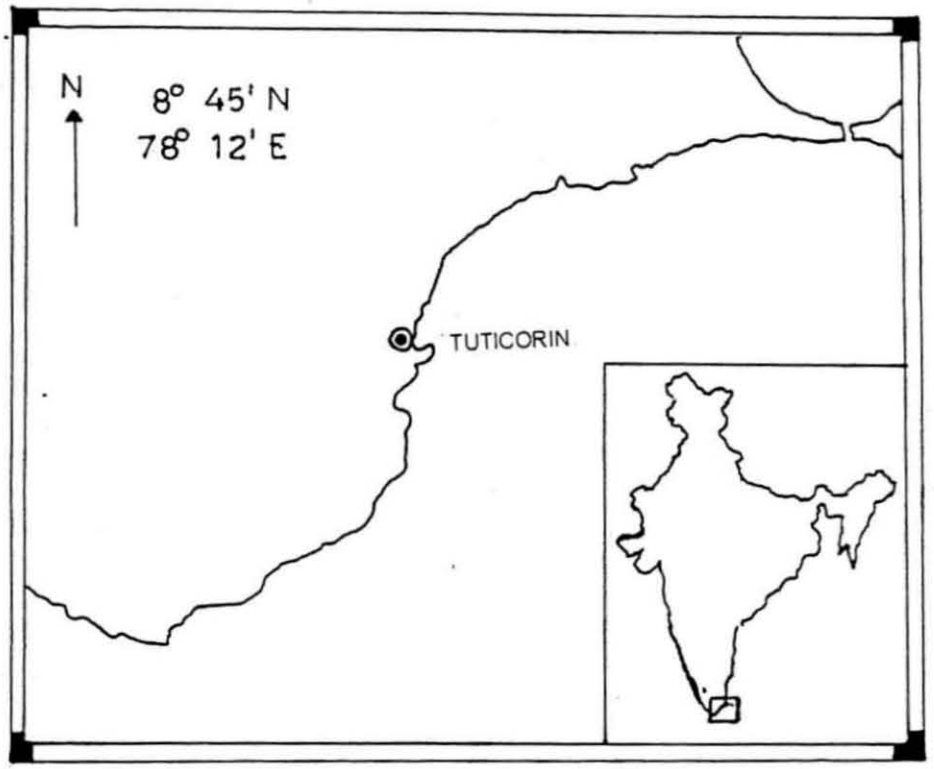


Fig. 1. Study area : Tuticorin, Southeast coast of India

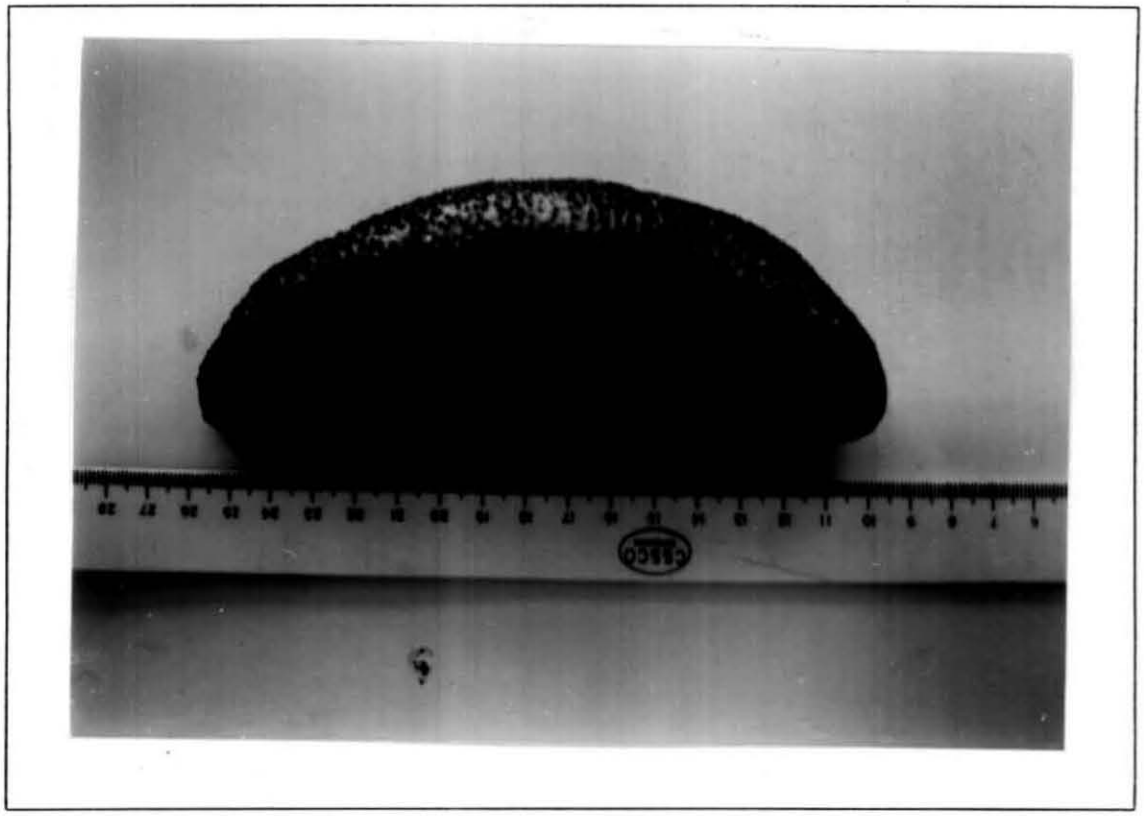


Plate I. Full specimen of *Holothuria atra*

Plate II. A view of the sampling site

The water samples for dissolved oxygen estimation were preserved with Winkler A and B solutions and later estimated in the laboratory following 'Modified Winkler method' as given by Strickland and Parsons (1968). To the fixed oxygen bottles were added two ml of concentrated Hydrochloric acid and were shaken till the precipitate formed is completely dissolved. Ten ml of this sample was pipetted out into a conical flask and titrated against Sodium thiosulphate solution, till the yellow colour fades. Then, a few drops of starch solution were added, which imparts a blue colour. The titration was continued till the blue colour disappears at the end point.

The sample sizes varied from 30 to 94 every month, with an average of 55 numbers per month. The samples were then taken to the Karapad Field Centre of Tuticorin Research Centre of Central Marine Fisheries Research Institute, eight km away. Twenty litre plastic bucket with sufficient quantity of sea water was used for transportation. After reaching the laboratory, the specimens were immediately transferred to one tonne FRP tanks filled with 750 litres of clean, filtered sea water. The sea water in the tank was given constant aeration. The specimens were kept. there overnight for emptying the gut and the following day they were dissected.

To start with, one specimen of *H. atra* was taken and placed on a wooden dissection board. It was allowed to relax and the total length (TL) of the specimen was measured using a measuring tape. The body of *H. atra* is elastic in nature and hence the total length of the specimen could not be measured accurately. Though the length measurements were recorded to the possible accuracy, it was not taken into consideration in this study as a body parameter. The specimen was subsequently

blotted dry and placed over an Ishida monopan weighing balance of 500g capacity and its whole wet weight (Ww) was noted, to the nearest of two g. Then, the animal was removed from the balance and using a scissors, a gentle incision was made at the posterior end passing through the anus. This will facilitate the draining of the coelomic water present inside the specimen. After complete draining, the drained weight (Wd) of the specimen was recorded.

Later, the specimen was cut open in the mid-dorsal line from the mouth to the anus to expose the viscera fully. The gonad, if present. was immediately traced and removed. The separated gonad was kept in a petridish with seawater for further examination. The gut portion starting from the pharyngeal bulb to the posterior part of the intestine and the respiratory trees, were removed. The weight of the remaining bodywall was taken as gutted weight (Wg). The gut weight (gw) including the gonad was also noted.

Jones and James (1970) reported a gastropod parasite. *Stilifer* sp., from the cloacal chamber of *H. atra*. They recovered 13 gastropods after examining 1359 specimens. Eight specimens were found to be infested with gastropod parasites. This gastopod, according to Waren (1983), belongs to the genus *Megadenus* sp. It is interesting to mention that the candidate came across two parasitic gastropods of *Megadenus* sp. in the cloacal chamber of one *H. atra*. The parasites were noticed during September,1998, embedded in the tissue of the wall of the cloacal chamber. The shell breadth of the gastropods were 2.8mm and 2.2mm, the smaller one being male.

3.2 GONAD MORPHOLOGY

In holothurians, the sexes are separate, except for a few cases. In the family Holothuriidae, gonad consists of a single tuft of tubules, whereas in the family Stichopodidae, gonad has two tufts of tubules. The gonadal tubules may be branched or unbranched, short or elongated, depending on the stage of maturity. The tubules are hollow and they open to a collective gonad base, situated on the left side of the dorsal mesentary. From the gonad base a gonoduct runs towards the anterior end along the dorsal mesentary, which opens out at the mid-dorsal line through a gonopore. Sometimes two or even three gonopores are also seen. The gametes are released outside through the gonopores at the time of spawning.

H. atra is dioeceous, with both male and female specimens were present in the population. A maturity scale in five stages were recognised for *H. atra* based on morphological and microscopical examinations on a pattern formulated and followed by Conand (1981). The stages identified were stage I and II (indeterminate stage), stage III (maturing), stage IV (ripe) and stage V (spent). The gonads were elongated and enlarged when they are fecund, but when gametogenic activity does not take place, it is represented as a tiny tuft of tubules.

The excised gonad was spread on a wooden dissection board and the number of gonadal tubules (Ngt) were counted. The tubules of smaller gonads were counted with the help of a dissection microscope after they were mounted and spread on a petridish or on a glass slide. The maximum and minimum tubule lengths (tl) were recorded nearest to one mm by stretching the gonad tubules. Shorter tubules

were measured with the help of a camera lucida. The width of the gonad tubules (tw) varied according to the maturation process. Thinner tubules were measured using an occular micrometer, while thicker tubules were measured using the camera lucida.

Since the gonad colour varies in respect of sex as well as maturity stage in *H. atra*, the colour of the gonad was also noted. The gonad was blotted dry and its weight (GW) was taken on a Sartorius electronic weighing balance, to the nearest of 10mg. The sexes and maturity stage was confirmed through microscopical examination using a Ernst Leitz Wetzlar microscope.

3.3 GONAD INDEX

The gonad indices provide a critical data on the maturity stage of the specimen. The gonad indices increase when the maturation process starts, rises to a peak when the gonad is fully grown and drops when the spawning or reproductive activity is over. The gonad indices with respect to wet weight, drained weight and gutted weight of the animal was estimated as follows :

$$\text{Gonad Index 1} = \frac{\text{Wet gonad weight (GW)}}{\text{Whole wet weight of the animal (Ww)}} \times 100$$

$$\text{Gonad Index 2} = \frac{\text{Wet gonad weight (GW)}}{\text{Drained weight of the animal (Wd)}} \times 100$$

$$\text{Gonad Index 3} = \frac{\text{Wet gonad weight (GW)}}{\text{Gutted weight of the animal (Wg)}} \times 100$$

3.4 HISTOLOGY

A portion of the male and female gonads in stage III, IV and V were selected for histological slide preparations. Freshly excised tissues were immediately transferred to 10% Neutral Buffered formalin and fixed for a period of 24 - 48 hrs. before further processing. Properly fixed tissues were later dehydrated through ascending grades of alcohol series (30 to 100%) and the tissues were kept in a mixture of alcohol and xylene (1:1 ratio), before clearing in xylene. The tissues were then put in a mixture of xylene and paraffin wax (1:1 ratio) for cold impregnation overnight. Subsequently, the tissues were transferred to molten wax (Paraffin wax with ceresin, Ranbaxy, melting point 58 - 60° C) for hot impregnation. After two more changes of 15 minute duration each in fresh molten wax, tissue blocks were prepared using L – blocks (Clark, 1981).

The prepared blocks were trimmed and serial sections were taken at an appropriate thickness of 5 - 7μ, using a rotary microtome. The sections were fixed on clean glass slides using fresh Meyer's albumen. The sections were flattened on a slide warmer with few drops of distilled water. Subsequently, the water was drained off and the slides were allowed to dry.

The slides were selected for their quality before staining. Routine staining was carried out using Harri's Hematoxylene stain, with 1% aqueous Eosin as the counter stain. Sections to be stained were de-paraffinised in two changes of xylene, and then passed through a descending series of alcohol grades for rehydration. Hydrated tissues were stained in Hematoxylene and turned blue in tap water before

counter staining with aqueous eosin. Eosin stained slides were then passed through 70% alcohol and again stained with alcoholic Eosin. The sections were again dehydrated in two changes of absolute alcohol and cleared in xylene before mounting in DPX mountant, with 0.1mm cover slips. The mounted slides were air dried and then stored in a wooden slide box for critical examination under the microscope and for taking photographs later.

The photographs of the gonad and the animal were taken using a Nikon FG20 camera. The film used was Kodak Gold colour. The indeterminate gonads were too small for histological preparations, so photographed by mounting on a glass slide using a Nikon AFX-DX II microscope fitted with a Nikon FX-35 camera, with photomicrographic attachment. The gametes were also photographed in a similar manner. The film used was an Orwo B/W film. The histological sections were photographed on a AFX-DX II microscope fitted with a Nikon FX-35 camera, with photomicrographic attachment. Nova B/W negative film was used for taking photographs of histological sections.

3.5 WEIGHT AT FIRST MATURITY

Weight at first maturity is referred to as the size at which an individual reproduce for the first time. It is an important parameter in stock assessment. In *H. atra*, the frequency of individuals with gonad were recorded in size classes of wet weight (Ww). As holothurians are very elastic, the total length was not taken into account in assessing the size at first maturity. Samples at stage I and II of gonad maturity were also included. Then, the percentage of specimens with gonad at stages

III, IV and V of maturity, which are considered to be in the gametogenic process, was calculated and plotted against the mid-value of the size classes. The point on the curve at which 50% of the animals possessed gonads undergoing gametogenic activity was taken as the weight at first maturity.

3.6 FECUNDITY

Fecundity is regarded as one of the most important parameters in relation to recruitment. The fecundity estimation was done only in ripe female gonads. A portion of the ripe female gonad was separated, drained and weighed on the electronic balance. This weighed tubule was fixed in a glass vial with a known volume of Gilson fluid. The Gilson fluid helps to disintegrate the ovarian stroma and harden the oocytes. The separated oocytes are volumetrically sub-sampled from a homogeneous suspension and the number of oocytes (n) were counted. The absolute fecundity was calculated as :

$$Fa = n \times \frac{\text{Wet gonad weight (GW)}}{\text{Known ovary weight}}$$

The relative fecundity (Fr) was the ratio of absolute fecundity of a specimen to the weight of the ovaries, or its gutted weight. In the present study, the relative fecundity was taken as the ratio of absolute fecundity to the gutted body weight (Wg) of the specimen as :

$$Fr = \frac{Fa}{\text{Drained weight (Wd)}}$$

3.7 OOCYTE DIAMETERS

The oocyte is a good indicator of reproductive activity. The female gonad bears oocytes of different diameters at different stages of maturity. Oocyte measurements made from histological sections may turn out to be an underestimation of actual diameter as the tissue shrinks during processing. Hence to record the oocyte diameters, a portion of the female gonad tubule was teased out into 1% formalin on a glass slide. Then the diameters of atleast 50 oocytes were measured using an occular micrometer, pre-caliberated with stage micrometer. As oocytes strongly deviate from spherical shape, the largest and smallest axis of the oocyte diameter, passing through the nucleus was taken and the average was used as the actual diameter.

3.8 GUT-GONAD RATIO

This was the ratio of gut to gonad. It was considered to be an indicator of the maturity stage of gonad. As the gonad matures the gut-gonad ratio decreased and reached a minimum when the gonad attained maximum development.

3.9 REPRODUCTIVE CYCLE AND ENVIRONMENT

Environmental parameters such as salinity, temperature, pH, dissolved oxygen and moonphase were collected from November 1997 to April 1999 and tried to correlate with mean values of gonad indices of the species, to find out whether these parameters hold any influence on the reproductive cycle of *H. atra*.

3.10 ASEXUAL REPRODUCTION

Asexual reproduction in *H. atra* is seen in the form of binary fission. The specimens taken for the study of reproductive cycles were carefully checked for any external signs of fission. If there was a fission, the specimens were examined thoroughly for their anatomy, to assess at which state of fission and subsequent regeneration the specimen belong to. Such asexually reproducing specimens of *H. atra* are categorized under Fissioning (F), Recently fissioned anterior part (A), Recently fissioned posterior part (P), Regenerating anterior part (Ap) and Regenerating posterior part (Pa). The specimens were recorded of their wet, drained, gutted and gut weights, stage of maturity of the gonad, if present, and their state of fission or regeneration were also noted.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 REPRODUCTIVE CYCLE

4.1.1 GONAD MORPHOLOGY

As a rule sexes are separate in holothurians. However, in some cases, they are bisexual. The gonad consists of a single tuft of many blind-ending tubules in the family Holothuridae. The tubules are united at the anterior end in a fleshy, saddle-shaped gonad base in the dorsal suspensor mesentary of the gut. The developed gametes are sent out through a gonopore, situated at the anterior end of the body, on the dorsal side. The gonopore, at the tip of a genital papilla become evident only at the time of spawning and occasionally more than one gonopore is seen.

4.1.2 MATURITY STAGES OF HOLOTHURIAN GONAD

The holothurian gonad is divided into different stages of maturity to assess the progression of the sexual cycle. The sexual stages are indicative of the reproductive characteristics and biology of the species. Many authors have tried different classifications for the holothurian gonad maturity stages. The classifications were largely based upon the variations in the morphological characters such as colour, size, weight, volume, length and thickness of tubules, branching *etc.* of the gonad. Progression of gametogenesis through histological examinations were also relied upon to confirm the stages classified through morphology, in the present study. The candidate has followed the classification formulated by Conand (1981) to differentiate the gonad maturity stage, as it is simple and clear. According to this,

the gonad of a holothurian is divided into five stages of maturity. In the present study, the gonad maturity stages of *Holothuria atra* are classified as follows:

Stage	Sex	Maturity Status of gonad
I	Indeterminate	Immature
II		Resting
III	Male / Female	Maturing
IV	Male / Female	Ripe
V	Male / Female	Spent

Stages I and II represent immature and resting phases of gonad maturity respectively. In these stages, the morphological as well as histological structure follow almost similar pattern, making the differentiation of these stages difficult. Moreover, since gametogenesis does not take place, it is almost impossible to make out the sex of the gametic cells at these stages. So the sex was considered as indeterminate in this case.

Some *H. atra* were found to have gonad with three distinct cohorts of tubules on the gonad basis : the smallest immature tubules at the anterior end was followed by maturing tubules in the middle, and the largest ripe tubules at the posterior, or maturing, ripe and spent tubules in the series of progression, as observed by Pearse (1968) in the same species. The same author also stated that, the tubule cohorts are not consistently seen in all individuals. This type of cohorts was observed in both male and female specimens in the present study too. Tubule cohorts varied in every species, with *H. atra* having three cohorts. Cohort observations were also made in *H. leucospilota*

(Viet-Nam and Britaev, 1993), *H. forskali* (Tuwo and Conand, 1992) and in *Parastichopus californicus* (Smiley and Clony, 1985).

4.1.3 GONAD CLASSIFICATION AT DIFFERENT MATURITY STAGES

4.1.3.a. Morphological Characters

The morphological characters of the different gonad maturity stages in *H. atra* are detailed below :

4.1.3.a.i. Stage I and II

The gonad was small in size, pale and translucent. The tubules were budding and unbranched, sometimes showing developing branches or slight branching. The number of tubules were also less. The tubules did not show the presence of any gametes. The tubule tips were often seen club-shaped (Plate III).

The number of gonad tubules were around 18 at this stage of maturity. The length of the tubules varied from 1 mm to 57 mm with a mean of 7.74 mm. The thickness of the tubules ranged from 45.6 μ to 1300 μ, with 366.5 μ as mean. As these stages gave no evidence of gametogenesis, it is difficult to make out the sexes and was considered as indeterminate. The mean gonad weight at these stages was 0.0465 ± 0.08 g. The mean gonad index with respect to the wet weight of the specimens (G1) was 0.037 ± 0.0624 at these stages.

The morphological characters of the male and female gonads at different maturity stages are described below separately:

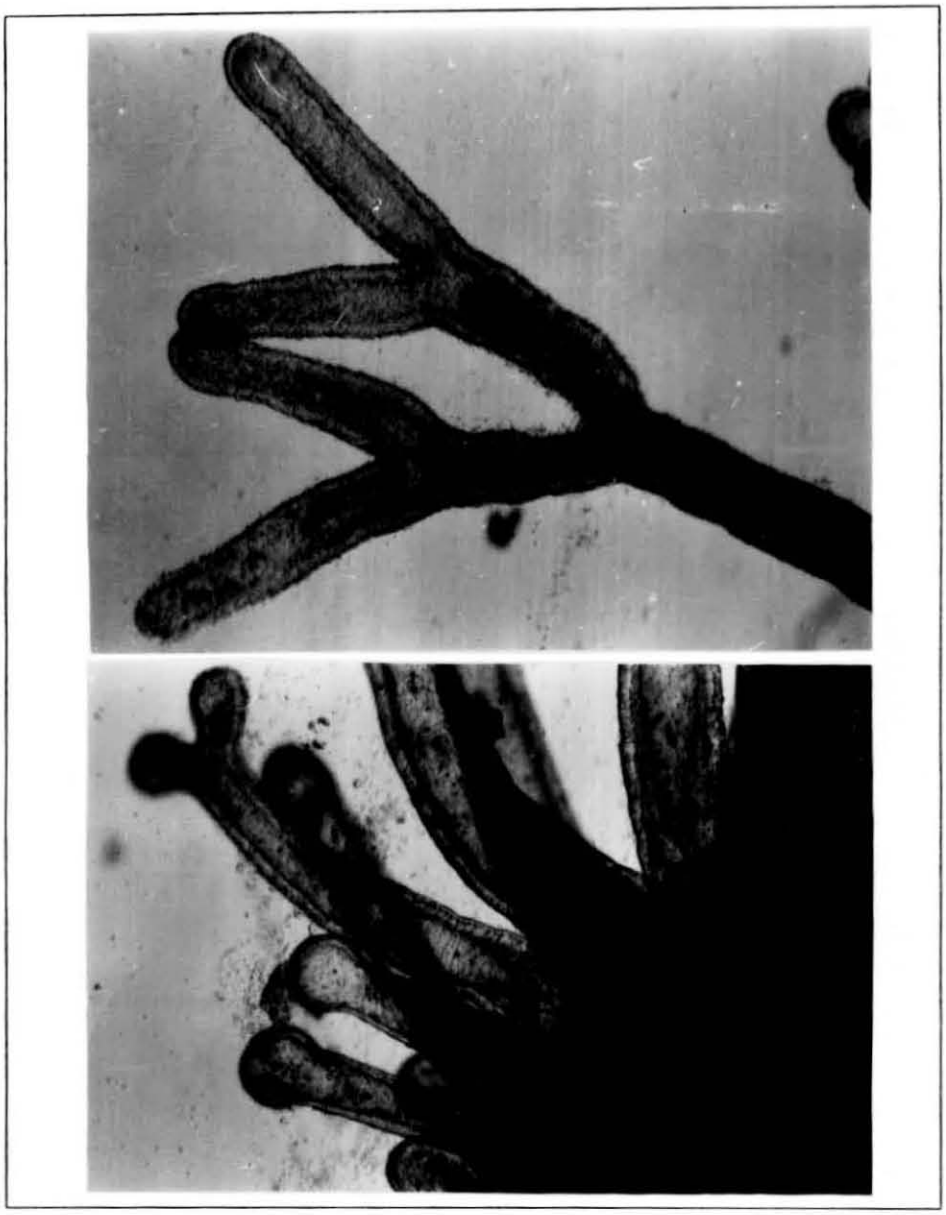


Plate III. Indeterminate gonad tubules

4.1.3.a .ii. MALE GONAD

4.1.3.a.ii.1. Stage III

The male gonad was slightly enlarged and white in colour (Plate IV). The tubules get extended, with a mean length of 27.17 mm at a range from 10 mm to 146 mm. The thickness of the tubules also increased to 797.53 μ, with a range from 114.7 μ to 2700μ. The tubules showed developing male gametes, getting filled up in the lumen. In some portions, the developing spermatogonia were seen as thin white or ivory-coloured streaks. The number of tubules increased to 20 and the tubules were always branched with pointed tips. Each tubule showed two to three branches with more branchlets. The mean gonad weight increased to 0.524 ± 0.718 g, and the mean G1 was about 0.385 ± 0.459.

