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32. REPRODUCTIVE BIOLOGY OF THE WEDGE CLAM, *DONAX CUNEATUS* LINNAEUS

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ABSTRACT

Donax cuneatus **of Madras coast has a distinct annuai reproductive cycle. Almost In all tha clams the gametoganic cycle starts in November-December. Spawning commences in February and extends upto July with intermittent extrusion of gametes. Subsequent to spawning, the Initiation of gimetogenesis does not commence immediately but begins after a distinct inactive period of three months. Temperature and salinity influence the breeding of the clams.**

INTRODUCTION

The reproductive biology of marine molluscs has been studied in India extensively during the last three decades. Though considerable work has been done on the gametogenesis of a few commercially important bivalve molluscs such as edible oysters, green mussel, clams like /Were-*trix casta and Katelysia opima* (Rao 1950, 1956; Durve 1964, 1965; Rao 1975). Our knowledge on the reproductive biology of the genus *Donax* is scanty. Nayar (1955) has made observations on the stages of maturity and breeding of *Donax* cwneafus of Palk Bay. Alagarswami (1966) has studied in detail the reproductive cycle of *D faba* of Mandapam coast and Rao (1967) of *D. cunea*-rws of Madras coast. In this work Oonax cunea-fws were collected from three places along Mad-ras coast and the annual reproductive cycle studied.

MATERIAL AND METHODS

To study the reproductive cycle of *Donax cuneatus,* samples were collected on fortnightly intervals from three stations namely Marina, Thiruvaniyur and Mahabalipuram along the Madras coast. Following collection, the clams werebroughttothelaboratory, shucked and the soft bodies were taken out. After noting the macrostructure of the gonad, the sex and stages of maturity were ascertained by examining fresh smears of gonad under microscope. Gonadal tissues were fixed in Bouin's fixative and 10% neutral buffered formaldehyde. It was then processed for paraffin embedding. The sections were cut at 6m thickness and were stained in Ehrlich's haematoxylin and counterstained with ^^^.^ J^^^ gametogenic cycle of *D. cuneatus* ^^g j^^j^^^ j^^^^^ ^^ ^^^^ ^^.^^^.^ ^^^p^^^ ^^ Tranter (1958), Alagarswami (1966), Rao (1967), Dinamani (1974) and Nagabhushanam and Talikhedkar (i977). in all, a total of 6116 clams were examined and studied for the reproductive cycle.

RESULTS

|^ £, *cuneatus* from the histological examination of the gonad five stages of development could be distinguished (Pi. 1, 2, and 3). The criteria by which these stages were defined are gjven in Table 1. in stage la (early active phase) the gonad is small, inconspicuous and colourless In stage I b (late active phase) the gonad become slightly larger in size, thick and firm, jj^e gonad is colourless and transparent. In stage II (Ripe phase) the gonad is full and plumpy and attain the maximum size, creamy in colour with very little connective tissue sorrounding it. In stage 111 (Post spawned phase) the gonad is f'abby, loose in consistency, slightly greyish in colour and few phagocytes are present among gonadal cells. In stage IV (Spent phase) the 9°"^^ is transluscent and shrunken. Relict oocytes and phagocytes are present. In stage V (Indeterminate) the follicles are completely collapsed. Sex cannot be differentiated by smear examination.

Annual reproductive cycle

The percentage frequency of gonadal phases in different months was observed in males and

TABLE 1. *External features, histological and cytological details of gonadal phases in* Donax cuneatus

