## Chapter 3 Recruitment

### 3.1 Introduction

Recruitment links the planktonic larval and the benthic adult components of

the life cycle acting as a filter between life history stages. Barnacles are one of the important components of intertidal community and also of the marine macrofouling community. Balanus amphitrite, an acorn barnacle is a dominant fouling organism found all over the world. The larval phase includes planktotrophic nauplii, followed by a pre-settling non-feeding cypris larva. The planktotrophic larvae may remain planktonic for several days to 2 months (Sheltema and Carlton 1984). This in turn permits larval dispersal. The key factors that influence final destination achieved by the larva during dispersal and before settlement is the duration it exists as a plankton. Studies have shown that the relationship between settlement and recruitment is set by larval behavior at the time of settlement, which is strongly influenced by the substratum characteristics (Raimondi 1988,1991; Strathmann et al. 1981).

Thus recruitment of $B$. amphitrite is governed by events during its planktonic larval development and the cues offered by the substratum. Recruitment is reported to vary with space and time (Jenkins et al. 2000, 2001). The larval abundance, which is considered as an important cause of variation in the recruitment, can be influenced by the variation in the levels of reproduction in adult populations (Wellington and Victor 1988). Recruitment is influenced by the variations in physical factors such as water temperature, salinity, current patterns that may disperse the planktotrophic larvae and biological factors such as phytoplnakton abundance which is a source of food for the developing larvae (Richardson and Brown 1990).

Considerable amount of work has been carried out on the biological aspects of marine foulers from European and Pacific waters (Barnes 1962; Crisp 1984). In India the work related to recruitment of $B$. amphitrite has been carried out with respect to its importance in the fouling community (Anil 1986; Nair 1965; Menon et al. 1977; lyengar et al. 1957; Daniel 1954; Karande 1965; Venugopalan and Wagh 1990) and on its taxonomy by Fernando 1990. However there is lack of recruitment related studies in the natural environment from this tropical habitat. Evaluation of temporal variation in the recruitment of B. amphitrite was undertaken at the Dona Paula bay situated at the mouth of the Zuari estuary, West Coast of India. The effect of environmental parameters, larval abundance and development, adult reproduction on the recruitment was evaluated.

### 3.2 Materials and Methods

### 3.2.1 Study location

The intertidal area of Dona Paula bay ( $15^{\circ} 27.5^{\prime} \mathrm{N}, 73^{\circ} 48^{\prime} \mathrm{E}$ ) was selected as the study site, which is located along the central West Coast of India. This site is situated at the mouth of the Zuary estuary. Tides range from 0.25 m during neap to 2.5 m during spring tide. Perennial connections of this estuary with the Arabian sea results in a rhythmic ingress and egress of marine and estuarine plankton populations (Devassy and Goes 1989). The study site is influenced by southwest monsoon. The estuary is almost homogenous during nonmonsoon months. During monsoon it experiences increased land run-off that lowers the salinity and increase the nutrient flux. Based on the influence of monsoon a year is broadly classified as pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January) seasons. The surface water temperature during the study period ranged from $22^{\circ} \mathrm{C}$ to $31^{\circ} \mathrm{C}$ and the salinity ranged from $4 \%$ to $36 \%$. Earlier investigations at this estuary also indicated similar temperature and salinity ranges (Devassy and Goes 1989; Mitbavker et al. 1997).

### 3.2.2 Recruitment

The investigations on the recruitment of barnacle, $B$. amphitrite at two tidal levels, mid-tide (one meter from the lowest low water mark (LLWM)) and hightide (two meters from the LLWM) was carried out from June'98 to Sept'99. Concrete boulders used to protect the jetty were used as substratum. Quadrats of $25 \mathrm{~cm}^{2}$ (in triplicate) were marked each month on the concrete blocks at both the tidal levels and these surfaces were then cleaned for the
existing fauna using scraper and nylon brush. The marked quadrats were observed for recruitment at the beginning of each month. In total 90 quadrats were marked (45 at each tide level). Fiber glass and acrylic panels were also used to study the recruitment. Recruitment on these substratum was monitored by exposing the panels of $15 \times 10 \mathrm{~cm}^{2}$ from the jetty at Dona Paula. Observations on recruitment of $B$. amphitrite on panels was made by exposing panels for one, two and three months duration. The number of barnacles both live and dead (shell remains) were counted. Spats were counted by using a magnifying lens.

### 3.2.3 Growth

The sizes (rostro carinal basal diameter) of the live and dead B. amphitrite were taken with the help of vernier caliper. The barnacles were grouped in size ranges of 2 mm . Those with a rostro carinal basal diameter of less than 2 mm were considered as spats. The percent area covered by the barnacles was calculated by using the individual's basal diameter assuming that the basal diameter to be circular. For observations on growth, solitary ( $\sim 5$ Nos.) and crowded individuals ( $\sim 5$ Nos.) were marked on the November'98 quadrat exposed for 1 month and these barnacles were monitored for growth every month. At the end of six to seven month all the marked barnacles were dead. The mortality of these can be attributed to the increase in surface water temperature and also due to rainfall during late pre monsoon month (May '99;

Fig. 3.19).

### 3.2.4 Gonads

Observations on gonads were carried out from June '98 to Sept '99. Individuals of $B$. amphitrite were collected, from the intertidal area and their gonad stages were classified. In this paper the percent of barnacles which possessed mature ovary (where ovary is dark brown in color and nauplii with visible eye spots are present) is presented.

### 3.2.5 Collection of cirripede larvae

Plankton samples were collected once in a month during June'98 to Sept'99. Horizontal plankton tows were made using a conical Heron-Tranter net of $100 \mu \mathrm{~m}$ mesh fitted with a flow meter. The amount of water filtered ranged from 25 to $170 \mathrm{~m}^{3}$. The samples were brought back to the laboratory and the cirripede larvae were counted. The larvae were quantified as abundance $100 \mathrm{~m}^{-3}$. Some of the larvae isolated from plankton were used for rearing in the laboratory.

