

Larval development and metamorphosis of *Balanus albicostatus* (Cirripedia: Thoracica); implications of temperature, food concentration and energetics

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The influence of food concentrations (0.5, 1 and 2×10^5 cells ml⁻¹) and temperatures (20 and 30°C) on the survival, development, organic carbon and nitrogen content of *Balanus albicostatus* larvae was evaluated. The effect of food concentration on the subsequent ageing and metamorphosis of cypris larva was also determined. At lower food concentration and temperature, naupliar development duration was prolonged and the rate of metamorphosis of nauplius to cyprid was low. The rearing food concentration affected organic carbon and nitrogen content of the nauplii, which was also reflected in non-feeding cyprids. A decrease in the carbon content was observed with cyprid ageing at 5°C. Metamorphosis was higher in 8-d aged cyprids when compared to 2-d aged cyprids, and was positively influenced by the natural biofilm.

INTRODUCTION

Larval ecology is important in the recruitment of marine invertebrates and plays an important role in determining the adult population and distribution. The growth and survival of larvae in the plankton and the duration of the planktonic phase depends on a complex suite of physical, chemical and biological processes, such as water temperature, salinity, dissolved oxygen concentration, food quality and quantity (for planktotrophic larvae) and abundance of key predators (Knowlton, 1974; Sandifer & Smith, 1979; Day & McEdward, 1984; Young & Chia, 1987; Rumrill, 1990; Hart & Strathmann, 1995; Eckman, 1996; Anil & Kurian, 1996; Anil et al., 2001; Dattesh & Anil, 2000, 2002). Surface chemistry is very important to larval settlement and plays an important role in the distribution of adults (Strathmann et al., 1981; Chabot & Bourget, 1988; Le Tourneux & Bourget, 1988; Roberts et al., 1991; Holm et al., 1997). Miron et al. (2000) showed that habitat selection in barnacle cyprids results from a complex relationship involving substratum type, hydrodynamics and larval age (i.e. physiological condition and competency).

Temperature is considered as one of the most important factors determining larval growth and changes in temperature can influence both physiological and physical (swimming activity and/or motion) means of the microscopic larvae (Rumrill, 1990; Podolsky & Emlet, 1993; Boidron-Métarion, 1995; Hart & Strathmann, 1995; Anil et al., 2001). Laboratory studies on larvae of many benthic marine invertebrates have shown that availability of food affects the growth and survival (Sulkin, 1975; Pechenik & Fischer, 1979; Lucas, 1982; Anil & Kurian, 1996; Qiu & Qian, 1997, 1999; Anil et al., 2001; Dattesh & Anil, 2002). Mullin & Brooks (1976) indicated that the

average food concentration in the natural environment is often less than what is required for maximum growth and survival. Thus it is important to understand the larval growth and development under different environmental conditions.

Balanus albicostatus Pilsbry, an acorn barnacle is abundant in the intertidal areas of Japan, Korea and China (Utinomi, 1967; Newman & Ross, 1976; Lee & Kim, 1991). Larval development is planktotrophic with six naupliar and a non-feeding, settling cyprid instar. Earlier reports have indicated that the planktotrophic larval development determines cyprid metamorphosis and post-metamorphosis development in barnacles (Pechenik et al., 1993; Jarrett & Pechenik, 1997; Anil et al., 2001). Barnacle cyprids, prefer to settle on substrata that possess a well-developed biofilm (Crisp, 1984; Clare et al., 1992). Bacteria isolated from biofilms have also been assessed by several investigators on the larvae of choice in the laboratory (Maki et al., 1988, 1990, 1994; Avelin Mary et al., 1993; O'Connor & Richardson, 1996; Khandeparker et al., 2002). Biofilms generally inhibit settlement of *B. amphitrite* cyprids (Olivier et al., 2000; Maki et al., 2000), although some biofilms were inductive or had neutral effect (Wieczorek et al., 1995). Previous studies have reported increases in invertebrate larval recruitment with aged and more heavily filmed surfaces (Wieczorek et al., 1995). In contrast Maki et al. (1990) demonstrated that a 3-d old natural biofilm, but not 1-d old biofilm, inhibited barnacle cyprids. Our previous study with *B. albicostatus* revealed that the 2-d old natural biofilm promoted cyprid metamorphosis in the laboratory (Khandeparker et al., 2005). Thus the present study included 2-d old natural biofilm as a control.