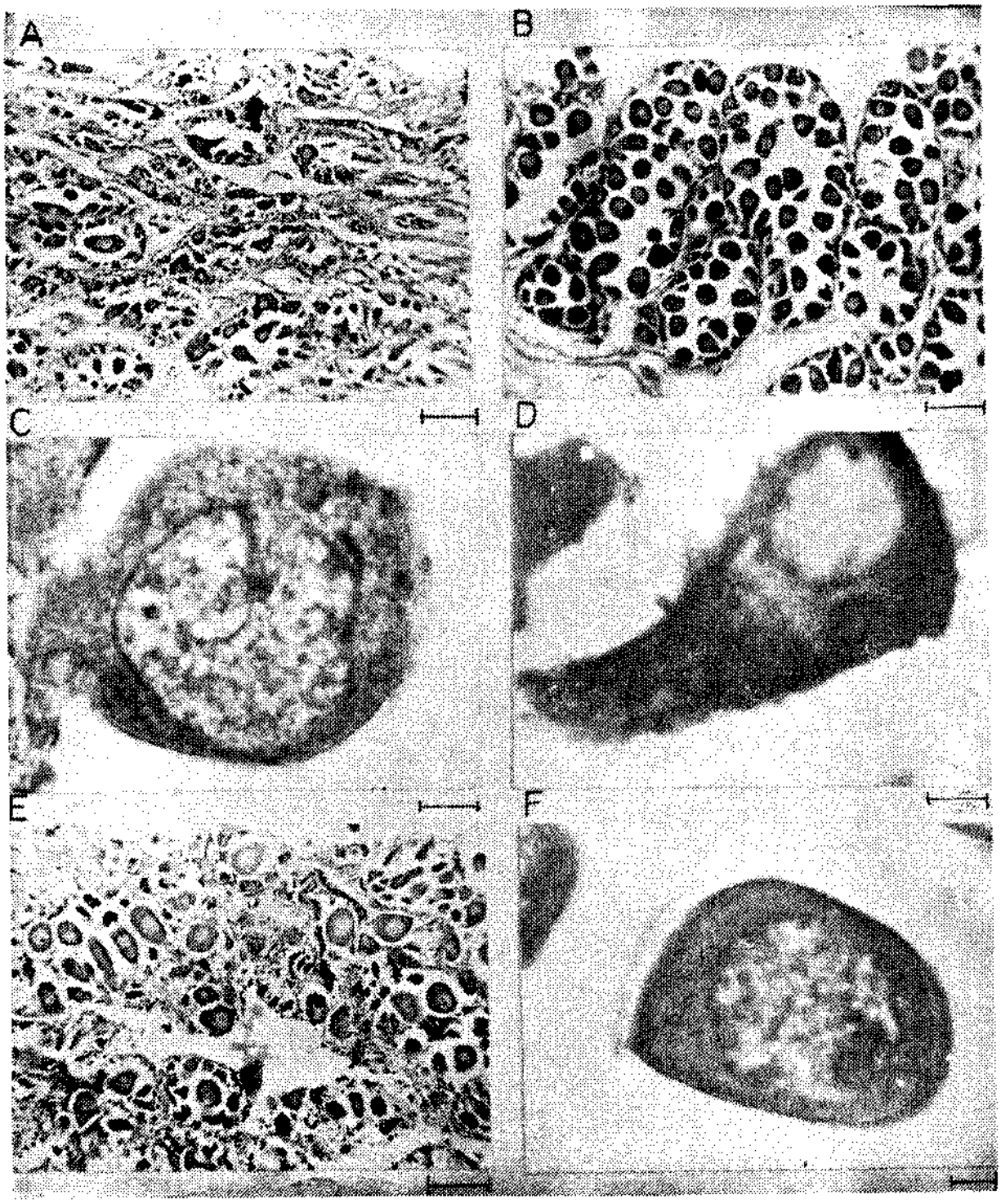
Gonad phase	Male	Female
1 a Early active	Increase in gonad size limiting the digestive gland to a restricted space; gonad somewhat flabby. Follicles contain mainly spermatogonia and spermatocytes; no spermatozoa.	Gonad size increases restricting the digestive gland to a limited space; gonad somewhat flabby; oogonia arising from stem cells in the follicle wall; a few attached to primary oocytes; no free oocytes
1 b Late active	Gonad thicker and firm, follicles larger and becoming packed together. Follicles contain predominantly spermatids and spermatozoa; characteristic swirling pattern of spermatozoa, with tails towards lumen. Follicles do not occupy entire gonad area.	Gonad thicker and firm, follicles larger and packed closely. Secondary oocytes are attached to the follicle wall by slender stalks. Few free oocytes in the lumen with distinct nuclei. Follicles increase in size.
II Ripe	Gonad becomes creamy in colour and full and piumpy. Bunches of spermatozoa with tail oriented towards the large follicle lumen. In fully ripe specimen, spermatozoa fill up the lumen.	Gonad becomes creamy and piumpy. Predominantly, large free oocytes in the lumen with distinct nucleus and nucleolus, rounded to oval; follicles are closely packed without interspace; no interfollicular tissue seen.
III Post spawned (Partially spent)	Gonad flabby and loose in consistency. Colour greyish; many follicles discharged; mass of spermatozoa separated from follicular walls; phagocytes present in the interfollicular space.	Gonad flabby and loose in consistency. Colour turned to greyish. Many follicles discharged. Few phagocytes present among gonadal cells.
IV Spent	Gonad loose and translucent. Follicles collapsed; residual sperms and phagocytes present.	Gonad loose and translucent. Residual oocytes occasionally present. Follicles collapsed, but relict oocytes present
V Indeterminate	Indeterminate phase is characterised by collapsed or shrunken follicles, with indifferent germ cells lining the walls in the early stage. Phagocytes distinctly present.	

