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REPRODUCTION IN *HOLOTHURIA (MERTENSIOTHURIA) LEUCOSPILOTA* (BRANDT) FROM ANJUNA, GOA

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ABSTRACT

Holothuria (Mertensiothuria) leucospilota spawns more than once in a year *i.e.* during the postmonsoon (October to January) and in monsoon periods (June to September). An increase in the gonad index is observed during September and December 1985 and again during April and May 1986. The macroscopic and microscopic features of gonads have been described. The tubules are found to be longer and narrower in males when compared to the females. The ripe oocytes measured upto 220 μ. The spawning of *Holothuria (Mertensiothuria) leucospilota* appeared to be influenced by low temperature and salinity. The relationships between the gonad index, temperature and salinity are not significant during the study period.

INTRODUCTION

Sexes are separate and there is no sexual dimorphism. The gonad usually consists of numerous tubules united into a single tuft attached to the left side of the dorsal mesentery. Except for the dorsal attachment the gonad hangs freely in the coelom (Hyman, 1955).

Spawning of holothurians indicates that each species breeds during one or more months at a definite time during a year. Usually, it is during spring and summmer in temperate latitudes (Selenka, 1876; Clark, 1889; Ludwig, 1898; Clark, 1899, 1910 a; Remiers, 1912; Ohshima, 1918; Runstroms, 1921; Courtney, 1927; Mortensen 1937, 1938). Nyholm (1951) and Edwin (1948) have suggested that the holothurians usually spawn in the late afternoon or in the evening or at night, a response to dimlight. Temperature also plays a significant role in influencing spawning. The general tendency in holothurians from temperate waters is to spawn during summer and during spring and it was related to an increase in temperatures (Hyman, 1955).

The present knowledge on reproduction and breeding of tropical echinoderms is scanty. Though holothurians display diverse reproductive habits (Hyman, 1955; Krishnaswamy and Krishnan, 1967; Bakus, 1973; Emson and Wilike, 1980), much needs to be done on reproductive pattern. Important contribution on the reproductive seasonality of tropical holothurians have also been made (Krishnaswamy and Krishnan, 1967; Conand, 1981; Harriott, 1985). Spawning was confirmed by data on the histological status of the gonads (Harriott, 1985).

During the present investigation the reproductive cycles of *Holothuria leucospilota* (Brandt) have been studied. An attempt was made to see if any correlation existed between the reproductive cycle, and the temperature and salinity.

MATERIAL AND METHODS

The animals were collected from the intertidal area at Anjuna. Every month 35 to 40 animals were collected from August 1985 to October, 1986. The gonads were preserved in buffered formalin (AR) (7% dilution). The method used for classifying sexual stages in fishes (Conand, 1975) was employed in the present study. This method was earlier followed

for some holothurians like *Thelenota ananas*, *Microthele nobilis*, *Microthele fuscogilva* (Conand, 1981), *Holothuria atra*, *Holothuria edulis* and *Holothuria impatiens* (Harriott, 1985). The method employs the macroscopic characteristics, gonad form, colour and consistency and the microscopic features of the preserved gonad in formalin. A part of the gonad was spread on a slide and examined for sex. For females, the oocyte diameters were measured by using an ocular micrometer and their frequency was noted.

Gonads and sexual stages

In *Holothuria leucospilota* the ovaries and testes are composed of one tuft of tubules (Pl. I A, B). In the females the gonadial tubules are shorter and wider, whereas in males they are longer and narrower. The Stage I and II corresponded to individuals with undetermined sex, with tubules 1 to 5 cm long and 0.5 mm to 0.8 mm in diameter. Stage III corresponded to the growing stage. In Males, the tubules 10 to 15 cm long and 1 mm in diameter were considered under this stage. Female tubules 5 to 12 cm long and 1 mm to 1.5 mm in diameter also represented this stage. Stage IV represented a stage when the male gonadial tubules were 15 to 25 cm long and with 2 to 5 mm diameter. In the same stage, the female gonadial tubule was 10 to 15 cm long and 12 mm in diameter. In Stage V, male gonadial tubules ranged from 15 to 18 cm in length and from 1 to 1.5 mm in diameter; the female tubules ranged from 8 to 12 cm and from 6 to 9 mm respectively.

The microscopic features in *Holothuria leucospilota* revealed that Stage I and II comprised the undetermined stage. The germinal cells were not distinguishable. The germinal cells had a diameter of 50 μm. In Stage III, the oocytes had diameters ranging from 140 to 159 μm (Pl. I C, D). Numerous spermatozoa were present in males. In females, dark brown coloured spherical oocytes with diameters of 150 to 220 μm were observed in the mature stage (Stage IV) (Pl. II A). Yellowish cells of a few spermatozoa in males and in females, a few oocytes of dark brown colour (diameter 120 to 200 μm) were present in post spawning stage V (Pl. II B, C).

