

**Reproduction in *Balanus amphitrite* Darwin (Cirripedia: Thoracica); influence of
temperature and food concentration**

Dattesh V. Desai, A. C. Anil* and Venkat K
Marine Corrosion and Materials Research Division
National Institute of Oceanography, Dona Paula, Goa 403 004, India

*Corresponding Author; Email: acanil@nio.org

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Abstract

Balanus amphitrite, an acorn barnacle is distinctly euryhaline, eurythermal and a dominant fouling organism found in warm and temperate waters through out the world. In this study, the influence of temperature and food concentration on the reproductive biology of this species collected from a tropical habitat was evaluated. Adult barnacles were maintained at 20, 25 and 30°C temperatures at different concentrations of food (50, 100, 150 and 200 *Artemia* ind⁻¹ day⁻¹). In this previously believed obligatory cross-fertilizing hermaphrodite, self-fertilization was observed. The rise in temperature from 20 to 30°C resulted in a longer interbreeding interval (6 to 7 days, 200 *Artemia* ind⁻¹ day⁻¹; 11 to 13 days, 50 *Artemia* ind⁻¹ day⁻¹). Computed carbon gained through feeding during the interbreeding interval indicated an inverse relationship to the temperature. At 20°C, although a greater amount of carbon was gained through feeding, the numbers of larvae produced were five fold less when compared to those raised at 30°C. At 20°C, 2.3µg C was required to produce a single larva, whereas at 30°C it was 0.4µg C. A rise in rearing temperature also influenced the molting rate positively. Observations on temporal variation in the gonad development of this species in a tropical coastal environment influenced by the monsoons indicated gonad development to be related positively to chlorophyll *a* concentration.

Introduction

Balanomorph barnacles are distributed worldwide and are found in all the marine environments from high intertidal zone to the depth of the ocean. Barnacles have gained economic importance owing to their presence in the hard fouling community and have been widely used in studies of inter-specific competition, habitat selection by planktonic larva and life histories (Crisp, 1954; Barnes and Barnes, 1967; Hurley, 1973; Crisp, 1974; Wethey 1979).

Most of the balanomorph barnacles are hermaphrodites (Charnov 1987), fertilization is internal and they are usually capable of producing eggs and sperms at the same time. Exchange of sperms between adjacent individuals, leading to cross fertilization, appears to be the rule among acorn barnacles. However, incidences of self-fertilization have also been reported (Barnes and Crisp 1956; Furman and Yule 1990; El-Komi and Kajihara 1991).

Several species of cirripedes in warm temperate and subtropical regions characteristically produce numerous small broods in rapid succession during summer. Temperature is considered to determine the length of the brooding season (Crisp 1950; Patel and Crisp 1960a, b; Hines 1978). Some cirripedes breed over a wide range of temperature whereas others breed until a certain critical temperature is reached and are termed as eurythermic or stenothermic respectively. Thus, temperature is an ecologically important component in determining the latitudinal distribution of cirripedes (Barnes and Barnes 1975; Lewis 1975; Barnes 1989). Within the optimum temperature range and in relatively stable conditions there is some evidence of seasonal breeding periods superimposed on a general continuous low level of reproduction, for example in *Elminius modestus*, *E. plicatus*, and *Chthamalus anisopoma* (Malusa 1986, Barnes 1989). It is also reported that the amount of food plays an important role in the breeding cycle of the cirripedes. Barnes and Barnes (1967, 1975) have shown that feeding in barnacles can control the time at which egg lamellae are produced; also poor nutritional conditions can cease breeding.

B. amphitrite is distinctly euryhaline and eurythermal in its tolerance. The study site Zuari estuary is a tropical estuary and experience a considerable amount of fresh water during monsoon thereby lower the salinity. We have presented the data relevant to recruitment of this species in this estuary (Desai and Anil, 2005) and elucidated the importance of monsoon and salinity changes.

In this paper we quantified the feeding rate and evaluated its influence on breeding. The frequency of molting was observed to evaluate its relationship with the breeding pattern. Observations on the incidences of self-fertilization in the laboratory were made in this species, which is represented as an obligatory cross-fertilizing hermaphrodite. Temporal variations in the ovarian development of barnacles collected from the nature were observed to draw inferences on the influence of ambient temperature and the amount of chlorophyll *a*.

Materials and Methods

Breeding and molting

In order to determine the effect of breeding (breeding interval and number of broods) and molting (molting interval and number of molts) several laboratory experiments were carried out. To study the breeding and molting, *B. amphitrite* was collected from the intertidal area of Dona Paula bay. Barnacles with a rostro carinal diameter (RCD) ranging between 8-12mm were sorted out in the laboratory, cleaned of the epibiotic growth with a nylon brush and used to carry out different experiments. These barnacles were mounted on acrylic plates (8x3 cm) as solitary and in pairs (5 replicates) using commercially available glue (Super Wiz). Acrylic sheets with barnacles mounted on them were immersed in filtered seawater (GF/C) in 250ml capacity glass beakers. Seawater (35‰) in the beakers was changed daily. These barnacles were fed with newly hatched *Artemia*. The number of molts and/or the larvae released was quantified prior to change of medium and food everyday. Everyday the unconsumed *Artemia*

nauplii were also counted.

Experiment 1

Observations on breeding, molting and food consumption were made for 75 days at different temperatures (20, 25 & 30°C) and food concentrations (50, 100, 150 and 200 *Artemia* ind⁻¹ day⁻¹). Through the experiment the barnacles were maintained in the incubator at respective temperatures.

Experiment 2

The experiment I demonstrated at a given food concentration (200 *Artemia* ind⁻¹ day⁻¹) the breeding is frequent at lower temperature (20°C) whereas at higher temperature (30°C) the number of larvae produced during experimental duration was higher and this was coupled with higher frequency of molting. Taking into consideration the ambient water temperature in the surrounding waters experiment II was designed to elucidate whether the production of higher number of larvae is sustainable over a longer period. Thus, experiment II was carried out considering all the food concentrations (50, 100, 150 and 200 *Artemia* ind⁻¹ day⁻¹) at 30°C for a longer duration (203days). The barnacle *B. amphitrite*, also gets exposed to this temperature for a longer duration in this tropical environment compared to other two temperatures (20 and 25°C). Food and seawater were changed everyday as mentioned above.

Experiment 3.

In order to understand the breeding of *B. amphitrite* raised in the laboratory from cypris instar and to compare it with those barnacles collected from the field this experiment was carried out. The nauplii released by the paired barnacles maintained at 30°C (fed with 200 *Artemia* ind⁻¹ day⁻¹) were collected and reared till they developed into the cypris stage in the laboratory using

Chaetoceros calcitrans (unicellular diatom) as food at a cell concentration of 2×10^5 cells ml^{-1} . These cyprids were transferred into 5 ml capacity polystyrene multiwells for settlement. The settled cyprids (spat) were provided with unicellular algae (*Chaetoceros calcitrans*) and *Artemia* as food. Once these juveniles attained a size of 5mm (rostrum carinal diameter) they were separated as solitary and paired individuals and maintained at 30°C at a food concentration of 100 *Artemia* $\text{ind}^{-1} \text{day}^{-1}$. The numbers of molts and broods were monitored every day prior to change of food and seawater.

