Chapter 5
Summary
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*Balanus amphitrite* has euryhaline and eurythermal capacity of survival and breeding. Year round breeding is observed in this species indicates its potential for continuous recruitment. In this organism cross-fertilization is the thumb rule, however incidences of self-fertilization was observed. The individuals reared in solitary condition released larvae. However the breeding interval for self-fertilization was longer than that observed in case of cross-fertilization. Thus crowding may not be an essential requirement for reproduction in this species. Laboratory experiments revealed molting and breeding at all the temperature (20, 25 and 30° C) and food concentration (50, 100, 150 and 200 *Artemia* ind⁻¹). However their frequency varied with the variations in these parameters. Molting rate decreased with decrease in temperature and feed provided. Maximum number of molts at higher temperature and food concentration can be attributed to adequate energy allocation for growth. The increase in food concentration positively influenced the molting and breeding at all the temperatures. Thus the nutritional condition and temperature play an important role in breeding and molting process in this organism.

Low molting and breeding was observed at low food concentration. However maximum number of larvae were observed at 50 *Artemia* ind⁻¹ at 20° C. It was observed in *Balanus balanoides* that presence of egg mass in the mental cavity delayed molting (Patel and Crisp 1961). Thus the energy gained between a longer breeding interval seem to be utilized for sustaining the egg masses and this situation also favored higher number of larval release.
Observations on the gonad development revealed that the minimum size at maturity is about 3.9 mm (rostro carinal basal diameter) during post-monsoon season. However during monsoon season the minimum size of barnacle with mature ovary was 6.2 mm. Variations in minimum maturity size can serve as a potential indicator of adult conditioning in the environment. The growth of *B. amphitrite* in the wild did show seasonal variations with minimal growth during monsoon season.

Recruitment of organisms in the boreal regions vary greatly according to the season of initiation, whereas in tropical 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. The study site is situated at the mouth of the Zuary estuary, with considerable tidal range (0.25 to 2.5m). During monsoon season it experiences increased land run-off and precipitation that lower salinity and increase nutrient flux. Recruitment of this organism is generally low during monsoon exceptions being monsoon break period. The number of cirripede larvae in the locality were also low during monsoon months. Gonad conditions indicated reproduction is low but not completely halted during the monsoon season. A spike in recruitment was observed during August 1998. During this period number of larvae were comparatively low in spite of considerable number of barnacle with ripe
ovaries were present. Thus the lack of synchrony in the gonad condition, larval abundance and recruitment observed, can be attributed to physical factors such as stratification and currents. The study site Zuari estuary being a tidal stream is more or less homogenous during non-monsoon months. However with the onset of monsoon, fresh water inflow results in stratification between the upper and subsurface layers. This stratification and currents may increase the chance of larval dispersal.

Peak in recruitment during 1999 was observed in July, and can be related to conditions such as break in monsoon. Generally phytoplankton blooms occur along the west coast of India during monsoons (Bhimachar and George 1950; Subrahmanyan 1954, 1959; Prakash and Sarma 1964; Devassy 1983). Larval release in barnacles has been found to be synchronized with the phytoplankton blooms (Starr et al. 1991). The larvae of barnacles become planktonic soon after release and it would be favorable if the larvae are released in the food rich environment. However in a situation like the burst of phytoplankton in the middle of the monsoon and the subsequent recurrence of unfavorable conditions can stress new recruits. Such recruitment spikes during the monsoon (August '98) also indicated maximum mortality among new recruits 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.
Larvae in the plankton were more during September and November 1998, however recruitment did not peak during these months. Differences in the development and survival of the larvae collected during different season was observed indicating adult conditioning inter brood variation to be important factors in the larval ecology of this species.

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 during 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 varied with the seasons. 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.

Recruitment is the link between adult and larval ecology. Larval processes have a strong effect upon recruitment success and determine adult population distribution and abundance. The most obvious factor to determine recruitment success is larval mortality.
The naupliar development of this species is planktotrophic. Under optimal rearing conditions the naupliar development is achieved in 4-6 days. Several factors such as temperature, salinity, nutritional conditions influence the duration of larval development (Knowlton 1974; Sandifer and Smith 1979; Anil et al. 1995; Anil and Kurian 1996; Anil et al. 2001). Starvation is an important factor that determines larval mortality, and susceptibility to starvation depends upon the species, age of the larvae and on environmental factors (Anger and Dawirs 1981; Fenaux et al. 1988; His and Seaman 1982). *B. amphitrite* larvae starved at higher temperature showed relatively lower ultimate recovery point (URP) than those starved at lower temperature, indicating faster degradation of energy reserves and the metabolic demands of the larvae are greater at higher temperature (Hodgson and Bourne 1988). It was observed in the present study that at lower temperature larvae are generally inactive owing to which utilization of energy would be less when compared to the larvae starved at higher temperature.

The effect of temperature on starvation varied significantly with the age of the larvae. When starved at low temperature (5° C), a decrease in the ultimate recovery point was observed with the larval age. This decrease can be attributed to the sudden change in temperature from 25° C (rearing temperature) to 5° C (starving temperature). However such a decrease in the URP was not evident in case of larvae starved at 15 and 25° C.