4.1.3.a.ii.2. Stage IV

Compared to the maturing stage, the ripe gonad showed a maximum advancement in the gonad maturity and morphology. The male gonad turned its colour into creamy-white. The gonad got extended in length, broadened and became turgid due to fullness of male gametes (Plate V). The number of gonad tubules increased to 37. The mean tubule length increased to 69.9 mm with a range of 19 mm to 365mm. The tubule thickness also increased to a mean of 1257.05 μ and a range of 327.8 μ to 3600 μ. With slightest disturbance, the sperms ooze out of the tubules rupturing the tubular wall at this stage. The gonad showed moniliform appearance, with bead like saccules in the tubule branches, filled with spermatozoa.

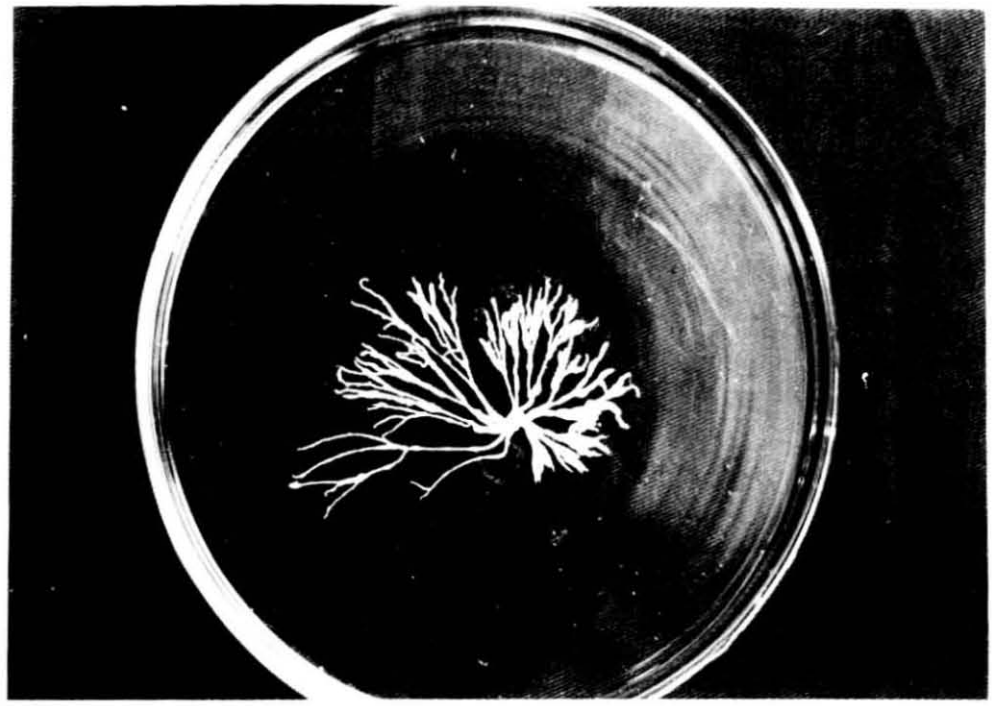


Plate IV. Maturing Male gonad

Plate V. Ripe Male gonad

The saccules were thicker than the tubules. The mean gonad weight was 5.395±5.155 g. The mean G1 was 2.995±2.027.

4.1.3.a.ii.3. Stage V

The tubules shrunk and the colour changed to pale cream. The tubules became flabby, showed empty saccules with some undischarged sperms (Plate VI). The number of gonad tubules reduced to 31. The tubule length decreased to a mean of 44.7 mm with length range from 6 mm to 303 mm. The thickness of the tubule varied from 131.1 μ to 1700 μ with a mean of 777.02 μ. The mean gonad weight at this stage was 1.948±2.501 g. The mean G1 was also low at 0.947±0.94.

4.1.3.a.iii. FEMALE GONAD

4.1.3.a.iii.1. Stage III

The maturing female gonad was light orange in colour. The gonad tubules showed developing oocytes in the lumen (Plate VII). The tubules of the female gonad also extended to a range of 8 mm to 162 mm with a mean at 34.72 mm. The number of gonad tubules also increased to 25. The tubules thickened due to the development of oocytes, and the mean thickness of the tubules at this stage was 1098.96 μ, with a range between 196.6 μ and 2900 μ. The tubules showed two or three branches with branclets, terminated with rounded tips mostly. The mean gonad weight at this stage was 1.794±2.988 g and the mean G1 was 1.156±1.741.

4.1.3.a.iii.2. Stage IV

The female gonad also showed maximum development when it was ripe. The colour of the gonad turned to reddish orange. The tubules got elongated and

Plate VI. Spent Male gonad

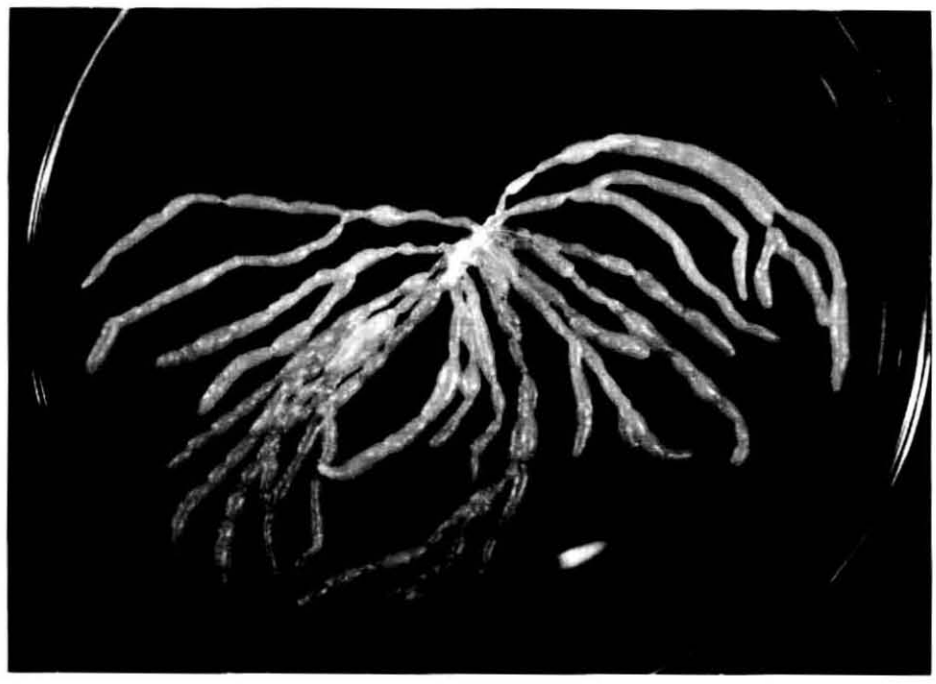


Plate VII. Maturing Female gonad

enlarged lodging fully ripe oocytes in the lumen (Plate VIII). The ripe oocytes (Plate IX) posses a micropyle protuberance which is characteristic of holothurians. This apical protuberance of the oocytes make them among the most visibly polarized in the animal kingdom, with the area of protuberance representing the animal pole (Smiley and Cloney, 1985; Maruyama, 1980). The number of gonad tubules increased to 31. The mean tubule length increased to 59.8 mm with a range from 15 mm to 208 mm. The tubule thickness also showed an increment to a mean of 1581.48 μ with a range of 442.5 μ to 4400μ. The mean gonad weight was 8.681$\pm$8.219 g and the mean G1 was 4.486$\pm$3.706.

4.1.3.a.iii.3. Stage V

The reduction in gonad morphology was observed in the female gonad also. The tubules shrunk, became flabby and the colour of the gonad changes to pale orange (Plate X). The number of gonad tubules was about 26. The empty lumen space of the gonad tubules showed a few unreleased eggs. The tubule length of the gonad varied from 7 mm to 100 mm with a reduced mean at 24.6 mm. The thickness of the gonad tubules had a mean of 822.6 μ within a range from 239.4μ to 4000μ. The mean gonad weight was lower at 1.449$\pm$2.16g. The mean G1 was 0.583$\pm$0.904. The female gonads weighed heavier than the male gonads, producing higher gonad index values, especially in stages III and IV.

The colour of the ripe gonad varied with species. In *H. atra*, the ripe gonad colour was creamy white and reddish orange for males and females respectively (James *et al.*, 1995; Present study). For *H. scabra*, it was creamy yellow for both the sexes

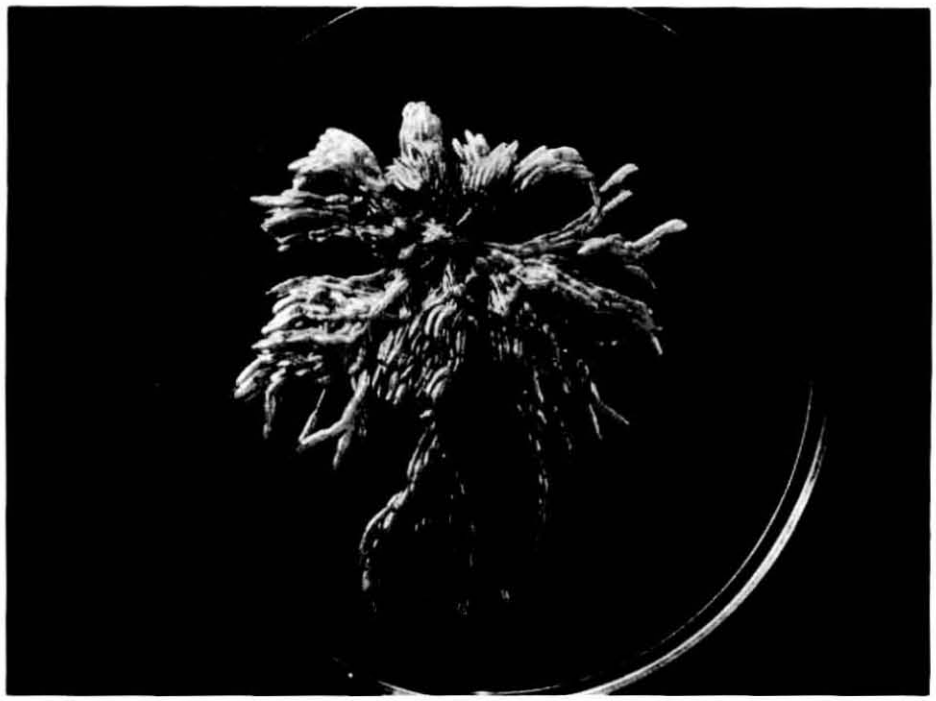


Plate VIII. Ripe Female gonad

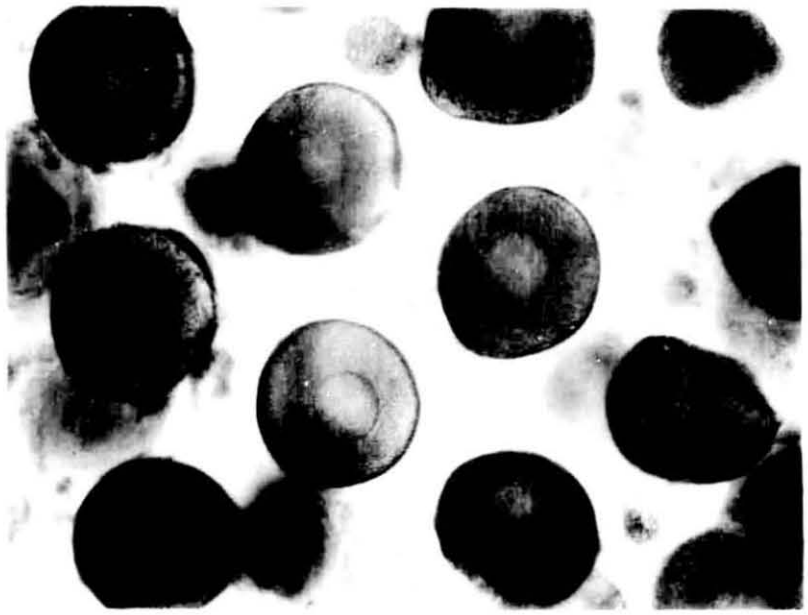


Plate IX. Ripe Oocytes

Plate X. Spent female gonad

(Baskar, 1994). Male gonads were brownish white and female gonads were white at ripeness in *H. nobilis* (Kandan, 1994) or off-white (Conand, 1981). It was again off-white for *H. fuscogilva* and purple for *T. ananas* in both the sexes (Conand, 1981). In *H. forskali*, the male ripe gonad was orange and female ripe gonad was bright red (Tuwo and Conand, 1992), while in *A. echinites*, the ripe male gonad was white and female gonad was pink in colour (Conand, 1982; Chao *et al.*, 1995). The male and female gonads were red and creamy white respectively in *H. leucospilota* (Chao *et al.*, 1995). The same author also reported creamy white colour ripe gonads in *H. cinerascens* and light yellow for *H. difficilis*. The male gonad was creamy white, while it was bright orange in female *P. californicus* (Cameron and Fankboner, 1986). The female gonad was dark brown and male gonad was pink in *A. africana* and it was orange, light green, white and orange respectively for *S. maculata*, *O. grisea*, *P. taiwanensis* and *P. rufescens* (Chao *et al.*, 1995). Hamel and Mercier (1996b) reported light pink male and dark red female ripe gonads in *C. frondosa*. The ripe gonads were yellow and green respectively, in *R. stephensoni* and *P. sykion* and the male gonad was translucent with yellowish tinge and female gonad was opaque white in *N. grammatus* (Foster and Hodgson, 1995). The eggs were golden yellow in *P. bullatum* and bright red in *P. chitinoides* (Mc Euen and Chia, 1991). The testis in *P. bullatum* was white and ovary was tan, and that were light pink or white and dark red respectively, in *P. chitinoides*. In *P. fragilis*, the testis was creamy white and ovary was green (Chao *et al.*, 1993b).

4.1.3.b. Histology of Gonad Maturity Stages

Like morphology, the histology of the maturity stages of *H. atra* gonad, also showed distinctive features at each stage. The phases of gonad maturation could be separated easily based on histological examinations. Histological classification was taken to confirm each stage of gonad maturity.

4.1.3.b.i. Stage I and II

Histologically, the tubules showed very minute development at this stage, like the morphological features. The primary germ layer was visible, but determination of the sex of the gametes generated from the germ layer was difficult at this stage. This was due to the fact that gametogenesis either does not taken place or it was not active. The tubules were very minute at this stage, hence it was difficult to take histological sections of the gonad. The sex was considered as indeterminate.

4.1.3.b.ii. MALE GONAD

4.1.3.b.ii.1. Stage III

A cross section of the male gonad clearly displayed gametogenesis. The primordial germ layer developed into spermatogonia, which underwent further development into spermatocytes. The secondary spermatocytes thus produced were released into the lumen of the tubule. The spermatocytes are non-motile. The process of gamete production carried on till the entire lumen got filled up with spermatocytes. The tubule wall noticed to be thicker at this stage (Plate XI).

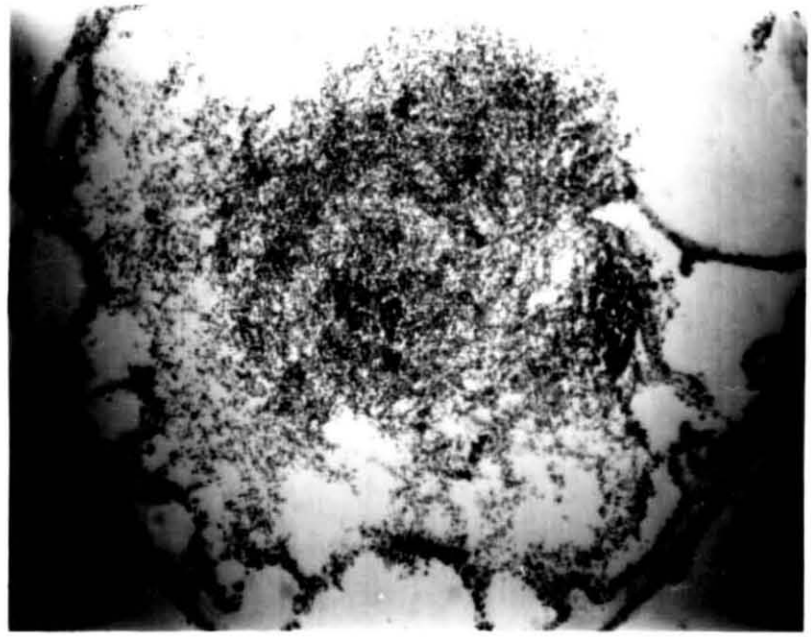


Plate XI. Cross section of maturing male gonad tubule

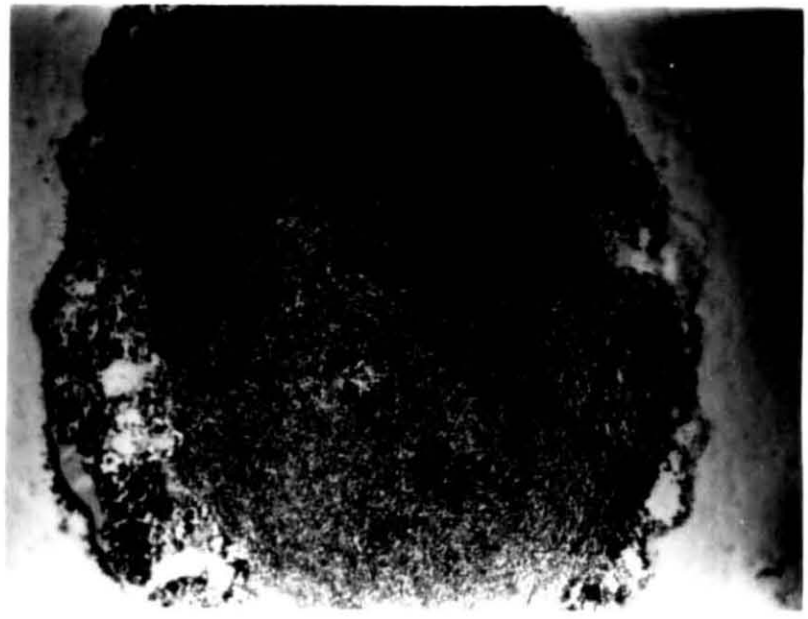


Plate XII. Cross section of ripe male gonad tubule

4.1.3.b.ii.2. Stage IV

The cross section of the ripe male gonad tubule showed a thinner tubule wall, with spermatozoa completely filling up the entire lumen (Plate XII). A smear preparation of spermatozoa with sea water clearly showed motile sperms under microscopic examination under high power.

4.1.3.b.ii.3. Stage V

The shrunken gonad had a reduced lumen, with unreleased sperms attached to the wall of the tubule. The tubule wall became thick. Sometimes, the unreleased sperms showed motility. Mostly, they were found dead (Plate XIII).

4.1.3.b.iii. FEMALE GONAD

4.1.3.b.iii.i. Stage III

The female gonad showed developing oocytes connected to the ovarian stroma in some cases. The oocytes varied in their diameters. Mostly the oocytes were circular in nature. Nucleus may or may not be present. The oocyte diameter varied at a range of 110-120 μ or even smaller. The ovary wall was thick and the oocytes do not entirely fill up the lumen (Plate XIV).

4.1.3.b.iii.2. Stage IV

The ripe female gonad showed closely packed oocytes in the lumen, which are ready for release. The mean oocyte diameter increased to 130-40 μ. The oocytes were polymodal with a definite nucleus (Plate XV). The ovary wall was visible as a thin streak. The oocytes remained free in the lumen of the tubule.