Carbon Quantification

Carbon quantification was done by calculating the amount of food intake by counting the unconsumed *Artemia* and subtracting it from the total number of *Artemia* provided as feed in each of the beakers every day. The *Artemia* nauplii used for hatching to feed the barnacles were from same batch of cysts and all were hatched for similar duration, it was assumed that they would have almost similar carbon. As single *Artemia* nauplius corresponds to $\sim 0.52 \mu\text{g C}$ (Omori and Ikeda 1984), the total number of nauplii fed were multiplied by this factor to get the energy gained in terms of carbon and was expressed as $\mu\text{g C}$ assimilated.

Field Observation of Gonads

Studies on temporal variations in the ovarian development were carried out for the *B. amphitrite* population at Dona Paula bay from March 1997 to September 1999. Observations on gonads were taken every fortnight. Collection of the barnacles was done as solitary (having no other barnacle in the vicinity of 5 to 6cms as cross-fertilization would not be possible) and crowded. Barnacles with ripe ovaries were measured for their size (RCD) before taking the observations on the status of the ovary.

Results

Breeding, Molting and food consumption (Experiment 1)

Breeding

The paired individuals produced higher number of broods when compared to the solitary individuals at all temperatures and food concentrations (Fig. 1 a,b,c). In the case of solitary individuals at 30°C, breeding was observed at all the food concentrations (Fig. 1c). In solitary individuals breeding interval was comparatively longer than the paired ones. For solitary individuals the maximum breeding interval was 43 days at 30°C (50 *Artemia* ind⁻¹ day⁻¹) (Fig. 1f), and the minimum interval was 19 days at 20°C (150 *Artemia* ind⁻¹ day⁻¹) (Fig. 1d). In case of paired individuals, an increase in food concentration resulted in an increase in the number of broods at almost all the temperatures (Fig. 1 a-c). However, the maximum numbers of broods are observed at 20°C (Fig. 1a). Two-way ANOVA results indicated that the mode of occurrence as solitary and paired individuals significantly influenced the breeding interval at 100, 150 and 200 *Artemia* ind⁻¹ day⁻¹ (Table 2). Similarly, temperature also significantly affected the breeding at all the food concentrations. Multiway ANOVA results for the influence of mode of occurrence, food concentration and temperature indicated that mode of occurrence as solitary and paired individuals with respect to temperature significantly affected the breeding interval ($p \leq 0.025$). The paired individuals released maximum number of larvae at 200 *Artemia* ind⁻¹ day⁻¹ at 30°C (Table 1). However, at 20 and 25°C at similar food concentrations, the number of larvae released was less (Table 1). A similar situation was evident at the other food concentrations (150, 100 and 50 *Artemia* ind⁻¹ day⁻¹) at all of the temperatures (Table 1) except at 50 *Artemia* ind⁻¹ day⁻¹ at 20°C, where maximum larvae per brood were observed. In case of solitary individuals the maximum number of larvae (2700 brood⁻¹) was released at 150 *Artemia* ind⁻¹ day⁻¹ (20°C) but no larvae were produced at the other food concentrations at this temperature (Table 1). At this temperature, the number of molts between the two broods was

less compared to 25 and 30°C (Table 1).

Regression analysis between the numbers of larvae released vs. the number of days (experimental schedule) for which the barnacles were raised indicated that the number of larvae released increased with the age of the barnacles. This increase was significant in case the of barnacles maintained at 30°C fed with 150 ($m=24.4$, $p\leq 0.02$) & 200 ($m=37.5$, $p\leq 0.02$) *Artemia* ind⁻¹ day⁻¹ and at 25°C fed with 200 ($m=22.99$, $p\leq 0.01$) *Artemia* ind⁻¹ day⁻¹.

Molting

The maximum number of molts was found at 30°C (Fig. 2c) and decreased with the decrease in the temperature (Fig. 2a,b), however, the number of molts increased with an increase in food concentration (from 50 to 200 *Artemia* ind⁻¹ day⁻¹) irrespective of the rearing temperatures (Fig. 2a-c). In general, solitary individuals had less molts compared to paired individuals (Two-way ANOVA; $p\leq 0.025$). At 20°C, the molting interval was longer (Two-way ANOVA; $p\leq 0.025$) (Fig. 2d) compared to 25 and 30°C (Fig. 2e,f). The results indicated a significant influence of temperature and mode of occurrence (solitary/paired) on the number of molts at all the food concentrations (Table 3), however the interaction between mode of occurrence and temperature significantly influenced the molting at 100, 150 and 200 *Artemia* ind⁻¹ day⁻¹ (Table 3).

Food Consumption

The food consumption rate was similar for both solitary and paired individuals at all the food concentrations. Feeding rate was not uniform throughout the experimental duration. At several instances, prior to/or during larval release generally there was a decrease in the food consumption rate and this was more prominent at higher temperatures.

The number of larvae released and molts between successive breeding varied with the amount of carbon gained through feeding (Table 1). The amount of carbon gained between the

successive broods was maximum at 200 *Artemia* ind⁻¹ day⁻¹ at 20°C (Table 1). At 20°C, maximum number of larvae was released at 50 *Artemia* ind⁻¹ day⁻¹ even though the carbon gained was more at 200 *Artemia* ind⁻¹ day⁻¹ (Table 1). It was observed that at 50 *Artemia* ind⁻¹ day⁻¹ 6 broods were produced, however at 200 *Artemia* ind⁻¹ day⁻¹ it was 13 broods (Table 1). Regression analysis between the carbon gained vs. number larvae and number of molts produced between successive breeding indicated a significant increase in the production of larvae and molts with an increase in the carbon gain and this was generally observed at higher food concentration (Table 4).

Breeding, Molting and food consumption (Experiment 2)

Breeding

The observations on breeding for a longer duration (203 days) at 30°C indicated an increase in the breeding rate with an increase in the food concentration (Two-way ANOVA; $p \leq 0.001$) (Fig. 3) and the results (Fig. 3a,c) were similar to those obtained in experiment 1 (Fig. 1c,f). Regression analysis indicated a significant increase in the number of larvae released with the age of the barnacle (1-203 days) only at 200 *Artemia* ind⁻¹ day⁻¹ ($m=10.854$, $p \leq 0.02$). The maximum numbers of larvae released were 7000 brood⁻¹ at 150 *Artemia* ind⁻¹ day⁻¹. However in general, numbers of larvae released were more at 200 *Artemia* ind⁻¹ day⁻¹.

Molting

The barnacles maintained for a longer duration (203 days) did not show any marked difference in the molting interval with reference to food concentration (Fig. 3b,d), and the interval was similar to that observed in experiment 1; 30°C (75 days) (Fig. 2f).

