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REPRODUCTIVE BIOLOGY OF THE SAND CRAB *EMERITA HOLTHUISI*

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ABSTRACT

Histological monthly observations of the gonads revealed that the *Emerita holthuisi* is a continuous breeder. Mature and spent gonad stages were observed throughout the year. The male *E. holthuisi* enters puberty upon attaining a carapace length of 2-3 mm and the female at 4 mm. The berried females of *E. holthuisi* were found throughout the year. The breeding cycle has been studied for one year (September 1973 to August 1974). There was an increase in the number of berried females in the months March and September. The number of females was always greater than that of males

INTRODUCTION

REPRODUCTIVE cycle of Crustacea have been studied by many workers (Rasmussen, 1958; Knudsen, 1964; Boolootian, 1965; Rahaman, 1966; Chandran, 1968; Pillai and Nair, 1970; Rice and Armitage, 1974). Cox and Dudley (1968) demonstrated the seasonal pattern of reproduction in the sand crab, *Emerita analoga*. One of the important aspects of reproductive physiology is the machanism of control of reproductive activity. Clark (1965) emphasized that two types of control, exogenous and endogenous are implicated in the control of reproduction. Boolootian *et al.* (1959) worked out the effect of environmental parameters on reproductive cycle of brachyuran and anomuran crabs of Monterey Bay.

Ali (1955) described the breeding behaviour of the crab *Paratelphusa querini*. While Weat (1965) studied the breeding cycle of *Petrolisthes-elongatus*. The continuous breeding in the population of tropical crustaceans *Mysidium columbie* and *E. protoricensis* was studied by Goodbody (1965). Sarojini (1966) studied the breeding habitats of *Diogenes bicristimanus*. Chandran (1968) observed breeding cycle of *Charybdis variegata*. Griffin (1968) recorded breeding and moulting cycles of two Tasmanian grapsid crabs.

Since very little information is available on the reproductive biology of Indian anomurans, the present investigation was aimed at studying the histology of gonads, sexual maturity, sex ratio and annual breeding cycle of the sand crab *E. holthuisi*.

MATERIAL AND METHODS

The adult specimens of *E. holthuisi* were collected from Mirya Bay, Ratnagiri at monthly intervals from September 1973 to August 1974. The crabs were maintained in glass troughs containing adequate sea water. Only healthy and intermoult adult animals were taken. The tissues were fixed in Bouin's fluid, dehydrated in alcohol, embedded in paraffin wax (M.P. 50-60°C) and were sectioned at 7 - 8 μ. The sections were stained with Delafield's haematoxylin and counterstained with eosin.

For studying the breeding cycle, the crabs were fixed in 10% formalin. The percentage of berried crabs was determined during the breeding season and the sex ratio was noted along with the sexual maturity of the crab.

RESULTS

Maturation process

On the basis of histological studies, the following maturity stages in the development of gonads, are distinguished in the male and female *E. holthuisi*.

Male (Plate I A - D)

Nature of sperm ducts : The sperm ducts of the males were examined for the mature spermatozoa. This probably is a valid indicator of puberty in male crabs, but exceptions apparently do exist as was observed by Haley (1969) in ghost crab.

A. *Immature :* The posterior extensions of testes of immature males are small and translucent.

B. *Maturing :* Follicles get compact with spermatozoa which are free in the lumen of the follicles; these often form 'plugs' in the lumen.

C. *Mature :* The posterior extensions of testes in mature males are opaque, white and swollen with stored spermatozoa.

D. *Spent :* The testes considerably reduced in size and with large spaces remaining after sperm liberation. Intestinal coils usually visible. Majority of the follicles are empty except for few residual sperms and spermatids.

E. *Spent recurring :* This stage follows the post-spawning period. Spermatogonia and stem cells appear on the follicle wall with clear cytoplasm.

TABLE 1. *Histological classification of the gonad (testis) stages of male* Emerita holthuisi *for the year September 1973 to August 1974*

Month	No. of animals observed	Spent recurring %	Maturing %	Mature %	Spent %
September 1973	18	—	65	12	42
October	21	—	47	11	37
November	25	2	56	5	32
December	19	3	59	6	32
January 1974	26	1	66	3	30
February	31	1	58	9	23
March	18	—	64	10	44
April	33	—	48	8	35
May	28	—	59	6	30
June	22	5	60	5	32
July	18	3	66	8	26
August	26	2	58	8	23

Female (Plate II A - D)

A. *Immature :* Ovary thin and translucent, without ripening oocytes.

B. *Maturing :* Ovary with ripening oocytes, but showing no evidence of ovulation, colour ranges from opaque white through yellow to orange.