females separately at all the three stations. However, the frequency of gonadal phases showed insignificant differences between the three stations. Hence the data for all the three stations were pooled together and presented in Table 2.

Gonadal condition in pre-monsoon

July

The gonad appeared loose and translucent. The gonads of both sexes were in the spent condition. The percentage frequency of partially spawned phases were 12.6 and 21,3 in bot^j



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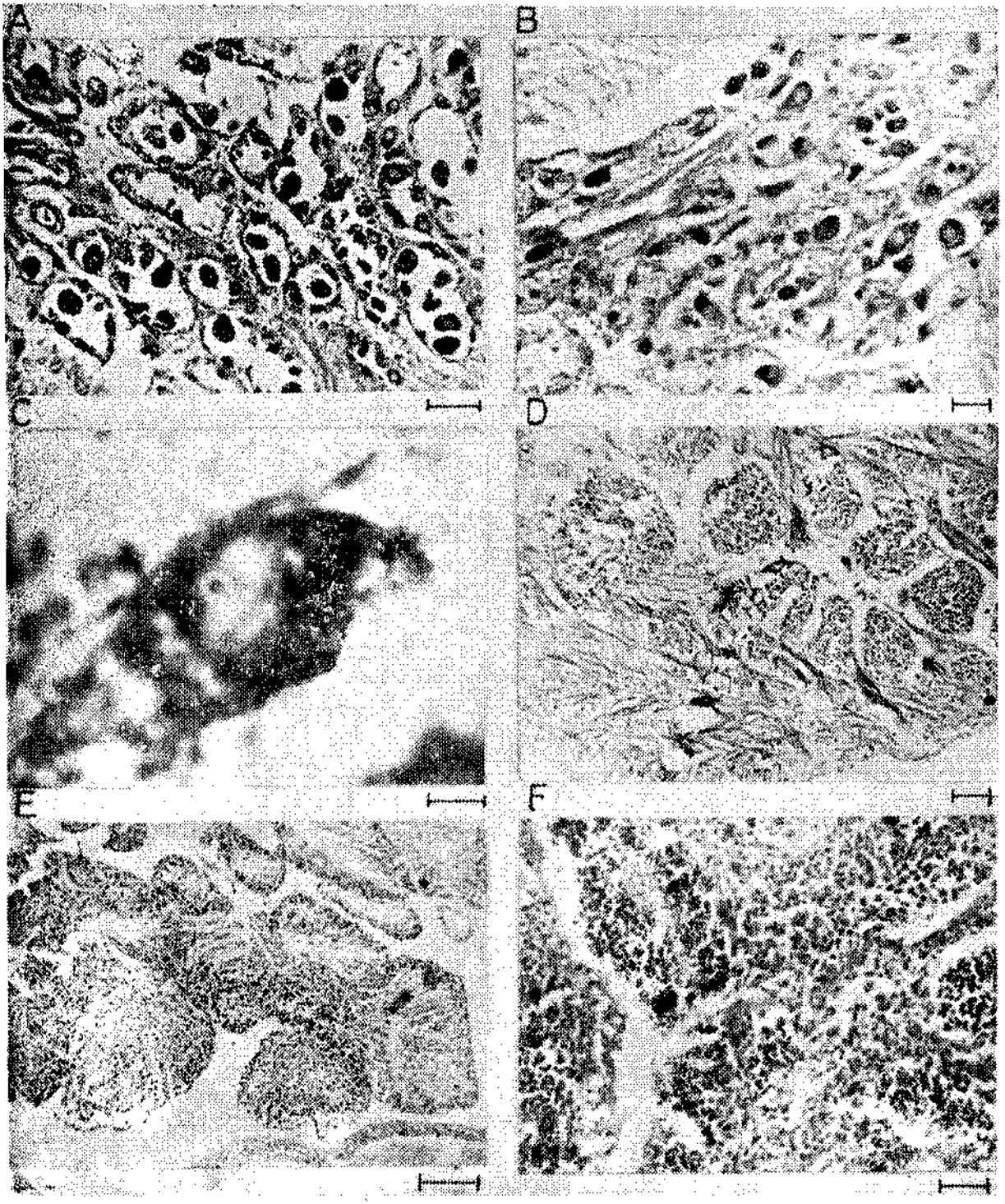