Production of molts was significantly influenced by the mode of occurrence and the food concentration (Two-way ANOVA; $p \leq 0.01$). In case of paired individuals a maximum of 14

molts were observed at 50 *Artemia* ind⁻¹ day⁻¹ between two successive breeding (48days interval) and number of molts were less at higher food concentrations (Table 1; Expt 2). Whereas, in the case of solitary individuals a maximum of 22 molts (90 days) were observed between successive breeding at 100 *Artemia* ind⁻¹ day⁻¹.

Food Consumption

The food consumption at all the concentrations was similar for both paired and solitary individuals. The results of the interbreeding carbon gain at different food concentrations indicated that it decreased with the food concentration (Table 1). Food concentration significantly influenced the breeding interval (Two-way ANOVA; $p \leq 0.001$) (Fig. 3c) and also the numbers of larvae released were less (Table 1; Expt. 2). A significant increase in the number of molts and number of larvae was observed with an increase in the amount of carbon gain (Table 4; Expt. 2).

Breeding and Molting (Experiment 3)

The observations on the breeding of barnacles raised in the laboratory indicated that in the case of solitary individuals the interval of self-fertilization was comparatively higher (Fig. 4c) than the barnacles used in experiment 1 (collected from wild) (Fig. 1f). The breeding interval was 54 days for the laboratory-raised individuals and 43 days for the individuals collected from the field and kept solitary. However, in the case of paired individuals the breeding interval was slightly shorter compared to experiment 1 (Figs. 4c and 1f). For the laboratory-reared barnacles in the case of paired individuals, the numbers of larvae released were more (3000 brood⁻¹) compared to solitary individuals (One-way ANOVA; $p \leq 0.025$).

Barnacles raised in the laboratory from cyprids showed a shorter molting interval (2.7 ± 1.6 days) (Fig. 3d) when compared to those individuals brought from the field (3.2 ± 1.9)

(Fig. 2f).

Field observations of gonads

Barnacles with ripe ovaries were found throughout the year, however the percentage varied with the seasons (Fig. 5). The percentage of individuals with ripe ovaries was high during late post-monsoon and early pre-monsoon months. During monsoon months this percentage was low. Maximum percent of ripe ovary were found during Dec'98 to March '99 (50 to 37%) followed by Dec'97 and Jan'99 (31 and 33%). Percentage of ripe ovaries was minimum during late pre-monsoon (April and May; 1997: 4 and 9%, 1998: 12 and 17%, 1999: 9 and 8%) and early monsoon months (June and July; 1997: 14 and 18%, 1998: 4 and 0%, 1999: 12 and 7%). It was observed that barnacles occurring as solitary individuals also showed ripe ovaries, however their percentage was comparatively lower than those observed in pairs (One-way ANOVA; $p \leq 0.025$) (Fig. 5). In summary, relatively high percent of ripe ovaries are found in pre-monsoon months (29-50%) while the rest of the months showed lower values (4-23%). It was observed that the average minimum size of individuals at which mature broods are produced varied with the seasons. It was 4.35mm RCB in post-monsoon, 5.65 during monsoon and 6.3mm RCB during pre-monsoon (Fig. 6). When the effect of temperature and chlorophyll on the size at maturity was analyzed, it was observed that during low temperature and high chlorophyll *a* content the size of the barnacles to produce mature broods was small (Fig. 6). Regression analysis indicated a significant decrease in the size at maturity of barnacles with an increase in the chlorophyll *a* content of the sea water (Fig. 7). The regression analysis between the temperature and the size at maturity of barnacles did not show a significant relation, however it indicated an increase in size with rise in temperature (Fig. 7).

Discussion

B. amphitrite has euryhaline and eurythermal survival and breeding capability, and can breed at

temperatures as low as 15° C (Crisp and Costlow, 1963; Iwaki 1981; Anil 1991; Anil et al., 1995). In Indian waters, *B. amphitrite* breeds round the year (Karande 1965). Though exchange of sperm between adjacent individuals leading to cross-fertilization generally occurs as seen in the present study (El-Komi and Kajihara, 1991) incidences of self-fertilization are also observed. The individuals that were kept solitary in this study released larvae. However, the breeding interval varied between the laboratory-raised and the field-collected individuals. The solitary individuals collected from the field showed ripe ovaries after an interval of 43 days, whereas this interval for laboratory-raised barnacles was 54 days. Such self-fertilization is stated to occur under circumstances when gametogenesis is completed without the occurrence of copulation (Barnes and Crisp 1956; Patel and Crisp 1961).

Molting was observed at all of the rearing temperatures and food concentrations. The number of molts was high at higher temperatures and food concentration, indicating food concentration along with temperature to affect the molting process. The breeding frequency also increased with an increase in food concentration, however, with an increase in temperature the breeding rate decreased. It has been shown that embryonic development is a function of temperature in several barnacles, *Balanus amphitrite* var. *denticulata*, *Elminius modestus*, *Semibalanus balanoides* (Crisp 1959; Patel and Crisp 1960a,b; Barnes 1963; Crisp and Costlow 1963). Thus the nutritional condition and temperature seems to play an important role in the breeding and molting processes in barnacles.

While studying the energy budget in *Balanus glandula*, Wu and Levings (1978) reported a loss of a large amount of energy in respiration, the second most important budget item was for egg production, followed in decreasing order by shell production, production of body tissue and molting. These observations indicate that the energy allocated for reproduction is less at higher temperatures and explains the faster breeding observed at lower temperatures in this study. In the present investigation it was observed that at lower temperature the carbon gained through

feeding was greater compared to higher temperature. At 200 *Artemia* ind⁻¹ day⁻¹ the carbon gain was 688µg C and they could produce 300 larvae. This translates to 2.3µg C to produce single larva (and molt twice during the inter breeding period averaging to 6 days). Whereas at 30°C, at similar food concentration, the carbon gain was 576µg C, and could produce 1500 larvae (five fold more compared to 20°C) translating to 0.4µg C per larva (and molt three times during the interbreeding period averaging to 7 days).

Breeding potential at any given temperature was regulated by food concentration. At 20° C, more larvae were released at 50 *Artemia* (9290; Table 1) than at 200 *Artemia* ind⁻¹ day⁻¹ (2829; Table 1). However the numbers of broods produced at higher food concentration were more. The release of more number of larvae at low food concentration can be attributed to the greater amount of energy accumulated by the animal during the longer inter-breeding interval. Thus the longer breeding interval at the lower temperature may help the individuals to accumulate more energy through feeding to produce more larvae. Such a phenomenon may be of importance to cold-water species to sustain the diversity by producing more larvae with less number of broods. It was observed for *Semibalanus balanoides* that the energy available for egg production is distributed among some 4000-10000 small eggs, according to the size of the adult, which hatch into planktotrophic nauplii. Egg size is a compromise between two extremes: the smaller the egg, the greater the fecundity, yet the greater the energy reserves it contains, the greater the probability of its survival (Crisp 1986).