### 3.2.6 Rearing of larvae

Larvae isolated from the plankton samples and those collected from the adult broods every month were reared individually ( $\mathrm{n}=24$ ) in multiwells (Nunclon delta-143982) at $25\left( \pm 1^{\circ} \mathrm{C}\right)$ temperature using Chaetoceros calcitrans, a unicellular diatom as food at a cell concentration of $2 \times 10^{5}$ cells $\mathrm{ml}^{-1}$. Each larva was reared with 2 ml of media with an antibiotic dosage of $0.3 \mathrm{ml} \mathrm{L}^{-1}$ of crystamycin (300mg of Penicillin G and 500 mg of Streptomycin in 4 ml of distilled water). Antibiotic dosage level is adopted from Harmes (1986) and Anil et al. (1995). Observations on the molts were taken every day prior to change of food. The total naupliar duration and percentage survival of nauplii
to cyprids was measured. To calculate the total naupliar duration of the plankton collected larvae which were in III, IV, V and VI instar, a day (which is minimum duration required to molt into the next instar under laboratory rearing) was added to each of the earlier instars. The cyprids as and when they appeared were put in the multiwells individually containing 2 ml of filtered sea water and the settlement of these cyprids was observed to evaluate the percentage metamorphosis of cyprids to juvenile.

### 3.2.7 Data analysis

The absolute recruitment is the fresh recruitment occurring each month, obtained by subtracting the number of barnacles recruited between two successive months. The cumulative abundance includes total number of barnacles observed from the beginning of quadrat exposure. The numbers are expressed as individuals $\mathrm{dm}^{-2}$.

## Clustering and multi dimensional scaling

The data on recruitment of barnacles during different months on fresh quadrats and the panels exposed for one month was subjected to cluster analysis. This was followed by non-metric multi dimensional scaling (NMDS) to produce two-dimensional ordinates. The data was log transformed before subjecting to cluster analysis and for plotting the NMDS ordination plot. A twoway ANOVA (months $x$ number of recruits for one month on fresh quadrat) was carried out for assessing the variations in recruitment of barnacles with different exposure months and also between the months and tidal heights. Two-way ANOVA was also carried out to see the variations in recruitment with different substratum. Similarly, two-way ANOVA was also carried out for the
percentage survival and metamorphosis of plankton collected larvae and those collected from the broods.

The data on the abundance of cirripede larvae and the percentage of barnacles with the ripe ovaries was subjected to cluster analysis and nonmetric multi dimensional scaling (NMDS) to obtain two-dimensional ordinates.

### 3.3 Results

### 3.3.1 Recruitment

## Concrete Substratum

The pattern of recruitment of $B$. amphitrite on fresh quadrats exposed for one month from June 1998 to September 1999 at mid-tide level is shown in (Fig. 3.1). Two prominent clusters, one comprising of four months (July '98, Sept '98, Oct '98 and Feb '99) and the other with 10 months were evident. Aug '98 (43 No dm ${ }^{-2}$ ) was the most dissimilar month at the mid-tide level (Fig. 3.1). At high tide such a differentiation was not evident, and the intensity of recruitment was of similar order round the year (Fig. 3.7a,b). Two-way ANOVA indicated that the recruitment of barnacles significantly varied with months and tide levels (Table 3.1).

Table 3.1 Two-way ANOVA of the absolute recruitment of Balanus amphitrite at Mid-Tide and High Tide level.

|  | df | SS | MS | Fs |
| :--- | :--- | :--- | :--- | :--- |
| Months | 14 | 2.4 | 0.174 | $8^{* * * * *}$ |
| Tide Level | 1 | 14 | 13.9 | $679^{* * * * *}$ |
| Months *Treatments | 14 | 2 | 0.137 | $7^{* * * * *}$ |
| Within sub. Gr.err. | 60 | 1.2 | 0.020 |  |
| Total | 89 | 19.6 |  |  |

(***** $\mathrm{p} \leq 0.001$ )


Fig. 3.1 Dendrogram and multidimensional scaling (NMDS) diagram for the absolute recruitment at the mid-tide level.

## Mid-Tide

## Monsoon Season

During monsoon recruitment spurted in Aug '98 (Fig. 3.2c). However surfaces 2/3 month old (those initiated in June and July '98) did not experience recruitment in August. The newly recruited barnacles on the freshly exposed Aug '98 quadrat included a large number of spats (size range of 0-2 mm; Fig. 3.2 c ). A comparison of live and dead indicated that the number of dead spats exceeded the live ones (Fig. 3.2c).


Months


Fig. 3.2 Cumulative abundance of B. amphitrite during monsoon season at MidTide level. First block represents the live, second block represents the dead barnacles and their size range ( mm ). The Y1 axis (Histograms) represents the
 and the dead barnacles (Lines)


Fig. 3.3 Absolute recruitment of $B$. amphitrite during different months on the quadrates aged for different duration. (a) Monsoon (b) Post-monsoon (c) Pre-monsoon

## Post-monsoon Season

Absolute recruitment peaked during Jan'99 (15 ind $\mathrm{dm}^{-2}$ ) (Table 3.2). In October the absolute recruitment on surfaces exposed for different duration was almost similar (Fig. 3.3b). Whereas in Nov '98, Dec '98 and Jan '99 the absolute recruitment increased on 1 to 5 months old surfaces and decreased on older surfaces (Fig. 3.3b). The absolute mortality and dislodgment on the quadrats initiated in post-monsoon and pre-monsoon season was high during July month (Table 3.2). Cumulative abundance peaked during pre-monsoon season on Nov '98 quadrat followed by $\sim 70 \%$ decrease in the abundance with the onset of monsoon 1999 indicating dislodgment (84 ind dm ${ }^{-2}$. Fig. 3.4b; Table 3.2). The percent area coverage remained uniform for 1-3 months and further it increased in case of live ones indicating lower mortality of higher size class individuals (Fig. 3.4a-d). The cumulative abundance indicated a similar trend for Oct, Dec '98 and Jan '99 (Fig. 3.4a,c,d).



