5. REPRODUCTION

5.1. INTRODUCTION

Marine bivalves mollusks, which are used as source of food and raw material for ornamental industry are inhabitants of freshwater, brackish and freshwater around the world. The littoral clams *Atactodea striata; Gafrarium tumidum;* and *Anadara scapha* are gathered for food in many countries of the Indian ocean (Nayar and Rao, 1985) and south east Asia (Nielsen 1976; Purchon and Purchon 1981; Davy and Graham 1983). An understanding of their reproductive biology is an important prerequisite for assessing the regeneration capacities of natural stocks and interpreting growth patterns.

Preliminary and detailed reproductive cycle of bivalve mollusks have already been carried out on few Indian bivalves. Some of the earlier important contributions are on *Anadara tuberculosa*, Cruz (1984); *Anadara grandis*, Cruz (1987); *Anadara broughtonii*, Dzyuba and Maslennikova (1982); *Anadara trapezia*, Hadfield and Anderson (1988); *Meretrix meretrix* Jayabal and Kalyani (1987); *Donax cuneatus* (Nagabhushanam and Talikhedkar (1977); *Anadara rhombea*, Natarajan and John (1983). Other works done include that of Alagarswami (1966) on *Donax faba*, Thangavel and Sanjeevaraj (1983) on *Meretrix casta*, and Narasimham et al. (1988) on *M. meretrix*, Rao, (1952), on *Katelysia opima*; Abraham (1953) on *Meretrix casta* and Nayar (1955) on *Donax cuneatus*, which give considerable information on the reproductive biology of various clam species. In marine bivalves enzymes activities have been reported to be powerful tools to study the reproductive processes (Mori et al 1966; Peek and Gabbott, 1990; Tremblay, 1992; Matsumoto, et al., 1997).

The gametogenic cycles in marine invertebrates are influenced by exogenous factors studied by Giese, (1959); Fretter and Graham, (1964); Wilson, (1969); Sastry, (1970); Adiyodi and Adiyodi, (1983). In addition to the geographical variation in the
reproductive cycle of clams many investigation have reported annual variations in factors such as timing of the cycle, timing and duration of spawning and number of spawning per year (Chipperfield, 1953; Mac Donald and Thompson 1998; Laruelle et al., 1994) . The reproductive biology of the Baby clam, Marcia opima from two geographically separated areas of India (Suja and Muthiah, 2007)

Few studies on growth and production of the Venerid bivalve exist. Population dynamics of Gafrarium tumidum have been investigated by Kurihara (2003). Reproductive cycle of the bivalves mollusks Atactodea striata, Gafrarium tumidum and Anadara scapha in New Caledonia studied by Baron (1992). Sahin et al. (2006) studied the seasonal variations in condition index and gonadal development of the introduced blood cockle Anadara inaequalvis in the southeastern Black sea coast. The life cycle and sexual strategy of Gafrarium pectinatum (Bivalvia: Veneridae) in Hong Kong mangrove was studied by Morton (1990). Cheung et al. (2007) has been studied the growth responses of the filter-feeding clam Gafrarium tumidum to water flow: A field manipulation experiment.

Bivalves occupy an important position in the commercial molluscan exploitation and culture due to their high food value and low price. Besides the above understanding the reproductive pattern of any organism is more important for mass scale production Bivalves exhibit various type of spawning i.e., single breeding period to year round spawning. The information on sex ratio, sexual maturity and breeding season of any organism is helpful in finding out the annual recruitment, assessment of growth and age that way in judicial exploitation. Knowledge of the reproductive cycle of the species is fundamental for developing management strategies (Shaw, 1965; Manzi et al., 1985; Sbrenna and Gampioni, 1994) and is crucial for establishing successful hatchery base production (Gribben et al., 2004, Peharda et al., 2006). Though the extensive studies are made available on the commercially important clams of Venerid, both in tropical and temperate waters. However, no detailed works on the reproductive biology of Venerid clams of Gafrarium pectinatum and Gafrarium
divaricatum have been carried out in Palk bay area and hence, the present study covering the various reproductive aspects such as gonadal maturation, condition index, sex ratio and spawning season of both species of Gafrarium. The reproductive biology of the species is mainly used for developing management strategies for the development of sustainable fisheries. The present study aim to determine some management strategies of clam by examining the reproductive biology.

