CHAPTER III
Chapter III

REPRODUCTIVE BIOLOGY

Introduction

A thorough knowledge on different aspects of reproduction is important and essential for understanding the annual recruitment to the population and to formulate suitable managerial measures to sustain the population in nature. Information on maturation process, breeding habits and the biotic and abiotic factors influencing the reproduction is imperative to develop suitable technologies for hatchery production of seed and for the culture of the species.

The process of reproduction in bivalves involves germ cell differentiation, gonad development, maturation, spawning, fertilisation, larval development and seed production. The pattern of reproduction differs from species to species according to various intrinsic and extrinsic factors. They may occur in a regular pattern resulting in annual or semi-annual cycle and continuous spawning can also occur with prominent peaks in particular seasons. In addition, environmental variables mainly salinity and temperature, are relied upon as synchronisers of the basic seasonal rhythm of gametogenesis.
Production of gametes in most of the marine bivalves requires a great deal of energy, suggesting a close relationship between the reproductive cycle and energy availability for growth (Bayne, 1985). Gonochorism is seen in majority of bivalves. The gametes are discharged through gonadal duct into the mantle cavity and from there into the surrounding water along with the exhalent water.

After the external fertilisation, larval development takes place in the ambient medium. Some bivalves exhibit hermaphroditism, sex reversal, incubation of developing young ones, etc. Failure in the reproductive activity may result in serious damage to the population structure thereby productivity. Thus, an understanding of the reproductive biology of the bivalves is essential for the proper management, culture and judicious exploitation of the resources. Several methods of assessing the course of reproductive cycle in marine bivalves have been employed. This includes direct observations on spawning in natural or laboratory populations or the occurrence of mature gonad in the population. Observations on gonadal smears and histological studies are resorted to understand the maturation process and to delineate the maturity stages. Relative abundance of developing, mature and spent population and larval abundance yield valuable information on spawning season, spawning intensity and spawning success.
REVIEW OF LITERATURE

Information on the breeding cycles of bivalves is extensively available from Indian waters. Abraham (1953) made detailed observations on the biology of the clam *Meretrix casta* regarding growth, breeding habits, longevity and mortality in the Adayar Estuary at Chennai (formerly Madras) backwaters. A comprehensive study on the reproduction of Australian pearl oyster *Pinctada albina* was conducted by Tranter (1958 a,b,c) which included primary gonad development, gametogenesis, breeding and sexuality and provided valuable information on the cytological aspects of reproduction. Mason (1958) reported the gonadal development, spawning, fertilisation, development of larvae and spat of *Pecten maximus*.

Durve (1964, 1965) made investigations on the seasonal gonadal changes and spawning in *Meretrix casta* and in the edible oyster, *Crassostrea gryphoides* from Bombay waters. A detailed investigation on the growth and reproduction of the clam, *Donax faba* in the Gulf of Mannar was carried out by Alagarawami (1966). Annual reproductive cycle of *Donax cuneatus* of the Madras Coast was studied by Rao (1967) based on the seasonal gonadal changes. Reproductive cycle of estuarine bivalve, *Musculista arcullata* was described by George and Nair (1973). Nagabhushanam and Mane (1975) reported on the reproductive biology of
mussel *Perna viridis* from Bhatia creek, Ratnagiri. Rao *et al.* (1975) studied the spawning, fertilisation and larval settlement of *Mytilus (=Perna) viridis*. Studies were conducted on the seasonal gonadal changes in the clam *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977). Salih (1977) gave a detailed account on the breeding activity of the clam *Meretrix casta* off Cochin barmouth. Studies conducted on the reproductive biology of the wedge clam *Donax cuneatus* by Nagabhushanam and Talikhedkar (1977a) revealed an extended spawning cycle with no resting period.