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Fig. 3.4 Cumulative abundance of $B$. amphitrite during post-monsoon season at Mid-Tide level. First block represents the live, second block represents the dead barnacles and their size range $(\mathrm{mm})$. The Y 1 axis (Histograms) represents the abundance ( $\mathrm{No} \mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent area covered by the live and the dead barnacles (Lines)

## Pre-monsoon Season

In the month of March peak recruitment was observed on a five month exposed surface, whereas in May the recruitment was almost uniform irrespective of the exposure duration (Fig. 3.3c). The absolute recruitment peaked during Mar '99 (2 month old; 31 ind dm ${ }^{-2}$ ) followed by Apr '99 (3 month old; 15 ind $\mathrm{dm}^{-2}$ ) on the quadrats initiated in Feb '99 (Table 3.2). However such peaks were not observed on any other quadrats. Cumulative abundance peaked on quadrats initiated in Feb '99 (Fig. 3.5a), whereas the subsequent month initiations maintained a lower profile (Fig. 3.5b-d). Similar results were highlighted by the percentage area coverage. It was observed that during late pre-monsoon month (May '99) the early setting of monsoon resulted in mortality.


Months


Fig. 3.5 Cumulative abundance of B. amphitrite during pre-monsoon season at Mid-Tide level. First block represents the live, second block represents the dead barnacles and their size range. The Y 1 axis (Histograms) represents the abundance (No $\mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent area covered by the live and the dead barnacles (Lines)

Monsoon 1999
(a)

(b)


Months

|  |  |  |  |  |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 0 |  |  |  |  |  |  |  |  |  |  |



Fig. 3.6 Cumulative abundance of $B$. amphitrite during monsoon 1999 season at MidTide level. First block represents the live, second block represents the dead barnacles and their size range (mm). The Y1 axis (Histograms) represents the abundance (No $\mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent area covered by the live and the dead barnacles (Lines)

Table 3.2 Absolute recruitment, mortality and dislodgment of barnacles during different months at Mid-Tide level.

| $\begin{aligned} & \text { Exp } \\ & \text { Dur } \end{aligned}$ | osure ation |  | June'98 | July | Aug | Sept | Oct | Nov | Dec | Jan'99 | Feb | Mar | Apr | May | June | July | Aug | $\begin{aligned} & \hline \text { Sept } \\ & \text { '99 } \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 |  | L | 8 | 3 | 43 | 3 | 2 | 8 | 6 | 15 | 3 | 9 | 12 | 8 | NS | 17 | 17 | 8 |
|  |  | D | 1 | 1 | 17 | 0 | 0 | 0 | 1 | 8 | 2 | 3 | 3 | 2 | NS | 5 | 3 | 2 |
|  | 2 | L | 21 | 11 | 39 | 2 | 6 | 10 | 2 | 0 (-4) | 31 | 5 | 8 | NS |  | 14 | 9 |  |
|  |  | D | 5 | 7 | 3 | 1 | 2 | 4 | 3 | 0 (-4) | 9 | 0 | 5 | NS |  | 5 | 3 |  |
|  | 3 | L | 0 (-7) | $0(-3)$ | 0 (-19) | 1 | 10 | 40 | 0 (-1) | 7 | 15 | 5 | NS | 12 |  | 28 |  |  |
|  |  | D | 0 (-4) | 0(-4) | 2 | 0 | 4 | 12 | 0 (-2) | 0 | 0 (-3) | 4 | NS | 1 |  | 5 |  |  |
|  | 4 | L | 0 | 1 | 14 | 36 | 4 | 16 | 15 | 5 | 11 | NS | $0(-12)$ | 4 |  |  |  |  |
|  |  | D | 6 | 0 (-1) | 5 | 22 | $0(-1)$ | 5 | 2 | $0(-1)$ | 14 | NS | $0(-7)$ | 0 |  |  |  |  |
| $\begin{aligned} & \text { ᄃ } \\ & \hline 0 \\ & 0 \\ & 0 \\ & \text { O } \\ & \text { E } \\ & 0 \end{aligned}$ | 5 | L | 3 | 2 | 0 | 12 | 0 (-2) | 49 | 8 | 5 | NS | 0 (-3) | 4 | 13 |  |  |  |  |
|  |  | D | $0(-3)$ | 1 | 2 | $0(-11)$ | 1 | 11 | 3 | 6 | NS | 0 (-4) |  | 3 |  |  |  |  |
|  | 6 | L | 1 | 18 | $0(-12)$ | $0(-4)$ | 14 | $0(-13)$ | 9 | NS | $0(-43)$ | 5 | 8 |  |  |  |  |  |
|  |  | D | 3 | 4 | 3 | 3 | 1 | $0(-8)$ | 8 | NS | 0 (-17) |  | 0 |  |  |  |  |  |
|  | 7 | L | 2 | 5 | 16 | $23$ | $0(-5)$ | 2 | NS | $0(-16)$ | 8 |  |  |  |  |  |  |  |
|  |  | D | $0$ | 10 | 8 | $9$ |  | 4 | NS | 0 (-5) |  | $0(-1)$ |  |  |  |  |  |  |
|  | 8 | L | $0(-5)$ | 12 | 6 | $11$ | $6$ | NS | $0(-26)$ | $3$ | $14$ |  |  |  |  |  |  |  |
|  |  | D | 0 (-4) | 1 | $10$ | 7 | 3 | NS | $0(-9)$ |  | $1$ |  |  |  |  |  |  |  |
|  | 9 | L | 20 | 11 | $10$ | 5 | $\overline{\mathrm{NS}}$ | $0(-84)$ | $11$ | $7$ |  |  |  |  |  |  |  |  |
|  |  | D | 21 | $0(-2)$ | $0(-2)$ | 21 | NS | $0(-13)$ | $2$ | $1$ |  |  |  |  |  |  |  |  |
|  | 10 | L | $0$ | $13$ | 4 | NS | $0(-19)$ | $16$ | $19$ |  |  |  |  |  |  |  |  |  |
|  |  | D | $2$ | $0(-2)$ | $6$ | NS | $0(-5)$ | $0(-1)$ | $0(-1)$ |  |  |  |  |  |  |  |  |  |
|  | 11 | L | $0(-9)$ | 6 | NS | $0(-56)$ | 7 | $16$ |  |  |  |  |  |  |  |  |  |  |
|  |  | D | $0(-9)$ | 12 | NS | $0(-38)$ | 0 (-1) | 17 |  |  |  |  |  |  |  |  |  |  |
|  | 12 | L | $\begin{aligned} & 0(-1) \\ & 2 \end{aligned}$ | $\begin{aligned} & \text { NS } \\ & \text { NS } \end{aligned}$ | $\begin{aligned} & 0(-60) \\ & 0(-35) \end{aligned}$ | $\begin{aligned} & 6 \\ & 3 \end{aligned}$ | $\begin{aligned} & 11 \\ & 0 \end{aligned}$ |  |  |  |  |  |  |  |  |  |  |  |
|  | 13 | L | $\begin{aligned} & \text { NS } \\ & \text { NS } \end{aligned}$ | $\begin{aligned} & 0(-44) \\ & 0(-18) \end{aligned}$ | $\begin{aligned} & 9 \\ & 18 \end{aligned}$ | $\begin{aligned} & 9 \\ & 0(-1) \end{aligned}$ |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1.4 | L | $\begin{aligned} & 0(-27) \\ & 1 \end{aligned}$ | $\begin{aligned} & 6 \\ & 0(-2) \end{aligned}$ | $\begin{aligned} & 10 \\ & 0(-7) \end{aligned}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 15 | L | $\begin{aligned} & 25 \\ & 0(-11) \end{aligned}$ | $\begin{aligned} & 8 \\ & 7 \end{aligned}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 115 | L | $\begin{array}{r} 4 \\ 2 \\ \hline \end{array}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