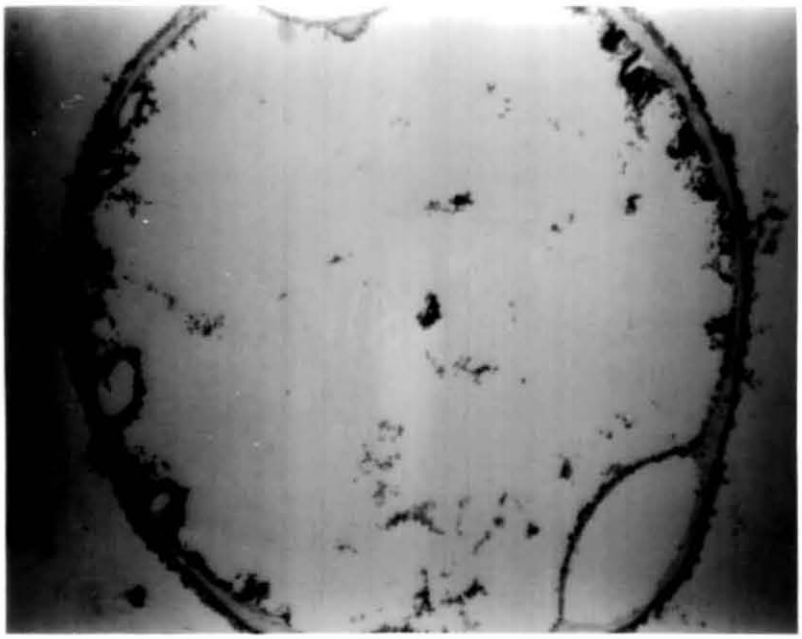


Plate XIII. Cross section of spent male gonad tubule

Plate XIV. Cross section of maturing female gonad tubule

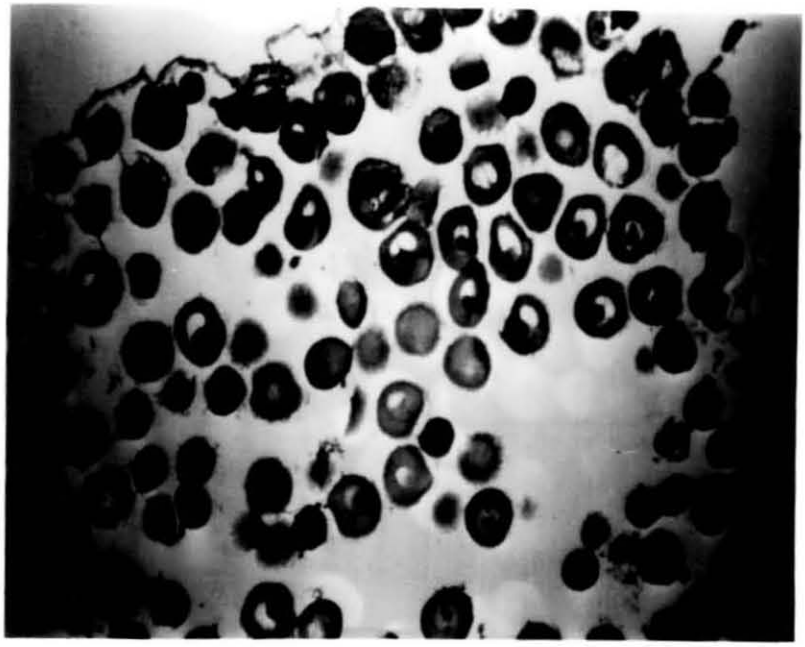


Plate XV. Cross section of ripe female gonad tubule

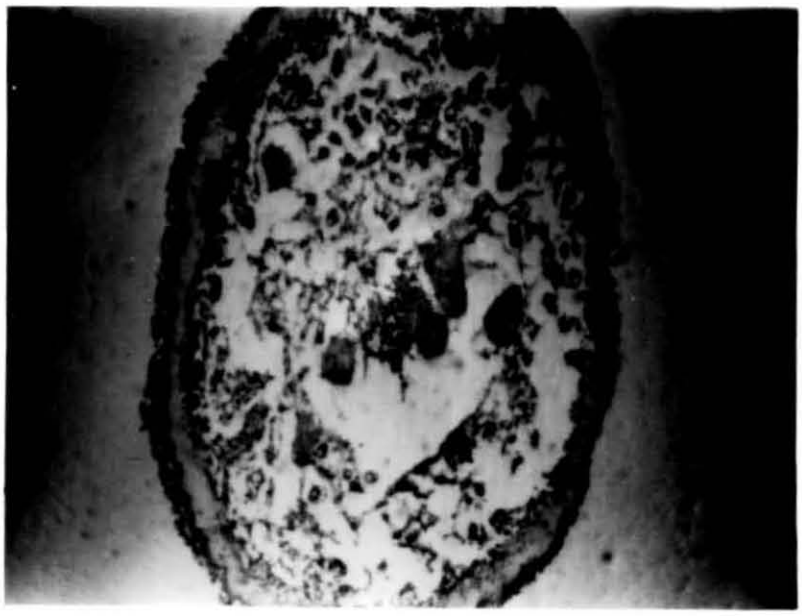


Plate XVI. Cross section of spent female gonad tubule

TABLE I a.

Morphological and microscopic features of Holothuria atra gonad at different maturity stages

STAGE – SEX	MORPHOLOGICAL FEATURES	MICROSCOPIC FEATURES
Indeterminate Stage I - **Immature** Stage II - **Resting**	Small gonad, pale and translucent. Tubules lesser in number, short, budding, unbranched or slightly branched.	Primary germ layer could be seen, but no sex cells.
Stage III - **Maturing** Male	Slightly enlarged gonad, white coloured. Tubules elongated and branched with pointed tips. Tubule number increased.	Showed non-motile secondary spermatocytes, filling up the lumen. Tubule wall is thick.
Female	Enlarged gonad, light orange in colour showing developing oocytes. Tubule branches had rounded tips and the number of tubules increased.	Developing oocytes, mostly non-nucleated, circular with a diameter of 110-115 μ, filling up the lumen. Tubule wall is thick.
Stage IV - **Ripe** Male	Gonad maximum developed, moniliform, creamy white with elongated tubules. Tubule numbers maximum.	Thinner tubule wall, lumen completely packed with fully grown, motile sperms.
Female	Gonad attains maximum size, reddish orange in colour, turgid. Number of tubules also reached maximum.	Tubule wall thin, with lumen packed full of ripe nucleated oocytes of polymodel shape with 130-135μ, diameter.
Stage V - **Spent** Male	Tubules shrunken, with gonad colour changing to pale cream. Tubules become flabby with empty saccules, retaining undischarged spermatozoa.	Reduced lumen with unreleased sperms attached to the tubule wall. Sperms are mostly dead.
Female	Gonad shrunken, flabby and became pale orange, showing empty spaces and unreleased eggs.	Reduced lumen with phagocytic residue; the unreleased oocytes, 110-140 μ, were atretic. Oocytes mostly lacked nucleus and sometimes cell wall also.

4.1.3.b.iii.3. Stage V

A reduced lumen area could be seen in the female spent gonad. The tubule wall thickened considerably. The lumen of the gonad tubule bore a few atretic oocytes, which were unreleased. The oocyte diameter varied in range 110-140 μ, and they were mostly irregular in shape. The oocytes lacked a nucleus and also an indefinite cell wall in most of the cases. Phagocytic residues were also seen dispersed in the lumen (Plate XVI).

The morphological and microscopic features of gonad at different maturity stages are compared in Table Ia.

4.1.4 MONTHLY COMPOSITION OF SAMPLED POPULATION

The sampling for the study on the reproductive cycle of *H. atra* was carried out for a period of 18 months, from November 1997 to April 1999. The composition of sampled population varied all these months. There were variations in the composition of sexes, maturity stages and also regarding the reproductive modes. The details were shown in Table I b. A major share of the population sampled comprised of animals which lacked a gonad. This is largely due to shallow water sampling. In some cases, bigger animals too lacked a gonad. In two instances, quite larger animals, had just gonad bases, swollen and devoid of gonad tubules. A similar case was reported by Chao *et al.* (1994) and in *H. atra*. These larger specimens which lacked a gonad are in the resting phase of their reproductive cycle. In *Actinopyga echinites* and *A. mauritiana* (Conand, 1993a) and *Stichopus japonicus* (Tanaka, 1958) also gonad was sometimes absent after spawning.

TABLE I b.

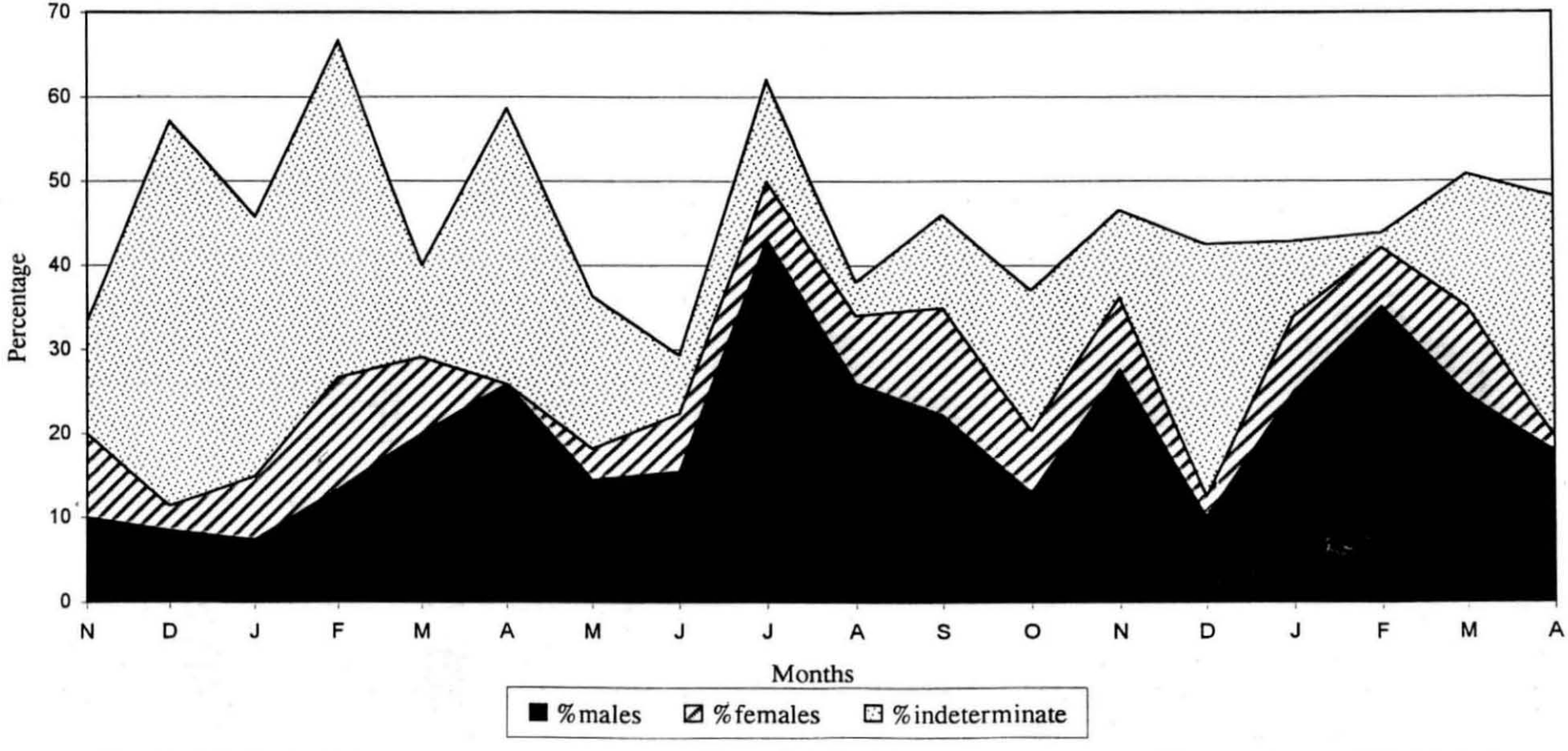
Percentage composition of monthly samples of H. atra

MONTH	TOTAL	WOG	WG	M III	M IV	M V	MALES	F III	F IV	F V	FEMALE	M+F	ID	FS
N	60	63.33	33.33	3.33	5.00	1.67	10.00	0.00	3.33	6.67	10.00	20.00	13.33	3.33
D	35	34.28	57.14	5.71	0.00	2.85	8.56	2.85	0.00	0.00	2.85	11.41	45.71	8.57
J	94	46.80	45.74	3.19	3.19	1.06	7.44	0.00	3.19	4.25	7.44	14.88	30.85	7.44
F	30	23.33	66.66	0.00	13.33	0.00	13.33	0.00	6.66	6.67	13.33	26.66	40.00	10.00
M	55	50.91	40.00	9.09	10.91	0.00	20.00	3.64	5.45	0.00	9.09	29.09	10.91	9.09
A	58	41.37	58.62	13.80	12.06	0.00	25.86	0.00	0.00	0.00	0.00	25.86	32.75	0.00
M	55	58.18	36.36	10.91	1.82	1.81	14.54	1.81	1.82	0.00	3.63	18.17	18.18	5.45
J	58	58.62	29.31	10.34	5.17	0.00	15.51	1.72	3.45	1.72	6.89	22.40	6.89	12.06
J	58	34.48	62.06	17.24	25.86	0.00	43.10	0.00	6.89	0.00	6.89	49.99	12.06	3.44
A	50	52.00	38.00	8.00	16.00	2.00	26.00	2.00	2.00	4.00	8.00	34.00	4.00	10.00
S	63	46.03	46.03	11.11	7.94	3.17	22.22	6.35	1.58	4.76	12.69	34.91	11.11	7.93
O	54	48.14	37.03	5.55	1.85	5.56	12.96	5.55	0.00	1.85	7.40	20.36	16.66	14.98
N	58	36.20	46.55	3.45	12.06	12.07	27.58	5.17	3.45	0.00	8.62	36.20	10.34	17.24
D	40	35.00	42.50	2.50	0.00	7.50	10.00	0.00	0.00	2.50	2.50	12.50	30.00	22.50
J	56	44.64	42.85	14.28	7.15	3.57	25.00	5.36	1.78	1.78	8.92	33.92	8.92	12.50
F	57	47.36	43.85	17.54	7.02	10.52	35.08	3.51	1.75	1.75	7.01	42.09	1.75	8.77
M	57	40.35	50.87	17.54	3.51	3.51	24.56	3.51	3.51	3.50	10.52	35.08	15.78	8.77
A	56	51.78	48.21	8.92	0.00	8.93	17.85	1.78	0.00	0.00	1.78	19.63	28.57	0.00
Mean	55.22	45.16	45.84	9.03	7.38	3.57	19.98	2.40	2.49	2.19	7.09	27.06	18.77	9.00
s.d.	13.13	10.15	10.11	5.52	6.76	3.82	9.63	2.20	2.21	2.31	3.68	10.62	12.76	5.69

WOG - Specimens without gonad
WG - Specimens with gonad
M III - Male specimens at stage III
M IV - Male specimens at stage IV
M V - Male specimens at stage V
F III - Female specimens at stage III
F IV - Female specimens at stage IV
F V - Female specimens at stage V
M + F- Male and female specimens
ID - Indeterminate specimens
FS - Fissions specimens

Fig. 2.

Percentage Composition of males, females and indeterminate sexes in the monthly samples of Holothuria atra



The percentage of animals without gonad was highest during November 1997 (63.33%) and least during February 1998 (23.33%). Higher percentages of animals without gonad mostly recorded during post-spawning months. These group included resting as well as newly recruited population, which were in the growing phase.

The animals with gonad comprised of males, females and indeterminate sexes. The indeterminate sexes included specimens in immature and resting stages of maturity. The percentage of animals with gonad was maximum in February 1998 (66.66%) and a minimum in June 1998 (29.31%). Moderate to higher percentage composition of animals with gonad was noticed during reproductive months.

The spawning population included males and females. The percentage of males and females varied over months (Fig. 2). But when both the sexes are considered together, it was observed that the percentage of males and females were higher during the reproductive periods, with maximum in July 1998 (49.99), and recorded a closer 42.09% in February 1999. Lower percentage of males and females were recorded during the non-reproductive period, a lowest 11.4% in December 1997 and a closer 12.5% in December 1998. The mean monthly composition males and females was $27.06 \pm 10.62\%$.

The percentage of male population also varied over months. As the samplings recorded a predominance of male population, the percentage of males were significant even during the non-reproductive periods. Males were present maximum in July 1998 (43.1%) and then recorded a high 35.08% in February 1999. They were

least in January 1998 (7.44%), with a lower percentage of 8.56% recorded in December 1997 too. Monthly a mean of 19.89 ± 9.63% of males were present in the samplings.

The female population throughout maintained a lower percentage during study period, with a mean of 7.09 ± 3.68 monthly. A maximum was recorded during February 1998 (13.33%) with closer values in September 1998 (12.69%). None were reported during April 1998, which could be due to the resting stage of gonad.

The maturing males (stage III) recorded a peak during February and March 1999 (17.54 each). Higher composition was also noted during July 1998 (17.24%) and January 1999 (14.28%). None were reported in February 1998 and the percentage was low during post-reproductive periods. The ripe males (stage IV) were maximum during November 1998 (12.07%). They were represented in mild numbers during the study period, except for some months when none were present in the samples.

The maturing females were present maximum during September 1998 (6.35%). Mild percentage of maturing females were present during many months. Ripe females were maximum during July 1998 (6.89%) and a closer 6.66% during February 1998. Spent females were maximum in November 1997 and February 1998 (6.67%). All the three stages of females were not present during certain months in the samples.

It is worth mentioning that, during some months males and on two occasions (August 1998 and February 1999) females, spawned after collection, altering the maturity stage as well as gonad index of the specimens.

The indeterminate specimens were higher in the composition of samples during non-reproductive periods. The percentage of indeterminate sexes, comprising immature (Stage I) and resting (Stage II) individuals, were maximum in December 1997 (45.71%). Higher percentage of indeterminate specimens were present during January 1998 (30.85%). February 1998 (40%), April 1998 (32.75%), December 1998 (30%) and April 1999 (40%), which were mostly non-reproductive months. The minimum of 1.75% was recorded during February 1999 and a closer 4% during August 1998. These were spawning months. Also, the percentage of indeterminate sexes were comparatively less during March 1998 (10.9%) and November 1998 (10.34%) when a rise in gonad index values were noted.

4.1.5 GONAD INDEX VARIATIONS

The gonad index is considered to be an indicator of the sexual activity of the animal. The gonad index rises with the onset of gametogenic activity, reaches a maximum when the gonad is at ripe stage, and declines after the spawning or gamete release occurred and remain low in the followed spent and resting phases.

In *H. atra*, the gonad index varied over months depicting a clear picture of the gametogenic process in the sampled population. In the present study, three types of gonad index values were estimated. The gonad index G1 was based on the wet body weight of the animal, G2 was based on the drained body weight and G3 based on

TABLE I c.

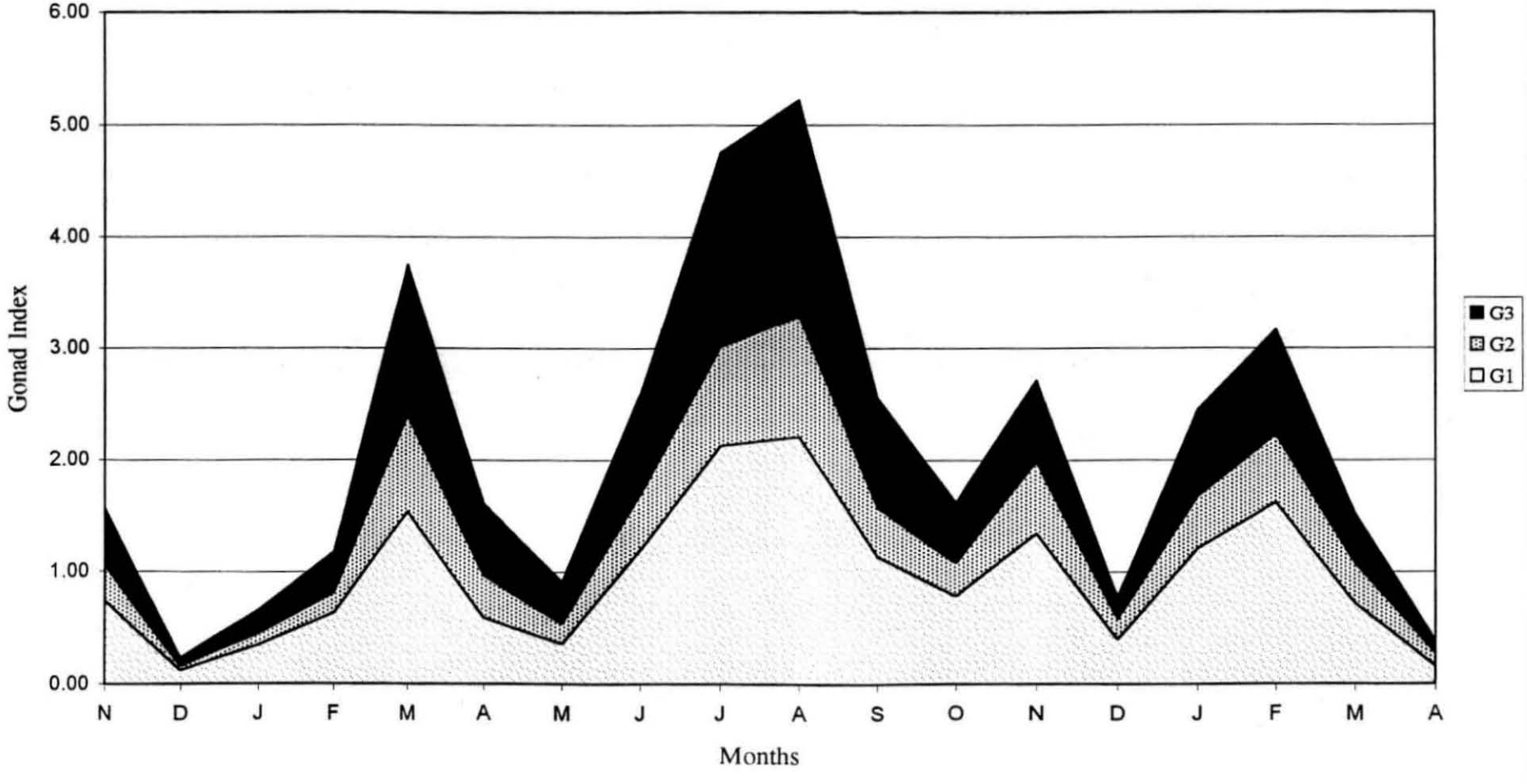
Monthly variations in gonad indices

MONTH	G1	s.d.	G2	s.d.	G3	s.d.
N	0.75	0.82	1.06	1.14	1.56	1.75
D	0.12	0.14	0.16	0.19	0.23	0.28
J	0.35	0.70	0.45	0.87	0.66	1.26
F	0.64	1.11	0.82	1.44	1.18	2.02
M	1.54	1.98	2.40	2.97	3.75	4.80
A	0.59	1.21	0.98	2.13	1.61	3.59
M	0.36	0.96	0.54	1.43	0.91	2.59
J	1.20	2.08	1.70	2.76	2.59	4.24
J	2.13	3.55	3.02	5.00	4.75	8.40
A	2.21	2.57	3.29	3.83	5.22	6.53
S	1.13	2.29	1.58	3.12	2.55	5.51
O	0.78	1.60	1.08	2.13	1.62	3.37
N	1.35	1.82	1.99	2.75	2.71	3.63
D	0.40	0.93	0.58	1.33	0.77	1.75
J	1.21	1.74	1.68	2.38	2.45	3.51
F	1.62	1.99	2.23	2.50	3.16	3.69
M	0.72	0.96	1.08	1.44	1.51	2.01
A	0.16	0.27	0.27	0.48	0.38	0.67

G1 - Gonad index with respect to the wet weight of the specimens
G2 - Gonad index with respect to the drained weight of the specimens
G3 - Gonad index with respect to the gutted weight of the specimens

Fig. 3.