The gonad index (GI) is expressed as the ratio of wet gonad weight to the total drained wet animal weight (Geise, 1959). The mean value of gonad index was calculated separately for the males and the females. Temperature and salinity of the sea water, from where animals were collected were recorded. Correlations, if any, between the gonad index, and temperature and salinity were examined based on regression and correlation analysis.

RESULTS

Male gonad index showed in increase during September (0.25), November (0.17) and December 1985 (0.29). There was a decline in the gonad index during October 1985 (0.10), January (0.05), February (0.03) and March 1986 (0.06). The gonad index in males increased again during June (0.13), July (0.16) and August 1986 (0.19).

The female gonad index values showed an increase in September (0.32), December (0.38) 1985 and April (0.26) 1986. During October (0.16), November 1985 (0.1) and January 1986 (0.11), a decrease in the gonad index was recorded. The variations in the gonad index values are high in Septmber 1985 (0.435), December 1985 (0.475) and May to July 1986 (0.16, 0.14 and 0.14 respectively). This indicated the high variations in the gonad state of the holothurian maturity. The decline in the gonad index from February to May 1986 (0.25 to 0.16) suggested that the animals might have attained the spent or the resting phase.

The monthly percentage of male, female and animals with undetermined sex were obtained by microscopic observations of gonads. The undetermined individuals sampled during January, February and March 1986. Males and females of *Holothuria leucospilota* represented 35.9% and 43.5% respectively. Maturing individuals were found (Stage III) only during August and September 1985 and 1986. They were in large numbers during September 1985. Maturing individuals were found again during April and May 1986. The spawning (Stage IV) period was during October, November 1985 and January 1986 with a maximum in June, July and August 1986. Post-spawning individuals