Detailed observations on the gametogenic stages, reproductive cycles, spawning periodicity, size at first maturity and sex ratio of the oyster *Crassostrea madrasensis* were made by Joseph and Madhyastha (1982, 1984) from Mangalore Coast. Observations on gametogenesis and breeding of oyster *Ostrea edulis* were reported by Wilson and Simons (1985) on the west coast of Ireland. They formulated an equation for predicting the onset of maximum ripeness of oyster populations with the help of histological studies. Jayabal and Kalyani (1986a) reported the reproductive cycles of three commercially important bivalves *Meretrix meretrix*, *M. casta* and *Katelysia opima* of Vellar Estuary. A comparative study of the reproductive cycle of the soft- shelled clam *Mya arenaria* in Long Island Sound were done by Brousseau (1987). Discussing on the reproductive cycle of the hard clam
Mercenaria mercenaria in Wassan Sound, Georgia, Hefflerman et al. (1988) found a synchronised polymodal breeding pattern. Narasimham (1988a) reported that the blood clam Anadara granosa in Kakinada Bay spawns throughout the year with two to four reproductive peaks.

Aspects of gametogenesis and spawning of the carpet-shell clam Ruditapes decussatus were reported by Shaffi and Daoudi (1991). Baron (1992) investigated the reproductive cycles of Stactidea striata, Grafrarium timidum and Anadara scapha. A comparative study was carried out by Xie et al. (1994) on the gametogenic cycles of the manila clam Tapes philippinarum and carpet shell clam Tapes decussatus. The gonadal developmental phases of the red clam Megapitaria aurantiaca were categorised into five stages using histological techniques (Garcia-Dominguez et al., 1994). Etim (1996) elucidated that Egeria radiata spawns once in a year during the peak of the rainy season in the Nigerian waters. Sebastian (1997) observed that the black clam Villorita cyprinoides at Cochin backwater breeds twice a year with peak spawning activity in June-July and January-February.

Environmental differences resulted in different physiological responses with respect to timings of development and developmental pattern. Nagabhushanam and Mane (1975) correlated seasonal variation of
reproductive cycle of *Mytilus viridis* with fluctuations in temperature and salinity of the area. Increase in temperature and salinity soon after the monsoon appeared to promote gametogenesis and initiate spawning in *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a). Nagabhushanam and Dhamne (1977) observed that the spawning stimulation in *Paphia laterisulca* appeared to be due to the sudden increase in salinity. Stephen (1980a, b) revealed that the influence of salinity during different seasons synchronised the gametogenic pattern in *Crassostrea madrasensis*. In *Saccostrea cucullata*, Sukumar and Joseph (1988) opined that an increasing salinity triggered the maturation while low saline condition initiated spawning. On the other hand, Victor and Subramoniam (1988) noticed an influence of low salinity and temperature on active gametogenesis in *Donax cuneatus* in Madras waters and high salinity and temperature on spawning. According to Robinson (1989), in *Crassostrea gigas*, optimum temperature and optimum salinity for larval rearing were 26°C and 25 ppt respectively after studying the reproductive cycle and conditioning trials. Newell *et al.* (1989) studied the factors regulating reproduction and recruitment in populations of *Crassostrea virginica* and found that the low salinity has an adverse effect on survival rate. Sphigel (1989) observed that gametogenesis in *Ostrea edulis* occurred in salinity as high as 41 ppt.
Though *Donax incarnatus* is a species with considerable importance as a nutritious and proteinous food, very little work on the reproductive biology has been carried out so far. Hence, the present investigation has been taken up to elucidate the gametogenic pattern and the influence of environmental factors on the reproductive biology. More emphasis has been given to the studies of gonadal smears and histology.

**MATERIAL AND METHODS**

Samples were collected every month during the year 1991, except during monsoon period when no samples were available from the collection ground at Malippuram coast. Specimens of different size groups were used for the study. The clams were maintained in the laboratory conditions for 24 hours in filtered (environmental) seawater collected from the sampling site. The detailed microscopic observations of the individual gonad were made for both sexes for the description and classification of the developmental stages of the gonad.

Standard histological technique was used to assess the reproductive cycle. Twenty animals arbitrarily selected with respect to age and visible stage of gonad development were excised and fixed in Bouins fixative for 24 hr. The tissue was then washed for 5 minutes in running water, dehydrated in graded ethanol, embedded in paraffin wax, serial sections of 7μ were made
and spread on slides smeared with Mayer's albumen, stained in Mayer's hematoxylin, counterstained with eosin and mounted in the D.P.X mounting medium. Slides were examined under light microscope and classified into different developmental stages. Examination of the sections made from monthly samples and on different maturity stages furnished detailed information on the reproductive cycle including the actual period of spawning in the study area. The male - female ratio was recorded in each month and Chi-square test was conducted.