[^0]High tide

The recruitment pattern at high tide level on the quadrats exposed for one month is presented in Figure 3.7. Recruitment of barnacles at high tide level was comparatively lower than the mid-tide level. There was a spurt in recruitment during Aug '98 (Fig. 3.2c) at mid-tide level, such was not a case at high tide level (Fig. 3.8c). Among all the high-tide quadrats the maximum recruitment was observed on June '98 quadrat (Fig. 3.8a). At high tide level mortality and dislodgment was maximum during pre-monsoon months, whereas at mid-tide level it was during early monsoon months.

The percent area coverage did not show any variation indicating a low recruitment and slow growth (Fig. 3.8a-o). Continued exposure (aging of the surface) did not influence the abundance (Table 3.3).


Fig. 3.7 Dendrogram and the non-metric multidimensional scaling (NMDS) diagram for the absolute recruitment at the high tide level

Table 3.3 Absolute recruitment, mortality and dislodgment of barnacles during different months at High-Tide level.


[^1]

Fig. 3.8 (a-d) Cumulative abundance of Balanus amphitrite during monsoon 1998 at high tide level.First block represents the live, second block represent the dead barnacles and their size range ( mm ). The Y1 axis (Histograms) represents the abundance ( $\mathrm{No} \mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent areas covered by the live and dead barnacles (lines)


Fig. 3.8 (e-h) Cumulative abundance of Balanus amphitrite during post monsoon at high tide level. First block represents the live, second block represent the dead barnacles and their size range. The Y1 axis (Histograms) represents the abundance (No $\mathrm{dm}^{-2}$ ) and Y2 axis represents the percent area covered by the live and dead barnacles (lines)


Months
Fig. 3.8 (i-l) Cumulative abundance of Balanus amphitrite during pre-monsoon at high tide level. First block represents the live, second block represent the dead barnacles and their size range (mm). The Y1 axis (Histograms) represents the abundance ( $\mathrm{No} \mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent area covered by the live and dead barnacles (lines)


Fig. 3.8 (m-o) Cumulative abundance of $B$. amphitrite during monsoon 1999 season at Mid-Tide level. First block represents the live, second block represents the dead barnacles and their size range ( mm ). The Y 1 axis (Histograms) represents the abundance ( $\mathrm{No} \mathrm{dm}^{-2}$ ) and Y 2 axis represents the percent area covered by the live and the dead barnacles (Lines)

Fiber Glass
The cluster dendrograms and the NMDS plots showed two prominent clusters for absolute recruitment during different months (Fig. 3.9).



Fig. 3.9 Dendrogram and non-metric multidimensional scaling (NMDS) diagram for the absolute recruitment on the fiberglass panel.