5.2. MATERIALS AND METHODS

5.2.1. Sample collection

Monthly samples of about 100 clams of both species of Gafrarium were collected from the natural bed at Thondi coast-Palk Bay (Lat. 11°30’N and Long 79°46’E) from Jan 2006 - Dec 2006 for the period of one year and used for studying the various aspects of maturation (Photos 7a & b; 8a & b). The specimens were collected monthly and kept in the aquarium tank for the observation of their reproductive conditions and the maturity stages were recorded based on the colour of the gonad. Fresh gonad smears were also studied under the microscope to ascertain the sex and maturity of gonad.

5.2.2. Determination of maturity stages

The sex and the stage of ova developments were ascertained by taking fresh smears of gonad by making a small cut on the surface of the gonad with a fine surgical needle at the middle of the gonad. The smears of clam were then observed under light microscope and 50 ova (early and late maturing) were taken in two axis and the averages of the clams were observed continuously for their percentage occurrence throughout the period of study. The ovarian stages of different ova in the present study were classified and the method followed by Ropes (1968).

5.2.3. Histology

For assessing the exact state of gonad and to supplement the data obtained by ova diameter progression, histological preparations were made and studied.
Approximately 25 individuals, arbitrarily selected with respect to age and the stage of gonad development were excised, fixed in Bouins fixative and prepared for sectioning by dehydration in ethanol and by embedding in paraffin wax at 60-62° C (Humason, 1972, Kripa, 1997) Sections were cut at 8 um thickness and stained with eosin and examined under microscope and classified into different developmental stages. Examination of the sections at regular intervals furnished detailed information on the reproductive cycle including actual spawning.

5.2.4. Sex ratio

To test homogeneity (Snedecor and Cochran 1967) Chi-Square test was done.

5.2.5. Condition index

The condition index (Cl) was calculated as (1) percentage of wet flesh weight in total weight and (2) as percentage of dry flesh weight in wet flesh weight (Narasimham, 1988).

5.2.6. Wet weight

Whole body tissues were separated and weighed to the nearest 0.1mg in electronic monopan balance after removing the excess moisture by blotting with filter paper. Percentage of meat weight was then calculated using the formula

\[
\frac{\text{Wet flesh weight of clam}}{\text{Total weight of clam}} \times 100
\]

5.2.7. Dry weight

The whole body tissues were dried in an electric oven at 60° C till constant weight was obtained and then weighed in an electronic monopan balance to the nearest 0.1 mg. Percentage of dry weight was then calculated using the formula

\[
\frac{\text{Dry flesh weight of clam}}{\text{Wet meat weight of clam}} \times 100
\]
5.3 RESULTS

Sexes are separate in *Gafrarium pectinatum* and *Gafrarium divaricatum*. External differentiation could not be made as well as any recognizable colour difference in the gonad of male and female. In all, four stages in female and two stages in male could be categorized from the observations made on the smear and histological sections.

5.3.1 Maturity stages

Based on the observations on the gonadal status of females the following maturity stages were categorized in both species of *Gafrarium* (Photos 9 – 12).

a) Early maturing

In early maturing clams, the ova were irregular in shape. The average size of the ova was less than 45µm in size.

b) Late maturing

In the late maturing stage clams, the ova measured between 45 and 58µm. Most of the ova tend to assume spherical shape. Some of the ova are still irregular in shape.

c) Matured

In the matured clams, almost all the ova assumed perfect spherical shape. The ova measured 72µm and above. Very few ova were found to be in near spherical shape.

d) Spent

In the spent clam, the smear contained very few ova. The size of the ova were 72 µm and above.

Regular observation on the histological preparations yielded valuable data on the progression of the ova. For different gonadal stages were determined among females from the histological observations such as early maturing, late maturing,
matured and spent and two stages, matured and spent, among males. Photomicrographs of various stages are given in Photos 9 - 12.

5.3.2. Percentage of maturity stages

The percentage occurrence of various stages of gonads in both species of Gafrarium based on the ova diameter during the study period is presented in Fig. 9 - 14.