RESULTS

Histology

Development of Gonad

In *Donax incarnatus*, the gonad envelops the ramified digestive gland and the loops of intestine. It develops seasonally to greater proportions, swelling the visceral mass. The ripe gonad is cream or yellowish in appearance.

*Donax incarnatus* is found to be gonochoristic with no sign of sexual dimorphism. There is no indication of the existence of sex reversal or hermaphroditism in samples observed during the course of the study. The maturation process of gonad is classified into five main stages in addition to
an indeterminate stage and termed as (i) early gametogenesis, (ii) late gametogenesis, (iii) mature, (iv) partially spent and (v) spent stage based on the cytological examinations of the gonad. Both male and female clams follow a similar pattern in the gonadal change.

*Indeterminate Stage (I₀)*

No gametogenesis is discernible and the sex is indistinguishable. Most of the gonad consists of interfollicular connective tissue between the follicles. The follicle is usually expanded and the follicular wall is dominant.

*Male: Early gametogenesis (MD₁)*

The proliferation and differentiation of the small earliest cells form stem cells which are distributed around the follicular wall is seen. Proliferation of follicles become more conspicuous and interfollicular tissue is present, but reduced (Fig. 3.1). Follicles contain definitive spermatogonia, and spermatocytes separate from follicle walls.

*Late Gametogenesis (MD₂)*

Follicles larger, become packed together. The follicles deeply penetrate the visceral mass and the follicular walls contain predominantly spermatogonia (Fig. 3.2) with spermatocytes and a few spermatids radiating into the lumen of follicles.
Fig. 3.1. Micrograph of early gametogenic stage of male gonad with proliferation of follicles and interfollicular tissue - MD₁

Fig. 3.2. Micrograph of late gametogenic stage of male gonad with spermatogonia (SG) - MD₂
Mature (MD₃)

The follicles contain closely packed sperm masses making appearance as streaks at various places in the gonad tissue (Fig. 3.3). They contain mainly spermatozoa. Spermatozoa aggregate in bands projecting into the lumen with their basophilic heads directed towards the periphery and sperm tails directed away from the wall towards the centre.

Partially spent (MR₁)

Follicles at various degrees of fullness are seen. In some follicles, the lumen is often seen empty due to the discharge of sperms while in other follicles, gametogenesis continues and the central part of the follicle is still filled with spermatozoa. Connective tissue starts developing between the follicles (Fig. 3.4).

Spent stage (MR₂)

In the spent condition the gonad is characterised by contracted follicles and the lumen of the follicles contain residual spermatozoa, which are partially cytolyzed by phagocytes (Fig. 3.5). Interfollicular tissue appears to occupy the space between the follicles.

Female: Early Gametogenesis (FD₁)
Fig. 3.3. Micrograph of mature male gonad with fully packed sperm masses (SPZ) - MD₃

Fig. 3.4. Micrograph of partially spent stage of male gonad with moderate quantity of spermatozoa - MR₁
This process in many respects is very similar to that of the male. Sex differentiation starts with the differentiation of the germ cells in the connective tissue. Follicles appear as scattered patches in the gonad. Follicular wall is lined with oogonia, primary and secondary oocytes (Fig. 3.6). Oogonia are the initial female germ cells proliferated from the large resting cells 'the stem cells' found around the follicular wall.

**Late Gametogenesis (FD₂)**

Follicles contain half-grown oocytes in the lumen and are attached to the wall by stalks; thin vitelline membrane is seen around some oocytes (Fig. 3.7). Follicular wall is lined with very a few oogonia and young oocytes; interfollicular tissue is seen.

**Mature (FD₃)**

Gonad is thick and attains larger size. Follicles contain full-grown and nearly round oocytes in the lumen of the follicles (Fig. 3.8). Free ova with nuclei are also found in the lumen. Pedunculated secondary oocytes are few and attached to the follicular wall.