It was observed that early instar larvae (II to IV instar) showed better development when reared with *C. calcitrans* than those reared with *S. costatum* even though the URP was either similar or more. Whereas, in the
advanced instars (V and VI instar) lower total naupliar development duration was observed when fed with *S. costatum*. The starvation tolerance and metamorphic success of the larvae varied with the age of the larvae and the food organism.

The influence of starvation, can be viewed as an interactive effect of type of food and larval age. Both the diatoms used in the present investigation were successful diets. It is also reported that *C. calcitrans* has similar food value as that of *S. costatum* (Kado 1991). The differences in the rate of larval development can be attributed to variations in the size of diatom cells, nutritional value and their ability to be in suspension. One of the reasons for such a difference can be the difference in the grazing rates owing to the variations in the size of diatoms.

The carbon gained through feeding is minimum in zero day old larvae when fed with *S. costatum* and it gradually increased with the larval age. However when fed with *C. calcitrans* maximum carbon gain was observed in two day old larvae and it decreased with larval age. This substantiates that with development the utility of the feed change.

The duration of all the instars increased after starvation. The lengthening of these periods can be viewed as an additional period necessary to accumulate enough energy to molt successfully to next instar and to the cyprid instar. Energy is allotted first to survival, then to molting and finally to development (Knowlton 1974). Molting was also observed at times when the larvae were undergoing starvation. This can be viewed as stress induced molting. The stress induced molting varied with the food organisms provided and also with
the age of the larvae. Thus the difference in the stress induced molting can be attributed to changes in food quality.

Cyprids like other invertebrate larvae derive their energy from stored lipids. The extent of this energy reserve in cyprids depends on the condition under which the nauplii grow (Lucas et al. 1979). The results from this investigation also reflected these observations. Naupliar development duration was prolonged at lower temperatures. It was also observed that the size of the larvae also varied with the rearing conditions. The nutritional status of the larvae indicated that it changes with the rearing food concentration and temperature.

The DNA and RNA content in the newly hatched nauplii showed considerable variation. However, with development the variations in RNA:DNA ratio narrowed down in each of the rearing conditions. This further indicates that even though initially different, under a given rearing condition this variability will have insignificant impact on the quality of cyprids.

The total naupliar duration lasted 9-12 d at 20° C as compared to 5-6 d observed at 30° C. The increase in food concentration appeared irrelevant at 30° C where as at 20° C the mortality rate was substantially reduced at higher food concentrations. At lower temperature increasing food concentration could compensate for decreased feeding efficiency and resulted in enhanced survival and shorter development period.

The temperature at which larvae were raised and the food concentration had variable influence on the starvation capability. Exposure to increased
starvation temperature eliminated the effect of doubling food concentration on starvation threshold levels. The larvae reared at 20° C had comparatively lower nucleic acid content. Even the prolonged development duration at lower temperature did not compensate for nutritional build up. This implies that the rearing conditions have a clear impact on the energetics of the larvae and food compensation and prolonged larval development at lower temperature may not correspond equally with larvae raised at higher temperature.

The RNA content and the RNA:DNA ratio of the larvae which were starved, decreased considerably. The minimum RNA content of the larvae, which could survive the starvation stress, was 0.4 to 0.5 μg larva⁻¹ and the RNA:DNA ratio was 0.3±0.2 μg larva⁻¹.

Antifouling assay is often the purpose of raising the barnacle larvae in the laboratory. However in the laboratory the larvae are provided with a rich diet of monoalgal culture, which do not mimic the conditions in nature. In the field rearing experiment it was observed that chlorophyll a content of the seawater was 0.3 - 4.0 μg L⁻¹. Even at the lowest laboratory rearing concentration the chlorophyll a content is 15 times higher than the maximum value found in the field. It is also to be noted that the natural phytoplankton population includes forms, which are of no value as cirripede larval feed. Owing to such differences the status of the larvae in the wild and those raised in the laboratory can be widely different. The difference in the total naupliar duration of the field-raised larvae and those raised in the laboratory was 2 d. However the larvae were smaller in size and had a lower RNA:DNA ratio.
The pre-settlement non-feeding cyprid instar is dependent on nutritional reserves stored during the naupliar development for its survival and exploration. The discriminatory capability of these larvae for choosing a substrate suitable for metamorphosis can also be widely different.

A comparison of the RNA:DNA ratio of the field and laboratory reared and those which are starved for 24 h indicated that the early instars (up to 3 d) of the field reared larvae (ca. 30° C) were most nearly similar to those raised at 20° C in the laboratory. Subsequently this ratio fell much below this level and was ~ 0.5 to 0.6 larva⁻¹. The VI instar nauplii raised in the laboratory and those in the field show that the field raised larvae had 1.4 (20° C) to 1.7 (30° C) folds lower RNA:DNA ratio. Careful considerations to balance this would be an essential prerequisite in standardizing antifouling assay protocols that will mimic events in nature. The larvae in the nature experience nutritional stress, however this does not proportionately translate to the well fed larvae raised in the laboratory. Thus it could be possible for the larvae to source their energy by feeding on organisms which are not a part of chlorophyll a pool and their roles need to be quantified.

In summary the events such as break in monsoon, quantum of nutrient influx, occurrence of blooms and recurrence of unfavorable conditions are important in reproductive and recruitment ecology of this organism. The larval rearing experiments indicated that rearing conditions do influence the nutritional status of the larvae, owing to which the resultant cyprids may respond differently to similar cues.