Monthly gonad index variations of Holothuria atra



the gutted body weight of the animal. The three gonad indices were taken to prove the confirmatory nature of the observations. (Fig. 3). The mean monthly gonad indices were estimated by taking the gonad indices of the male, female and indeterminate sexes into consideration. This was to represent the whole population with gonad. The indeterminate population comprised the resting animals also.

Though, the three gonad indices were estimated, more emphasis was given to G1 in this study to depict the reproductive periodicity of *H. atra* because the size at first maturity and frequency distributions were estimated by taking the wet weight of the specimens. The other gonad indices, G2 and G3, does not deviate from the trend followed by the G1 values (Table. I c).

The G1 started at 0.74 ± 0.82 in the first month of sampling, November 1997. Then it slumped to a least value of 0.119 ± 0.14 in December 1997. A recovery and maturation phase followed in January 1998 and February 1998, to reach a higher G1 of 1.54 ± 1.98 in March 1998. The spent and resting phase in the following two months, brought the G1 down. Again, another maturation phase started in June 1998 and reached a maximum G1 value of 2.21 ± 2.57, showing a peak reproductive activity in August 1998. The post-reproductive period was observed with lower G1 values in the following two months. November 1998 showed a minor increment in the G1 showing some reproductive activity. December 1998 recorded lower G1 denoting the resting period. Another maturation period started in January 1999 and reached a higher index in February 1999 indicating the reproductive activity. Spent and resting stages followed in March 1999 and April 1999, recording lower G1.

The G1 was higher during March 1998, July 1998, August 1998 and February 1999. Moderate values were recorded during November 1997, June 1998, September 1998, October 1998, November 1998 and January 1999. G1 was lower during December 1997, January 1998, April 1998, May 1998, December 1998, March 1999 and April 1999. Similar trend was noticed in the case of G2 and G3, a peak G2 was recorded in the month of August 1998 (3.291 $\pm$ 383) and a least in December 1997 (0.158 $\pm$ 0.19). G3 was maximum in August 1998 (5.217 $\pm$ 6.53) and minimum in December 1997 (0.231 $\pm$ 0.27).

4.1.6 OBSERVATIONS ON SPAWNING*

Spawning of *Holothuria atra* was observed on a number of occasions in the holding tank as well as in the transporting container. Males spawned predominantly throughout the period of observation. Altogether, 35 male specimens and just 3 female specimens spawned. Only males spawned in the months of March '98, July, September, October, November '98 and April '99. Both males and females spawned during August '98 and February '99. Month-wise spawning patterns and spawning behaviour are below:

a. **March '98** : Two male specimens spawned during forenoon in the holding one tonne FRP tank. The water temperature was 30° C and salinity was 36 ppt. No peculiar spawning behaviour was noticed. The sperms were sent out as a creamy white continuous streak. The spawning lasted for ½ - 1 hour and occured three days before Newmoon (**NM-3**).

* A part of this topic has been published. Please see Annexure.

b. **July '98** : Single male specimen spawned in the transporting container at a water temperature of 27° C and a salinity of 34 ppt. No peculiar spawning behaviour could be noticed. The spawning took place during the morning hours and lasted about 15 minutes. The crowding stress during transportation might have induced spawning. The spawning occurred a day prior to Newmoon day **(NM-1)**.

c. **August '98** : A total of five males spawned at 30° C and 36 ppt salinity in the holding tank. The spawning time varied from 5-40 minutes. Some specimens lifted their anterior portions, but no side-wise swaying movements were observed. After sometime, single female specimen started spawning by discharging reddish orange eggs intermittently as strong jets. This animal showed no peculiar spawning behaviour and the spawning duration was about 3½ hours. The spawned fecundity was estimated at 13.2 lakhs. The moonphase was three days before First quarter (¼ - **3**).

d. **September '98** : Five males spawned, three during transportation and two in the holding tank. The spawning lasted ½ - 1 hour at 27-28° C and at 34 ppt salinity. The spawnings occurred respectively 4 and 5 days before and after the New Moon period (**NM-4** and **NM+5**). Two specimens showed swaying movements and one animal had two gonopores, about 3mm apart from each other.

e. **October '98** : Three male specimens spawned at 29°C and 33 ppt salinity in the holding tank during afternoon. One animal had 3 gonopores, which were 1mm apart from each other. The spawning time varied from 45 minutes to 2 hours.

Slight swaying movements with lifting of anterior end was noticed. Spawning was observed a day after Newmoon (**NM+1**).

f. **November '98** : One male spawned during transit in the container and continued in the holding tank at 30° C. The spawning lasted for 1 hour at 35ppt salinity. No peculiar spawning behaviour was noticed. Moonphase was two days prior to Last quarter (¾ **-2**).

On another occasion, two males spawned during transit and continued to spawn in the holding tank at 30° C and 35 ppt. Again, four more male specimens spawned in the holding tank One animal had two gonopores, 2mm apart from each other. A few animals lifted their anterior portion and showed swaying action. The spawning duration varied from 30 minutes to 2 ½ hours.

g. **February '99** : Altogether ten males and two females spawned at 26-27° C and 35 ppt salinity. Spawning was observed during afternoon. Spawning of males varied from 30 minutes to two hours. One male had two gonopores, situated about 1cm apart from each other. A few males showed swaying movements. Females started spawning late and lasted about 30 minutes each. Both the animals lifted their anterior portions and swayed while releasing the eggs. The spawned fecundity was estimated at about 7.3 lakhs. The moonphase was four days prior to First quarter (¼- **4**), when eight male specimens and two female specimens spawned. Later, two more males spawned on the Newmoon day (**NM**).

h. **April '99** : Two male specimens spawned at 31° C and 33 ppt salinity. One specimen had two gonopores placed about 2mm apart from each other. Spawning period varied from 30 minutes to 1 hour. No peculiar behaviour was observed during spawning, which occurred a day before Full moon **(FM-1)**.

The spawning duration of male *H. atra* varied from five minutes to 2½ hours and that of female was about ½ to 3 ½ hours. One to three gonopores were observed in males. Three gonopores were observed once and two were noticed on four occasions. Females had only one gonopore whenever they spawned.

The trend analysis of the gonad index variations of *H. atra* from November 1997 to April 1999 shows that the species has peak reproductive activity during certain months of year at Tuticorin. Monthly samplings mostly contained animals at all gonad maturity stages. But the percentage composition of such animals varied over months, and the percentage of mature animals were higher when reproductive activity increased.

It could be observed that *H. atra* at Tuticorin, got comparatively higher gonad index values during July - August, November and in February - March period. When considering the occurrence of spawning, which highly seasonal, it could be inferred that *H. atra* follows a bimodal breeding season at the place of study, during August and February. Although mature specimens were present in the samples during other months also, elevating the mean gonad index values, the act of spawning clearly defines the reproductive months of this species at Tuticorin. Each reproductive peak is followed by a resting and recovery phase, which could be very short, completed within

a month or may prolong over 3 - 4 months. Yearly variation could be there in a small scale due to the variations in environmental parameters, as observed in the shifting of a minor peak from March 1998 to February 1999. Peak reproductive period was preceded by a maturing phase and was followed by a spent phase. The occurrence and duration of maturing as well as spent phase may be related to the intensity of reproductive activity taking place. The spawning observations, especially that of female specimens, also substantiate the observations on reproductive cycle. The female specimens spawned without any stimulation only during August 1998 and February 1999, although mature females were present in the samples during certain other months also. Most of the male specimens spawned during transportation itself.

Reproductive activity is known to vary with latitude in several echinoderm species including holothurians. When the environmental factors are more stable over a wider area, the patterns of reproductive periodicity of organisms are more similar in nature. The tropical waters show minor variations in the degree of variability of reproductive periodicity and because of the constancy of environmental parameters. Different parameters have been used to calculate echinoderm gonad indices. For holothurians, total wet weight and drained weight have been most commonly used (Tanaka, 1958; Conand, 1981; Harriot, 1985; Sewell and Bergquist, 1990). Lawson (1966) reported an increase in the gonad size of holothurians as the spawning period approaches. Reproductive period or the breeding season is the time which mature fertilizable gametes are present. During sexual maturity, gonads undergo 12-35 fold increase in development (Tanaka, 1958).

In the North-West Redsea, *H. atra* had a breeding season from early July to early September (Pearse, 1968). He stated that *H. atra* appeared to spawn throughout the year when near the equator, but many have restricted spawning periods, when they occur in temperate region. Pearse also observed some ripe specimens in the population throughout the year, when they were near the equator. This view has been supported in the present study by the presence of ripe specimens even during non-reproductive periods. And, male specimens were found to spawn irrespective of reproductive seasons. Variations among individuals in reproductive activity showed little relation to size or depth difference, according to Pearse; small specimens with ripe gonad were found in shallow waters, and often very large specimens from deeper areas contained minute undifferentiated gonads. Moreover, in some specimens, new gametogenesis began even before the there was no gametogenesis followed after spawning, leading to a reduction in gonad size to a minimum. Many holothurians including *H. atra* showed absence of gonad tubules after spawning showing resorption. Conand (1981) formulated a five stage maturity scale for the gonads of holothurians. The stages were immature, resting growing, mature and post-spawning. James *et al.* (1995) recognised five stages of gonad maturity in *H. atra* , and observed a peak reproductive activity during July-August, with minor activity during January - February also, at Tuticorin. This observation closely follows the bimodal breeding season observed in the present study. James *et al.* (*op. cit.*) observed a maximum average gonad weight of 8.257g and 13.257g respectively for males and females. The gonad index reached a maximum of 10.754 and a minimum of 0.079. Hiremath and Desai (1994), recorded partial spawning of

H. atra in January and February at Karwar, South west coast of India. A complete spawning was recorded in May. The gonad indices were 6.62±0.6 for males and 6.47±0.01 for females. Chao *et al.* (1994) recognised five stages of maturity for gonad in *H. atra* and observed mature gonads from March to September in southern Taiwan. Spawning and post - spawning specimens with a wet weight of 351-1400g spawned from June to September while small specimens with less than 190g wet weight had mature gonads in May, June and September, but showed no signs of spawning. The mean weight of female gonad was 27.2±2.4g (24-26g) and that of male gonad was 20 ± 43g (14-26g). The gonad indices respectively for female was 9.43 respectively for males and females were at a maximum of 9.72±11.2 and 9.12±0.86. The mean gonad index in the month of June was 9.43±1.01. Seeto (1994) observed a main breeding period in summer for *H. atra* (September - December) in Fiji. He stated that the spawning was asynchronous and although the increase in gonad mass was periodic, the ripeness of the gonads was prolonged. Ripe oocytes were present all around the year. Conand (1993a) recorded a mean gonad weight of 5.5±5.0g and 7.4±6.4g respectively for male and female *H. atra*. Harriot (1985) observed biannual spawning of *H. atra* in summer (November) and winter (May) at Heron reef, Great Barrier reef. There were variations in gonad index with site for *H. atra* indicating that spawning was asynchronous. The mean gonad index was 2.7% for *H. atra*. *H. impatiens* spawned during late spring or summer at the same site, with a maximum mean gonad index of 2.6%. *H. edulis* at the same site had a smaller gonad index of 0.4%, and showed asynchronous spawning with no annual reproductive pattern. Harriot recognised there gonad maturity stages for all the three species studied.

A five stage maturity chart was followed by Baskar (1994) in studying the reproductive biology of *H. scabra*, which Krishnaswamy and Krishnan (1967) differentiated four maturity stages for the same species. Krishnaswamy and Krishnan observed breeding of *H. scabra* during July and October at Mandapam, South east coast of India, while Baskar observed spawning in June and October in the Gulf of Mannar region, South east coast of India, with maximum gonad indices during April to June for males and October to November for females. Krishnaswamy and Krishnan (*op. cit.*) observed a maximum gonad index of 1.95 in July, and the number of mature and gravid specimens were higher during breeding months . According to Tuwo (1999), *H. scabra* had two spawning periods in Indonesia, the first during March - April and the next during September - October. In this study, a five stage gonad maturity scale was followed. Battaglene *et al.* (1998) observed a peak reproductive period from October towards the end of dry season, in the Solomon Islands for *H. scabra*. Kandan (1994) recognised six stages of gonad maturity for *H. nobilis* in Minicoy island of Lakshadweep Islands in the Arabian sea, and observed 100% mature specimens during July and August and spawning in September. *H. leucospilota* appeared to spawn twice in a year at Goa , West coast of India, during post-monsoon (October-January) and monsoon (June-September) periods (Jayasree and Bhavanarayana,1994). Five stages of maturity were assigned for the gonads in the study. The percentage of undetermined specimens were higher during January, February and March. *H. leucospilota* got biannual spawning season in Vietnam, a spring one in February-March and a Summer one in June - August. Spring spawning is brief, while summer spawning is extended (Viet-Nam and Britaev, 1993). Tuwo and

Conand (1992) in Penfret Island recognised five stages of maturity of gonad for *H. forskali*. The development of gonad was synchronous in both sexes. Pre-spawning gonads were present maximum during January - March. Gonad indices of female specimens were observed generally higher than males. In her study, Conand (1981) observed that *T. ananas* and *M. fuscogilva* reproduced during warm reason while *M. nobilis* reproduced during cold season. Monthly percentage of indeterminate sexes were higher during July and August for *T. ananas*, in December for *M. nobilis* and in April for *M. fuscogilva*. Gonad cycle index of *A. echinites* increased for September to November-December in New Caledonia (Conand,1982). The percentage of indeterminate specimens were low from September to January while it was higher from April to August. Spawning was largely observed in January and February. Bulteel *et al.* (1992) observed occurance of spawning at the end of summer (August - September) for *H. tubulosa* in the Mediterranean. For *A. mauritiana*, the gonad indices peaked during spring and summer in Guam, depicting a bimodal breeding season. The percentage of indeterminate specimens was greatest in October (Hopper *et al.*, 1998). Although detailed studies are lacking, some tropical holothurians appear to show more prolonged or continuous spawning throughout the year as their proximity to equator increases. Spawning activity of *A. mauritiana* was highly seasonal, although mature gonads were seen throughout the samplings. Taking this case into consideration, *H. atra* has been observed to spawn only twice during the present study. So it could be better to position the reproductive cycle of *H. atra* at Tuticorin with a bimodal breeding season, during July- August and February-March. This view coincides with the pattern observed by James *et al.* (1995) at Tuticorin. Chao *et al.*

(1995) observed annual spawning period lasting two to four months in spring or summer in Taiwan for nine species of sea cucumbers. The species studied included *Afrocucumis africana* (Dendrochirota), *Actinopyga echinites*, *H. leucospilota, H. cinerascens, H. difficilis* (Aspidochirota), *Synapta maculata, Opheodesoma grisea, Patinapta taiwanensis* and *Polycheira rufescens* (Apoda).

Cameron and Fankboner (1986) observed an annual reproductive cycle with spawning in late spring through summer in British Columbia, for *Parastichopus californicus*. Spawning was partially synchronous. The gonad index based on gutted weight was highest for female than male specimens. Ripe specimens of *S. variegatus* was found from November to March. The percentage of indeterminate specimens were more in New Caledonia during February to September, which mainly comprised resting population (Conand, 1993b). It spawned during warm seasons. The gonad index was highest in December. A five stage maturity scale was followed in this study. Sewell and Bergquist (1990) recorded a synchronous gonad development in both male and female population of *S. mollis* in New Zealand. It spawned in summer, and gonad or gonad basis disappeared during winter months, due to resorption (Sewell,1992). Tanaka (1958) recognised five stage gonad maturity scale for *S. japonicus* and observed a maximum reproductive activity during June to August. He stated that fertilizable ova were available only for a short period compared to mature spermatozoa, which were present from February to the time of spawning. Arakawa (1990) observed maximum gonad development during January and February in this species. *Isostichopus fuscus* got a peak reproductive period from November to March. Absence of juveniles at the study site suggested habitat preferences between

juveniles and adults (Martinez *et al.*, 1997). Perez-Plascencia (1997) reported that, maximum gonad index was noted in April for *Parastichopus parvimensis* in Mexico. A recruitment was observed between June and July. The reproduction was synchronous for both male and female specimens.

In *R. stephensoni* and *P. sykion*, the gonads never regressed completely after spawning, while it regressed in *N. grammatus* (Foster and Hodgson, 1995). Gonad index values does not varied much between males and females in *R. stephensoni* and *P. sykion*. These two species had well defined reproductive cycles in which there was a brief resting phase followed by long gametogenic and maturity periods. Maximum gonad growth was observed form May to August and spawning between December and January. *N. grammatus* had longer resting phase, long gametogenic and a short maturity period in October - December.

Spawning of *Cucumaria frondosa* was observed in mid - June in the laboratory as well as in the St. Lawrence estuary, Canada by Hamel and Mercier (1995b) and noted that recruitment of the same species was highest in June in the Gulf of Maine (Dorothy and Miles, 1997). Gametogenesis in St. Lawrence estuary was observed in early winter. Five gonad maturity stages were recognised by Hamel and Mercier (1996b). *Cucumaria pseudocurata* spawned during early winter (January) (Rutherford, 1973). Fish(1967) observed stage III eggs in *C.elongata*, but no reproduction. Costelloe (1985 and 1988) observed spawning of *Aslia lefevrei* between January and May in North west Spain. He followed a five stage gonad maturity chart and found that gonad index was maximum in February. The species spawned during

evening, night or early morning. The spawning behaviour was typical with lifting of anterior end and swaying movements prior to gamete release. Pearse (1908) and Colwin (1948) recorded the spawning behaviours of *Thyone briareus,* by lying horizontal and with waving of its tentacles gently in water. The spawning took place in June in Massachusetts according to Colwin (*op. cit.*). *P. fabricii* was reported to spawn in eastern Canada during Summer (Hamel *et al., 1993).* *P. chitinoides* and *P. bullatum* recorded spawning from February through May in Washington, with a peak during April and May (Mc Euen and Chia, 1991). Tyler *et al.* (1987) suggested spawning period in *Cherbonniera utriculus* to be prior to May. *Phyrella fragilis* breeds during April-May in Taiwan, with a peak gonad index in April (Chao *et al.*,1993). Sewell (1994) and Sewell and Chia (1994) recorded spawning of male *Leptosynapta clarki* in November and December, and subsequent internal fertilisation, followed by release of juveniles of 1-2 mm in early spring (April – May). Green (1978) assigned five stages of maturity of gonad for *L. tenuis* and observed that population in North Carolina spawned twice, once in the spring and again in the fall. Tyler *et al.*(1994) observed seasonal oocyte development in *Bathyplotens natans.* Tyler and Gage (1983) observed no apparent annual seasonality in *Ypsilothuria talismani*, but observed a slight synchrony in reproductive activity between males and females.

Krishnan (1968) observed accumulation of organic components in the gonads of *H. scabra* with the advancement of maturity. Deep invaginations of the germinal epithelium of the testis are formed during the period of active spermatogenesis (January-February), but are reduced in pre-spawning *Cucumaria japonica* (Reunov *et.al.*, 1994). The onset of ovulation was marked by dissolution of junction

complexes (Smiley and Cloney, 1985) in *S. californicus*. Smiley (1988) opined that the phagocytic action in spent tubules reduces the ovarian epithelium, making the gonad shrunken and develops a rust colour in *S. californicus*. Phagocytosis of residual sperms and oocytes facilitate the accumulation of nutrients which may be used for the next gametogenic cycle (Pearse,1969). Costelloe (1985) and Hamel *et al.*(1993) suggested the thickening of gonad tubule walls after spawning, may be due to accumulation of nutrients derived from phagocytosis. Tubule wall thickening was observed in *R. stephensoni* and *P. sykion*. In *N. grammatus*, resorbing tubules were seen (Foster and Hodgson, 1995). Resorbing tubules were also observed in *L. tenuis* (Green,1978) and *Ypsilothuria talismani* (Tyler and Gage, 1983). A comparison of reproductive seasons and the mean gonad indices of different sea cucumber species are given in Table I d and Table I e respectively. The timing of reproduction is largely determined by proximate factors, for example the ability of the adults to acquire food reserves enabling them to devote energy to reproduction and by ultimate factors, like the timing of spawning such that optimal conditions are available for settlement and development.

Spawning behaviours of holothurians have been observed and studied by a number of researchers (Pearse, 1908; Colwin 1948; Cameron and Fankboner 1986; James *et al.*, 1988; Minchin 1992; Conand 1993a and b; James, 1996a; Battaglene *et al.*, 1998 and Battaglene, 1999). The spawning behaviours of all the aspidochirote sea cucumbers followed a typical pattern. It included lifting up of the anterior end of the body to attain a sigmoid shape, followed by sidewise swaying movements keeping the anterior end like the hood of a snake. The swaying movement

TABLE I d.

Comparison of reproductive seasons of different sea cucumber species

Sl. No	Species	Season	Place	Author(s)
1	*Holothuria atra*	July-August January-February	Tuticorin	James *et al.* (1995)
		May January-February	Karwar	Hiremath and Desai(1994)
		September-December	Fiji	Seeto (1994)
		Summer & Winter	Heron reef, Great Barrier reef	Harriot (1985)
		March-September	Taiwan	Chao *et al.* (1994)
		July-September	Red sea	Pearse (1968)
		July-August February-March	Tutcorin	Present Study
2	*H. scabra*	July and October	Mandapam	Krishnasamy and Krishnan (1967)
		June and October	Gulf of Mannar	Baskar (1994)
		March – April September-October	Indonesia	Tuwo (1999)
		May & November	Solomon Islands	Battaglene *et al.* (1998)
		December-February	New Caledonia	Conand (1993a)
3	*H. tubulosa*	August – September	Mediterranean	Bulteel *et al.* (1992)
4	*H. forskali*	January-March	Penfret Island	Tuwo and Conand (1992)
5	*H. nobilis*	Cold Season	New Caledonia	Conand (1981)
		July - September	Minicoy	Kandan (1994)
6	*H. fuscogilva*	Warm season (December-February)	New Caledonia	Conand (1981)
7	*H. impatiens*	Late Spring / summer	Heron reef	Harriot (1985)
8	*H. edulis*	No Pattern	Heron reef	Harriot (1985)

(Cont'd...)