In *Gafrarium pectinatum* (fig.9), the occurrence of all the four maturity stages in all the months of observation in varying percentages was quite evident. Late maturing and matured stages of gonad ranged from 8.0% (Nov.) to 65% (Feb.) respectively. The lowest percentage (8.0%) occurrence of late maturing gonad along with highest percentage of spent (80%) was observed during November. Similarly a lower magnitude of matured gonad (12%) and moderate percentage (60%) of spent gonad were observed in April. Occurrence of spent gonad was observed throughout the study period in varying magnitude ranging from 9% (Aug.) to 80% (Nov).

In *Gafrarium divaricatum* (fig 10.) Late maturing and matured stages of gonad ranged from 6% (Nov.) to 68 % (Feb.) respectively. The lowest percentage (6.0%) occurrence of late maturing gonad long with highest percentage of spent (72%) was observed during November. Similarly a lower magnitude of matured gonad (8%) and moderate percentage (59%) of spent gonad were observed in April. Occurrence of spent gonad was observed throughout the study period in varying magnitude ranging from 7 % (Aug.) to 72 (Nov). In the present study ,in both species ,the matured stage of gonad were not observed in Jan and December and also the early maturing and late maturing stages were not observed in October.

5.3.3. Sex ratio

Data on the monthly sex ratio for the study period is presented in Tables.2
Table 2. Sex ratio of *Gafrarium pectinatum* during Jan 2006 – Dec 2006

<table>
<thead>
<tr>
<th>Month</th>
<th>Male (Nos)</th>
<th>Female (Nos)</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Ratio</th>
<th>Chi Square Value</th>
<th>‘P’ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>18</td>
<td>32</td>
<td>36</td>
<td>64</td>
<td>1:1.78</td>
<td>1.355</td>
<td>0.2444</td>
</tr>
<tr>
<td>Feb</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>60</td>
<td>1:1.50</td>
<td>0.0487</td>
<td>0.8253</td>
</tr>
<tr>
<td>March</td>
<td>19</td>
<td>31</td>
<td>38</td>
<td>62</td>
<td>1:1.63</td>
<td>0.2355</td>
<td>0.6275</td>
</tr>
<tr>
<td>April</td>
<td>21</td>
<td>29</td>
<td>42</td>
<td>58</td>
<td>1:1.38</td>
<td>0.5282</td>
<td>0.4674</td>
</tr>
<tr>
<td>May</td>
<td>22</td>
<td>28</td>
<td>44</td>
<td>56</td>
<td>1:1.27</td>
<td>0.1151</td>
<td>0.7344</td>
</tr>
<tr>
<td>June</td>
<td>21</td>
<td>29</td>
<td>42</td>
<td>58</td>
<td>1:1.38</td>
<td>0.3006</td>
<td>0.5835</td>
</tr>
<tr>
<td>July</td>
<td>22</td>
<td>28</td>
<td>44</td>
<td>56</td>
<td>1:1.27</td>
<td>0.1134</td>
<td>0.7363</td>
</tr>
<tr>
<td>August</td>
<td>17</td>
<td>33</td>
<td>34</td>
<td>66</td>
<td>1:1.94</td>
<td>0.0588</td>
<td>0.8084</td>
</tr>
<tr>
<td>September</td>
<td>21</td>
<td>29</td>
<td>42</td>
<td>58</td>
<td>1:1.38</td>
<td>0.0032</td>
<td>0.9545</td>
</tr>
<tr>
<td>October</td>
<td>23</td>
<td>27</td>
<td>46</td>
<td>54</td>
<td>1:1.17</td>
<td>0.3130</td>
<td>0.5758</td>
</tr>
<tr>
<td>November</td>
<td>21</td>
<td>29</td>
<td>42</td>
<td>58</td>
<td>1:1.38</td>
<td>0.0073</td>
<td>0.9321</td>
</tr>
<tr>
<td>December</td>
<td>21</td>
<td>29</td>
<td>42</td>
<td>58</td>
<td>1:1.38</td>
<td>0.0023</td>
<td>0.9615</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>354</td>
<td>41</td>
<td>59</td>
<td>1:1.46</td>
<td>3.081184</td>
<td>0.7043</td>
</tr>
</tbody>
</table>