C. *Mature :* Some of the oocytes are attached to the wall of follicles and others are free in lumen. Each oocyte is oval or round in shape and contains a large nucleus.

D. *Spent :* The ovaries considerably reduced in size, follicular wall shrunken. Ovary with large degenerating residual oocytes but, showing no signs of further maturation.

E. *Spent recurring :* The ovaries slightly increase in size overlapping the intestinal coils. As such they are not externally visible. Germ cells give rise to oogonia with clear cytoplasm.

TABLE 2. *Histological classification of the gonad (ovary) stages of the female* Emerita holthuisi *for the year September 1973 to August 1974*

Month	No. of animals observed	Spent recurring %	Maturing %	Mature %	Spent %
September 1973	20	2	68	10	39
October	18	6	46	9	35
November	15	5	53	7	30
December	21	9	55	6	28
January 1974	25	7	58	7	30
February	20	2	63	5	20
March	12	1	72	4	41
April	24	7	45	7	32
May	20	11	50	7	29
June	18	8	55	9	28
July	16	3	61	6	23
August	20	2	64	3	21

The stages of gonadal developments for one year (from September 1973 to August 1974) are shown in Tables 1 and 2 which are expressed in terms of percentage.

BREEDING CYCLE AND MATURITY

For observing the breeding cycle of the animal every month the berried females were counted in the collection for a period of one year (September 1973 to August 1974). To know whether the animals were sexually mature, their reproductive organs were examined. The data in Table 3 indicate that the male sand crab enters maturity upon attaining a carapace length of about 3 mm and the data in Table 4 show that the female crab enters maturity after attaining a carapace length of about 4 mm. The ovaries of most of the specimens are narrow and transparent or whitish strands in the nonbreeding season. In mature specimen, the ovary was thick and bulky in appearance, sometimes it was yellow or ruddy orange in colour. From Table 5 it is clear that the large number of

berried females were found during the months of September and October 1973, and February, March April and August 1974. Just before the extrusion of the eggs the mature individuals were recognisable by their bulging

TABLE 3. *The maximum length of the carapace and the maturity of the testis of* Emerita holthuisi *as observed upon dissection and sectioning. Number of animals observed* 57

Maximum length of carapace in mm	Immature in number	Mature in number
1	1	—
2	2	—
3	—	6
4	—	5
5	—	7
6	—	9
7	—	10
8	—	6
9	—	5
10	—	3
11	—	2

abdominal pleopods and the development of the feathery hairs on the exopodite of the pleopods. The eggs when extruded get attached to the exopodite with the help of sticky substance. The testes were also well developed in most of the specimens during the breeding season.

TABLE 4. *The maximum length of carapace and the maturity of ovaries of* Emerita holthuisi *as observed upon dissection and berried appearance. Number of animals observed* 93

Maximum length of carapace in mm	Immature in number	Mature in number
1	1	—
2	3	—
3	2	—
4	—	3
5	—	5
6	—	5
7	—	8
8	—	6
9	—	7
10	—	9
11	—	11
12	—	10
13	—	6
14	—	7
15	—	3
16	—	4
17	—	2
18	—	1

Sex-ratio

From Table 6 it can be clearly noted that the number of females always surpass the number of males throughout the year; however, during

the breeding peaks female percentage increases drastically with some minor exceptions.

TABLE 5. *The percentage of berried and nonberried female in the total female collection of* Emerita holthuisi *from September 1973 to August 1974*

Month	Percentage of berried female	Percentage of non berried female
September 1973	89.5	10.4
October	73.3	26.7
November	39.4	60.5
December	62.3	37.7
January 1974	56.8	43.2
February	60 0	40.0
March	89.1	10.9
April	62.0	37.8
May	71.2	28.8
June	66.7	33.3
July	66.2	33.8
August	71.2	28.8

TABLE 6. *Percentage of males and females in total collection of* Emerita holthuisi *from September 1973 to August 1974*

Month	Total collection	Percentage of males	Percentage of females
September 1973	284	23.70	76.30
October	223	26.90	73.10
November	186	15.05	84.95
December	211	23.22	76.78
January 1974	262	45.41	54.59
February	395	10.13	89.87
March	378	3.40	95.60
April	248	15.72	84.28
May	234	17.52	82.48
June	112	24.11	75.89
July	244	6.55	93.44
August	155	10.32	89.68