Sl. No	Species	Season	Place	Author(s)
9	*H. leucospilota*	February-March June – August June- September October-January June-September	Vietnam Taiwan Goa, India	Viet-Nam and Britaev (1993) Chao *et al.* (1995) Jayasree and Bhavanarayana (1994)
10	*H. cinerascens*	April-June	Taiwan	Chao *et al.* (1995)
11	*H. difficilis*	August-September	Taiwan	Chao *et al.* (1995)
12	*Actinopyga mauritiana*	Spring & Summer	Guam	Hopper *et al.* (1998)
13	*A. echinites*	January-February June – July	New Caledonia Taiwan	Conand (1982) Chao *et al.* (1995)
14	*Stichopus japonicus*	June – August	Japan	Tanaka (1958)
15	*S. variegatus*	November-March	New Caledonia	Conand (1993b)
16	*S. mollis*	Summer	New Zealand	Sewell (1992)
17	*Isostichopus fuscus*	November-March		Martinez *et al.* (1997)
18	*Parastichopus californicus*	Late Spring-Summer	British Columbia	Cameron and Fankboner (1986)
19	*P. parvimensis*	April	Mexico	Perez-Plascencia (1997)
20	*Thelenota ananas*	Warm season	New Caledonia	Conand (1981))

TABLE I e.

Comparison of peak gonad indices of different species of sea cucumbers

Species	GI	Author
Holothuria atra	6.6212 ± 0.6 (M) 6.4703 ± 0.01 (F)	Hiremath and Desai (1994)
	2.7 (F)	Harriot (1985)
	3.3 ± 2.6 (M) 3.3 ± 1.4 (F)	Conand (1993a)
	10.754 (Max.) 0.079 (Min.)	James *et al.* (1995)
	0.037 ± 0.064 (Min.) 2.995 ± 0.027 (M) 4.486 ± 3.706 (F)	Present Study
	9.12 ± 0.86 (F) 9.74 ± 1.2 (M)	Chao *et al.* (1994)
H. scabra	5.4 ± 2.4 (M) 7.2 ± 3.8 (F)	Conand (1993a)
H. leucospilota	0.29 (M) 0.38 (F)	Jayasree and Bhavananarayana (1994)
H. nobilis	2.89 ± 2.14 (M) 5.04 ± 3.42 (F)	Conand (1981)
	3.5 ± 2.1 (M) 5.3 ± 3.0 (F)	Conand (1993a)
H. scabra versicolor	4.0 ± 1.7 5.8 ± 2.8	Conand (1993a)
H. fuscogilva	0.83 ± 0.8 (M) 2.18 ± 1.74 (F)	Conand (1993a)
H. fuscopunctata	2.2 ± 1.1 (M) 4.0 ± 2.2 (F)	Conand (1993a)

(Cont'd...)

Species	GI	Author
H. edulis	1.3 (F)	Harriot (1985)
H. impatiens	2.6 (F)	Harriot (1985)
H. forskali	29.44 - 30.54 (M) 38.95 - 41.87 (F)	Tuwo and Conand (1992)
H.echinites	6.07 ± 3.10 (M) 7.84 ± 5.29 (F)	Conand (1982)
	6.9 ± 3.6 (M) 9.0 ± 4.7 (F)	Conand (1993a)
Actinopyga mauritiana	7.1 ± 4.2 (M) 7.2 ± 4.2 (F)	Conand (1993a)
	14.0 ± 5.4	Hopper *et al.* (1998)
Thelenota ananas	1.124 ± 0.525 (M) 1.584 ± 1.034 (F)	Conand (1981; 1993a)
Stichopus variegatus	2.8 ± 1.19 (M) 2.7 ± 1.5 (F)	Conand (1993a)
Neostichopus grammatus	2.56	Foster and Hodgson (1995)
Roweia stephensoni	2.78	Foster and Hodgson (1995)
Pseudocnella sykion	1.13	Foster and Hodgson (1995)
Aslia lefevrei	18% (M) 17% (F)	Costelloe (1985)
Phyrella fragilis	0.32	Chao *et al.* (1993)

may be lacking in some cases. The males spawn first, releasing the gametes in a continuous streak, followed by females, which release the eggs in a series of short powerful bursts. The observations made in *H. scabra* (James *et al.*,1988; Battaglene *et al.*, 1998 and Battaglene, 1999), *H. atra* (James, 1996a), *H. fuscogilva, A. mauritiana* and *A. miliaris* (Battaglene, 1999), *P. californicus* (Cameron and Fankboner,1986) and *S. variegatus* (Conand,1993b) supports this pattern. Conand (1990) observed the spawning *of A. miliaris, Bohadschia similis* and *Holothuria flavomaculata* with similar behavioural patterns. *H. atra* in the present study also observed to spawn in the acclimation tank with these typical behavioural characteristics. Pearse *et al.*(1988) observed spawning of *P. californicus* in August, a day after full moon in British Columbia. Altogether, three males and one female spawned. *Polycheira rufescens* in Japan spawned on day one or two before every full and new moon in the period from the second half of July until August or October (Kubota and Tomari, 1998). *H. atra* females spawned twice during the present study, on both occasions 3-4 days prior to first quarter. Even on other occasions, the ripe females present in the samples did not spawn. But males spawned irrespective of reproductive period or moon phase. Multiple gonopores were reported in *P. chitinoides* (4-19), *C. miniata* (upto 9), *C. piperata* (upto 7), *C. fallax* (5-6), *C. lubrica* (upto 4) and *Pentamera populifera* (upto 7), all in male specimens by Mc Euen(1988). Duration of spawning varied with species. Mc Euen opined that tentacle movements were characteristic of dendrochirotids and not aspidochirotids. Heaviest spawning of *P. bullatum* was observed by Mc Euen and Chia (1991) in April

and May. Spawning of *Aslia lefevrei* in the wild resulted in the occurance of pentactulae after seven days of fertilization (Costelloe,1988).

4.1.7 INDUCED SPAWNING AND ARTIFICIAL FERTILIZATION

Induced spawning was tried by the candidate in *H. atra* by way of thermal stimulation during July 1998 and August 1998. The animals were directly transferred to sea water of temperature higher by 4-5°C. But on both the occasions the animals failed to respond to the stimulus. Moreover, spawning of males specimens was observed predominantly on a number of occasions, without any inducement. They spawned spontaneously on a number of occasions while on transportation.

Artificial fertilisation was also tried by the candidate as an alternative method for larval rearing. It was done by stripping the male and female gonads into a dry container. The gametes were stripped and mixed well to effect fertilisation. This method was tried during July 1998, August 1998 and February 1999. In all the cases the fertilisation rate was very poor and the larvae developed were deformed. In July 1998, a few eggs developed into pre-auricularia stage after 24 hours, but failed to become auricularia larvae. In August 1998 and February 1999, the eggs perished without development after 24 hours.

4.1.8 LARVAL REARING TRIALS

In August 1998, both males and females spawned at 30° C. The spawning fecundity was estimated at about 1,320,000 eggs. The eggs reached blastulae stage after 15 hours of development. Of these, about 2,50,000 eggs at the blastula stage were separated and kept in a 100 L FRP tank @ 3 blastulae/ ml. Since it was

difficult to rear a large number of larvae in the hatchery, only 2,50,000 eggs in the blastula stage were taken for further rearing. The blastulae showed a characteristic clock-wise rotation. After 24 hours, the eggs developed to pre-auricularia stage. On the third day, the larval rearing tank showed infestation of copepods as well as ciliates. Efforts to control them proved futile and ultimately it resulted in the mortality of most of the auricularia larvae. The eggs reached auricularia stage after a period of 36-48 hours. The survival declined to about 25% on the third day, which further declined the next day to nil.

In February 1999 too, both males and females spawned and the spawned fecundity of both the females was estimated at 7,30,000 eggs. Here, the fertilisation rate was very low at 25% . This could have been due to a prolonged time lag between the spawning of males and females. All the pre-auricularia perished by the third day due to copepod infestation. In both the trials, microalga *Isochrysis galbana* was given as food. Copepods seemed to prey upon larvae and consume the algal food given to them.

James (1996a) described early development of *H. atra* at Tuticorin. Well developed auricularia were attained on several occasions. A pentactula was also observed on one occasion. James *et al.* (1988) could successfully induce spawning by way of thermal stimulation in *H. scabra* and reared the larvae in the hatchery up to juveniles. Ramofafia *et al.* (1995) induced spawning in *H. atra* by way of heat stress (raising 2-3°C) at Solomon islands. Larvae were fed with *Tetraselmis* sp. algae, Frippak feed and Lelco yeast. Doliolaria was attained on the 20th day and by 30th day,

all the Doliolaria larvae died. Battaglene *et al.* (1998) and Battaglene (1999) induced spawning by thermal stimulation (raising 3-5°C) in *H. scabra* in the Solomon Islands. The pentactulae settled in 12-14 days at 28°C. Battaglene (1999) could also induce spawning in other species like *H. fuscogilva*, *A. mauritiana* and *A. miliaris*, by adding dried algae to static sea water. According to Cameron and Fankboner (1989) the larval cycle in *P. californicus* lasted about 66 -127 days. *S. japonicus* reached juvenile stage in 22 days (Arakawa,1990). Hamel and Mercier (1996a) observed development of fertilised eggs of *C. frondosa* into pentactulae after nine days of development. In *L. clarki,* the fertilised eggs reached pentactula stage in two weeks. Settlement was achieved in *P. chitinoides* in twelve days (Mc Euen and Chia,1991). They could also settle *P. bullatum* in 25 days.

Ito and Kitamura (1997 and 1998) induced metamorphosis of *S. japonicus* larvae using periphytic diatoms for mass production. The larvae reached pentactula stage in 13-14 days. Artificial induction of oocyte maturation was tried with success in *H. leucospilota* and *H. pardalis* and after insemination the eggs developed to typical auricularia larvae (Maruyama, 1980).

In the present study, the fouling of the seawater with ciliates and copepods, created problem for the successful rearing of larvae of *H. atra*. The adults spawned without any induction in the holding tanks.

4.1.9 SEX RATIO

During the samplings made, it was found that the sampled population comprised predominantly male specimens when compared to females. In April 1998,

the female population was not represented in the samplings. This could be attributed to the resting phase of the animals. Moreover, the number of indeterminate sex was much higher during the same month. Altogether, there were 200 males and 71 females in the total 994 animals sampled. The observed sex ratio was 2.9 : 1.

To test the significance of the observed sex ratio with respect to the expected one, Chi-square test was performed, and it revealed that during the months of April 1998 (15.0), July (15.21), August 1998 (4.76), November 1998 (5.76), May 1999 (4.26), February 1999 (10.67) and April 1999 (7.36), it was significantly higher than the expected ratio (3.48) at N -1 degrees of freedom.

To test the homogeneity of the sex ratio over the months sampled, Chi-square test was performed and it was found that Chi-square value at 17 degree of freedom (N=18) at 5% level of significance was 27.587, which was significantly higher than the observed value of 19.78. Hence it could be inferred that, there is no significant variation in the sex ratio of the sampled population between months, and the population does not deviate from the expected sex ratio of 1:1.

Viet-Nam and Britaev (1993) observed no hermaphroditism in *H. leucospilota* and at Nha Trang Bay, a sex ratio of 0.87 :1 was observed for females to males. Males and females were 35.9% and 43.5% respectively in *H. leucospilota* (Jayasree and Bhavanarayana,1994). A sex ratio of 1:1 was observed for *H. forskali* (Tuwo and Conand, 1992), *A. echinites* (Conand,1982), *C. frondosa* (Hamel and Mercier,1996b), *P. californicus* (Cameron and Fankboner, 1986), *S. variegatus* (Conand, 1993b), *R. stephensoni*, *P. sykion* and *N. grammatus* (Foster and Hodgson, 1995), *P. fabricii*

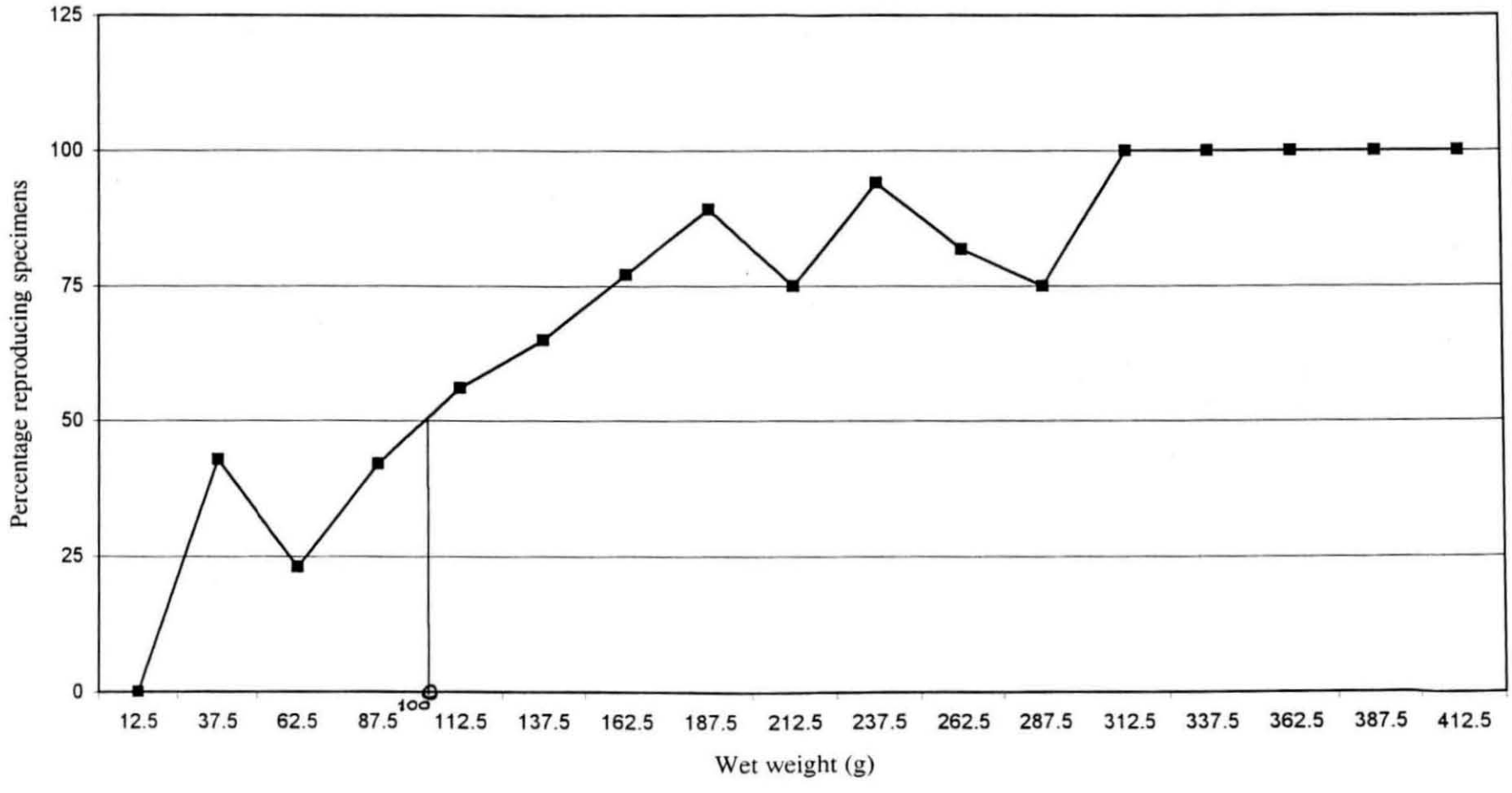
(Hamel *et al.*, 1993), *P. fragilis* (Chao *et al.*,1993) and *A. lefevrei* (Costelloe, 1985 and 1988). Of these, the number of females were more in *P. fabricii*, *C. frondosa* and *S. variegatus*, while the number of males exceeded the female population in *A.lefevrei*. Conand (1981) observed a male to female ratio of 1.08:1.0 for *T. ananas*, 1.02:1.0 for *M. nobilis* and 1.14:1.0 for *M. fuscogilva* in New Caledonia. *H. scabra versicolor* also showed slight deviation in sex ratio at New Caledonia (Conand, 1993a). Although *L. clarkii* showed protandry, the sex ratio was observed to be 1:1 for specimens above 500mg total weight (Sewell, 1994). Critical size for sex change in *L. clarki* was at a range of 200-400mg. Kandan(1994) observed a sex ratio of 1:1 for *H. nobilis* at Minicoy island. Baskar (1994) observed an average sex ratio of 1:0.89 for males to females, although it was 1:1 during the breeding season for *H. scabra*. It could be inferred that, the deviation in the sex ratio of *H. atra* observed in the present study was a temporary phenomenon, as observed in other species also. The statistical analysis approved this view and the population maintain the normal sex ratio of 1:1.

4.1.10 WEIGHT AT FIRST MATURITY

The percentage of specimens, with gonad at either III, IV or V stage of maturity was plotted graphically against mid-value of wet weight size classes, and the point at which 50% of the specimens possessed such gonad was considered as the weight at first maturity. The specimens included males and females in the maturing, ripe or spent stage of maturity. In the present study, *H. atra* was found to reach maturity at 100 g in terms of the wet weight of the body (Fig. 4).

Fig. 4.

Weight at first maturity (g) for H. atra



4.1.11 FECUNDITY

Absolute fecundity (Fa) is defined as the number of oocytes of the principal mode in the pre-spawning or ripe stage of the gonad. Oocytes in the stage IV are considered to be equivalent to the oocytes spawned in one reproductive season.

Altogether, 18 ripe female specimens of *H. atra* were dissected for their gonads, to estimate the absolute fecundity, which are given in Table I g. The absolute fecundity of these specimens varied from 7,45,650 to 14,787,900 eggs, with a mean of 3,175, 938 eggs. The weight of ripe female gonads, which used for fecundity estimations, varied from 2.265 g to 40.52 g, with a mean of 9.833 g. The mean wet weight of the animals studied for fecundity was 182 (181.4)g with range from 122 to 258 g.

Relative fecundity (Rf) is the ratio of absolute fecundity of a specimen to the weight of the ovary or to its gutted weight, expressed in terms of the number of oocytes per gram of ovary or number of oocytes per gram of gutted weight respectively. In the present study, the relative fecundity was estimated based on the gutted body weight. It was estimated by dividing absolute fecundity (Fa) by mean gutted body weight (Wg). The mean absolute fecundity (Fa) was 31.76×10^5 oocytes and the mean gutted body weight was 84 g. The relative fecundity (Rf) was calculated as 3.78×10^4 oocytes per gram of gutted body weight.

The absolute fecundity was tried to correlate with other body factors like wet weight, drained weight, gutted weight and gonad weight. The correlation coefficient was insignificant when tried with wet weight (0.420), drained weight (0.436) and

TABLE I g.

Estimated Absolute Fecundity (Fa) of ripe female

TL (mm)	Ww (g)	Wd (g)	Wg (g)	GW (g)	Fa
170	152	96	64	2.48	938200
210	240	160	90	40.52	14787900
220	138	118	90	10.16	3352140
220	196	116	66	15.72	5185250
205	176	130	72	20.30	5684390
205	180	140	100	3.20	1598129
175	142	88	62	4.32	1204796
120	140	96	64	7.50	1475864
190	246	172	118	13.26	4218540
165	182	156	104	15.63	3450520
175	250	164	122	7.42	2475740
160	250	170	120	6.56	2953460
160	122	76	50	2.46	810650
185	122	62	52	2.26	745650
220	258	160	106	3.94	1319740
150	124	86	62	6.75	2228050
195	176	120	80	7.17	2309545
195	172	116	82	7.36	2428320
Mean 184.44	181.44	123.67	83.56	9.83	3175938
s.d. 27.49	48.27	34.78	23.69	9.24	3238805

TL - Total length
Ww - Wet weight
Wd - Drained weight
Wg - Gutted weight
Gw - Gonad weight
Fa - Absolute Fecundity

gutted weight (0.192). But absolute fecundity showed significant positive correlation to the gonad weight (0.978). The correlation coefficient for gonad weight was significantly higher than the table values of 0.468 and 0.590 at 5% and 1% level of significance respectively. This relation was evident when larger gonads showed higher absolute fecundity.

TABLE VALUE		CALCULATED VALUE			
Correlation coefficient (r) at 16 d.f		Ww	Wd	Wg	Gw
5%	1%	0.420 ns	0.436 ns	0.192 ns	0.978 **
0.468	0.590				

Ww - Wet weight
Wd - Drained weight
ns - not significant
** - highly significant
Wg - Gutted weight
GW - Gonad weight

4.1.12 OOCYTE DIAMETERS

The diameter of the oocytes varied in respect to the maturity stages of the female gonad. The oocytes were smaller in diameter when they were maturing, reached maximum size when ripe, again get reduced in size when spent. To determine the mean oocyte diameter, class intervals were defined at intervals of 5.0 μ each with a range from 30.0 μ to 230.0 μ, and the frequencies at each class intervals were noted. Atleast, 50 oocytes were measured using pre- calibrated ocular micrometer to record their respective diameters at each stage of maturity.

The modal class for oocyte diameter at each stage of maturity of the female gonad is given in Table I h. At stage III, the oocyte diameter ranged from 35 μ to 180.0 μ and the maximum frequency was in 110.0 -115.0 μ. A good number of

TABLE I h.

Oocyte diameter frequencies of female gonad of Holothuria atra at different maturity stages

Oocyte Diameter (μ)	Stage III	Stage IV	Stage V
30 – 35	8	0	0
35 - 40	0	0	0
40 - 45	0	0	0
45 - 50	27	0	10
50 - 55	0	0	0
55 - 60	0	0	22
60 - 65	0	5	0
65 - 70	47	4	23
70 - 75	1	0	0
75 - 80	0	0	14
80 - 85	69	3	5
85 - 90	0	0	0
90 - 95	10	1	21
95 -100	168	25	31
100 -105	18	0	11
105 -110	7	6	7
110 -115	495	199	94
115 -120	0	0	0
120 -125	28	32	27
125 -130	21	0	19
130 -135	300	506	78
135 -140	19	38	18
140 -145	0	0	0
145 -150	75	408	45
150 -155	0	0	0
155 -160	0	31	1
160 -165	54	192	26
165 -170	0	0	0
170 -175	0	27	0
175 -180	0	0	0
180 -185	10	71	3
185 -190	0	3	0
190 -195	0	24	0
195 -200	0	11	1
200 -205	0	0	0
205 -210	0	11	0
210 -215	0	4	0
215 -220	0	3	0
220 -225	0	0	0
225 -230	0	7	0
Total Oocytes	1357	1611	456

oocytes were also there in 130.0 -135.0 μ range. A total of 1357 oocytes were measured for their diameter.

When the animal matured further, the oocytes became enlarged, and at the ripe stage (stage IV), they attained more or less polymodal shape. The oocyte diameter had a range from 62.0 μ to 230.0μ. The modal class range was at 130.0-135.0 μ and a good number were also seen at 145.0 -150.0 μ and 160.0 -165.0 μ ranges. The total number of oocytes recorded for their diameter was 1611.