*Grafrarium divaricatum*

<table>
<thead>
<tr>
<th>Month</th>
<th>Male (Nos)</th>
<th>Female (Nos)</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Ratio</th>
<th>Chi Square Value</th>
<th>‘P’ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>32</td>
<td>51</td>
<td>38.5542</td>
<td>61.4458</td>
<td>1:1.59</td>
<td>0.9489</td>
<td>0.3300</td>
</tr>
<tr>
<td>Feb</td>
<td>30</td>
<td>53</td>
<td>36.1446</td>
<td>63.8554</td>
<td>1:1.77</td>
<td>0.0952</td>
<td>0.7576</td>
</tr>
<tr>
<td>March</td>
<td>33</td>
<td>50</td>
<td>39.759</td>
<td>60.241</td>
<td>1:1.52</td>
<td>0.0361</td>
<td>0.8493</td>
</tr>
<tr>
<td>April</td>
<td>28</td>
<td>55</td>
<td>33.7349</td>
<td>66.2651</td>
<td>1:1.96</td>
<td>2.193</td>
<td>0.1387</td>
</tr>
<tr>
<td>May</td>
<td>29</td>
<td>54</td>
<td>34.9398</td>
<td>65.0602</td>
<td>1:1.86</td>
<td>0.1222</td>
<td>0.7267</td>
</tr>
<tr>
<td>June</td>
<td>33</td>
<td>50</td>
<td>39.759</td>
<td>60.241</td>
<td>1:1.52</td>
<td>0.2928</td>
<td>0.5884</td>
</tr>
<tr>
<td>July</td>
<td>33</td>
<td>50</td>
<td>39.759</td>
<td>60.241</td>
<td>1:1.52</td>
<td>0.0012</td>
<td>0.9724</td>
</tr>
<tr>
<td>August</td>
<td>31</td>
<td>52</td>
<td>37.3494</td>
<td>62.6506</td>
<td>1:1.68</td>
<td>0.5569</td>
<td>0.4555</td>
</tr>
<tr>
<td>September</td>
<td>35</td>
<td>48</td>
<td>42.1687</td>
<td>57.8313</td>
<td>1:1.37</td>
<td>1.360</td>
<td>0.2435</td>
</tr>
<tr>
<td>October</td>
<td>27</td>
<td>56</td>
<td>32.5301</td>
<td>67.4699</td>
<td>1:2.07</td>
<td>0.0007</td>
<td>0.9787</td>
</tr>
<tr>
<td>November</td>
<td>32</td>
<td>51</td>
<td>38.5542</td>
<td>61.4458</td>
<td>1:1.59</td>
<td>0.07183</td>
<td>0.7887</td>
</tr>
<tr>
<td>December</td>
<td>29</td>
<td>75</td>
<td>27.8846</td>
<td>72.1154</td>
<td>1:2.59</td>
<td>2.648</td>
<td>0.1037</td>
</tr>
<tr>
<td>Total</td>
<td>372</td>
<td>645</td>
<td>36.76146</td>
<td>63.23854</td>
<td>1:1.75</td>
<td>8.3269</td>
<td>0.5777</td>
</tr>
</tbody>
</table>
From the tables (2), it is evident that the female clams in both species were more dominant during the month of September and October with an average value of 59 & 63.2%. In *Gafriarium pectinatum* – Males were found more number in May, July and October and in *Gafriarium divaricatum* it was found more number in March, June, July and September. The Chi square values were not significant in both species. In both species the sex ratio of 1:1.75 and 1:1.46 differed significantly from 1:1 ratio.

### 5.3.4. Condition index based on dry and wet flesh weight

The monthly average values (%) of the condition index (Cl) for all groups combined based on dry meat weight and wet meat weight were calculated and presented in both species of Gafriarium. In *Gafriarium pectinatum* the male wet flesh weight varied between 7.50% (Nov) and 13.25% (Sep) and the female it was 8.35% (Nov.) and 15.72% (Sep.). For the dry weight of male it was between 7.5% (Oct.), 15.30% (Sep.) and in female it was 9.5% (Oct.), 16.8% (Aug.) respectively (Figs 11&12). Whereas in *Grafriarium divaricatum* in male the wet flesh weight varied between 8.5% (Nov) and 14.5% (Sep) and the female it was 8.0% (Nov.) and 15.9% (Sep.) and for the dry weight of male it was between 8.8 (Oct.), 14.8% (Sep.) and in female it was 9.2 (Oct.), 16.0% (Aug.) respectively (Figs 13&14). During the study period the monthly average values of Cl showed the trends in the fluctuations of Condition index (Cl). The high values of Cl value may be due to moderately or well developed gonads and the low value may coincided with spawning period.