TABLE 7. *Temperature and salinity variations during the year September 1973 to August 1974*

Month	Temperature °C	Salinity ‰
September 1973	27.75	28.32
October	29.00	30.34
November	28.00	30.54
December	26.50	31.00
January 1974	24.50	32.50
February	26.80	33.00
March	30.20	33.40
April	31.20	34.20
May	33.00	34.50
June	30.00	29.00
July	27.00	25.30
August	26.35	22.60

Correlation between environmental parameters and annual reproductive cycle

In Table 7 monthly variations in temperature and salinity are profiled. From December onwards the temperature as well as salinits increased upto May 1974. The variations in these environmental factory have been fonnd to affect the reproductive activity of the sand crab which has been described in the later part of the discussion

DISCUSSION

The histological study indicated that the testes displayed two distinct peaks in the mature and spent stages (September and March). Whereas only spent stage of ovary showed two peaks in September and March. During the entire study, both male and female gonads showed fluctuation in all developmental stages. Full grown ova and spermatozoa are generally noted in gonads throughout the year showing increase in their number during the breeding peaks. After spawning the size of the gonad was decreased and developing oocyte and spermatogenic cells were found to be increasing in number. It is, therefore, concluded that *E. holthuisi* is a continuous breeder, showing two peaks of spawning.

Stephenson (1934) found that the species inhabiting the tropical water exhibit several types of breeding cycle such as continuous breeders around the year; discontinuous breeding; two spawning periods, and one single breeding season. Further, many investigators have shown the effect of salinity on the reproductive cycle of many marine animals. Giese (1959) stated that the life cycle of an animal is usually so timed by some environmental factor/factors that the young once are produced at a period favourable for their survival.

In *Emerita asiatica*, Menon (1934) reported that the male crabs range from 3.5 to 7.5 mm in carapace length during summer and after just they arrive on beach they get matured. The females are seen throughout the year and are in 22-30 mm in body size range. The male *E. talpoida* is sexually matured at 2.5 - 3 mm carapace length and grew to maximum length of 7 - 12 mm while the female crab grew to a maximum length of 28 mm (Wharton, 1942). However, Barnard (1950) in *E. astroafricana* observed males to reach 35 mm maximum length whereas female attain maximum length of 23-37 mm. Recently, Goodbody (1965) while working on *E. portoricensis* from Jamaica Coast detected maximum size of 8 mm in males and 17 mm in females. He further stated that female reached first sexual maturity at the length of 8 mm. Except the crab *E. portoricensis* all the above mentioned female *Emerita* species reached their first sexual maturity when they are 12 mm in body length. Compared to above findings it can be seen that in the present study on *E. holthuisi* revealed some interesting features. The female *E. holthuisi* reached its first sexual maturity at 4 mm and reached a maximum size of 18 mm which is somewhat related to the female *E. portoricensis*. The male *E. holthuisi* reached its sexual maturity at 3 mm showing similarity with male *E. talpoida* but attained a maximum size upto 10-12 mm showing similarity with *E. analoga*.

Jackson (1913) observed that *Eupagurus* breeds throughout the year in the Irish Seas. Carayon (1941) had collected berried females only from June to August from Northern Norway, March to summer from

Bohuisian (Norway) and November to February from French Coast. From the data it is clear that the breeding was not continuous in these regions.

Wharton (1942) reported that the berried Females of *E. talpoida* were seen from June to September. Lloyd and Yonge (1947) and Pike (1953) stated that the spawning is clearly seasonal in *Crangon* and *Pandalus* respectively. Boolootian *et al.* (1959) reported that *Pachygrapsus crassipes, Hemigrapsus nudus, E. analoga* breed synchronously whereas *Pugettia producta* and *Petrolisthes cirictipes* breed continuously. *Emerita protoricensis* and *Mysidium columbiae* are continuous breeders (Goodboy, 1965). The females of *Charybdis variegata* of Tondi region on the east coast of India have two distinct peaks in the gonad index, during March and September. It is, therefore, concluded that the species are biannual breeder (Chandran, 1968).