It was observed at many instances that the liberation of brood is accompanied by a molt. The intermolt period of ovigerous barnacles is longer than the unfertilized ones owing to the presence of egg masses in the mantle cavity (Patel and Crisp 1961; Fyhn and Costlow 1977). Molting while embryos are present may therefore be disadvantageous. Thus, extending the intermolt period while the egg masses are developing minimizes the danger. Low molting and breeding was observed at 50 *Artemia* ind⁻¹ day⁻¹ food concentration, however, the maximum

number of larvae was observed at this food concentration at 20° C. Hence the decrease in molting can be attributed to the presence of egg masses in the mantle cavity, for which the animal may delay its molting.

It was observed that during or prior to larval release the feeding rate decreased indicated by the presence of more number of unconsumed *Artemia* nauplii. There could be two possible explanations. (1) There may be actual halting of feeding activity during breeding or prior to larval release (2) The interval of observation period was 24h and within this period the adult could have fed on its own larvae. However, the incidence of reduced feeding a day prior to release of larvae indicates the earlier reason for reduced feeding a greater probability.

The observations on the gonad index of the field-collected *Balanus amphitrite* showed seasonal changes and also varied between solitary and crowded individuals. The percentage of ripe ovaries was low in solitary individuals when compared to those from the crowded ones. Low percentages of ripe ovaries were observed during monsoon season and can be attributed to low salinity. The study site being a tropical estuary receives comparatively large amount of fresh water run off during monsoon season that lowers the salinity down to 4‰, and this could be a reason for low breeding in monsoon. Barnes (1989) also suggested that the effect of salinity on the breeding season is particularly important in estuarine habitats where fresh water run-off due to river discharge is excessive. Pillay and Nair (1972) indicated restricted brooding of *B. amphitrite communis* in the monsoon period and attributed this to low salinity and food availability. In cirripedes those producing more than one brood per year, the time between the laying down of egg masses is important. In warm-water species producing several broods a year, those settling early in the year will reach maturity and will themselves reproduce during that year. Age can also affect the number of eggs/larvae produced by an adult. It was observed that the minimum size of adults at which mature broods (stage IV ovary) observed varied with the seasons. This size was minimum during post-monsoon followed by monsoon and

maximum during pre-monsoon. In general, the surface water temperature was slightly lower during post-monsoon months compared to pre-monsoon months, however there is difference in the size at maturity of the individuals, indicating that temperature plays an important role in the growth and reproduction in this species. However, a significant decrease in the size at maturity of barnacles with an increase in the chlorophyll *a* content of seawater was observed. Inter annual seasonal averages (March 1997-September 1999) showed that the size at maturity was minimum (4.35mm) when chlorophyll *a* was 4.39 mg m⁻³ and temperature was 27°C in post-monsoon season and the average maximum size (6.3mm) was during pre-monsoon season when chlorophyll *a* was 2.7mg m⁻³ and temperature was 31°C. Earlier studies have reported that phytoplankton abundance is more during post-monsoon compared to other seasons at this study site (Patil, 2003). It was observed that the minimum size at maturity of the laboratory-raised barnacle is 6.7mm. The difference in the maturity size of laboratory-raised and the field-collected barnacles can be attributed to the available diet. Nichole (2005) suggested that growth of filter feeding benthic invertebrates (*Polycipes polymerus*, *Balanus glandula* and *Mytilus californianus*) to be largely dependent upon the productivity. Dattesh and Anil (2005) reported low growth rate of *Balanus amphitrite* during monsoon season compared to post and pre-monsoon and correlated to low food availability during monsoons. In the laboratory they were raised with a mono-species animal diet (*Artemia* sp.), whereas in the field the adults are exposed to a mixed diet (phytoplankton and zooplankton) that could have possibly rendered faster growth and maturity. This indicates that the quality of food and seawater temperature determines the growth in this species and needs further validation.

Recently satellite derived environmental data is used to forecast the climatic changes. Issues related to remote sensing of optically-complex waters are frequently encountered, for examples in coastal regions and in lakes. Water masses of this kind are often designated as “Case 2” waters. It is generally recognized that Case 2 waters are more complex in their composition and

optical properties and interpretation of optical signal from these water can therefore be rather difficult. To date, remote sensing of ocean colour has focused largely on the relatively simple Case 1 waters, and it is well recognized that the standard algorithms in use today for chlorophyll retrieval from satellite data break down in Case 2 waters (Sathyendranath 2003). Observations of the present study showed a significant relation between the chlorophyll *a* values and the size of this barnacle species. With further refinement, the reproduction index and growth can be used as a proxy for changes in the environmental parameters.

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References

- Anil AC (1991) Studies on macrofouling ecology of cirripedes in Hamana Bay (Japan), D. Agr. Thesis, Faculty of Agriculture, Univeristy of Tokyo, Tokyo, Japan
- Anil AC, Chiba K, Okamoto K, Kurokura H (1995) Influence of temperature and salinity on larval development of *Balanus amphitrite*: implications in fouling ecology. Mar Ecol Prog Ser 118: 159-166
- Barnes H (1963) Light, temperature and the breeding of *Balanus balanoides*. J Mar Biol Ass UK 43:717-727
- Barnes H (1989) Egg production in cirripedes. Oceanogr. Mar Biol Annu Rev 27: 91-166

- Barnes H, Barnes M (1967) The effect of starvation and feeding on the time of production of egg masses in the boreo-arctic cirripede, *Balanus balanoides* (L.). J Exp Mar Biol Ecol 1:1-6
- Barnes H, Barnes M (1975) The general biology of *Verruca stroemia* (O F Muller). V. Effect of feeding, temperature and light regime on breeding and moulting cycles. J Exp Mar Biol Ecol 19:227-232
- Barnes H, Crisp DJ (1956) Evidence of self-fertilization in certain species of barnacles. J Mar Biol Ass U K 35:631-639
- Charnov EL (1987) Sexuality and hermaphroditism in barnacles: a natural selection approach. In: Southward AJ (Ed.), Crustacean issues 5. Barnacle biology. AA Balkema, Rotterdam, pp: 89-104
- Crisp DJ (1950) Breeding and distribution of *Chthamalus stellatus*. Nature Lond 166:311
- Crisp DJ (1959) The rate of development of *Balanus balanoides* (L.) embryos in vitro. J Anim Ecol 28:119-132
- Crisp DJ (1974) Factors influencing the settlement of marine invertebrate larvae. In: Grant PT, Machie AN (Eds.), Chemoreception in marine organisms, Academic Press, London, pp: 177-265
- Crisp DJ (1986) Biology of benthic marine organisms. In: Thompson MF et al. (Eds.), Indian ocean, AA Balkema, Rotterdam, pp: 69-84
- Crisp DJ, Costlow JD Jr (1963) The tolerance of developing embryos to salinity and temperature. Oikos 14:22-34
- Dattesh DV, Anil AC (2005) Recruitment of the barnacle *Balanus amphitrite* in a tropical estuary: implications of environmental perturbation, reproduction and larval ecology. J Mar Biol Assoc U K 85: 909-920