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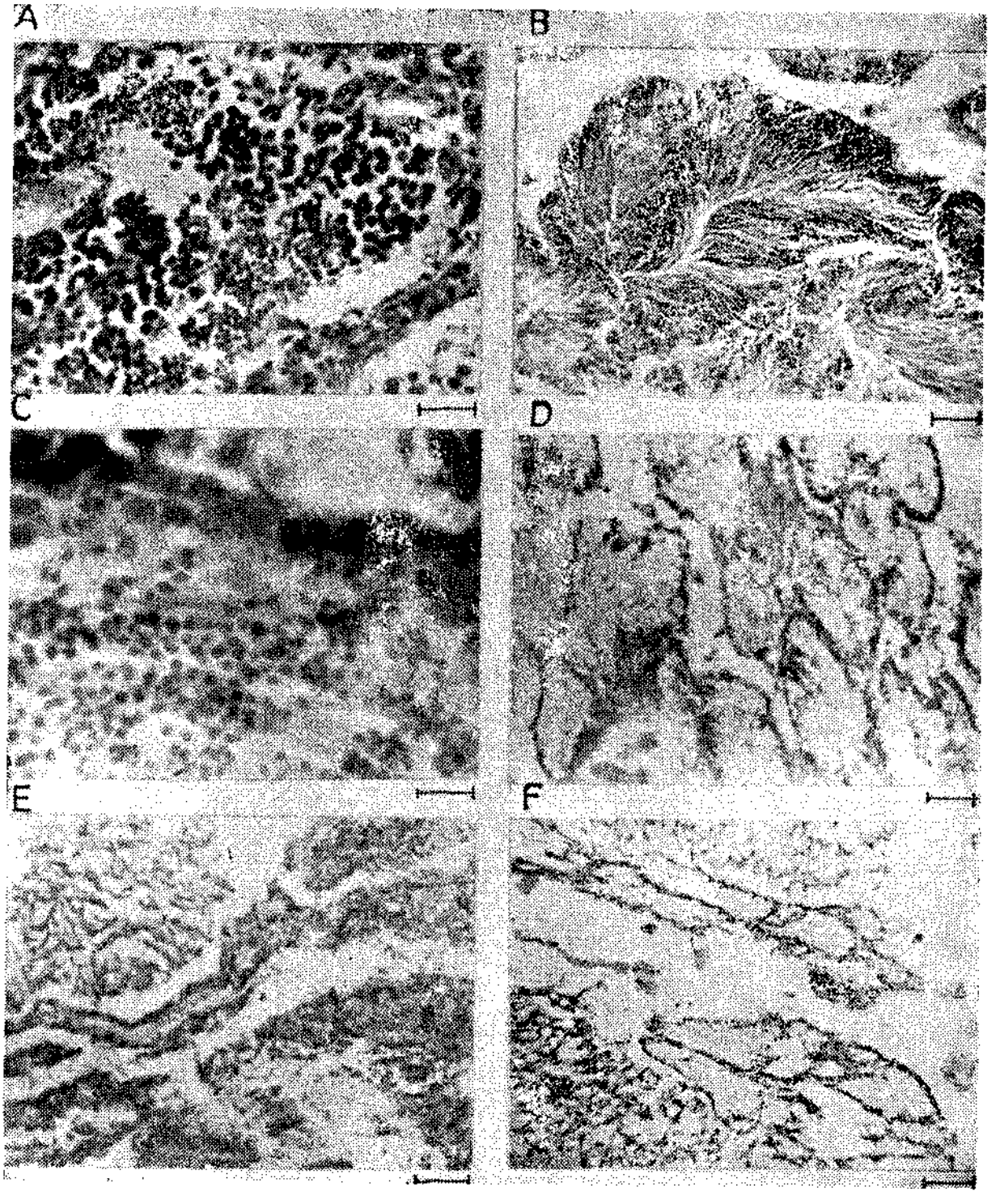