August'98 with the post monsoon months (Oct, Nov '98 and Jan '99) grouped into one cluster, where the recruitment was low (6-14 No $\mathrm{dm}^{-2}$ ) (Fig. 3.9). Recruitment was high during pre-monsoon months (Fig. 3.10a). July '98 month showed comparatively high recruitment (126 No dm ${ }^{-2}$ ) (Fig. 3.10a). The second cluster was of all the pre-monsoon and the monsoon months (Fig. $3.9 a, b)$. During all the months maximum number of barnacles were in the size range of $0-2 \mathrm{~mm}$ (spats). Recruitment was high in August and September 1999 compared to that of 1998. The percentage area covered by barnacles remained almost similar, accept a slight rise during pre-monsoon months (Fig. 3.10a).


Fig. 3.10 Recruitment of $B$. amphitrite on fiberglass panel. First block represents the live, second block represents the dead barnacles and their size range ( mm ). The Y 1 axis (Histograms) represents the abundance ( $\mathrm{No} \mathrm{dm}{ }^{-2}$ ) and Y 2 axis represents the percent area covered by the live and the dead barnacles (Lines)

The fiberglass panels exposed for two months showed maximum recruitment during monsoon months (July-Aug and Aug-Sept '98) followed by premonsoon months (Feb-Mar, Mar-Apr and Apr-May) (Fig. 3.10b). However maximum mortality was observed during monsoon months. The percent area coverage of dead barnacles exceeded that of the live ones (Fig. 3.10b). The maximum size attained by barnacle was $18 \mathrm{~mm}(R C B)$. On the panel exposed for three months, maximum recruitment was observed during pre-monsoon (Jan-Mar '99). During July to September and Aug to Oct '98 though the number of barnacles were high, during these months maximum mortality was observed (Fig. 3.10c). The percent area coverage peaked during Aug-Oct '98 and during pre-monsoon months. During late pre-monsoon and early monsoon months mortality was high (Fig. 3.10c).

On one month panel the maximum size attained by barnacles was in the range of $16-18 \mathrm{~mm}$. However during one month exposure maximum number of barnacles were in the range of $0-4 \mathrm{~mm}$. On the panels exposed for two and three months the maximum size attained was $18-20 \mathrm{~mm}$ and 22 mm respectively.

Acrylic

On acrylic surface absolute recruitment was high during pre-monsoon months. The cluster dendrograms showed three prominent clusters (Fig. 3.11).


Fig. 3.11 Dendrogram and the non-metric multidimensional scaling (NMDS) diagram for the absolute recruitment of $B$. amphitrite on acrylic panels

The post-monsoon months Oct '98 (10 No dm ${ }^{-2}$ ), Nov '98 (10 No dm ${ }^{-2}$ ) and Jan '99 with Aug ' 98 formed the first cluster. The second cluster comprised of pre-monsoon and monsoon months (Fig. 3.11). The number of individuals ranged from (11 to 103 No $\mathrm{dm}^{-2}$ ). June and Dec ' 98 with Mar '99 were grouped into the third cluster. The maximum recruitment was observed during March '99 (123 No dm ${ }^{-2}$ ) (Fig. 3.12a). As observed on fiberglass, on acrylic surface also the maximum number of recruits were in the size range of $0-2 \mathrm{~mm}$ of RCB (Fig. 3.12a). Percentage area coverage was almost similar except during pre-monsoon months (Fig. 3.12a). Cumulative abundance on the panels exposed for two months was maximum during pre-monsoon months (Fig. 3.12b). Percent area coverage was also high during these months. This was followed by post-monsoon and monsoon months.

The abundance on three month exposed panels was high during May-July '99 (Fig. 3.12c). The percent area coverage was high for live when compared to dead barnacles (Fig. 3.12c). Most of the recruits were in a size range of 1-4 $\mathrm{mm}(\mathrm{RCB})$ (Fig. 3.12c), indicating their settlement at the end of the exposure period (July '99). Maximum size attained was 18 mm RCB.


Fig. 3.12 Recruitment of $B$. amphitrite on acrylic panel. First block represents the live, second block represents the dead barnacles and their size range (mm). The Y1 axis (Histograms) represents the abundance (No $\mathrm{dm}^{-2}$ ) and Y2 axis represents the percent area covered by the live and the dead barnacles (Lines)

Two-way ANOVA results indicated that recruitment significantly varied with the exposure months on different substratum (Table 3.4).

Table 3.4 Two-way ANOVA of the absolute recruitment of $B$. amphitrite on different substratum (Fiber Glass, Acrylic and Concrete)

| Factor | df | SS | MS | Fs |
| :--- | :---: | :---: | :---: | :---: |
| Months | 15 | 22 | 1 | $0.001^{* * * * *}$ |
| Substratum | 2 | 3 | 2 | $0.001^{* * * * *}$ |
| Months * Substratum | 30 | 31 | 1 | $0.001^{* * * * *}$ |
| Within subgr. Err | 96 | 6 | 0.1 |  |
| Total | 143 | 62 |  |  |

(***** $\mathrm{p} \leq 0.001$ )

### 3.3.2 Growth

Barnacles attained a maximum size of 6-8 mm on surfaces exposed for two to three months irrespective of the season. Further growth varied with seasons and the time of their recruitment (Fig. 3.2c,b). The individuals recruited during monsoon took a longer duration (8-10 months) to attain a size of $16-20 \mathrm{~mm}$, whereas those recruited in post-monsoon took 6-8 months. The maximum size attained by the adults ranged between 20-22 mm. The larger size groups were found in comparatively lesser numbers. The growth rate of barnacles at mid-tide level was comparatively faster and can be attributed to the feeding efficiency. Individuals that occurred solitary showed higher growth rate compared to those that were crowded. At high tide level individuals attained a maximum size of 14-16 mm (Fig. 3.8a). Solitary individuals grew at a faster rate $\left(0.17 \pm 0.03 \mathrm{~mm}\right.$ day $\left.^{-1}\right)$ and in case of crowded individuals the growth rate was slower ( $0.1 \pm 0.06 \mathrm{~mm} \mathrm{day}^{-1}$ ) (Fig. 3.13).