**Partially Spent Stage (FR₁)**

This stage is characterised by the reduction in density of ova and rounding off as the pressure within the follicles is reduced (Fig. 3.9). Active
Fig. 3.5. Micrograph of spent stage of male gonad with residual spermatozoa and interfollicular tissue - MR₂

Fig. 3.6. Micrograph of early gametogenic stage of female gonad with developing oocytes (OGM) - FD₁
Fig. 3.7. Micrograph of late gametogenic stage of female gonad with pedunculated oocytes - $FD_2$

Fig. 3.8. Micrograph of female gonad with fully grown and nearly round oocytes (OC) - $FD_3$
discharge of ripe ova takes place and as it proceeds, the central portion of the follicles remain vacant

Spent Stage (FR₃)

Gonad remarkably shrunkens in this stage and is in different stages of phagocytosis. Spawning in females is not complete. Interfollicular tissue is seen (Fig. 3.10). Phagocytes present in this stage are for the resorption of the relict eggs by cytolysis.

In the present study the males were found to be in abundance only in April. In all other months, females were predominated (Table 3.1). As the clams reach 13-14 mm., they become sexually mature.

Annual Reproductive Cycle

In the annual reproductive cycle of Donax incarnatus studied over a year period has shown some sort of similarity in the development of the gonads in the two sexes. The percentage distribution of different stages of the gonads in different months is given in Table 3.2 and Fig. 3.11 and 3.12.

In January, the male clams were in late gametogenesis phase. Most of the clams were in the developing (45%) and mature condition (30%) while a few of them were still found in the spawning and spent phase. In February and March, most of the males were in mature and partially spawned phase.
Fig. 3.9. Micrograph of partially spent female gonad with reduction in density of ova - FR₁

Fig. 3.10. Micrograph of spent female gonad showing regression of oozytes with connective tissue - FR₂
Table 3.1. Distribution of male and female *D. icarnatus* during 1991
(Samples not available from May to August)

<table>
<thead>
<tr>
<th>Month</th>
<th>Male</th>
<th>Female</th>
<th>Chi-square</th>
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<tbody>
<tr>
<td>January</td>
<td>35</td>
<td>40</td>
<td>0.33</td>
</tr>
<tr>
<td>February</td>
<td>30</td>
<td>34</td>
<td>0.25</td>
</tr>
<tr>
<td>March</td>
<td>35</td>
<td>27</td>
<td>0.56</td>
</tr>
<tr>
<td>April</td>
<td>38</td>
<td>42</td>
<td>0.20</td>
</tr>
<tr>
<td>May - August</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>September</td>
<td>35</td>
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</tr>
<tr>
<td>October</td>
<td>42</td>
<td>46</td>
<td>0.18</td>
</tr>
<tr>
<td>November</td>
<td>31</td>
<td>41</td>
<td>1.39</td>
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<tr>
<td>December</td>
<td>41</td>
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<tr>
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<td>287</td>
<td>311</td>
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</table>
Table 3.2. Percentage distribution of the different stages of gonad development in male and female of *D. incarnatus* in different months
(Samples not available from May to August)

<table>
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<tr>
<th>Month</th>
<th>IND</th>
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<th>MD₂</th>
<th>MD₃</th>
<th>MR₁</th>
<th>MR₂</th>
<th>IND</th>
<th>FD₁</th>
<th>FD₂</th>
<th>FD₃</th>
<th>FR₁</th>
<th>FR₂</th>
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</thead>
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<td>30.00</td>
<td>10.00</td>
<td>5.00</td>
<td></td>
<td>9.09</td>
<td>50.00</td>
<td>22.7</td>
<td>13.64</td>
<td>4.55</td>
<td></td>
</tr>
<tr>
<td>February</td>
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<td>30.00</td>
<td>55.00</td>
<td>10.00</td>
<td></td>
<td></td>
<td>4.55</td>
<td>31.8</td>
<td>50.00</td>
<td>13.64</td>
<td></td>
<td></td>
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<tr>
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<td>27.77</td>
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<td></td>
<td>5.00</td>
<td>15.00</td>
<td>20.00</td>
<td>50.00</td>
<td>10.00</td>
<td></td>
</tr>
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<td>9.09</td>
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<td>18.18</td>
<td>45.45</td>
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<td>4.16</td>
<td>12.5</td>
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<td>21.74</td>
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<td>50.00</td>
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<td></td>
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<td>4.16</td>
<td>33.33</td>
<td>50.00</td>
<td>8.3</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.11 Percentage frequency of gonadal phases in *D. incarnatus* (Male)
(Samples not available from May to August)
Figure 3.12. Percentage frequency of gonadal phases in *D. incarnatus* (Female) (Samples not available from May to August)
Individuals with partially spawned condition were more (55%) in February and March, followed by mature phase (30%). Almost same percentage of mature and spawning phases was observed during February and March; the spawning phase was at its peak during these months. It was indicated by the presence of few spent clams (10% and 5.55%) with residual gametes. During April, 45.45% of the males were in spent phase with relict spermatozoa and spermatids in the lumen of the follicles. Others were partially spawned (18.18%) and mature (18.18%) condition.