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PLATE I. A. Cross section of the active phase ovary of *Donax cuneatus* showing the proliferation of oogonia and oocytes. Bar represents lOO(Ji. B. Cross section of the ripe phase ovary showing fully packed follicles. While few of the oocytes are attached to the inner wails of follicles by stalk like connections, majority of the oocytes are free. Bar represents 100/*. C. Magnified oocyte of ripe phase ovary. Nucleus with nucleolus is noticed Bar represents lO/i. D. Magnified stalked oocyte of ripe phase ovary. Bar represents 10 i^. E.Cross section of post spawned phase ovary showing unspawned oocytes, shrunken follicle cell noticed. Bar represents 80 \^. F. Magnified oocyte of post spawned phase ovary. Bar represents 10 *v-.*

PLATE II. A Cross section of the spent phase ovary showing contracted follicles containing some relict ova. Bar represents SO/'. B. Cross section of the spent phase ovary showing the process of cytolysis of oocytes. Bar represents 80 M. C. Magnified degenerating oocyte showing the disintegration of nucleus and the process of cytolysis. Bar represents 10 Z'. D. Gross section of the early active phase testis showing divisions of spermatogonia upto secondary spermatocytes. Bar represents 100 Z'. E.Cross section of the late active phase testis showing clusters of spermatids outside the core of spermatozoa. Bar represents 100|it. F. Magnified late active phase testis showing the occurrence of Spermatocytes, spermatids and spermatozoa. Bar represents 50 *[>•.*

PLATE III. A. Magnified late active phase testis showing the occurrence of spermatocytes, spermatids and spermatozoa. Bar represents 10 [A. B.Cross section of ripe phase testis showing streams of spermatozoa arranged more or less radial columns with their tails radiating towards the centre of follicular lumen. Bar represents 100 t^. C. Magnified ripe phase testis showing the tailed spermatozoa. Bar represents 10 (A. D. Gross section of post spawned phase testis showing the shrunken follicles. Bar represents 100 i^. E. Cross section of spent phase testis showing the highly shrunken follicles with empty follicular space. Bar represents 100//. F. Gross section of gonad in sexually indeterminate phase. Bar represents lOOl*.