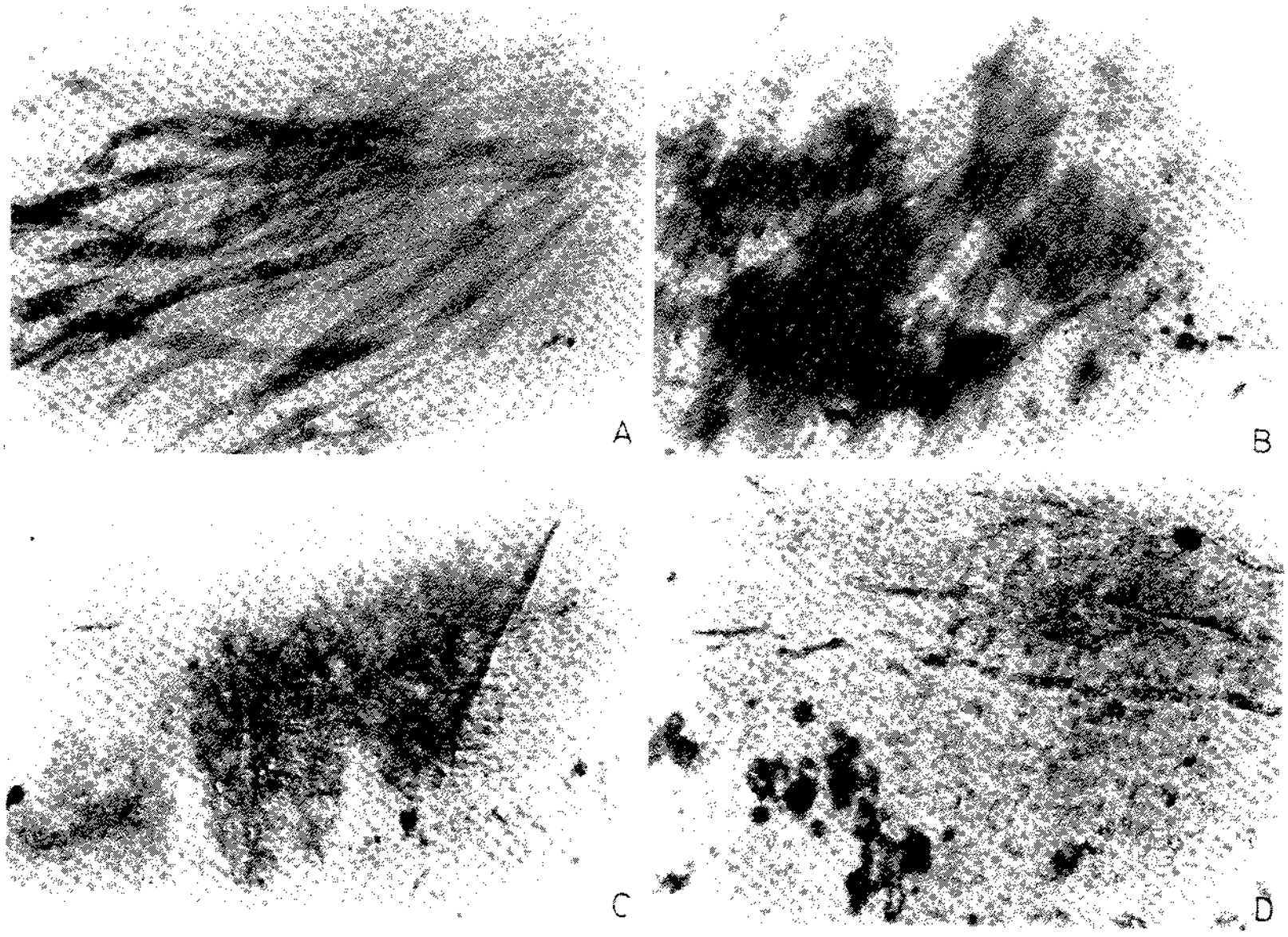

Plate I A and B. Gonadial tubules, and C and D. Germinal cells of *Holothuria (Mertensiothuria) leucospilota*.