The spent stage recorded fewer oocytes compared to the other two maturity stages. At this stage (V), the oocytes reduce in size, some times lacked a nucleus and a definite cell wall, because of the onset of atresia. The oocytes, 456 numbers, at spent stage had a range from 49.0 μ to 197.0 μ. The oocytes were maximum at 110.0 -115.0 μ class and a significant number was also observed to have 130.0 -135.0 μ diameter. Ovaries at the post-spawning or spent stage often contain oocytes from a smaller - diameter batch, thought to be resorbed; sometimes oocytes from the principal mode also remain (Conand, 1990).

Oocyte sizes are very important in formulating reproductive strategies of organisms. Harriot (1985) opined that *H. atra* had a large gonad index, reproduced twice a year, and gonad contained large number of small eggs. In the present study, it was found that the mean fecundity of *H. atra* was about 3.176 million eggs. The oocytes are comparatively smaller at 130 - 150μ. This observation failed to agree with the mean oocyte diameter of 88μ quoted by Harriot, but agreed with that observed by Conand (1993) at 150μ in *H. atra*. According to Harriot (*op. cit.*), *H. impatiens*

TABLE I i.

Comparison of maximum oocyte diameter and absolute fecundity of different species of holothurians

Sl. No	Species	Oocyte diameter	Absolute Fecundity	Author(s)
1	*Holothuria atra*	88μ (68 – 108)	-	Harriot (1985)
		150μ	-	Conand (1993)
		130-150μ	31.76×10^5	Present Study
2	*H. scabra*	-	$9 - 12 \times 10^6$	Conand (1990)
		190μ	17313×10^3(max.)	Conand (1993a)
			9207×10^3(min.)	
		120 -156μ	104688 - 1004160	Baskar (1994)
3	*H. scabra versicolor*	150μ	2.18×10^6	Conand (1990)
		210μ	18708×10^3(max.)	Conand (1993a)
			2296×10^3(min.)	
4	*H. leucospilota*	112.8-144.0μ	-	Viet-Nam and Britaev(1993)
		120±5μ	-	Chao *et al.* (1995)
		220μ	-	Jayasree and Bhavanarayana (1994)
5	*H.nobilis*	180μ(162-198)	931071	Kandan (1994)
		150μ	78517×10^3(max.)	Conand (1993a)
			13281×10^3(min.)	
		150μ	22800×10^3	Conand (1981)
6	*H. fuscogilva*	170μ	7350×10^3	Conand (1981)
		170μ	14219×10^6(max.)	Conand (1993a)
			6387×10^3(min.)	
7	*H. fuscopunctata*	210μ	13172×10^3(max.)	Conand (1993a)
			295×10^3(min.)	
8	*H. cinerascens*	100±10μ	-	Chao *et al.* (1995)
9	*H. diffcilis*	75±5μ	-	Chao *et al.* (1995)
10	*H. edulis*	103μ (21-128)	-	Harriot (1985)
11	*H. impatiens*	184μ(155-209)	-	Harriot(1985)
12	*H. forskali*	180μ	-	Tuwo and Conand(1992)
13	*Actinopyga echinites*	175μ	-	Conand (1982)
		165μ	25044×10^3(max.)	Conand (1993a)
			3831×10^3(min.)	
		110±5μ	-	Chao *et al.* (1995)
14	*A. mauritiana*	170μ	33790×10^3(max.)	Conand (1993a)
			23683×10^3(min.)	
		110 -135μ	33.63×10^3	Hopper *et al.* (1995)

(Cont'd...)

Sl. No	Species	Oocyte diameter	Absolute Fecundity	Author(s)
15	*Stichopus variegatus*	180μ 180μ	10×10^3 17585×10^3(max.) 7243×10^3(min.)	Conand (1993b) Conand (1993a)
16	*S. japonicus*	150μ	-	Arakawa (1990)
17	*S. mollis*	180μ	-	Sewell (1992)
18	*Parastichopus californicus*	150μ	-	Cameron and Fankboner (1986)
19	*Neostichopus grammatus*	300 -350μ	-	Foster and Hodgson (1995)
20	*Thelenota ananas*	200μ 200μ	4750×10^3 7861×10^3(max.) 2239×10^3(min.)	Conand (1981) Conand (1993a)
21	*Cucumaria ferrari*	2mm	-	Gaschen *et al.* (1993)
22	*Molpadia blakei*	200μ	-	Tyler *et al.* (1987)
23	*Cherbonniera utriculus*	200μ	-	Tyler *et al.* (1987)
24	*Leptosynapta tenuis*	200μ	-	Green (1978)
25	*Afrocucumis africana*	$30 \pm 50\mu$	-	Chao *et al.* (1995)
26	*Ypsilothuria talismani*	350μ	<50	Tyler and Gage (1983)
27	*Roweia stephensoni*	400 - 450μ	-	Foster and Hodgson (1995)
28	*Psudocnella sykion*	300 - 350μ	-	Foster and Hodgson (1995)
29	*Synapta maculata*	$70 \pm 5\mu$	-	Chao *et al.* (1995)
30	*Opheodesoma grisea*	$100 \pm 5\mu$	-	Chao *et al.* (1995)
31	*Patinapta taiwanensis*	$65 \pm 10\mu$	-	Chao *et al.* (1995)
32	*Polycheira rufescens*	$110 \pm 10\mu$	-	Chao *et al.* (1995)
33	*Psolus fabricii*	400 - 600μ	-	Hamel *et al.* (1993)
34	*P. chitinoides*	627μ	34700	Mc Euen and Chia (1991)
35	*Psolidium bullatum*	300μ	3074	
36	*Phyrella fragilis*	$350 \pm 50\mu$	-	Chao *et al.* (1993)
37	*Peniagone azorica*	300μ	1500 – 5000	Tyler *et al.* (1985)
38	*Aslia lefevrei*	250 – 340μ	-	Costelloe (1985)

reproduced only once, with a similar gonad Index and gonad containing small number of large eggs (184μ). *H. edulis* produced intermediate number of medium sized eggs (103μ). Conand (1993b) recorded an absolute fecundity of 7-12x10^3 and a relative fecundity of 9 X 10^3 per gm drained weight of the animal in *S. variegatus*. *A. echinites* had relatively large oocytes but low fecundity (Conand, 1982). The absolute fecundity ranged from 1,04,688 to 10,04,160 in *H. scabra* (Baskar, 1994) and an average of 9,31,071 was observed in *H. nobilis* (Kandan,1994). Baskar observed that fecundity and gonad weight correlated significantly.

The number of eggs was a function of body size in *C. pseudocurata* (Rutherford, 1973). The larger the animal, the greater the number of eggs produced. The fecundity ranged from one to 340 eggs. The fecundity in *C. frondosa* was 9000 mature oocytes /year for individuals of 170g. (Hamel and Mercier, 1996b). Table 1 i gives a detailed comparison of oocyte diameter and fecundity of different species of sea cucumbers. In the present study, only gonad weight was found to have any correlation to the absolute fecundity and not to any other morphological parameters.

4.1.13 WET WEIGHT FREQUENCY OF *Holothuria atra*

The wet weights of sampled *H. atra* specimens varied from 14 to 402g. Wet weight of the sampled specimens were arranged in classes of 25 g intervals and the frequency at each class was studied. The range given was from 1 g to 425 g (Table 1j). The sampled specimens were classified into Normal (N), Fission (F), Specimens with Gonad (WG), Specimens without gonad (WOG) and indeterminate (ID). The specimens

TABLE I j.

Wet weight distribution of sampled Holothuria atra

Ww (g)	N	WOG	WG	Male	Female	ID
1-25	10	10	0	0	0	0
26-50	89	82	7	3	0	4
51-75	157	118	39	6	3	30
76-100	211	104	107	32	13	62
101-125	123	57	66	28	9	29
126-150	113	33	80	43	9	28
151-175	59	11	48	28	9	11
176-200	53	16	37	23	10	4
201-225	26	6	20	8	7	5
226-250	19	2	17	10	6	1
251-275	13	2	11	8	1	2
276-300	9	1	8	5	1	2
301-325	6	0	6	5	1	0
326-350	3	1	2	2	0	0
351-375	1	1	0	0	0	0
376-400	1	0	1	0	1	0
401-425	1	0	1	0	1	0

Ww - Wet weight of specimen
N - Normal specimens
WOG - Specimens without gonad
Wg - Specimens with Gonad
ID - Indeterminate specimens

with gonad included males, females and indeterminate sexes. The weight frequency of fission specimens (F) is discussed in the Chapter on asexual reproduction.

The normal specimens showed a range form 14 to 402 g, with a maximum frequency in the 76-100 g class. The specimens without gonad showed a range from 14 to 368 g with a modal class of 51-75 g; and those with a gonad showed a maximum frequency in 76-100 g class. The wet weight range for specimens with gonad was form 36 to 402g. The indeterminate specimens also had a similar modal class with a range form 40 to 284 g. The wet weight of male specimens ranged from 36 to 330 g and the female specimens had a range from 64 to 402 g.

Kandan (1994) observed a length range of 200 – 390mm and a weight range of 500 – 2000g for *H. nobilis* in Minicoy island.

4.1.14 GUT – GONAD RATIO

This is the ratio of the gut to gonad. The gut- gonad ratio varied at each reproductive stage in males, females, as well as indeterminate specimens. The gut-gonad ratio was maximum in indeterminate specimens (2439.92). The maturing male specimens had a gut-gonad ratio of 209.14 and ripe males recorded a lower 10.37. The spent males recorded a higher gut-gonad ratio of 176.93. In female, the maturing animals had a gut-gonad ratio of 37.27, while ripe females recorded a least of 5.61. The spent females recorded a higher gut-gonad ratio of 306.73. The mean gut-gonad ratio for maturing specimens of gonads of both sexes, was 123.21 and that for ripe, specimens, was 7.99, but in was 243.33 for spent stage. It could be seen that the gut-

gonad ratio decreased with the maturation process, till the spawning. It increased afterwards in the spent stage.

4.1.15 RELATION OF ENVIRONMENTAL PARAMETERS TO REPRODUCTIVE CYCLE.

The mean values of temperature, salinity, pH and dissolved oxygen recorded during the sampling period from November 1997 to April 1999 is given in Table I k. water temperature recorded a maximum of 33°c during April 1998 and a minimum of 25°c during December 1998 and January 1999. When reproductive activity was there, the water temperature fell within a range of 26 -30°c, the mean was 28 $\pm$ 2.02°c.

The salinity during reproducing months varied in a range from 33 to 36 ppt. A maximum of 36 ppt was recorded in March 1998 and the least 25 ppt was recorded during December 1997 with a mean of 33 $\pm$ 2.48 ppt.

The pH showed a little fluctuation with a range form 7.5 to 8.4. pH recorded a maximum of 8.4 in July 1998 and a minimum of 7.5 in February 1999 with a mean of 1.97 $\pm$ 0.25. Mostly it fell within 7.6 to 8.2 range during the sampling months. And the dissolved oxygen ranged from 3.54 to 9.6 mg/l. A maximum was recorded (9.6 mg/l) in December 1997 and the minimum in May 1998 (3.54 mg/l) with a mean value of 5.99$\pm$1.46 mg/l, during other months it varied between 4.12-7.3 mg/l.

Correlation analysis was done to explore a relationship of environmental parameters such as water temperature, salinity, pH and dissolved oxygen to the gonad index variations during the annual reproductive cycle of *H. atra*. It was found that only

TABLE I k.

Environmental parameters recorded monthly at Tuticorin during the sampling period.

MONTH	AT. TEMP. (°C)	W. TEMP. (°C)	SALINITY (ppt)	pH	D.O. (mg/l)
N	29	28	35	8	4.2
D	28	27	25	8.2	9.6
J	27	26	31	7.6	7.3
F	29	27	33	8.2	6.2
M	32	30	36	7.7	7.6
A	34	33	34	8	7.4
M	31	29	33	8.1	3.54
J	32	29	34	7.9	4.12
J	31	28	33	8.4	5.21
A	32	29	35	8.1	5.82
S	30	28	33	8.1	5.48
O	30	27	34	8.1	5.96
N	31	30	35	8.2	7.12
D	27	25	31	7.7	6.42
J	27	25	31	7.6	5.78
F	28	26	35	7.5	5.22
M	28	27	34	8	5.93
A	30	30	33	8.2	4.92

AT. TEMP. - Atmospheric Temperature
W. TEMP. - Water Temperature
D.O. - Dissolved Oxygen.

salinity was marginally correlated in a significant manner (0.51) to the mean value of gonad index, GI. The correlation coefficient was 0.325 and 0.418 at 5% and 1% level of significance respectively, and at N-1 degrees of freedom. It could be inferred that *H. atra* at Tuticorin preferred comparatively higher salinity range of 34 - 36 ppt to get matured and reproduce. Other parameters such as water temperature, pH and dissolved oxygen were not at all influenced the gonad index variations and the reproductive cycle.

Harriot (1985) observed spawning of *H. atra* in summer as well as winter at Heron reef. Seeto (1994) opined that the reproductive seasonality of *H. atra* was probably influenced by temperature. But in the present study, the reproduction was marginally correlated with only one parameter, the salinity. An increment in salinity could be brought by an increment in temperature, probably bringing an indirect relation to temperature also, although temperature was not correlated here in this study. Baskar (1993) also could not derive any relation between gonad activity and environmental parameters at Tuticorin and Mandapam in his study on *H. scabra*. But Krishnaswamy and Krishnan (1967) in Gulf of Mannar presumed that salinity change induced breeding in *H. scabra*. According to Kandan (1994), lower temperature and salinity influenced spawning in *H. nobilis* at Minicoy island. The spawning of *H. leucospilota* was found to be influenced by low temperature and salinity, although relationships between gonad index, temperature and salinity were not significant (Jayasree and Bhavanarayana, 1994). Tuwo and Conand (1992) found a slight correlation of gonad development and temperature in *H. forskali*. Battaglene (1999) opined that in *H. scabra*, salinity and temperature appear to provide proximal cues that synchronize and regulate the seasonal

4.2.2 FISSION OBSERVATIONS

Fission by transverse division was observed on two occasions in the holding tank. First, the animal developed a constriction on the integument at a particular point, which later deepened. The head was occasionally raised and the posterior end was held static by attaching the tubefeet firmly to the substratum. Then, the integument was stretched by a forward pull of the anterior part and the split was effected. At this moment, the visceral parts were exposed, which soon discarded.

Afterwards, the division of the body wall and internal musculature was completed, dividing the animal into an anterior and posterior parts. The whole process lasted about 12-15 hours. Crowding in the holding tank would have stimulated evisceration here.

4.2.3 CLASSIFICATION OF FISSION SPECIMENS

A clear method to classify the fission specimens of *H. atra* was given by Conand (1996). In the present study, the candidate followed the same methodology for classifying the fission material of *H. atra* with some slight modifications.

Basically the fission specimens are divided into fissioned and regenerating. A more detailed classification for fission animals is given below:

- Fissioning (**F**)
- Recently fissioned anterior part (**A**)
- Recently fissioned posterior part (**P**)
- Regenerating anterior part (**Ap**) and
- Regenerating posterior part (**Pa**)

Each group of fission specimens could be described as follows.

4.2.3. (i) Fissioning (F)

Strictly speaking, these were specimens which are undergoing fission. The tegument could be seen stretched, with viscera hanging out through the fissioning area.

4.2.3. (ii) Recently fissioned anterior part (A)

This was the fissioned anterior portion. The animal bore a fission scar at its posterior end, in many cases. Mostly the anus was not perforated. The intestine sometimes was incomplete or connected to the posterior end as a straight line. The right respiratory tree was present, and appeared regenerating. The gonad tubules remained intact in some cases, but in some other, the gonad undergone depending on the time since fission (Fig. 6).

4.2.3. (iii) Recently fissioned posterior part (P)

The anterior end bore a scare or sometimes a pore in place of mouth. No organelle connected to the mouth were seen. The intestine remained as a cut-off piece, but found attached to the anterior end as a straight line in some cases. The right respiratory tree was seen extended upto the anterior end. Gonad, when present, found floating in the coelom (Fig. 7).

4.2.3. (iv) Regenerating anterior part (Ap)

The regenerating portion could be differentiated at the posterior as a cup-like or cone-like structure. The anus was perforated, but with an undeveloped or underdeveloped cloaca. The intestine seemed to be connected with the anal part, with

A	-	Anus
C	-	Cloaca
CR	-	Calcareous Ring
I	-	Intestine
LRT	-	Left Respiratory Tree
RRT	-	Right Respiratory Tree
OT	-	Oral Tentacles
PV	-	Polian Vesicle
PB	-	Pharyngeal Bulb
VOP	-	Vesciles of Oral Podia
RCR	-	Regenerating Calcareous Ring
ROT	-	Regenerating Oral Tentacles
RPB	-	Regenerating Pharyngeal Bulb
RPV	-	Regenerating Polian Vesicle
RVOP	-	Regenerating Vesicles of Oral Podia

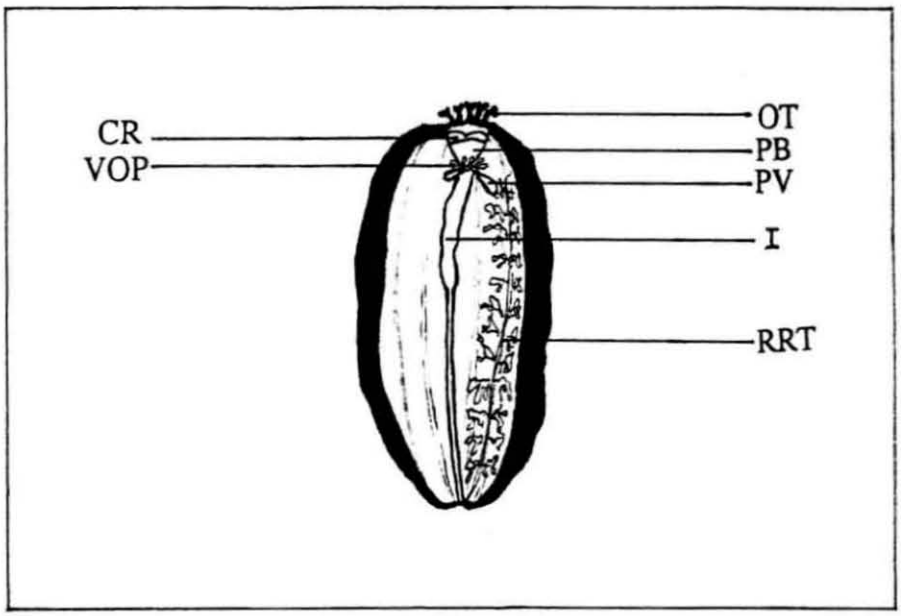


Fig. 6. Diagrammatic representation of the internal anatomy of Recently fissioned anterior part (A) of *H. atra*

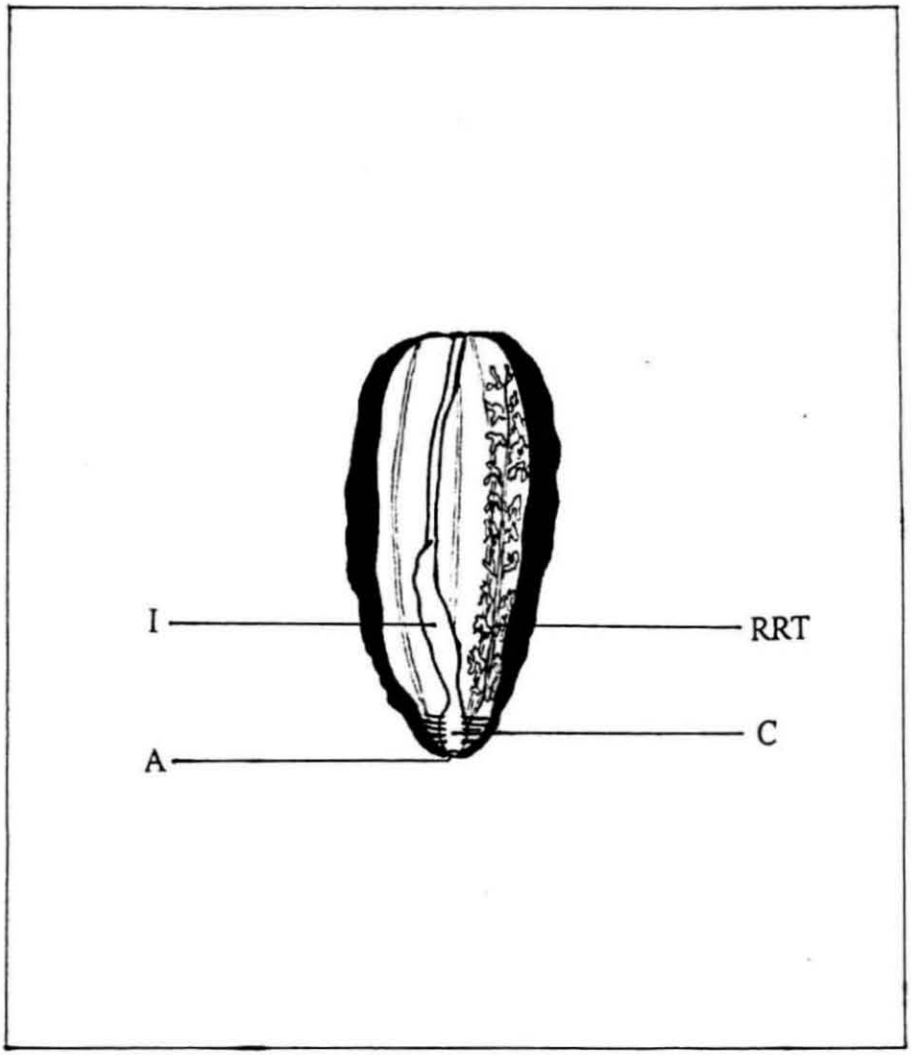


Fig. 7. Diagrammatic representation of the internal anatomy of Recently fissioned posterior part (P) of *H. atra*