After a break of few months, in September (Postmonsoon) most of the male clams collected were in indeterminate stage (47.83%). A few clams with mature (21.74%) and partially spawned (17.39%) conditions could be seen in the population. Small percentages of clams were in developing and spent stages. During October, males with mature gonad (45.83%) increased in number. Developing (16.66%) and spawning males (25%) were also observed. But in November, the late gametogenesis stage (57.14%) was more. Mature (19.05%) and partially spawned (9.52%) males were also observed in small percentage. During December, 50% of the population was in partially spawned, 31.8% in mature, 9.09 % in spent and 4.55% in early and late gametogenesis.
In the case of females, almost the same trend as in males could be observed. In January, 50% of the females in the population were in late gametogenesis phase. Individuals with mature (22.7%) and partially spawned gonads (13.64%) were also observed. A small percentage of early gametogenesis (9.09%), partially spawned (13.64%) and spent stage (4.55%) were also encountered in this month. During February and March, there was a rise in number of spawning females (50%) in the population while mature females registered only 31.8% and 20% respectively. In April, majority of the female clams were in spent condition (54.16%) in the population. This was followed by partially spawned (20.83%) and mature (12.5%) stages. Small percentage (4.16%) of developing and indeterminate clams was also encountered along with other maturity stages.

After the break of four months, during September most of the clams were in indeterminate condition (50%). Besides, 25% of the clams showed mature, 12.5% partially spawned state of gonad. About 4.16% of the clams were in developing (early and late gametogenesis) and post-spawned stages. During October, clams of mature stage constituted 42.86%. Clams with all the other stages of gonad could be seen in this month. During November, most of the clams were in late gametogenesis stage (48.14%). A few animals with mature (18.51%) and partially spawned (14.81%) gonads could be seen.
A small percentage of indeterminate and late gametogenesis stages could also be observed during the period. All the four stages were observed during December. Individuals with partially spawned (50%) followed by mature (33.33%) condition predominated in the population.

DISCUSSION

Bivalves are the group characterised by gonochorism and hermaphroditism. According to Coe (1943), about 96% of the species included in the Class Bivalvia have separate sexes. In the present study, the wedge clam *Donax incarnatus* is found to be gonochoristic and showed no signs of sex reversal and hermaphroditism. Similar observations were made by Nagabhusananam and Talikhedkar (1977a) in *Donax cuneatus* and in *Crassostrea madrasensis* (Stephen, 1980b; Joseph and Madhyasta, 1984).

The result of the test of variance for homogeneity revealed that the Chi-square value is found to be insignificant.

The classification of different stages in the reproductive cycle in *D. incarnatus* was very similar to the allied species of the bivalves. The maturation process of both male and female gonad involved five maturity stages, namely, early gametogenesis, late gametogenesis, mature, spawning and spent. This is in agreement with the classification of maturity stages
described by Nagabhushanam and Talikhedkar (1977a) and Victor and Subramonium (1988) in *D. cuneatus*.

The breeding habits and seasonal changes of many species of pelecypods from different parts of the world have been recorded: *Meretrix casta* by Abraham (1953); Durve (1964); Salih (1977); *Musculista arculata* Leela and Balakrishnan (1973); *Paphia laterisulca* Nagabhushanam and Dhamne (1977); *Crassostrea madrasensis* Joseph and Madhyastha (1984); *Anadara granosa* Narasimham (1988a), *Villorita cyprinoides* Modassir (1991) and *Marcia opima* by Maqbool (1993).