Fig. 3.13 Growth of $B$. amphitrite occurred as solitary and paired individuals

### 3.3.3 Cirripede larvae

Cirripede larvae were observed throughout the sampling duration. They were present in large numbers during Sept and Nov '98 (2300 and 2200 No 100m ${ }^{-3}$ ) (Fig. 3.14), while during Oct '98 (500 No $100 \mathrm{~m}^{-3}$ ) and Mar '99 (600 No 100m ${ }^{-3}$ ) they were considerably less. The minimum number of cirripede larvae were observed in late pre-monsoon and monsoon months (Apr '99 to Sept '99 and June '98 to Aug '98). The dendrogram and NMDS showed three clusters (Fig. 3.15). The first peak during Sep '98 and Nov '98 formed one cluster, followed by second peak during Oct ' 98 and Mar ' 99 . Rest of the months were grouped separately which had less number of larvae (Fig. 3.15).


Fig. 3.14 Monthly variation in the abundance of cirripede nauplii in the plankton


Fig. 3.15 Dendrogram and non-metric multidimensional scaling (NMDS) diagram for the cirriepede nauplii in plankton

### 3.3.4 Gonads

Percentage individuals with ripe ovaries were maximum during Dec '98 ( $50 \pm 16$ ). During Aug and Sept ' 98 months the percentage of ovaries was 23 and $22.5 \pm 5 \%$ respectively. Monsoon months showed comparatively low percentage of ripe ovaries (Fig. 3.16). The cluster dendrograms and the

NMDS plots indicated two clusters (Fig. 3.17). The July '98 month which did not indicate ripe ovaries was the most dissimilar. It was observed that the rest of the months which had percent ripe ovary ranging from $4-50 \%$ grouped into one cluster (Fig. 3.17).


Fig. 3.16 Monthly variation in the percemaye ui o. amphitrite with ripe ovary


Fig. 3.17 Dendrogram and non-metric multidimensional scaling (NMDS) diagram for the gonad condition

### 3.3.5 Rearing of larvae

The total naupliar duration was longer for the nauplii collected from plankton than those released from the broods (Fig. 3.18). The nauplii collected from plankton and reared in the laboratory showed lower percent survival to cyprid
when compared to those larvae released from the broods (Table 3.5; Fig.
3.19a).


Fig. 3.18 Monthly variation in the total naupliar duration of the larvae collected plankton and
Table 3.5. Two-way ANOVA of the percentage survival of nauplii to cyprids of plankton collected larvae and larvae collected from broods (treatments), reared in the laboratory.

|  | df | SS | MS | Fs |
| :--- | :--- | :--- | :--- | :--- |
| Months | 15 | 34871 | 2325 | $22112^{* * * * *}$ |
| Treatments | 1 | 7062 | 7062 | $67173^{* * * * *}$ |
| Months *Treatments | 15 | 38676 | 2578 | $24525^{* * * * *}$ |
| Within sub. Gr.err. | 64 | 7 | 0.10 |  |
| Total | 95 | 80616 |  |  |

(***** $\mathrm{p} \leq 0.001$ )



Fig. 3.19 Variations in the percentage survival of the nauplii to cyprid and metamorphosis of cyprid to juvenile of the nauplii collected from plankton and from broods during different months

### 3.4 Discussion

Recruitment of organisms in the boreal regions vary greatly according to the season of initiation, whereas in tropical region it may be largely non-seasonal, unless the environment is influenced by the meteorological factors such as fresh water run-off arising from monsoon conditions (Barnes 1972). The distribution of a species is in equilibrium involving many complex interactions between population and environment (Tait 1981). Estuarine environment has spatially and temporally varying physico-chemical factors. Hence in such an environment the role of environmental parameters in determining the population of organisms becomes more prominent.

Balanus amphitrite is a euryhaline and eurythermal species (Crisp and Costlow 1963; Iwaki 1981; Anil 1991). This species is also known to breed round the year in Indian waters (Karande 1967). Recruitment of Balanus amphitrite varied with the substratum, indicating variation in the substratum specificity exhibited by the settling larvae (Fig. 3.4.1). Substratum of different physical and chemical compositions might be expected to affect initial colonization in addition to the development of an assemblage. The absolute recruitment was more on fiberglass followed by acrylic and concrete. Fiberglass surface was more fibrous compared to acrylic and concrete. However more settlement on panels (both Fiber glass and acrylic) when compared to concrete substratum may be due to the quadrats on the concrete boulders were exposed during low tide, whereas the panels were suspended in such a way that they will not get exposed even during low tide. Thus the chances of microbial film formation would be faster on these surfaces.

Similarly the chances of desiccation would be low. However there is difference in the recruitment between fiberglass and acrylic. These results support the suggestions of Pomerat and Weiner (1942) that larvae generally prefer to settle on materials which are fibrous or porous, as opposed to hard and smooth surfaces. It was also reported that barnacle larvae settle in greater numbers on dark than on light surfaces. Harlin and Lindbergh (1977) stated that rough surfaces have greater algal colonization than smooth surfaces. Thus fiberglass being more rough than acrylic might have greater algal colonization, and further succession of macrofoluers.