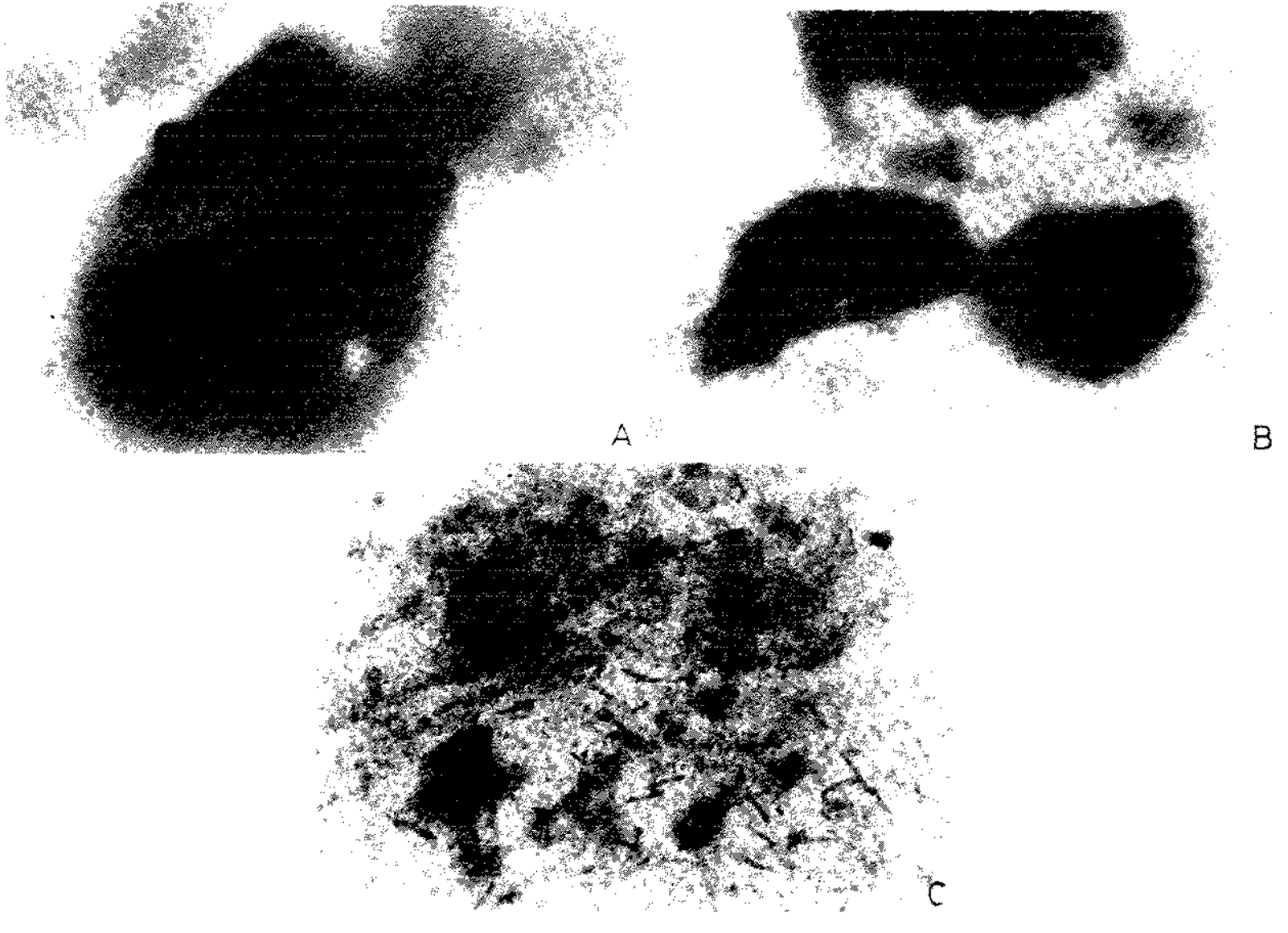

Plate II A - C. Mature oocytes of *Holothuria (Mertensiothuria) leucospilota*.

(Stage V) were found during January (40%) to March 1986 (25%).

During the study period, August 1985 to October 1986, water temperature ranged between 24.0° C and 31.5° C. The maximum temperature was recorded in March 1986 (31.5° C). The minimum temperatures were during August 1985 (24.0° C), July and August 1986 (26.0° and 25.0° C) respectively. The salinity values ranged from 35.1‰ - 23.6‰. The maximum salinity was recorded during October 1985 (35.0‰) and March to May 1986 (34.0 to 35.1‰). The minimum salinity was recorded during February (28.6‰), July 1986 (23.6‰) and November 1985 (29.23‰).

No relation was noticed between temperature and gonad index. The decrease or increase in gonad index did not correspond much with the increase or decrease of temperature. The coefficient of regression was - 0.22. Though not significant a positive relationship was noted between salinity and gonad index. The coefficient of regression ws 0.34.

When the sexual cycles were compared with temperature and salinity, maturation was observed to begin during August to september 1985. Spawning occurred in October to November 1985, followed by post-spawning in December 1985 to January 1986. Resting period occurred in February to March 1986. The reproductive cycle of *Holothuria leucospilota* was correlated with the gonad index variations, the cycle of different maturity stages and with the percentage of undetermined individuals. The period of Stage IV was dissociated into maturity (August 1985 and September 1986), pre-spawning (maximum peak of gonad index) and spawning (October and November 1985; beginning of the decline of the gonad index). The period of Stage V was divided into post-spawning (December 1985 to January 1986) or spent (during the decline of the gonad index) and resting (February to May 1986) corresponding to the maximum percentage of undetermined individuals. During spawning (October - November 1985) the temperature and salinity were 29.5° C, 29.0° C and 35.0‰, 29.23‰, respectively. In post-spawning (December 1985 - January 1986) the temperature and salinity were 28.0° C, 28.5° C and 34.0‰, 33.1‰ and during prespawning period (February to May 1986) the temperature and salinity were 29.5° C to 31.5° C and 28.6 to 35.0‰ respectively. The temperature and salinity during July and August 1986 (26.0° C and 23.6° C, 32.5‰) corresponded to the spawning period of *Holothuria leucospilota*. The variations in temperature (29.0° C to 29.5° C) during spawning period were very small, whereas the variations in salinity (29.23 to 35.0‰) were significant.

Discussion

Krishnaswamy and Krishnan (1967) have shown that *Holothuria scabra* spawned once during July and again during October. Green (1978) had reported bimodal breeding in *Leptosynapta tenuis*. He assumed that gametogenic growth reached a peak within a month either during summer of the fall. Conand (1981) had reported the sexual cycles in *Thelenota ananas*, *Microthele nobilis* and *Microthele fuscogilva*. The ripe oocytes in *Thelenota ananas* measured 200 μm. The ripe oocytes in *Microthele nobilis* measured from 140 to 160 μm and the ripe oocytes of *Microthele fuscogilva* measured 170 μm. In *Thelenota ananas* and *Microthele fuscogilva*, the annual reproductive cycle was represented only by a single spawning period during the warmer season. *Microthele nobilis* reproduced only during the cold season.

In the case of *Holothuria leucospilota*, there was an increase in the gonad index during September (0.40) and December 1985 (0.475). It decreased during January (0.035), February (0.025) and March 1986 (0.035). The gonad index exhibited increase again during April (0.125) and May (0.16). During the monsoon months a slight decrease (0.14 to 0.13) was observed. The macroscopic and microscopic features of the gonads could be correlated with those of the gonad index. The ripe oocytes measured upto 200 μm during August, September, October 1985, May, June, July and in August 1986. When spawning occurred during October and November 1985, (*i.e.* in Stage IV) the gonad index was low. Again during June, July and in August 1986, a decline in the gonad index was noticed. The Stage V (post-spawning period) corresponded to the resting or spent individuals

in January, February and March 1986 which indicates that the *Holothuria leucospilota* spawned more than once a year during the monsoon and post-monsoon season.