### 5.4. DISCUSSION

The present study in both species *Gafriarium pectinatum* and *Gafriarium divaricatum* clearly indicated the availability of spent specimens along with different stages of maturity indicating year round breeding of this species when conditions are favourable. However, based on the lowest percentage of late maturing clams along with the highest percentage of spent clams during November, it may be concluded that this represents the peak period of spawning in this region. A secondary peak in April was also observed when considerable percentage of clams is found to be in spent stage. The
spawning season determined for the present species almost agrees with the finding of Nagabhushanam and Mane (1976) for *Katelysia opima*. Nayar (1955) has observed that *D. cuneatus* has a single spawning seasons from Jan to April, the peak of spawning being in April. Coe (1955) has observed that the fist spawning takes place at the age of one year or one and half year in *D. gouldi* resulting from the late spawning. The spawning season extends from April to October or November.

Hornell, (1922) while studying the clam *Meretrix casta* concluded that spawning takes place during April-May and again in September. Abraham (1953) observed three breeding peaks of various magnitudes, one in July-Aug., the second in Oct. – Nov. and the third one in summer months of March – April in *M. casta* occurring in Adyar estuary. Durve (1964) and Parulekar et al. (1973), found this species to be a continuous breeder. (Salih 1973), inferred that there are two spawning peaks one in January and the other predominant one in October. Sreenivasan (1983a), indicated that *M. casta* spawns during April – Sep. in the Vellar estuary. Thangavelu and Poovannan, (1994) found that *M. casta* occurring at Muttukadu to be a continuous breeder with two intense spawning periods one during May and another during, Sep. – Oct. based on the high percentage of spent clam availability. Thangavelu and Sanjeevaraj (1983) found ripe clams in all the months showing spawning round the year as environmental factors are favourable. However, peak periods were observed during March – April, July – Aug. and Oct.-November.

Narasimham et al. (1988) observed *M. meretrix* as a prolonged breeder (about 9 months) in Korampallam estuary and concluded that spawning occur when the temperature variation is narrow. They further inferred that there was absence of spawning during Nov – Dec. When the salinity was very low < 10 ppt. In Vellar estuary, *M. meretrix* spawns from February-September (Jeyabal, 1984). Nagabghushanam and Mane (1976) has inferred that gametogenesis in *Katelysia opima*, to reach a peak in October with a secondary peak activity in March. They concluded that this species has two spawning period a major one during October – November and
a minor one in March. The term Percentage edibility has been used in the following account to indicate the percentage ratio of the wet weight to the whole weight of the clam, following works of Venkataraman and Chari (1951) and Durve (1964a). The same ratio has been denoted by the term “Condition Index” by Ansell and Loosmore (1963). But the latter term has been widely used by other authors (Baird 1958; Cooper and Marshall 1963; Durve 1964a) to indicate the ratio of the volume of the meat to the volume of the animal as a whole.
Photo.7a. Collection of *Gafrarium species*

Photo.7b. Collection of *Gafrarium species*
Photo 8a. *Gafrarium pectinatum*

![Gafrarium pectinatum](image1)

Photo 8b. *Gafrarium divaricatum*

![Gafrarium divaricatum](image2)
Photos 9 (a-d). *Gafrarium pectinatum*—Male

A - Early maturing  
B - Late maturing  
C - Matured  
D - Spent
Photos 10 (a-d). *Gafrarium pectinatum* - Female

A - Early maturing

B - Late maturing

C - Matured

D - Spent
Photos 11 (a-d). *Gafrarium divaricatum* – Male

A - Early maturing

B - Late maturing

C - Matured

D - Spent
Photos 12 (a-d). *Gafrarium divaricatum* – Female

A - Early maturing

B - Late maturing

C - Matured

D - Spent
Fig. 9. Percentage of maturity stages of *Gafrarium pectinatum*

Fig. 10. Percentage of maturity stages of *Gafrarium divaricatum*

Fig. 11. Condition index on dry and wet weight of *Gafrarium pectinatum-Male*
Fig. 12. Condition index on dry and wet weight of *Gafrarium pectinatum-Female*

Fig. 13. Condition index on dry and wet weight of *Gafrarium divaricatum-Male*

Fig. 14. Condition index on dry and wet weight of *Gafrarium divaricatum-Female*