Fig. 3.4.1 Absolute recruitment of Balanus amphitrite on concrete, fiberglass and acrylic surfaces

The observations made in this study showed that in general during monsoon months (exception being monsoon breaks discussed later on) the recruitment was low compared to non-monsoon months. The number of cirripede larvae in the plankton were also considerably low during monsoon season. However the gonad conditions indicated that the reproduction is not totally halted during
monsoon season. In fact during August and September'98 months percentage individuals with mature broods were considerable (23 and 22.5\%). Roughgarden et al. (1988) indicated that recruitment at a site is governed by the interactions among adult populations at that site or by limitations in larval supply reaching the site by regional pattern of circulation in coastal waters. It has also been stated that the isolation and integrity of estuarine populations are maintained by various hydrodynamic processes (Bousfield 1975; DeWolf 1973; Crisp 1984a; Anil et al. 1995). Anil et al. (1995) while studying the recruitment in a semi-enclosed bay, where water exchange with the adjacent open sea is limited, indicated that the settlement of cirripedes can be correlated to the larval abundance with a reasonable degree of certainty. However the environment under consideration is a bay, with considerable tidal influence and exchange of water with the open sea (Shetye et al. 1995). It experiences very high fresh water run-off during monsoon season thus lowering the salinity and increasing turbidity.

At mid-tide level the recruitment was very high whereas at high tide level it was low. This can be attributed to the substratum immersion duration. During both dry (non-monsoon) and wet (monsoon) seasons the amplitude of the semi-diurnal band remains unchanged. Thus the mid-tide quadrats would be submerged about 60 times a month (twice a day), whereas surfaces exposed at high-tide would remain immersed for a minimum of 16 to a maximum of 26 times (i.e. ones in 2 to 1.7 days) in a month. Hence the surface conditioning, time available for cyprids to explore the surface and subsequent settlement and recruitment would be at the mercy of time interval for which a surface remain immersed.

Recruitment did not peak (Fig. 3.1a) with the peak in larval abundance (Fig. 3.14a,b; Sept and Nov '98). A spurt in recruitment was observed in Aug '98 (Fig. 3.1a,b) whereas during the same month number of larvae were comparatively low (Fig. 3.14a,b) in spite of considerable number of barnacles with ripe ovary (Fig. 3.16a,b). Thus the recruitment, larval abundance and the percentage of barnacles with ripe ovaries did not synchronize (Figs. 3.1, 3.14 and 3.16; NMDS scatter plots). A lack in synchrony among the gonad condition, larval abundance and recruitment can be attributed to dispersion of larvae due to physical forces and biological processes. Over small spatial scale, variations in the density of settlers of benthic sessile species is the result of interactions among larval behavior, local hydrodynamic conditions and the physical, chemical and biological characteristics of the benthic habitat (Navarrete and Wieters 2000). This estuary being a tidal stream is more or less homogenous from surface to bottom during the non-monsoon months when fresh water flow is insignificant (NIO 1979). With the onset of southwest monsoon (June-October), increased fresh water inflow results in stratification between upper and subsurface layers. The stratification and upwelling may increase the chance of larval dispersal and could be one of the reason for low recruitment during monsoon.

Although there is a lull in the recruitment during monsoon season, a sudden spurt in the recruitment was observed in the month of August during 1998, whereas high recruitment during 1999 was seen in the month of July. On fiberglass panel such high recruitment was observed during July '98. This shift in the recruitment can be correlated with the conditions such as break in the monsoon. The rainfall data showed that during the year 1998 the peak
monsoon was during third week of July and during 1999 this was during mid June. After this there was a break in monsoon (Fig. 3.4.2).


Fig. 3.19 (a) Average of absolute recruitment on concrete, fiberglass and acrylic surfaces, percentage of barnacles with ripe ovaries and monthly variation in the abundance of cirripede nauplii (b) Daily rainfall during the year 1998-99

It is reported that the algal blooms occur along the west coast of India during monsoon season. These blooms are generally caused by factors associated with the rainfall (Bhimachar and George 1950; Subrahamanyam 1954,1959; Prakash and Sarma 1964; Qasim et al. 1972; Devassy 1983). By the advent of monsoon, blooms of Skeletonema costatum, Ceratium furca are reported in this estuary (Devassy 1983). Gopinathan (1974) reported the dominance of Skeletonema costatum immediately after or following a break in monsoon. Larval release in barnacles has been found to be synchronized with the phytoplankton blooms (Starr et al. 1991). This is also supported by Barnes (1962b) data for 15 years at Millport, Scotland; the release of Semibalanus
balanoides nauplii was closely associated with the spring S. costatum outburst. Barnacle larvae become plantkonic soon after release and it would be favorable if the larvae are released to food rich environment. In temperate regions where switch over of seasons is clearly demarcated such kind of signaling can work in favor of the successful recruitment. However in a situation like the burst of phytoplanlnkton in the middle of monsoon and the subsequent reoccurrence of unfavorable conditions can lead to confusing signals and also put new recruits into stress. This was observed on all the surfaces in the months of July and Aug '98 where the maximum number of spats ( $0-2 \mathrm{~mm} R \mathrm{RCB}$ ) were dead.