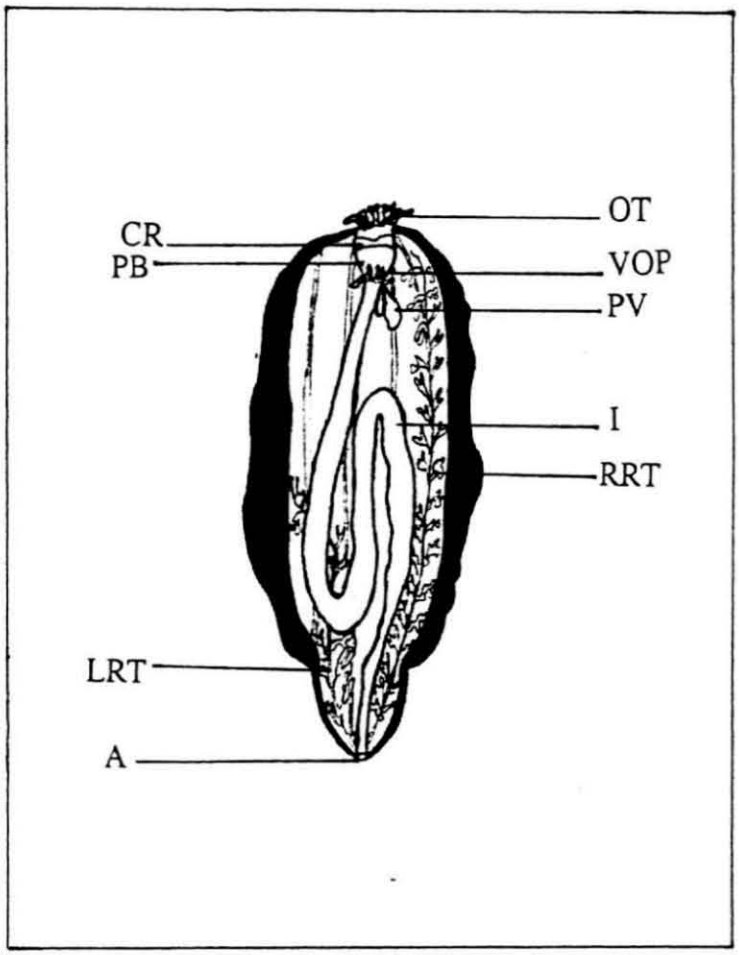


Fig. 8. Diagrammatic representation of the internal anatomy of regenerating anterior part (Ap) of *H. atra*

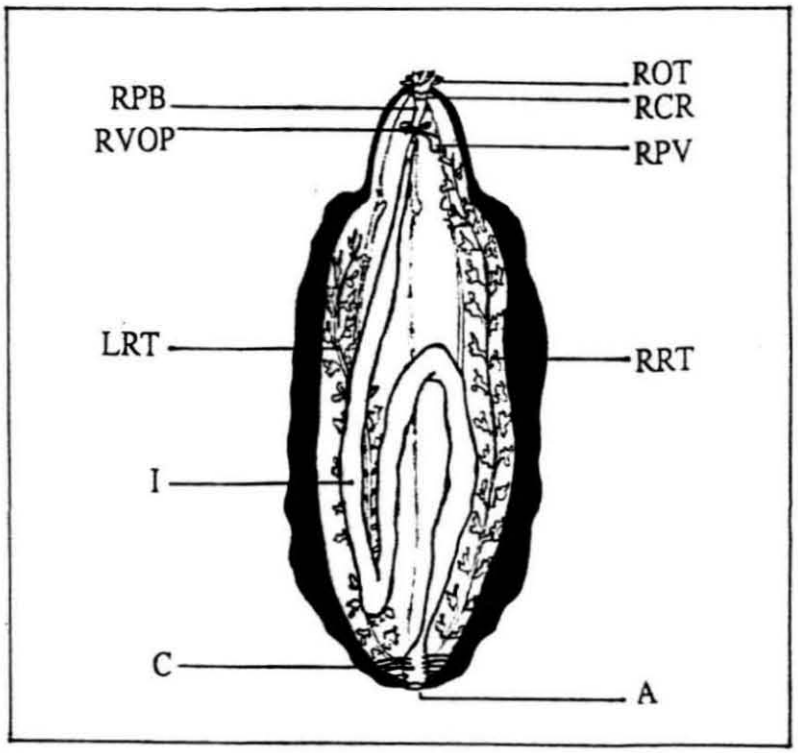


Fig. 9. Diagrammatic representation of the internal anatomy of regenerating posterior part (Pa) of *H. atra*

loops. The regenerated tegument was thinner. Respiratory trees elongated, with the right one running almost near the anterior end (Fig. 8).

4.2.3. (v) Regenerating Posterior part (Pa)

The regenerated muscle tegument was identified as thinner, extended anteriorly like a cone or a cup. The mouth was seen, with small regenerating oral tentacles. The right respiratory tree regenerated to the anterior end, while the left one regenerated to about three-fourth length. The intestine, with loops seen connected to the regenerating pharyngeal bulb at the anterior end. The pharyngeal bulb and calcareous ring were smaller than those in normal individuals, with regenerating vesicles of oral podia and polian vesicle(Fig. 9).

Of the 86 fissioned specimens, only one was observed fissioning (1.16%), 20 were recently fissioned anterior parts (23.26%), 21 each were recently fissioned posterior and regenerating anterior parts (24.42%) and 23 were regenerating posterior parts (24.74%). It could be seen that, the ratio of anterior as well as posterior parts, either fissioned or regenerating, was evenly distributed in the sampled population, thereby confirming that this mode of asexual process is a part of the population studied.

4.2.4 MONTHLY COMPOSITION OF FISSION PRODUCTS

On an average, about 9% of the total population of *H. atra* at the sampling site undergone asexual reproduction by way of fission every month. The percentage of fission specimens every month, was denoted by **S**, where $\mathbf{S = (F+A+P+Ap+Pa)}$.

The percentage composition of S and the percentage occurrence of different fission products or stages, every month were mentioned in Table II a.

Fissioning animals (F) were reported only once during the studying period, in January 1998 (1.06%). Recently fissioned anterior portions (A) were recorded a maximum in December 1998 (7.5%). Higher percentages were also reported during June 1998 (5.17%), October 1998 (5.5%) and March 1999 (5.26%). No recently fissioned anterior specimens were recorded during December 1997, February, March, April and September 1998, February 1999 and April 1999. Recently fissioned posterior parts (P) were not seen during November 1997, March 1998, April 1998, May 1998, July 1998, February and April 1999. A maximum was found during December 1998 (10%), with a closer percentage (5.17%) in November 1998 and October 1998 (5.55%).

Regenerating anterior portions (Ap) were found to be maximum during February 1999 (7.01%) with August 1998 reporting slightly higher percentage (4%). No such cases were reported during February 1998, April 1998 and April 1999. The regenerating posterior portions (Pa) were not noted during November 1997, April 1998, July 1998, October 1998, March 1999 and April 1999. A peak percentage was found in November 1998 (8.62%). February 1998, March 1998 and January 1999 recorded higher 6.67%, 7.27% and 5.35% respectively.

The anterior parts (A+Ap) recorded a maximum during December 1998 (10%) and none were reported during February 1998, April 1998 and April 1999. Higher percentage of anterior portions were also recorded during June 1998 (6.89%),

TABLE II a.

Percentage composition of fission specimens of Holothuria atra

Month	Total	N	F	A	P	Ap	Pa	A+P	Ap+Pa	S	A+Ap	P+Pa	F%	R%
N	60	96.67	0.00	1.67	0.00	1.67	0.00	1.67	1.67	3.33	3.33	0.00	0.83	0.88
D	35	91.43	0.00	0.00	2.85	2.85	2.85	2.85	5.71	8.57	2.85	5.71	1.43	2.86
J	94	92.55	1.06	1.06	2.12	2.12	1.06	3.19	3.19	7.44	3.19	3.19	2.13	1.60
F	30	90.00	0.00	0.00	3.33	0.00	6.67	3.33	6.67	10.00	0.00	10.00	1.67	3.33
M	55	90.90	0.00	0.00	0.00	1.82	7.27	0.00	9.09	9.09	1.82	7.27	0.00	4.55
A	58	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M	55	94.54	0.00	1.82	0.00	1.82	1.82	1.82	3.63	5.45	3.63	1.82	0.91	1.82
J	58	87.93	0.00	5.17	3.45	1.72	1.72	8.62	3.45	12.06	6.89	5.17	4.31	1.72
J	58	96.55	0.00	3.45	0.00	0.00	0.00	3.45	0.00	3.45	3.45	0.00	1.72	0.00
A	50	90.00	0.00	2.00	2.00	4.00	2.00	4.00	6.00	10.00	6.00	4.00	2.00	3.00
S	63	92.06	0.00	0.00	1.58	3.17	3.17	1.58	6.35	7.93	3.17	4.76	0.79	3.18
O	54	85.18	0.00	5.55	5.55	3.70	0.00	11.11	3.70	14.81	9.26	5.55	5.56	1.85
N	58	82.75	0.00	1.72	5.17	1.72	8.62	6.89	10.34	17.24	3.45	13.79	3.45	5.17
D	40	77.50	0.00	7.50	10.00	2.50	2.50	17.50	5.00	22.50	10.00	12.50	8.75	2.50
J	56	87.50	0.00	1.78	3.57	1.78	5.35	5.35	7.14	12.50	3.57	8.93	2.67	3.57
F	57	91.22	0.00	0.00	0.00	7.01	1.75	0.00	8.77	8.77	7.01	1.75	0.00	4.39
M	57	91.22	0.00	5.26	1.75	1.75	0.00	7.01	1.75	8.77	7.01	1.75	3.51	0.88
A	56	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	55.22	91.00	0.06	2.05	2.30	2.09	2.49	4.35	4.58	8.94	4.15	4.79	2.21	2.29
s.d.	13.13	5.68	0.25	2.36	2.66	1.73	2.74	4.56	3.19	5.71	2.98	4.30	2.28	1.59

August 1998 (6%), October 1998 (9.26%), February and March 1999 (7.01% each). The posterior portions (P+Pa) were maximum present during November 1998 (13.79%) with higher percentage during the months of March 1998 (7.27%), December 1998(12.5%) and January 1999 (8.93%). None were reported in November 1997, April 1998, July 1998 and April 1999.

In the present study, maximum percentage of fission products of *H. atra* were recorded during December, 1998 with a mean of 9.00 ± 5.69%. In the present study, the mean rate of fission was 2.21 ± 2.28% with the months of July 1998, and the period from October 1998 to March 1999, except February 1999, showing higher fission rates. No fission products were reported during the month of April in 1998 as well as 1999. The regeneration rate was higher than the mean value of 2.29 ± 1.59%, during December 1997, February - March 1998, August 1998 to February 1999.

The monthly incidence of fission in *H. atra* was higher from October to January and in June - July at Reunion Island (Conand, 1996). The same author also stated that fission does not result in an increase in the density of the population. Whereas, the fission of *H. atra* showed two distinct periods, first from November 1993 to June 1995, during which the rate was more than 3.7% and second, from November 1995 to November 1997, during which the rate was lower (<3.7%) with an average fission rate of 3.7% at Reunion Island on a fringing reef population (Jaquemet *et al.*, 1999). The back reef population are Reunion Island had a higher fission rate of 20% (Conand, 1996). Boyer *et al.* (1995) at Reunion Island reef, found that about 20.2% of the total surveyed population involved in asexual reproduction with a mean fission rate

of 4.7% and a mean regeneration rate of 10.6%. They noted a stable rate of fission from October to December, and the regeneration rates were on a decrease from October to February. The average monthly fission rate was 4.5% for *H. atra* in South Taiwan. The fission peaked in September and August. A maximum fission in *H. atra* population at Great Barrier Reef, Australia was noticed from May to July (1o to 26%) (Uthicke, 1997a).

A comparison of fission seasonality revealed that the seasonality of *H. atra* at Tuticorin closely resembled that at Reunion Island, as observed by Conand (1996) and Boyer *et al.* (1995) but it differed from that observed by Uthicke (1997a) and Chao *et al.* (1993a) in Great Barrier Reef and Southern Taiwan respectively. Seeto (1994) reported low fission rates for *H. atra* at Fiji. Bakus (1973) collected a specimen of *H. atra* which seems to have a bud. Many researchers reported the occurance of specimens with a double posterior end.

S. chloronotus had a seasonality in fission from March to October and *H. edulis*, between March and July at Great Barrier Reef (Uthicke, 1997a). Emson and Mladenov (1987) observed that fission in *H. parvula* was more frequent in summer at Bermuda. Viet-Nam and Britaev (1993) observed no asexual reproduction in *H. leucospilota* in Nha Trang Bay of Vietnam.

The asexual reproduction observed in the present study of *H. atra* was comparatively lesser than that observed elsewhere. A maximum between 16% and 26% observed by Uthicke (1997a) in Great Barrier Reef. The Reunion population had rates at 7.4% (Jaquemet *et al.*, 1999), 20.2% (Boyer *et al.*, 1995) and 20%

(Conand, 1996). The average monthly fission frequently in Southern Taiwan was 4.5% (Chao *et al.*, 1993a). The rate was 12.5% for *H. leucospilota* at Reunion Island (Conand *et al.*, 1997).

4.2.5 FISSION RATE AND REGENERATION RATE

Fission rate (F) and regeneration rate (R) were indicative of the asexual reproduction process undergoing in a sea cucumber population. The fission and regeneration rates, expressed as percentages, were calculated as follows in the present study.

Fission rate (F%) = **[(A+P) / 2T x 100]** and

Regeneration rate (R%) = **[(Ap+Pa) / 2T x 100]**

according to the methodology followed by Boyer *et al.* (1995) and Conand (1996). '(A+P)' corresponded to the specimens that have recently undergone fission. The fissioning specimens were also included under this category. '(Ap+Pa)' were the regenerating specimens. 'T' represented the total animals sampled.

The fission rate was the rate of asexual reproduction by way of fission undergone in the sampled population during a particular month, whereas, regeneration rate gave the rate of regeneration during a particular month, of the sample population, which have undergone fission sometime back. The rates showed minor differences over time.

The fission rate was maximum (8.75%) during December 1998 and higher rates were also observed during October 1998 (5.55%), June 1998 (4.31%), November

1998 (3.45%), January 1999 (2.68%) and March 1999 (3.51%). Fission rate was 0% during March 1998, April 1998, February 1999 and April 1999. The mean rate of fission was 2.21 ± 2.28%.

The regeneration rate was maximum in November 1998 (5.17%), Higher rates of regeneration was also recorded during December 1997(2.86%), February 1998 (3.33%), March 1998 (4.54%), August 1998 (3%), September 1998 (3.18%), December 1998 (2.5%) January 1999 (3.57%) and February 1999 (4.38%). The regeneration rates touched 0% during April 1998, July 1998 and April 1999. The mean regeneration rate was 2.29 ± 1.59%

The rates of fission and regeneration do not corresponded monthly. This was due to the fact that, regeneration was a much longer process when compared to fission. The mean fission rate per month was 2.21 ± 2.28%, while the mean regeneration rate was 2.29±1.59%. Since these values were closer, it could be inferred that the rate of fission as well as regeneration in the sampled population is more or less the same. And, the post-fission mortality is negligible. The monthly rates of regenerating individuals from the anterior (1.167%) does not differ much from that of posterior (1.278%). So, both anterior as well as posterior parts enjoyed identical survival rates after fission in the sampled population.

The rate of fission as well as regeneration in *H. atra* was also lower when compared to other populations. Conand (1996) observed a fission rate of 9.3% and regeneration rate of 9.8% at Reunion Island, whereas it was 9.5% and 10.6% respectively in the study by Boyer *et al.* (1995) and 3.7% each by (Jaquemet *et al.*,

1999). Fission rate was 5.2% and regeneration rate was 4.3% for *H. leucospilota* (Conand *et al.*, 1997).

4.2.6 PRESENCE OF GONAD IN FISSION PRODUCTS

Fissioned specimens comprised of animals with and without gonad. There were males, females and indeterminate specimens. Of the total 86 fission specimens collected, 61 had no gonad. There were 13 males, six females and six indeterminate specimens. There were six males with maturing gonads, three with ripe gonads and four with spent gonads. Seven females had ripe gonad, while one each had maturing and spent gonads. More or less every stage of the fission specimens showed the presence of gonad. A total of 25 specimens were found to have a gonad, accounting to 29.06.% of the total fission specimens.

The single fissioning specimen recorded, had a ripe female gonad weighing 5.637g. The anterior fissioned and regenerating parts had gonad of different maturity stages, which remained intact in the coelomic cavity. Recently fissioned anterior parts (A) had gonads on nine occasions, seven male and two indeterminate. No female gonads were seen.

The regenerating anterior parts (Ap) also had gonads on seven occasions. There were three males, one female and three indeterminate specimens. The posterior parts had gonad, always floating in the coelomic cavity. Sometimes, only a few tubules or a part of the gonad was observed. The rest of the gonad was assumed to be eviscerated out or locked up in the anterior part. Eight recently fissioned posterior parts (P) bore gonads, comprising of three males, three females and one indeterminate

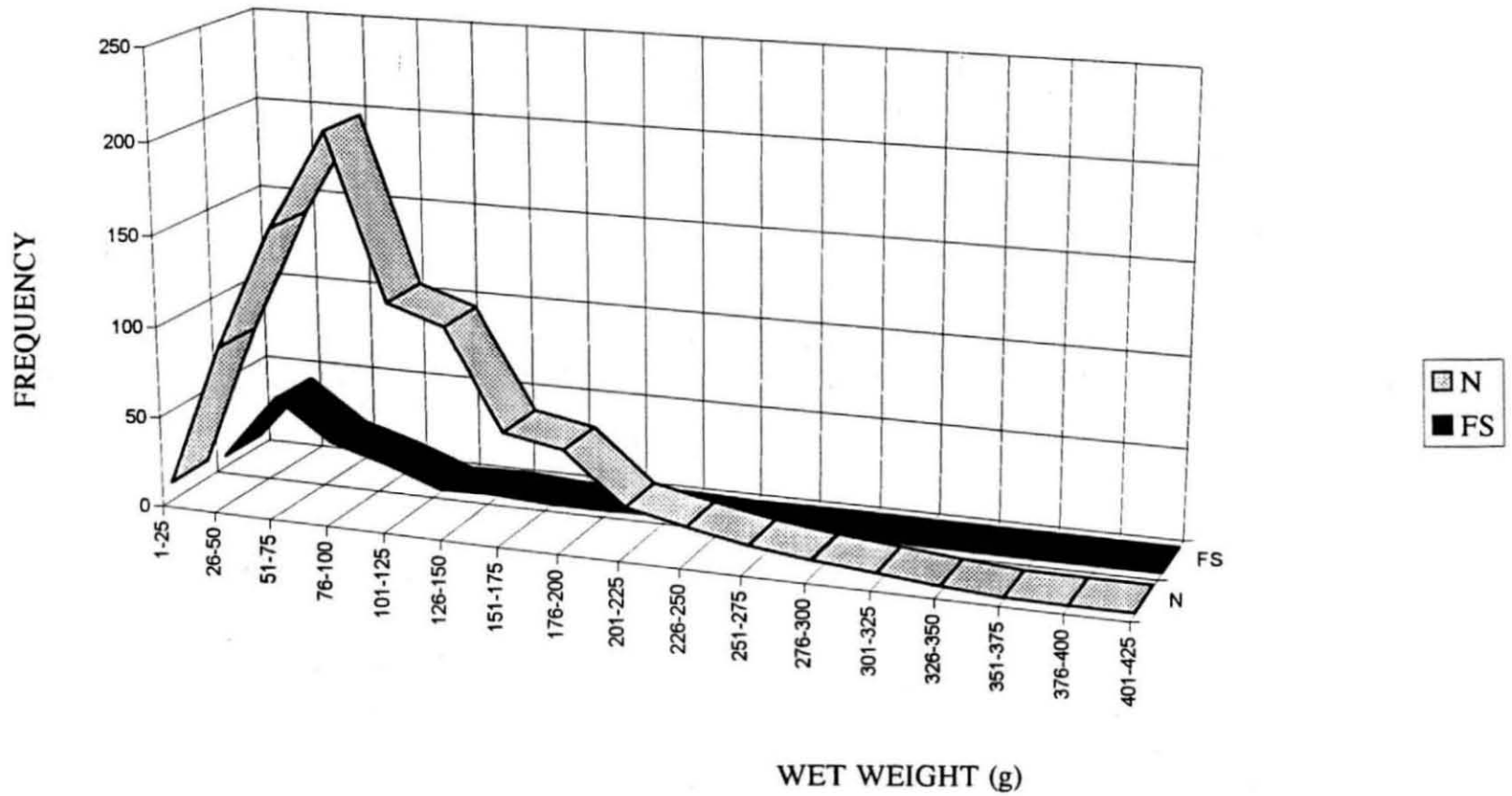
TABLE II b.

Comparison of wet weight distributions of normal (N) and fission specimens (FS)

Ww (g)	mid	N	FS
1-25	12.5	10	6
26-50	37.5	89	43
51-75	62.5	157	21
76-100	87.5	211	12
101-125	112.5	123	1
126-150	137.5	113	2
151-175	162.5	59	0
176-200	187.5	53	0
201-225	212.5	26	1
226-250	237.5	19	0
251-275	262.5	13	0
276-300	287.5	9	0
301-325	312.5	6	0
326-350	337.5	3	0
351-375	362.5	1	0
376-400	387.5	1	0
401-425	412.5	1	0

Fig. 10.

Comparison of wet weight frequencies of normal (N) and fission specimens (FS)



sex. One female gonad, retrieved entirely was ripe and had an absolute fecundity of 14,75,800 eggs. The regenerating posterior part (Pa) had gonad on just one occasion, a ripe female. In other such specimens, the gonads might have undergone, due to prolonged isolation.

4.2.7 WET WEIGHT FREQUENCY OF FISSION SPECIMENS

The wet weight of fission specimens of *H. atra* were recorded and their frequency at each weight class were plotted (Table. II b). The fission specimens had a wet weight range with a minimum of 16 g and a maximum of 206 g. The modal class for wet weight of fission specimens was at 26-50 g, where maximum frequency was recorded. A comparison of wet weights of normal and fission specimens is given in Fig. 10.

In the present study, the wet weight of fission specimens varied from 16 g to 206 g with a maximum number at 26-50 g class. Conand (1996) observed a range from 40 g to 310 g with modes at 30, 90 and 120 g for *H. atra* at Reunion Island. A decrease in wet weight mode was noticed by Jaquemet *et al.*(1999). Chao *et al.* (1993a) recorded a wet weight range from 6 g to 182 g for *H. atra* from Southern Taiwan. The modal weight for *S. chloronotus* fission specimens were 15 g (Conand *et al.*, 1998). The modal class was also low in fission population of *H. parvula* (Emson and Mladenov, 1987).

4.2.8 WET WEIGHT DISTRIBUTION OF FISSION PRODUCTS

The wet weight of fission products were seen and their respective frequencies at each weight class of 10 g intervals were noted (Table II c). The recently

TABLE II c.

Wet weight distribution frequencies of fission products of Holothuria atra

Wet Weight (g)	A	P	Ap	Pa
0 – 10	0	0	0	0
10 - 20	0	0	1	1
20 – 30	8	3	6	2
30 – 40	4	1	5	2
40 – 50	3	4	3	6
50 – 60	2	1	2	3
60 – 70	3	4	3	3
70 – 80	0	2	0	3
80 – 90	0	3	0	2
90 – 100	0	1	0	1
100 – 110	0	0	0	0
110 – 120	0	0	0	0
120 – 130	0	1	0	1
130 – 140	0	1	0	0

fissioned anterior portions (A) were present maximum in the 20-30 g range, while the recently fissioned posterior parts (P) were larger with a modal class of 40-50 g and also 60-70 g. This may be due to the point at which the fission occurred, which is not exactly at the middle portion, but shifted a little towards the anterior end (Conand, 1996).