TABLE 2. *Percentage frequency of gonadal phases in Monthly Samples of* Donax cuneatus *from Marina, Thiruvanmiyur and Mahabalipuram (pooled data) during the period July 1982 to August 1984.*

Month		MALE Gonadal phase					FEMALE Gonadal phase				
Year		I	II	III	IV	V	I	II	III	IV	V
July	1982			24.05	47.66	28.29			25.78	45 93	28.29
Aug				8.60	44.90	46 49			6.77	46 73	46.49
Sep					23.00	77.00				23.08	77 00
Oct		16.72				83.28	16.72				83.28
Nov		56 84	22.97			20.19	60.78	19.02			20 19
Dec		9.36	79.81	6.91		3.92	16.73	72.60	6.76		3.92
Jan	1983	5.12	58.97	24.83		11 08	6.14	54.85	27.92		11.08
Feb		1.04	28.70	65.87	2.30	2.08	1.19	32.54	60.40	3.80	2 08
Mar			13.09	70.18	13 28	3.45	1.17	15.81	62.01	17.56	3.45
Apr			5.30	47.32	46 29	1.10		8.72	56.00	34.18	1.10
May			1.59	35.88	61.79	0.73		3.33	38.73	57.21	0.73
June				17.57	77.14	5.29			27.36	67.35	5.29
July				8.89	50.50	40.61			6.55	52.83	40.61
Aug				2.57	46.44	50.99			2.10	46.90	50.99
Sep					10.57	89.43				10.57	89.43
Oct		1.68			1.12	97.20	1.06			1.12	97.82
Nov		56.28	4 89			38 84	55.46	5.70			38.84
Dec		28.11	64.29	5.84		1.76	32 66	61.27	4.31		1.76
Jan	1984		76.64	19.98	0.47	2.90		82.49	14.61		2.90
Feb			24.63	65.87	9.50		1.52	18.90	70.58	9 01	
Mar			15.14	71.48	13.39		2.38	16.18	67.14	14.30	
Apr			3.33	61.81	34.86			1.89	65.78	32.33	
May				47.31	52.69				47.77	52.23	
June				13.65	86.35				16.66	83.34	
July					92.49	7.51			92.49	7.51	
Aug					18.56	81.44			18.56	81.44	

I = Active phase; II = Ripe phase; III = Partially spawned phase;
IV = Spent phase; V = Indeterminate phase.

males and females respectively. On the other hand in males the spent phase increased to 61.5% as against 52.5% in females. The percentage of indeterminate phase was 26.2 in both males and females. The fully spent clams were characterized by the presence of negligible numbers of residual oocytes and spermatocytes and more of vesicular tissue and condensed connective tissue. The follicles collapsed and the follicle lumen showed varying degrees of emptiness. In the spent gonad unshed oocytes or spermatozoa which were large in number in the lumen were in the cytolysed condition.

August

In this month majority of the clams had entered the indeterminate phase. The percentage of spent males and females was 39 and 44 respectively and that of the indeterminate clams was 56. The follicles were completely disrupted leaving a hieroglyphic appearance, In this stage

the gametes which were not yet released undergo resorption by being enveloped with connective tissue around the follicles. A large number of invading phagocytic cells appeared. During this time, the nucleus disappeared, the cytoplasm oozed out, the oocytes disintegrated, becoming transparent, and the constituents of the gametes were resorbed completely. When the process of cytolysis and resorption was completed, the follicles became empty. The gonad became translucent. It was at this stage that the sex of the individual become indeterminate.

September

In September the percentage frequency of indeterminate phase gonads was on the increase (72%) when compared to spent phase gonads which constituted only 28%. The residual reproductive elements were completely resorbed and the follicles were very much shrunken.

Gonadal condition in Monsoon

October

Though the percentage of gonads in the indeterminate phase increased furthermore to 88%, gametogenesis had commenced in 12% of the clam; the intensity of activity was considerably low when compared to the peak period of activity. In general, the indeterminate phase clams did not show any marked difference from those observed in the previous month. In the indeterminate phase, no trace of the follicular tissue was found and the gonad was completely resorbed.

November

The gametogenic activity initiated during the previous month became intense and as a result, the percentage of clams in the active phase increased to 54 in males and 66 in females. Only 5% of the clams were in the indeterminate phase. Due to active gametogenesis, about 40% of males and 27% of females had entered the ripening phase.