Naupliar cultures raised using different food concentrations have been reported to affect the amount of energy

reserves in cyprids (West & Costlow, 1987; Hentschel & Emlet, 2000). Cyprids like other invertebrate larvae derive their energy from stored lipids. These competent larvae delay metamorphosis in the absence of stimuli. However, after postponement, larvae will settle in a less discriminatory manner (Rittschof et al., 1984). This signifies the importance of energy reserves and/or nutritional stress of the larvae that are used.

To date studies relating to *B. albicostatus* have been focused on larval description, growth, their phylogeny and reproduction and also for their presence in the fouling community (Iwaki, 1981; Lee & Kim, 1991; Nakamura, 1997; Puspasari et al., 2000, 2001). However, studies addressing the naupliar development, energetics and subsequent metamorphosis of cyprids are limited in this species. A detailed understanding of larval development and metamorphosis was required to evaluate the effects of different environmental factors. The objectives of the present study were: to provide the basic information on larval development and metamorphosis of *B. albicostatus*; and to understand how temperature and food concentration influence larval development and energetics. Effect of cyprid ageing on their metamorphosis and subsequent changes in their energy content were also evaluated.

MATERIALS AND METHODS

Rearing of Balanus albicostatus nauplii

Adult *Balanus albicostatus* were collected from the intertidal area near the Seto Marine Biological Laboratory, Wakayama Prefecture, Pacific coast of Japan and were brought to the laboratory and exposed to air for 1–2 h and then immersed in filtered seawater, which triggered the release of larvae. The I instar nauplii do not feed and moulted into II instar within a few hours and these larvae were used to carry out different experiments. Instars II to VI are phytoplanktonotrophic. Rearing experiments were carried out using three food concentrations as low, medium and high (0.5 , 1 and 2×10^5 cells ml^{-1} respectively) and two temperatures 20 and 30 (± 1)°C under 12 h:12 h, L:D cycle. *Chaetoceros calcitrans*, a unicellular diatom was used as food. The diatom cultures were grown in *f*/2 medium (Guillard, 1975). The medium contained three stock solutions. Stock 1 was prepared dissolving NaNO_3 , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in distilled water. Stock 2 contained five different trace metal solutions ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 5\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) prepared in distilled water with $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$. Stock 3 was vitamins, containing vitamin B_{12} , thiamine HCl and biotin. Sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) was dissolved in distilled water and used as stock 4. The working solution was prepared adding stock solutions 1 to 4 in filtered autoclaved seawater. Instar II nauplii ($N=24$) were transferred into multiwells (24-well Nunclon Delta 143982) with respective food concentration and reared individually. The rearing wells were placed in incubators at respective temperatures. Each larva was reared with 2 ml of food with an antibiotic dosage of 3×10^{-4} ml well^{-1} Crystamycin (Harms, 1986; Anil et al., 1995). The rearing of these nauplii was carried out until they reached the cyprid

instar. Observations were made on the percentage metamorphic success of nauplii to cyprids, larval instar duration and the total naupliar duration (TND). Everyday nauplii were transferred to new wells containing fresh food. Observations were also made on larval mortality and moulting everyday. The total length of the larvae was measured using a microscope equipped with an ocular micrometer.

The power function regression (PFR) analyses were carried out to gauge the influence of variables such as temperature and food concentration (v) on instar and total naupliar duration (D) as $D = bv^m$ (where b , intercept; m , slope). Two-way analysis of variance (ANOVA) (Sokal & Rohlf, 1981) was carried out to evaluate the combined interaction of both the variables (temperature and food concentration) on metamorphic success, instar duration, TND and size of the larvae. Before ANOVA, the data in the form of percentage of larval metamorphosis were arcsine transformed to ensure normality of means and homogeneity of variances.