Maximum larval abundance was observed during September and November 1998, however recruitment did not peak during these months. The larvae collected from plankton during Sept '98 and reared in the laboratory showed low naupliar survival to cyprid and metamorphosis of cyprid to juvenile. The total development duration was also longer (Fig. 3.18). However same was not true when larvae were collected from the adult broods and reared in the laboratory, and the results were vice-versa. It would be possible that the quality of the larvae collected from plankton and those collected from broods could belong to different brood stock. Qui and Qian (1997) reported that the larvae from single brood responded to food concentration in the same manner as larvae from multiple broods through their laboratory experiments. In the present experiment the larvae collected during different months responded in a different manner. For example in the month of September '98 the larvae from the broods were collected on $18^{\text {th }}$ September and those form plankton were collected during $7^{\text {th }}$ September '98. B. amphitrite breed frequently (5-9
day brood $^{-1}$ ), low breeding interval will have impact upon the larvae released as there might be change in the quality and the quantity of the food available during the inter-breeding interval. It was observed in Balanus balanoides by Barnes and Barnes (1967) that the ovarian development is dependent upon the adequate food supply and interruptions in the food supply leads to regression of tissue. Lucas and Crisp (1987) while studying the energy metabolism of eggs during embryogenesis in Balanus balanoides indicated that when starved adults retain their mature egg mass beyond the normal term, egg metabolism occurs largely at the expense of the remaining lipid reserves. These would be exhausted in a further 6-7 weeks and the embryos are unable to survive. The ability of the adults to postpone hatching may therefore have important implications for the energy reserves and viability of the newly hatched larvae. Thus it is possible that poor ambient food availability during monsoon can influence the quality of the larvae released by this species at the end of the season. It was stated by Starr et al. $(1990,1991)$ that the spawning at the onset of the bloom might represent a trade-off between an adequate food supply for the larvae and the renewing of reserves in the adult. The larvae collected during other peak, Nov'98 were comparatively better than those of Sept'98. With the ending of monsoon season the environment returns to stable marine condition and this change over can lead to better conditioning of adults and the larvae released. Thus the differences in the development and survival of the larvae collected during different seasons indicate adult conditioning and inter brood variation to be important factors in the larval ecology of this organism.

Laboratory studies have shown that the naupliar development under optimal rearing conditions is completed in 4-6 days (Anil et al. 2001). The factors such as food concentration, temperature and salinity influence the duration of larval development and also the ability of the larvae to metamorphose (Anil et al. 2001). In an earlier work (Unpublished data) it was observed that the Balanus amphitrite larvae reared in the field took longer duration to reach the cyprid instar when compared to those reared in the laboratory. The difference in the larval development duration can be attributed to the food stress or starvation. Mullin and brooks (1976) stated that the average food concentration in the natural environment is often less than what is required for maximum growth and survival, which may result in nutritional stress to the growing larvae. The phytoplankton population is reported to be high during post-monsoon, moderately high during pre-monsoon and a sharp decline in the peak monsoon months is observed in this estuary (Devassy 1983). The differences in the phytoplankton abundance can change rate of larval development and metamorphosis and bring in temporal changes in recruitment.

The growth rate of barnacles was low during monsoon compared to post and pre-monsoon season. The seasonal changes in the water temperature and other environmental factors can also cause the differences in the growth rate. Slow growth during monsoon can be correlated to low food availability. It is also true that the higher land run-off during monsoon increases suspended load, which may interfere with the feeding mechanism and respiratory organs, thereby reducing the growth rate (Santhakumaran 1989). Higher growth rate during post-monsoon, can be attributed to stable marine conditions and the quantum of food available for larval growth. In this estuary the maximum
abundance of diatoms were reported during post-monsoon season (Mitbavker and Anil 2002). During summer the air temperature and the surface water temperature is raised about $10^{\circ} \mathrm{C}$ from post-monsoon. It was observed by Southward $(1955,1957)$ that the surface water temperature affects the growth rate of barnacles through their influence on the rate of cirral beat and feeding. Slow growth during summer can also be attributed to less plentiful food and rise in air and sea surface temperatures above the optimum for the animal (Ritz and Crisp 1970).

Temporal variation in the recruitment of Balanus amphitrite can be attributed to the variations in the physico-chemical and biological parameters mainly due to the monsoon conditions. With the onset of monsoon high concentrations of nutrients are brought into coastal waters, accompanied by rich growth of phytoplankton (Subrahamanyan 1959). It is reported that light penetration is an important factor in the productivity. During post monsoon period gradual repopulation of estuarine and coastal waters is observed and an increase in the light penetration is exhibited in tropical regions. At high latitudes growth of phytoplankton is limited by outstanding seasonal change in the incident radiation (Zenkevitch 1963). At middle latitudes, incident illumination is considerable and more or less exhibit regular changes over the year. Strickland (1965) mentioned that even moderately overcast sky could reduce the light intensity to one third or less than that found in clear weather. Pronounced differences in the weather from one year to another may, therefore, affect the time of the spring outburst in temperate areas, which will affect the productivity. In tropical areas overall incident radiation is very high than at middle and high latitudes. However during rainy season the light
intensity goes down by $50 \%$. But the land run-off during monsoon brings nutrient rich waters into the coastal areas, and a break in the monsoon increases the intensity of the incident radiation can induce phytoplankton blooms. During monsoon-months when nutrient concentrations are high and the salinity is low the diatoms are abundant. However, during pre-monsoon months when the nutrient concentrations are low there is a shift towards dinoflagellate dominance. Thus the change in the phytoplankton community can influence breeding in adult barnacle and larval feeding. In this environment blooms of $S$. costatum is observed during monsoon months especially during the break (Mitbavker and Anil 2002; Patil and Anil (Unpublished data)). Starr et al. (1991) identified coupling of nauplii release in barnacles with phytoplankton blooms: a parallel strategy to that of spawning in urchins and mussels. Spawning at the onset of the bloom may represent a trade-off between an adequate food supply for the larvae and renewing of reserves in the adult (Starr et al. 1991).

In the present context the recruitment of barnacles that occurred during monsoon break period were stressed with the recurrence of low saline and turbid condition indicating synchronization of releasing larvae to food rich environment may not be full proof in such environments. Impulsive release of larvae could be a shortsighted luxury. It is important to understand the impact of such environmental perturbances in sustenance of population. Probably temporal variation or recruitment failures in tropical environment such as one studied can be attributed to improper cue synchronization.


[^0]:    Numbers in the parenthesis indicate the number of barnacles dislodged, L - Live, D - Dead, NS - Not Sampled.

[^1]:    Numbers in the parenthesis indicate the number of barnacles dislodged, L - Live, D - Dead, NS - Not Sampled.