In the active phase, pronounced enlargement of follicles was due to the rapid increase of their size. In the testes, the reproductive follicles contained a large number of early stages of spermatogonia with spermatocytes and a few spermatids radiating into the lumen of the follicles. The gametogenic activity in the females proceeded rapidly. The number of follicels in the ovary increased and small oocytes with rounded distal ends protruding into the lumen, the other end being attached to the follicular wall by a slender stalk. A few follicles still in the process of proliferation have young oocytes in the follicular wall. In the ripe phase the gonads became plump and full and form the major part of the visceral mass. The follicles were enlarged and packed with reproductive elements.

December

As a result of the rapid growth of gonadal follicles during the previous month, the gonads had become full and plump and form the major part of the viscera. The percentage of ripe gonads in both males and females was 70 and 71 respectively. Concurrently, 20% of males and 21% of females had entered the partially spawned phase.

The follicles in the males as well as females were closely packed with ripe gametes without any interspace, the vesicular connective tissue being completely obliterated. Ripe male gonads were characterized by streams of spermatoza, with their tails directed towards the lumen. In the ripe female gonad, follicles filled the whole gonad with very little interspace between them. Large numbers of nearly round oocytes with distinct nucleus and nucleolus were found in the lumen of follicles.

Gonadal condition in post-monsoon

January 1983

In January, majority of the clams still remained in the ripe phase. The percentage of ripe phase gonad in both males and females were 53 and 43 respectively. The gonads in the partially spawned phase did not show any variation from those observed in the previous month. Clearly, there was a mild spawning activity in December; however, the ovary remained in the same condition till the end of January, 1983.

February

Spawning had become very vigorous, as evident from the appearance of a larger percentage of clams in the post-spawned phase. The gonads in the ripe phase showed a decrease from the previous month, as spawning started vigorously. The percentage of postspawned gonads increased to 62 and 74 in males and females respectively.

In the postspawned phase, the gonads gradually became flabby and slightly loose in consistency, showing a dull greyish colour indicating the commencement of spawning. Later on, the follicles became shrunken resulting in a marked reduction in the number of gametes within the lumen. The unspawned oocytes remained attached to the follicular wall; however, they were smaller in size. In some follicles of male gonad, the lumen appeared empty due to the discharge of spermatozoa.

March

Spawning had reached the peak as evident from the appearance of a large percentage of clams in the postspawned phase. The percentages of partially spawned gonads in males and females were 72 and 74 respectively.

Gonadal condition in summer

April

The percentage of postspawned gonads still remained high. However, the /appearance of a larger percentage of spent phase gonads in this month indicated that the spawning activity was still vigorous. The partially spawned phase gonads which formed 72% males and 74% females during the previous month declined to 47% and 58% respectively. Nevertheless the spent phase clams constituting 10% males and 4% females during the previous month increased to 40% and 25% respectively.

May

In May, spawning had far advanced and majority of the clams were found to be in the spent phase as indicated by the slow fall of postspawned phase gonads of both sexes. The percentage of spent phase gonads of males and females was 49 and 58 and that of postspawned ones was 40 and 25. The fully spent gonads appeared loose and translucent. The lumen contained residual oocytes and spermatozoa. The follicles were collapsed in some case, while in others, faint lines indicating the presence of follicular walls. Phagocytes appeared in large numbers both inside and outside the follicles. They cytolysed and devoured the remnants of undischarged residual gametes.

June

Spawning continues further and the clams in spent phase increased in number The percentage of spent phase gonads of males and females increased to 75 and 71 respectively. The fully spend gonads were characterised by the presence of a negligible number of residual gametes.

The same pattern of reproductive cycle was observed during the subsequent year from July 1983 to August 1984 with minor variations in the time, intensity of gametogenesis and spawning. Between July and August 1983 the major rity of gonads continued to remain in the spent phase. At the same time, the process of cytolysis and resorption was over in 40% of the gonads. Thereafter, these clams had entered the indeterminate phase. By September and October the residual reproductive elements were all resorbed and the sex of individual became indeterminate. From November onwards, gametogenic activity was initiated and the clams entered the active phase. In December and January as a result of the rapid growth of the follicles, the gonads had become fully ripe commensurate with the reproductive cycle for the corresponding period in 1982 and 1983. From February to May, spawning was vigorous as evident from the larger percentage of postspawned gonads. By June and July, spent phase gonads were on the increase owing to the continuation of the spawning activity. By August, the residual gametes were all absorbed and the clams entered the indeterminate phase.