- El-Komi MM, Kajihara T (1991) Breeding and moulting of barnacles under rearing conditions. *Mar Biol* 108:83-89
- Furman ER, Yule AB (1990) Self-fertilisation in *Balanus improvisus* Darwin. *J Expt Mar Biol Ecol* 144 (2-3):235-239
- Fyhn UEH, Costlow JD (1977) Histology and histochemistry of the ovary and oogenesis in *Balanus amphitrite* L. and *B. eburneus* Gould (Cirripedia, Crustacea). *Biol Bull Mar Biol Lab, Woods Hole* 152:351-359
- Hines AH (1978) Reproduction in three species of intertidal barnacles from Central California. *Biol Bull Mar Biol Lab, Woods Hole* 154:262-281
- Hurley AC (1973) Fecundity of the acorn barnacle *Balanus pacificus* Pilsbry: A fugitive species. *Limnol Oceanogr* 18 (3):386-393
- Iwaki T (1981) Reproductive ecology of some common species of barnacles in Japan. *Mar Fouling Tokyo* 3(1): 61-70
- Karande AA (1965) On cirripede crustaceans (barnacles) an important fouling group in Bombay waters. *Proc Symp Crust J Mar Biol Assoc India (Ser 4) 1945-1950*.
- Lewis CA (1975) Reproductive biology and development of the gooseneck barnacle, *Pollicipes polymerus*, with special emphasis on peristaltic constrictions in the fertilized egg. Ph. D. Dissertation, University of Alberta, Edmonton, Canada, pp. 320.
- Malusa JR (1986) Life-history and environment in 2 species of intertidal barnacles. *Biol Bull* 170(3):409-428
- Nicole EP (2005) Growth of filter feeding benthic invertebrates from a region with variable upwelling intensity. *Mar Ecol Prog Ser* 295: 79-85
- Omori M, Ikeda T (1984) Methods in marine zooplankton ecology. Chapter 7. A Wiley-Interscience Publication, John Wiley and Sons, pp: 143-145

- Patel B, Crisp DJ (1960a) Rates of development of the embryos of several species of barnacles. *Physiol Zool* 33:104-119
- Patel B, Crisp DJ (1960b) The influence of temperature on the breeding and moulting activities of operculate barnacles. *J Mar Biol Ass U K* 39:667-680
- Patel B, Crisp DJ (1961) Relation between the breeding and moulting cycles in cirripedes. *Crustaceana* 2:89-107
- Patil JS (2003) Studies on ecology of diatoms. Ph. D. Dissertation, University of Goa, Goa, India, pp. 188
- Pillay KK, Nair NB (1972) Reproductive biology of the sessile barnacle, *Balanus amphitrite communis* (Darwin), of the southwest coast of India. *I J Mar Sci* 1:8-16
- Sathyendranath S (2000) Remote sensing of ocean colour in coastal, and other optically-complex, waters. In: Sathyendranath S (Ed.) Chapter 1, Reports of the international ocean-colour coordinating group, Published by the International ocean-colour coordinating group, Dartmouth, Canada, pp. 5-21
- Wethey DS (1979) Demographic variation in intertidal barnacles. Ph.D Thesis, Univ. of Michigan, Ann Arbor, Michigan.
- Wu RSS, Levings CD (1978) An energy budget for individual barnacles (*Balanus glandula*). *Mar Biol* 45: 225-235

Table 1. Average carbon gained between successive breeding, average number of larvae produced per brood, average number of molts between successive breeding and total number of broods, larvae and molts produced at different temperature and food concentration by paired barnacles.

	Food Conc.	Carbon Gained ($\mu\text{g C}$)	No. of Larvae	No. of Molts	Total No. of broods	Total No. of Larvae	Total No. of Molts
Expt. 1 Observation for 75 days							
20° C	200	688 (± 356)	300 (± 250)	2 (± 1)	13	2829	21
	150	479 (± 350)	500 (± 290)	2 (± 1)	11	4556	17
	100	272 (± 200)	300 (± 225)	2 (± 1)	14	3752	15
	50	255 (± 150)	1500 (± 1900)	2 (± 1)	6	9290	12
25° C	200	603 (± 370)	800 (± 700)	2 (± 1)	12	8935	23
	150	513 (± 380)	460 (± 260)	2 (± 1)	9	4606	21
	100	392 (± 178)	1300 (± 500)	2 (± 1)	9	12005	17
	50	210 (± 108)	460 (± 350)	2 (± 1)	8	3410	12
30° C	200	576 (± 335)	1500 (± 1500)	3 (± 1)	10	14585	28
	150	660 (± 556)	1500 (± 750)	3.5 (± 2)	7	10690	23
	100	400 (± 172)	1400 (± 600)	2 (± 1)	8	11585	22
	50	303 (± 147)	750 (± 760)	3 (± 1)	5	3730	14
Expt. 2 Observation for 203 days							
30°C	200	808 (± 615)	1700 (± 1500)	4 (± 2)	23	34700	70
	150	622 (± 350)	1600 (± 1700)	3 (± 2)	21	31000	52
	100	424 (± 292)	1300 (± 1400)	4 (± 3)	19	21850	60
	50	340 (± 400)	700 (± 600)	6 (± 5)	11	8100	47

(Number in the parenthesis indicate the standard deviation from the average)

Table 2. Two-way ANOVA of the influence of temperature and mode of occurrence (solitary/paired individuals) on breeding interval at different food concentrations (df. Degree of freedom; SS. Sum of squares; MS. Mean of squares; Fs. Fischer constant) (****p \leq 0.001, ns – not significant)

Factor	50 <i>Artemia</i> ind ⁻¹ day ⁻¹			100 <i>Artemia</i> ind ⁻¹ day ⁻¹			150 <i>Artemia</i> ind ⁻¹ day ⁻¹			200 <i>Artemia</i> ind ⁻¹ day ⁻¹			
	df	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
A. (Solitary /Paired)	1	57	57	4.1 ns	1582	1582	101****	586	586	273****	512	512	1341****
B. Temperature	2	2252	1126	82****	1744	872	56****	1172	586	273****	701	351	919****
A*B	2	1646	823	60****	1336	668	43****	896	448	209****	614	307	805****
Within sub. Gr.err.	12	165	14		187	16		26	2.1		5	0.4	
Total	17	4120			4849			2680			1832		

Table 3. Two-way ANOVA of the influence of temperature and mode of occurrence (solitary/paired individuals) on number of molts at different food concentrations. (****p \leq 0.001, **p \leq 0.025, ns – not significant)

Factor	50 <i>Artemia</i> ind ⁻¹ day ⁻¹				100 <i>Artemia</i> ind ⁻¹ day ⁻¹				150 <i>Artemia</i> ind ⁻¹ day ⁻¹				200 <i>Artemia</i> ind ⁻¹ day ⁻¹			
	df	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
A. (Solitary /Paired)	1	24	24	24****	8	8	8**	20	20	23****	168	168	189****			
B. Temperature	2	13	6	6**	79	39	39****	119	60	67****	55	27	31****			
A*B	2	7	3	3 ns	67	33	33****	119	60	67****	59	29	33****			
Within sub. Gr.err.	12	12	1		12	1		11	1		11	1				
Total	17	56			166			269			293					