In the present investigation, the barried females of *E. holthuisi* were found throughout the year (September 1973 to August 1974). From Table 5 it may be inferred that *E. holthuisi* breed throughout year attaining peaks in September and March. Apart from the two peaks in spawning this crab showed variation in the intensity of spawning from month to month throughout the year. The rise and fall in percentage of ovigerous females during the period of September 1973 to August 1974 can be interpreted on the basis of data presented in Table 5. According to Sankolli (1965) the incubation period (fertilized egg to megalopa) of *E. holthuisi* is about 28 to 30 days. The adult females reberry themselves within 2-6 days after the escape of the zoeal larvae. For crustaceans in general, and anomurans in particular, there is no literature correlating the role of environmental factors in controlling reproductive activities. In case of *E. holthuisi* according to the results obtained in our study, it may be suggested that moderate salinity (28 to 34‰) and moderate temperature (26 to 31°C) ranges help to induce spawning. The spawning peak observed in March may be because of gradual increase in temperature followed by a slow enhancement in salinity. During rainy season the breeding activity was at low level as indicated by histological observations and at that time the salinity of sea water was declined. Whereas in September sudden rise in salinity and temperature may be the probable cause for increased spawning. But this increased spawning was less as compared to one occuring in March.

However, it should be remembered that the exogenous control of reproduction is not an absolute entity, but it is expected that the exogenous parameters may trigger the endogenous controlling machanisms (probably hormonal) which regulate the reproductive phenomenon.

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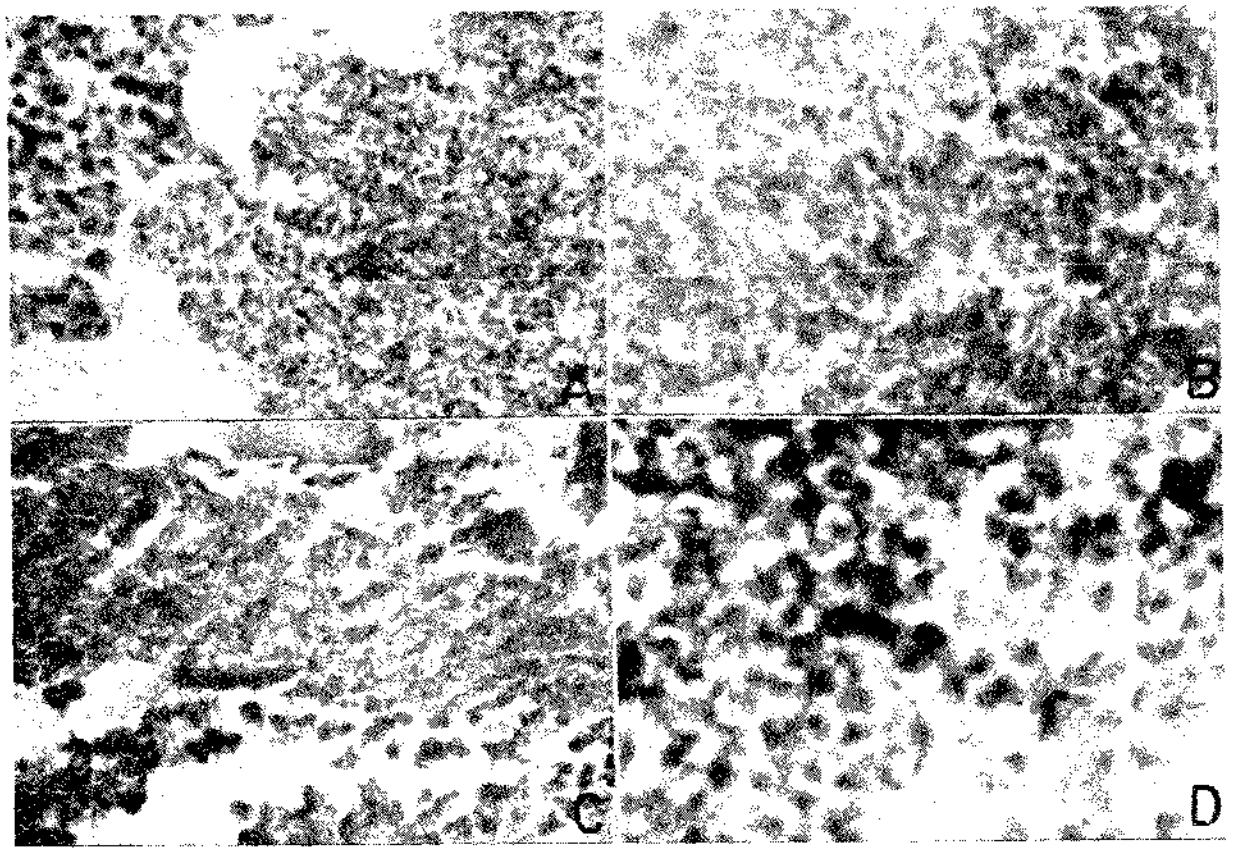

PLATE I. Gonadal stages of male *Emerita holthuisi* - A. Immature; B. Maturing; C. Mature and D. Spent.