The principal environmental factor that induces breeding of animals in temperate forms is generally considered to be the increase or decrease in temperature. This is well established in echinoderms and for other invertebrates (Stott, 1931; Giese *et al.*, 1959; Pearse, 1985; Sastry, 1966). As far as the tropical animals are concerned, it is generally believed that breeding is continuous (Giese *et al.*, 1964; Krishnaswamy and Krishnan, 1967). In *Stomopneustes variolaris*, *Oreaster hedemanni*, *Penaeus indicus* (Subrahmanyam, 1963; Durve, 1964; Giese *et al.*, 1964; Rehman, 1965; Rao, 1965), a well marked breeding season was reported. For holothurians, factors responsible for spawning have not clearly been said so far. Salinity (Krishnaswamy and Krishnan, 1967) and temperature (Tanaka, 1958) were considered responsible. Green (1978) reported that the reproductive cycle of *Leptosynapta tenuis* was regulated by temperature and salinity. The mid-summer spawning was correlated with the high temperatures (Rutherford, 1973). Mc Crary (1989) had suggested that the high temperature influenced spawning in the planktonic larvae of *Leptosynapta tenuis* (Ayres). Correlation was established between temperature and the reproducctive phenomenon in *Arbacia punctulata* and *Paracentrotus lividus* (Moore, 1966). The optimum temperature requirements for reproduction were considered for some tropical species (Yonge, 1940; Pearse, 1968). *Strechinus neumayeri* (Pearse and Giese, 1966), *Odontaster validus* (Pearse, 1965, 1966) and *Stylocidaris affinis* (Holland, 1967) have shown marked reproductive periodicities even though there is very little or no seasonal fluctuation in sea water temperature. Similar examples of little or no direct relation between fluctuations of the sea water temperature and reproduction can be found for other groups of marine animals (Galtsoff, 1961). In *Holothuria leucospilota*, the higher values of gonad index during September (0.435) and December 1985 (0.475) and during May to July 1986 (0.16, 0.14 and 0.14 respectively) suggest that spawning had taken place during these months. The temperatures recorded during above months were 25.0, 28.0, 29.5, 29.5 and 26.0° C respectively. This may imply that the low temperature influenced spawning. The maximum temperature was recorded during March 1966 (31.5° C); the gonad index recorded in that month was 0.03. It was also noted that the variations in temperature during August 1985 to October 1986 did not correspond much with the variations in gonad index during that period.

Orton (1920) stressed upon the influence of temperature over the breeding in temperate marine invertebrates, but the never discounted the role of salinity. The Ceylon Pearl Oyster, *Margaritifera vulgaris* was found to spawn twice in a year. The spawning season coincided with the southwest and northeast monsoons. Based on this, Mal'pas (1933) concluded that the oysters are induced to spawn with the changes in salinity. Similar trend was also reported for *Crassostrea madrasensis* by Hornel (1910). Stephenson (1934) found that the vast majority of species in the Great Barrier Reef spawned during the summer months and when the rainfall was maximum. Panikkar and Aiyar (1939) had suggested that the lowering of salinity, consequent upon the onset of the north-east monsoon, influenced the breeding of animals off the Madras Coast.

Salinity was considered to be the inducing factor for breeding in the tropical regions (Giese *et al.*, 1964; Durve, 1964). Giese *et al.* (1964) have suggested that decrease in salinity may induce breeding in *Stomopneustes variolaris*. Durve (1964) while working on *Meretrix casta* reported that neither an increase nor a decrease, but an optimum salinity is responsible for te breeding. It was presumed that in *Holothuria scabra* (Krishnaswamy and Krishnan, 1967) the salinity was responsible for initiating breeding. Other factors such as increase in the concentration of quantity of food during spawning periods are also to be considered (Jayaraman, 1954).

The ara of present investigation recorded the rainfall of about 3400 mm during the south-west monsoon of 1985. The rainfall recorded during the southwest monsoon in 1986 was 2650 mm. This was reflected in the low salinity values recorded (23.6 to 34.5‰). During October and

November, the heavy fresh water runoff from the land was reflected in the reduced salinities (Sankaranarayanan *et al.*, 1978). During October and November 1985, the salinity recorded was 35.0 and 29.23‰. Thus it may be possible that the low salinity of sea water had influenced the spawning in *Holothuria lucospilota* at Anjuna.

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