It may be seen from the foregoing account that *D. cuneatus* of Madras coast follows a definite pattern of annual reproductive cycle with a prolonged breeding period extending from January to July. The gonadal changes were cyclical with well-defined phases of gametogenesis such as ripening, spawning and regression. Gametogenesis commences between October and November. Ripening took place mainly In December-January, spawning activity continues from February to July, entering into the Indeterminate sexual phase from August-September.

DISCUSSION

D. cuneatus exhibits an extended annual breeding cycle, in which the initiation of gametogenesis does not commence soon after spawning, but begins after a distinct inactive period of three months. Interestingly, the time taken for the completion of gametogenesis is shorter than the spawning season. Spawning does not occur in one stroke but intermittently over a long period (Rao 1967; Mane and Nagabhushanam 1976). This strategy in spawning is helpful in the reduction of intra-individual competition among the young ones.

A comparision of data on the reproductive cycle of *D. cuneatus* inhabiting different beaches on the east and west coast of India reveals difference in their breeding cycle, especially with reference to spawning season. In the west coast, the spawning season of *D. cuneatus* extended from October to January (Nagabhushanam and Talikhedkar 1977) and on the south east coast it was from January to April (Nayar 1955). On the Madras coast as mentioned earlier, the spawning season extends from January to July. Rao (1967) has also recorded almost a similar spawning season, January to June in the species on the Madras coast. In marine invertebrates resorting to broadcast fertilization, the time of release is very important as the embryonic development is accomplished within a very short period and the larvae are planktotrophic. Therefore, the survival of larvae depends very much on the environmental conditions obtained at the time of spaivning. A subtle difference in the spawning season of *D. cuneatus* in various parts of the Indian coasts lends support to this supposition. An analysis of food preferences for these larvae in different beaches may throw new light on this aspect.

Whereas the causative factor for differential spawning period may be related to larval survival, the environmental factors such as the temperature and salinity may constitute the proximate stimulus for gametogenesis and spawning. In temperate regions many workers have shown a close relationship between fluctuation of the habitat media of marine invertebrates and their gonadal conditions. There, the chief spawning stimulus, in the case of oysters, is the rise in temperature (Loosanoof and Davis 1950).

The low surf water temperature recorded at Madras coast from November 1982 (27.6°C) to December (26.2°C) and November 1983 (27.4°C) to December 1983 (26 2°C) coincides with the period of active gamete development and ripening in D. *cuneatus.* During the period of peak spawning the temperature which triggers spawning lies between 27.6°C (February) and 28 2°C (March) in 19?2 and 26 rc in (February) and 27.5°C in (March) in 1983. The temperature in the preceding months of spawning was observed to be 25°C in January 1982 and 25.5°C in January 1983 This clearly indicates that a slight fall in the temperature during November and December indicates gamete development and ripening of gonad in *D. cuneatus.* A slight increase in the temperature during February and March induces the clam to spawn and this activity continues upto July. The data of Nayar (1955) and Rao (1967) show that in *D. cuneatus* spawning takes place whan the temperature is on the ascent. Therefore, the temperature appears to be an important factor regulating the gametogenesis and spawning in *D. cuneatus.* However, Nagabhushanam and Talikhedkar (1977) are of the opinion that temperature may not influence the spawning of this clam in the west coast.

Another important variation in the sea, under the influence of monsoon rains, is salinity. In the present investigation the low surf water salinity recorded on the Madras coast during October-December coincided with the period of active gonad development and ripening in *D. cuneatus* and a sudden increase in th6

salinity during February and March triggers the clam to spawning which continues till July. Thus in *D. cuneatus* active gametogenesis takes place in both sexes when the temperature and salinity are low and spawning occurs when the temperature and salinity are higher.

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