Table 4. Regression analysis between the carbon gained vs. number larvae and number of molts produced between successive breeding at different temperature and food concentration

	Number of larvae					Number of Molts			
	Food Conc.	m	r	n	p	m	r	n	p
Expt. 1	Observation for 75 days								
20° C	200	0.75	0.949	10	p≤0.001	0.005	0.45	10	Ns
	150	0.71	0.84	9	p≤0.01	0.005	0.65	9	p≤0.05
	100	0.442	0.43	12	ns	0.002	0.47	11	Ns
	50	10.22	0.772	6	p≤0.05	0.003	0.47	6	Ns
25° C	200	1.11	0.704	11	p≤0.01	0.002	0.56	10	p≤0.05
	150	0.24	0.35	9	ns	0.002	0.84	9	p≤0.01
	100	1.95	0.607	9	p≤0.05	0.002	0.62	9	p≤0.05
	50	0.92	0.265	8	ns	0.004	0.706	6	Ns
30° C	200	3.585	0.66	10	p≤0.05	0.002	0.6	9	p≤0.05
	150	0.999	0.723	7	p≤0.05	0.003	0.929	7	p≤0.001
	100	2.41	0.65	8	p≤0.05	0.002	0.524	8	Ns
	50	1.432	0.278	5	ns	0.002	0.315	5	Ns
Expt. 2	Observation for 203 days								
30° C	200	1.539	0.62	20	p≤0.001	0.003	0.739	20	p≤0.001
	150	2.426	0.5	20	p≤0.02	0.004	0.705	20	p≤0.001
	100	3.22	0.674	17	p≤0.01	0.007	0.7514	15	p≤0.001
	50	-0.692	0.481	11	ns	0.011	0.963	9	p≤0.001

(Ns – Not significant)

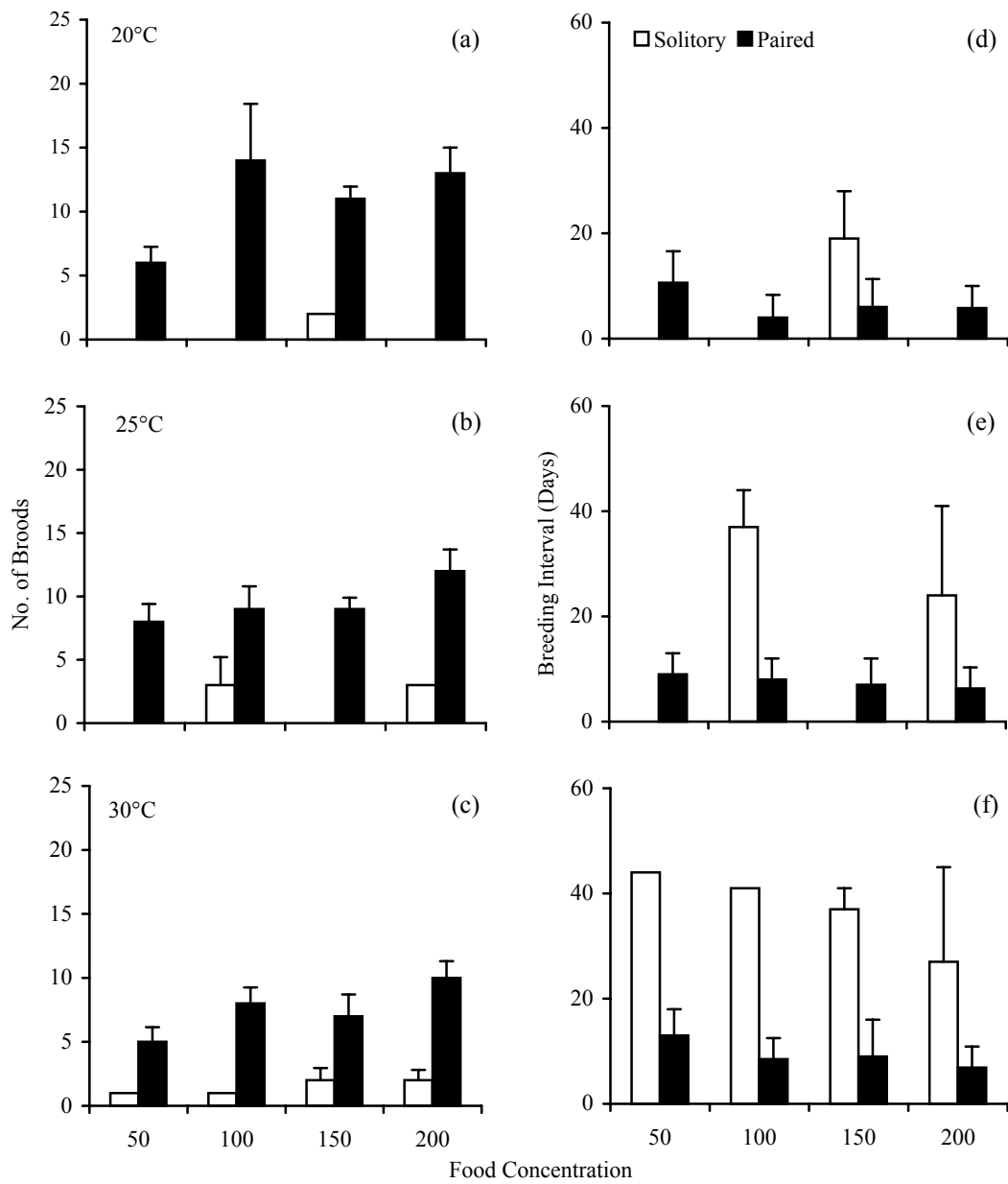


Figure 1. Number of broods (a,b,c) and breeding interval (d,e,f) for barnacles reared at different temperatures and food concentrations for 75days. Vertical lines indicate the standard deviation from the mean.