In the present investigation, *Donax incarnatus* showed a continuous breeding pattern with two spawning peaks in February-March and in December (Fig. 3.11 and 3.12). In tropical species, continuous breeding may not of the same intensity throughout the period and the fluctuations seen may probably be due to the environmental variations. The present findings closely agrees with Alagarswami (1966) in the species *Donax faba* from Mandapam area of the Southwest coast of India, but this species shows a prolonged breeding period with two peaks. The nature of reproductive cycle of a population of one locality is found to differ from that of another population of the same species occurring at a different locality.
(Nagabhushanam and Talikhedkar, 1977a; Victor and Subramonium, 1988) in *D. cuneatus*.

Narasimham (1988b) demonstrated two to four spawning peaks in *Anadara granosa* and the peaks were also noticed in *Mercenaria mercenaria* by Hefferman et al. (1988). Single extended annual reproductive cycle is shown by some bivalves like *Pinctada albina* (Tranter, 1958b), *Crassostrea gryphoides* (Durve, 1965), *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a), *Saccostrea cucullata* (Morton, 1990) and *Anadara sopha* (Baron, 1992).

The gonad of bivalve usually is seen in a resting stage after spawning (Loosanoff, 1962). But during the course of this study, germ cells of different developmental stages could be seen in the follicle of the gonad, showing apparently no resting or neutral stage. Similar observations were also made in *Donax faba* (Alagarswami, 1966), *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977), *Donax cuneatus* (Nagabhushanam and Talikhedkar, 1977a). In the present study, gametogenesis is initiated soon after spawning and in some females even before the follicles are cleared of cell debris. Braley (1982) attributed this because of a stable food supply, which restores depleted food reserves quickly. However, Victor and
Subramonium (1988) observed an inactive period of three months after spawning in *Donax cuneatus* along the Madras Coast.

The sequence and timing of events in the reproductive cycles of marine invertebrates are influenced by complex physico-chemical variables in the environment. The factors inducing spawning may be quite different from those inducing annual reproductive cycle (Giese, 1959). Under tropical conditions of the Indian Coasts, temperature is relatively high throughout the year and generally does not fall below the optimum requirements of many bivalves. Thus, it may be suggested that temperature may not play a direct role in the spawning of marine bivalves of Indian waters. But rapid changes in salinity are known to stimulate the spawning activity in bivalves of the Indian Coasts and this is dealt in detail in one of the following chapters. Some notable works on this aspect are those of Nagabhushanam and Talikhedkar (1977a) in *Donax cuneatus*, Joseph and Madhastha (1984) in *Crassostrea madrasensis*, Victor and Subramoniam (1988) in *Donax cuneatus*, Narasimham (1988a) in *Anadara granosa* and Maqbool (1993) in *Marcia opima*.

In the present investigation, during February, March and April, the salinity is found to be relatively higher (Table 1.1) and this promotes intense spawning activity during the period. After a break during monsoon season,
the gradual increase in salinity from September triggers the gametogenesis and during October-December, the increased salinity provides another congenial condition for intense spawning resulting in the second peak. Thus one of the main environmental factors inducing spawning is found to be the salinity of the ecosystem in which *D. incarnatus* inhabits. Increase in salinity has been found to stimulate spawning in many bivalves, viz. *Donax cuneatus* (Rao, 1967; Nagabhushanam and Talikhedkar, 1977b); *Paphia laterisulca* (Nagabhushanam and Dhamne, 1977); *Donax cuneatus* (Victor and Subramonium, 1988) and *Marcia opima* (Maqbool, 1993). However, in *Crassostrea madrasensis* (Stephen, 1980a) and *Saccostrea cucullata* (Sukumar and Joseph, 1988), the peak spawning was observed with the decline in salinity.

The present study on the reproductive cycle has revealed that the wedge clam *Donax incarnatus* is gonochoristic and shows five stages of gonad development in both male and female. Gametogenesis is initiated soon after spawning with no resting stage. The size at which the clam attains first sexual maturity is found to be 13-14 mm. It shows a continuous breeding pattern with two spawning peaks in February-March and December.