The regenerating anterior portions (Ap) had a modal class of 20-30 gm. A significant number were also present in the 30-40 g range. The regenerating posterior portion (Pa) had a modal class at 40-50 g range. Here also it appeared that the posterior part was slightly larger when compared to anterior portions.

4.2.9 FISSION AND ENVIRONMENTAL PARAMETERS

An attempt to correlate the monthly composition of fission specimens with environmental parameters such as water temperature, salinity, pH and dissolved oxygen failed of show correlation as the coefficients were not significant when compared to the expected values of 0.325 and 0.418 at 5% and 1% level of significance at N-1 degrees of freedom.

Attempts to correlate fission patterns to environmental parameters proved futile in the present study. A higher rate of fission in the Reunion population of *H. atra* during cooler months were reported by Jaquemet *et al.* (1999). A similar view was expressed for *H. atra* and *S. chloronotus* at Great Barrier Reef by Uthicke (1997a). But fission was more frequent in summer for *H. parvula* (Emson and Mladenov, 1987) when water temperatures were more than 25°C for *S . chloronotus*. Conand and Uthicke (1999) suggested that food availability and population density may be involved

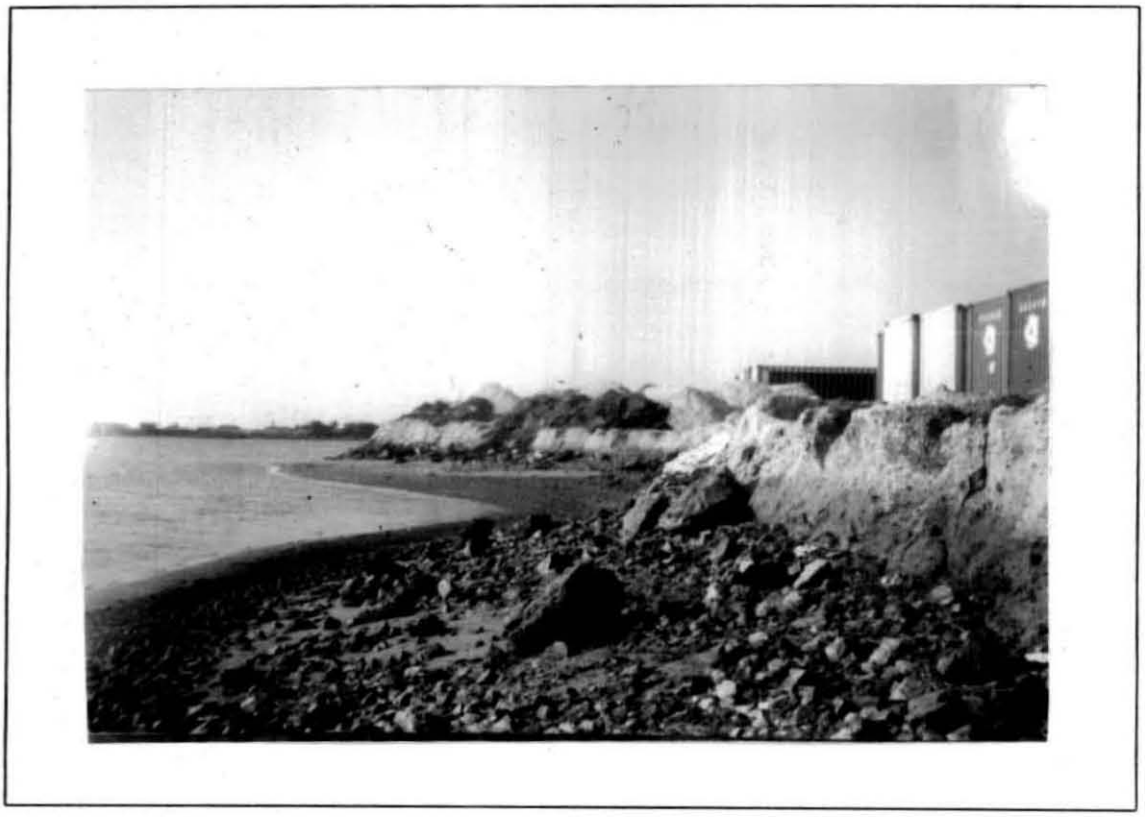


Plate XVII. A view of the sampling site reclamation

in the regulation of asexual reproduction. Pearse(1908) reported self mutilation of *T. briareus* by casting off anterior end of the body, when water become stagnant or conditions become unfavourable. While, *L. inharens* constricts off pieces at the posterior end of the body until only a small fragment is remaining (Pearse, 1909). Kille (1942) stated that asexual reproduction by transverse fission may be responsible for the dearth of distinct age classes in a locality. He found that there existed no correlation between the stage of gonad development and occurrence of fission in *H. parvula*. Human intervention and man-made factors were pointed as possible triggers in La Saline reef by Conand (1996). This view could be taken into account in the present study, that during the fag end of the sampling period, from September 1998 onwards, there were reclamation works going on at the sampling site (Plate XVII). This might have triggered higher fission rates as observed during November 1998 - January 1999. James (1982b) reported constriction and breaking of body into bits by *P. tuticorinensis* when kept in stale sea water. According to him, such behaviour was noticed in *Anapta gracilis* and *P. rufescens* also. Fission occurs in small individuals living in shallow tidal pools, suggesting that fission probably is triggered by a stressful environment resulting from solar radiation (Chao *et al.*, 1993a). This view can also be considered, as the sampling site was not much deep in the present study. Pearse (1968) opined more frequency of fission in surf-swept intertidal areas than in quiet, deeper waters, thus lowering the average sizes of the animals there. Conand (1990) opined about higher fission rates in reef flat and shallow water population. Triggers for asexual reproduction by way of fission mainly remain hypothetical and have to be experimentally verified, as they are presently derived from field observations.

Environmental exogenous factors from the habitat, anthropogenic, temperature or emission, or from the population itself such as density or size, probably regulate endogenous chemical or nervous factor. Reichenbach and Holloway (1995) induced fission by placing rubber band in the middle of the body of *T. ananas*, *H. fuscogilva*, *A. mauritiana, A. miliaris, S. chloronotus* and *S. variegatus*. Although all the species fissioned, only *T. ananas* and *S. chloronotus* had the ability to regenerate both anterior and posterior animals with more than 80% survival rate. *S. chloronotus* regenerated faster (3 months) than *T. ananas* (5-7months). Uthicke *et al.* (1998) proposed evidence to suggest that asexual reproduction is dominant only in areas where sexual recruitment was limited by other factors. An identical case was reported in *H. parvula* at Bermuda (Emson and Mladenov, 1987) where population has probably been maintained by fission.

The identical survival rates of anterior as well as posterior parts in fission and regeneration in the present study disputed the view expressed by Conand (1996), where posterior part had better survival rates in the Reunion population of *H. atra*. But Jaquemet *et al.* (1999) at the same site observed a zero mortality rate for both these parts. A higher rate of mortality for regenerating anterior portions were recorded in *H. leucospilota* by Conand *et al.* (1997). A similar trend was noticed in *S. chloronotus* also (Conand *et al.*, 1998). According to Conand *et al.*(1997), mortality of specimens resulting from fission in *H. atra* is much higher than mortality of recently fissioned anterior and posterior portions in *H. leucospilota*.

4.2.10 OBSERVATIONS ON EVISCERATION

Evisceration was observed on three occasions in the holding tank, one at a time. The specimens eviscerated through the anal opening, ejecting out the viscera including the gonad. As *H. atra* does not eviscerate readily like *H. scabra,* a few stimuli were tried out to induce the evisceration in *H. atra.* The stimuli were 0.1%, 0.2%, 0.4% and 1% solutions of Sodium hydroxide, Distilled water, desiccation and thermal stimulation. The specimens for the trials were carefully examined for any symptoms of fission and evisceration. Only defecating specimens were selected for the study.

One to two ml. 1% Sodium hydroxide was injected into the coelom of *H. atra* using a hypodermic syringe to induce evisceration and were exposed on a tray. The bodywall of the specimens turned turgid, then showed wriggling movements and contracted to the shape a ball. within 5-10 minutes there was an outrush of coelomic fluid followed by evisceration. This stimulus had a 95% success rate. But all the eviscerated specimens developed lesions at the point of injection upsetting the normal activities of the animal. Later, a weaker solution (0.1%) of Sodium hydroxide was tried to induce evisceration. About 5-10 ml. solution was injected into the coelom. The success rate was low at 15%, with the specimens showing same behavioural patterns. Evisceration was effected within 15-25 minutes, but a few specimens developed lesions on their body. Evisceration was also induced by injecting 0.2% and 0.4% Sodium hydroxide solutions into the coelom. Only 40% specimens eviscerated in 0.2% treatment, while a higher evisceration rate of 80% was recorded in 0.4% sodium hydroxide treatment. The behavioural patterns were

similar to that of other trials. Evisceration effected within 15 minutes to 4 hours after injection. About 20% specimens developed lesions on their body in 0.4% Sodium hydroxide treatment.

The third stimulus was distilled water injected into the coelom at the rate of 80-120 ml. depending on the size of the specimen studied. After injection, the specimens were released back to the holding tank. The specimens became turgid for a while, then showed wriggling movements, contracted like a ball, but regained normalcy within 10-15 minutes. About 3-4% of the injected specimens eviscerated after 15-30 minutes. The behavioural patterns during evisceration was same as in the previous cases.

The fourth stimulus was of distilled water followed by desiccation. Distilled water was injected at the rate of 80-100 ml. and the specimens were kept out of water in a tray for 1/2 - 1 hour. The specimens contracted and became turgid but retained their normal shape after 15-30 minutes. Only 4% of the specimens eviscerated.

Desiccation for 1/2 - 1 hour was also tried to induce evisceration in *H. atra*. The specimens contracted and remained in that position. They regained their normal shape when replaced in sea water and none eviscerated.

Thermal stimulation was also tried for evisceration. The specimens were directly transferred to sea water 9° C higher than the acclimatized medium.

Specimens showed stress reactions, but remained calm after 30-45 minutes. No evisceration was observed.

It could be inferred that 0.4% Sodium hydroxide solution could be better used to induce evisceration in *H. atra*. All other treatments either failed or provided defective results to be practised. This finding may help in studying the regeneration capabilities as well as regenerating time taken by this species, where fission followed by regeneration is supposed to be a common phenomenon in its populations.

Clark (1976) stated that *H. atra* may eject gut if provoked enough. Autotomy in holothurians could be either by evisceration or by splitting the body into two or more pieces by transverse division. Pearse (1909) induced evisceration in *T. briareus* with Strychine and Methylene blue (2%). The evisceration is effected by breaking of body wall just behind the calcareous ring, and throwing out the visceral organs. *L. inharens* is induced when lack of sand for burrowing or foul water conditions arise, for fission. The same author opined that autotomy may occur when any combination of the conditions causing the inner branches of the longitudinal muscles broken, in *T. briareus*. Kille (1936) induced autotomy of stomach, intestine and lantern in *T. briareus* by chemical (weak ammonia solution) or electrical stimulus. Ninety six per cent of the animals lived and regenerated lost parts. It was observed that the regeneration of the lantern was delayed. Kille (1939) could regenerate gonad tubules following extirpation in the same species. No generation was possible in complete gonadectomy. Mary Bai (1971) induced evisceration in *H. scabra* by injecting distilled water into the coelom. It was observed that the regeneration of the

alimentary canal as well as the haemal system started from mesentary, and that of respiratory tree from the ruptured end of its main stem. The same author also observed that the phenomenon of eviscertion is neither seasonal nor spontaneous, based on wild observations. The regeneration of alimentary canal of *H. scabra* occurred within seven days, which is quite rapid. Conand (1990) observed evisceration of *H. nobilis* and *H. fuscogilva* in New Caledonia lagoon. But no reaons were stated for the same. She also reported immediate evisceration of T. ananas on collection.

Fission in *H. atra* had been subjected to study in different parts of the world. But more serious studies on the role of asexual propagation in population dynamics of this species and the role of environmental factors with process are possible only through a study on a long term basis.

CONCLUSION

The present study throws some light on the reproductive characteristics of *H. atra* at Tuticorin for the first time. It was found that the species studied follows a biannual breeding season at the study area. The seasons for breeding were July-August and February - March, which were confirmed by the observations on the mean gonad index values, percentage composition of mature specimens in the samples and also on the basis of highly seasonal spawning activity. It is interesting to note that ripe specimens were present in the samples even during the non-breeding months. This is a characteristic feature of many tropical species, reported by other researchers too. In this study the gonad characteristics at different maturity stages were analysed, both morphological and microscopically. The gonad index was least for stage I and II, which is indeterminate. It reached a maximum at ripeness, in the stage IV in both male and female sexes. The fecundity and the weight at first maturity was estimated. The weight distributions of animals with gonad and without gonad varied. Interestingly, a number of large animals were found without a gonad it is assumed that, the gonad of these specimens might have regressed. Similar cases were reported by other workers earlier in the same species and some other species also.

The species was found to undergo fission at the study site, which is mode of asexual propagation. Recently fissioned as well as regenerating specimens were collected in the monthly samples. Still, a specific cycle for fission could not be derived from the observations. The fission specimen were divided into different fission products

according to their state of fission or regeneration. The monthly rates of fission and regeneration were also calculated. The wet weight of normal and fission specimens varied considerably. Fission and evisceration were observed in the holding times on a few occasions. After conducting some experiments, it was found that 0.4% Sodium hydroxide solution could induce evisceration in the species, without interfering with the activities of the specimens. This findings may help in studying the regeneration of internal organs of *H. atra*.

The reproductive cycles may show a little variation over years due to changing environmental parameters. It can vary with differences on latitude and longitude also. The reproductive cycles as well as reproductive output could be regarded as a function of environmental parameters. In the ever changing environment, there could be variations due to variation in the climatic and other related factors. These factors influence the breeding season, fecundity, maturity and asexual propagation. Although distributed at high densities in the wild, the larval cycle of this species is yet to be achieved under controlled conditions.

SUMMARY

1. The annual reproductive cycle of *Holothuria atra* at Tuticorin was studied for a period of 18 months from November 1997 to April 1999.
2. The gonad is divided into five stages of maturity *viz.*, immature, resting, maturing, ripe and spent.
3. The ripe gonad is creamy white in male and reddish orange in female.
4. Histological characteristics also support the morphological observations on gonad maturity.
5. Three types of tubule maturity cohorts at a time were observed in the present study. In some cases, gonad was absent even when the specimen was big, or gonad was reduced to a swollen base.
6. The mean gonad weight varied from 0.0465 $\pm$ 0.08 g at indeterminate stage to a mean maximum of 5.395 $\pm$ 5.155 g in a males and 8.681 $\pm$ 8.219 g in females.
7. The largest gonad weighed 40.52 g, which was a female.
8. The gonad indices were calculated in respect of wet weight, drained weight and gutted weight, all showing similar trends. The gonad index related to wet weight (G1) ranged from 0.037$\pm$0.0624 at indeterminate stage to a mean maximum of 2.995$\pm$0.027 in males and 4.486$\pm$3.706 in females.
9. It was observed that annually *H. atra* breeds twice at Tuticorin, during July–August and February – March, based on gonad index values and spawning observations.

10. The highest average gonad index (G1) was noticed during August 1998 (2.21±2.57) showing peak reproductive activity. It was higher during March 1998 and February 1999. The least G1 was recorded in December 1997 (0.119 ± 0.14).

11. The percentage of male and females were comparatively higher during the breeding months.

12. Ripe specimens were present even during non-breeding months.

13. Spawning observations in the holding tank were made during the months of August 1998 and February 1999 in both the sexes. The specimens showed typical behaviour pattern for spawning.

14. Male specimens spawned irrespective of breeding season and occasionally displayed multiple gonopores. Spawning of males in the holding tank was observed during March, July, August, September, November '98, February and April '99.

15. The fertilized eggs were reared up to auricularia larvae.

16. While induced spawning attempts by thermal stimulation failed, artificial fertilization yielded lower fertilization rate and deformed larvae, which perished after one day.

17. Observed sex ratio was about 2.9:1 for males to females. The samples contained 200 males and 71 females. But statistically, the population follows the 1:1 ratio.

18. The weight at first maturity for *H. atra* was observed as 100 g (approx.) based on the wet weight of the specimens.

19. The absolute fecundity varied from 7,45,650 to 14,787,900 eggs, with a mean of 3,175,938 eggs. The relative fecundity was estimated at 3.78 x 10^4 oocytes per gram of gutted body weight. The absolute fecundity correlated only to gonad weight.

20. The maximum oocyte diameter ranged around 130–150 μ in the ripe female gonad. The ripe oocytes were polymodal and bore a nucleus.

21. The wet weight of samples varied from 14 to 402 g. Normal specimens showed maximum frequency in 76 to 100 g class, while specimens without gonad were maximum in 51-75 g class. The maximum weight of a specimen without gonad was 368 g. Specimens with gonad ranged from 36 to 402 g with a maximum frequency in 76 – 100 g class.

22. The gut-gonad ratio was maximum at indeterminate stage and minimum when the gonad was ripe.

23. Among the environmental parameters, only salinity was marginally correlated to the reproductive periodicity at Tuticorin.

24. The percentage of fission specimens were maximum during December, 1998 and none were recorded during April 1998 and April 1999.

25. No specific season or pattern was observed in the occurrence of fission at the sampled area.

26. Evisceration in the holding tank was observed on three occasions.

27. Evisceration was induced by injecting 0.4% Sodium hydroxide solution into the body coelom without disturbing normal activities of the specimen.

28. A mean 9.00 $\pm$ 5.69% of the monthly samples comprised fission specimens.

29. Fission observations were made on two occasions in the holding tank, the process lasted for about 12-15 hours. Reason may be over crowding.

30. Fission specimens were classified into : Fissioning (F), Recently fissioned anterior (A) and posterior (P) parts; regenerating anterior (Ap) and posterior (Pa) parts.

31. Fission rate was calculated using the formula :

F(%) = [(A+P)/2T x 100] and Regeneration rate **R(%) = [(Ap+Pa)/2T x 100).**

F(%) was maximum in December 1998, while R(%) was maximum in November 1998.

32. The mean rates of fission and regeneration were 2.21 $\pm$ 2.28% and 2.29$\pm$1.59% respectively. These closer values indicate negligible post-fission mortality in the study area.

33. Nineteen fission products had gonad in them, 13 males, six females and six indeterminate.

34. The wet weight of fission specimens varied from 16 to 206 g, with a maximum frequency in the 26-50 g class.

35. None of the environmental parameters correlated with the fission rates or regeneration rates, proving that fission is a regular process within the population irrespective of other influences.

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ANNEXURE

NATIONAL SEMINAR ON

DEVELOPMENT AND TRANSFER OF FISHERIES TECHNOLOGY

3 - 5 FEBRUARY, 1999

AQP 5

CULTURE OF SEA CUCUMBERS IN SHRIMP FARMS - A TAKE OFF IN TECHNOLOGY

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The seed of *Holothuria scabra*, commercially most important sea cucumber was produced for the first time in the hatchery of Tuticorin Research Centre of CMFRI in 1988. Since then, the seed has been produced on a number of occasions. The juveniles of sea cucumbers produced can be grown with great advantage in shrimp farms since much of the feed given to the shrimps goes as a waste and settles down to the bottom of the farm enriching the soil. The sea cucumbers, being detritus feeders subsist on the organic matter present in the farm soil. They convert organic waste into body protein and grow fast. The presence of the sea sucumbers at the bottom of the farm is in no way affect the activities of the shrimps. In fact, the shrimps grow faster and the pollution in the farm is removed and the environment is kept clean. All the results regarding growth, mortality and organic content of the soil of the farm are presented.

Observations on fission and spawning

Communicated by Ram Mohan, Tuticorin RC CMFRI, Tamil Nadu. India 628 001.

1. Spawning observations

1. Date: 24.03.1998
   Time: 09:45
   Species: *Holothuria atra*
   Moon phase: NM-3
   Remarks: Two male specimens spawned one after the other in laboratory holding tanks at 30°C, for about 15–20 minutes. No peculiar spawning behaviour was noticed.

2. Date: 22.07.1998
   Time: 08:30
   Species: *Holothuria atra*
   Moon phase: NM-1
   Remarks: One male specimen spawned during transit in the container by slightly lifting its anterior end, for 12 minutes. The water temperature recorded was 27.5°C.

3. Date: 27.08.1998
   Time: 11:45
   Species: *Holothuria atra*
   Moon phase: 1/4-3
   Remarks: Four male specimens spawned in holding tanks by lifting their anterior end, but showed no swaying action. The spawning duration was 15–40 minutes. Later, two more male specimens spawned, but for a shorter duration. A single female specimen spawned in the same tank intermittently for about 4 hrs. No peculiar behaviour was observed. The water temperature recorded was 29.5–31°C.

4. Date: 15.09.1998
   Time: 17:50
   Species: *Holothuria atra*
   Moon phase: 3/4+2
   Remarks: One male specimen spawned for 30 minutes during transportation at 28°C water temperature. It erected its anterior end and showed swaying movements.

5. Date: 24.09.1998
   Time: 09:30
   Species: *Holothuria atra*
   Moon phase: NM+3
   Remarks: Two male specimens spawned in containers for 30 minutes to 1 hr. at 28°C by lifting their anterior end. One specimen had two gonopores; spawning time of 1 hour.

6. Date: 21.10.1998
   Time: 14:10
   Species: *Holothuria atra*
   Moon phase: NM+1
   Remarks: Three male specimens spawned in holding tanks, at about 29.0° to 29.5°C water temperature. The spawning duration extended from 45 minutes to 2 hrs. 15 min. One specimen had 3 gonopores. This particular animal lifted and swayed its anterior end.

2. Fission and regeneration observation

Species: *Holothuria atra*
Site: South Brezk Water, New Harbour, Tuticorin, Tamil Nadu, India.
Habit: Calm, loamy bay with beds of seagrass such as *Cymodocea* sp., and *Halophila* sp., along with some sea weeds and dead coral stones.
Date: November 1997 – October 1998.
State: Fissioned and regenerating anterior as well as posterior parts were observed. A maximum percentage of such specimens was noted during October, 1998 and minimum during April, 1998. The fission rate was higher at a temperature range of 25–27° C, at a steady salinity level of 34–35 ppt.
Behaviour variations: Not observed.