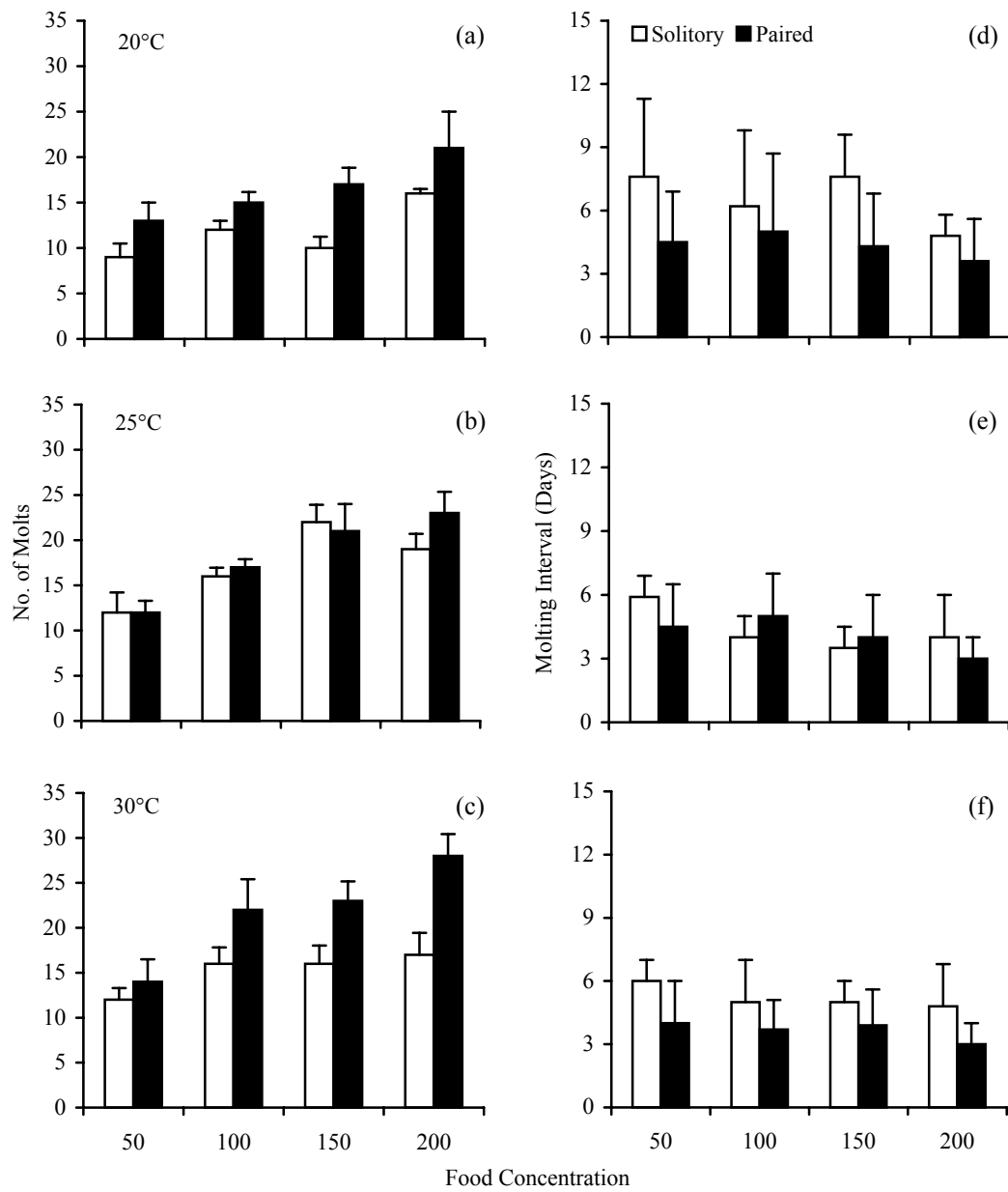


Figure 2. Number of molts (a,b,c) and molting interval (d,e,f) for barnacles reared at different temperatures and food concentrations for 75days. Vertical lines indicate the standard deviation from the mean.

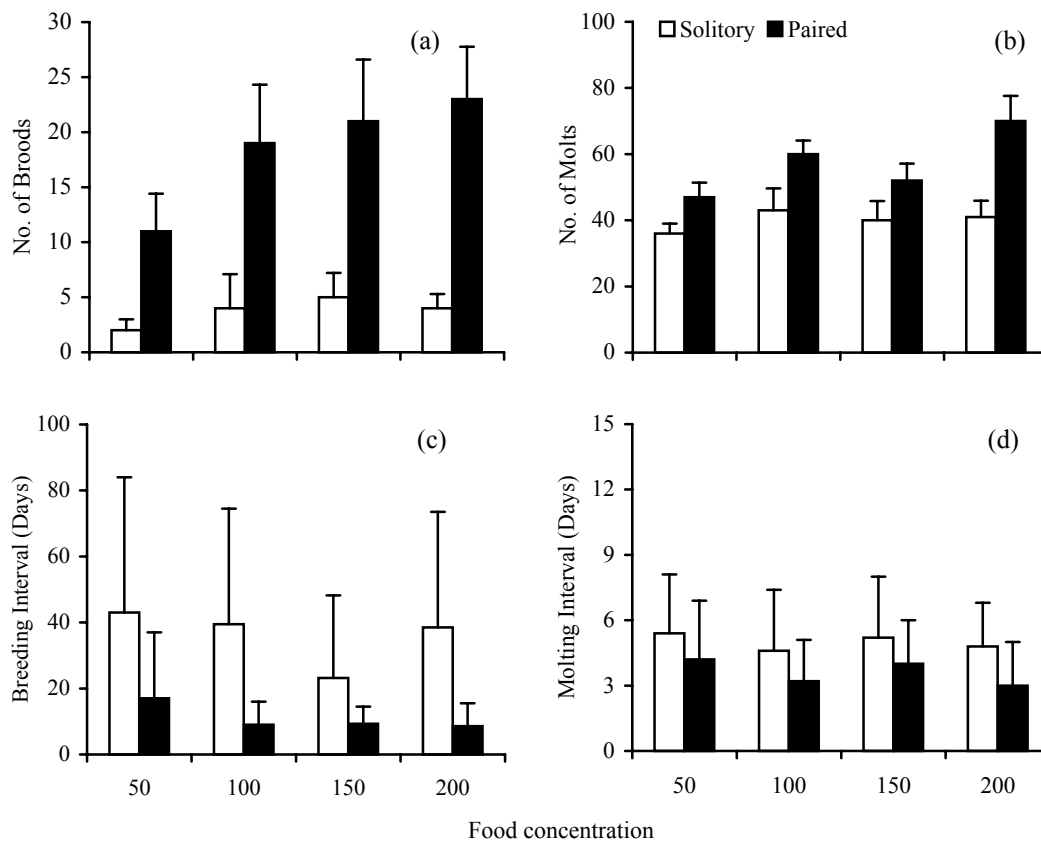


Figure 3. Variations in (a) number of broods (b) number of molts (c) breeding interval (d) molting interval of the barnacles reared for 203 days at 30°C at different food concentrations. Vertical lines indicate the standard deviation from the mean.

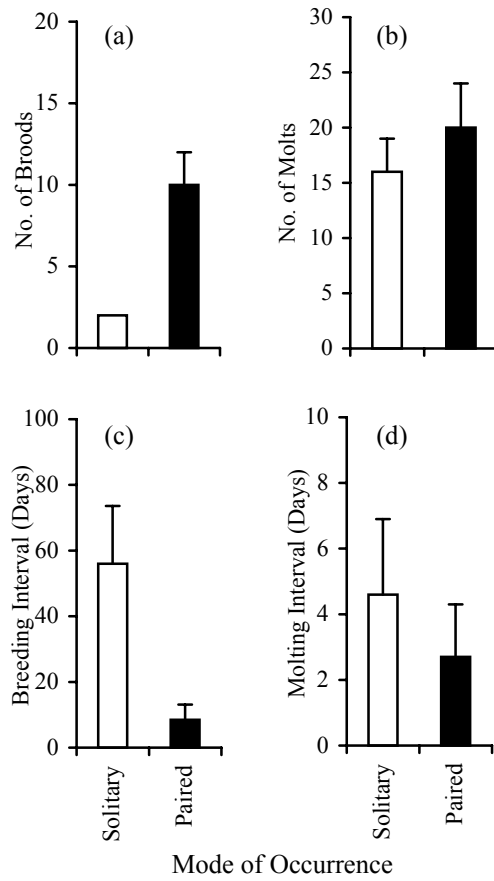


Figure 4. Variations in (a) number of broods (b) number of molts (c) breeding interval (d) molting interval for the laboratory-raised *B. amphitrite*. Vertical lines indicate the standard deviation from the mean.