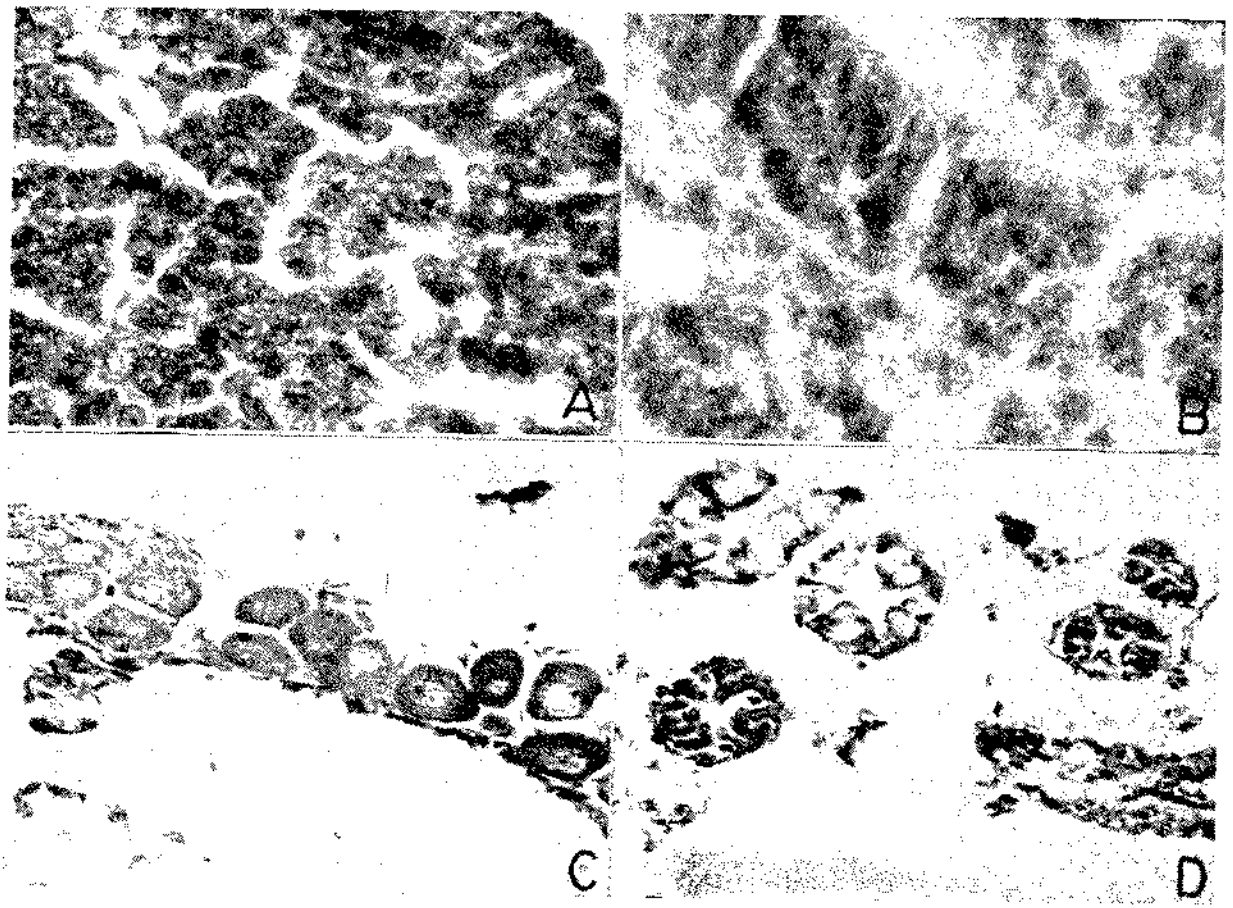

PLATE II. Gonadal stages of female *Emerita holthuisi* - A. Immature ; B. Maturing ; C. Mature and D. Spent.

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