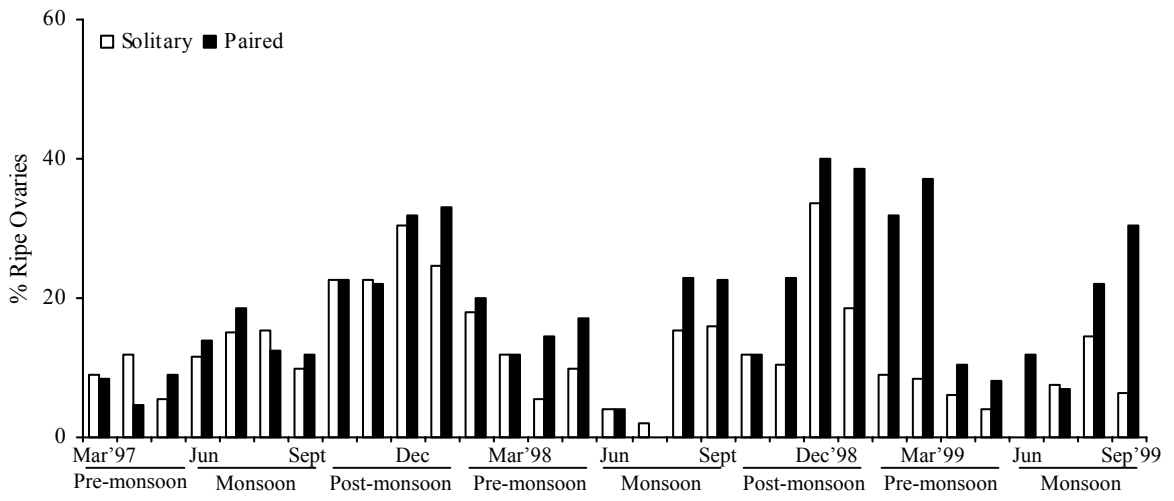


Figure 5. Temporal variation in the percentage of barnacles (solitary and paired) with ripe ovary

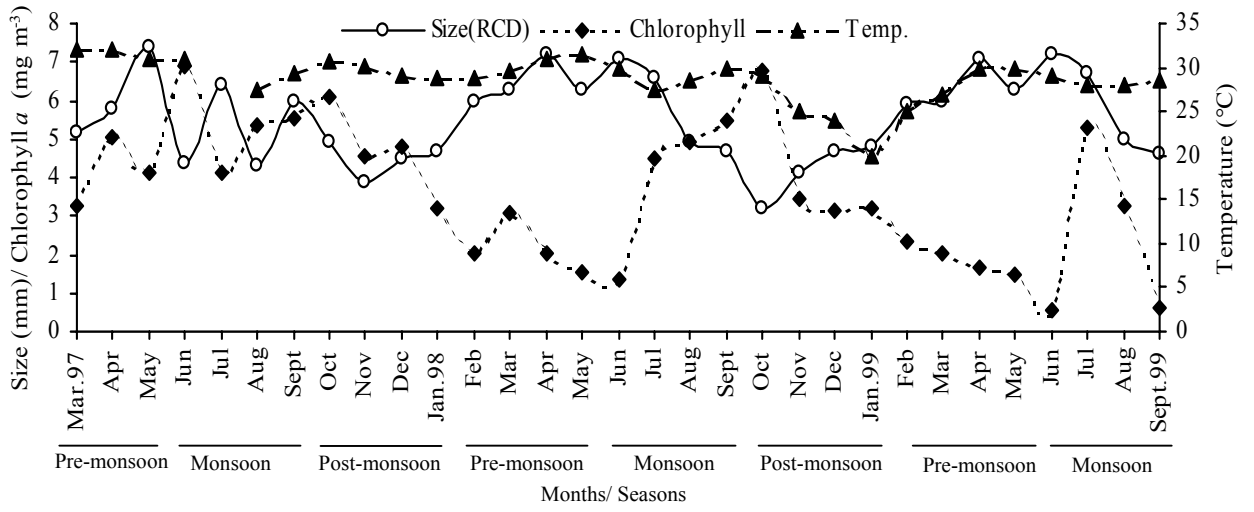


Figure 6. Temporal variation in the average minimum size of barnacles with mature broods, chlorophyll *a* content and temperature of seawater. RCD: Rostro Carinal Diameter

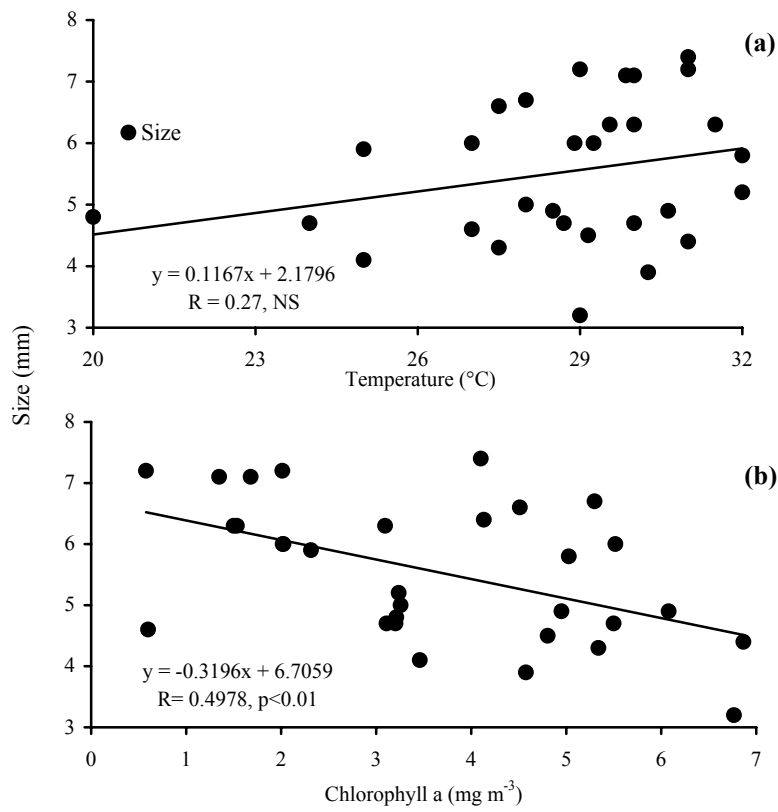


Figure 7. Relationship between seawater temperature and chlorophyll *a* content on the size at maturity of barnacles