Organic carbon, nitrogen and dry weight of the larvae

Organic carbon and nitrogen contents were estimated and dry weight of nauplii and cyprids were determined for the larvae reared using 1 and 2×10^5 cells ml^{-1} food concentration at 30°C . For this, nauplii were mass reared in 2-l glass beakers (1 larva ml^{-1}) at 1 and 2×10^5 cells ml^{-1} of *C. calcitrans* as food. Everyday prior to change of the food, larvae ($N=5$) were removed and immediately filtered on pre-combusted (500°C for 2 h) and pre-weighed Whatman GF/F filters. Samples were kept in the deep freezer at -84°C until analysis (in triplicate). Frozen samples were thawed at room temperature and then oven dried at 60°C for 48 h. Dry weights were measured using a microbalance. Weighed samples on the filters were grounded using mortar and pestle and were analysed for organic carbon and nitrogen by a CHN analyser (Elementar Analysensysteme GmbH, vario EL). Standards were prepared using acetanilide.

Biofilm development

Sterile multiwells (24-well Nunclon Delta 143982) were used to develop biofilms. They were immersed in fibreglass tanks with continuous flow of non-filtered natural seawater. Biofilms were allowed to develop for two days and such treated multiwells were used to carry out the settlement assays.

Assay protocol for evaluation of metamorphosis of Balanus albicostatus

Approximately 15–20 cyprids were introduced per well with 2 ml of filtered autoclaved seawater (35%) in polystyrene multiwells (24-well plates, Nunclon Delta 143982) (Maki et al., 1990). The multiwells with 2-d-old biofilms (positive control) were treated similarly and were assessed using six replicates for each of the treatment and repeated twice ($N=12$). Cyprids were stored in a refrigerator at 5°C for ageing and were used in the settlement assays as described by Rittschof et al. (1984). Ageing at 5°C was adopted, as the major cyprid protein content remains

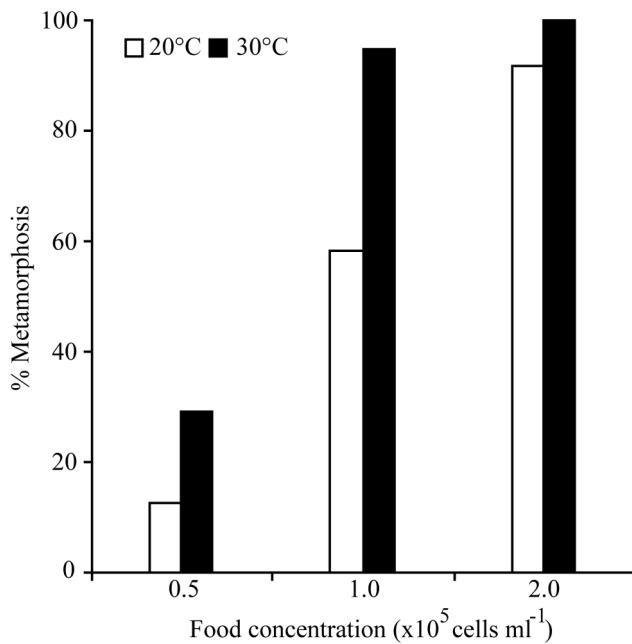


Figure 1. Percentage successful metamorphosis of the nauplii to cyprids reared at different food concentrations and temperatures.

constant at this temperature (Satuito et al., 1996). Bioassays were carried out for 2 and 8 d-aged cyprids. Assay wells were maintained at $26 \pm 1^\circ\text{C}$ on a 12h:12 h L:D photoperiod. They were monitored every 24 h for a period of four days and metamorphosed cyprids were counted.

RESULTS

Mortality

Mortality was not observed in II and III instars at any rearing conditions. Larvae showed a higher mortality rate

in the advanced instars and it varied with the rearing conditions. Larvae reared at higher food concentration did not show any mortality in the IV instar, however, those reared using low food showed 25 and 4% mortality at 20 and 30°C respectively. A similar trend was also observed in the case of V instar larvae, but with a higher per cent of mortality (54 and 17% at low food concentration, at 20 and 30°C respectively). The VI instar showed minimum mortality (8%) at low food concentration when reared at 20°C as most of the larvae were dead prior to VI instar. Whereas at 30°C rearing temperature mortality was maximum (50%) at low food concentration compared to just 4% at medium food concentration.

Percentage metamorphosis

The percentage of nauplii metamorphosing to cyprids varied with the rearing temperature and food concentration. The larvae reared at 20°C showed an increase in metamorphosis rate with an increase in the food concentration (Figure 1). However, the larvae reared at 30°C, at medium and high food concentration showed an almost similar metamorphosis rate, whereas those reared at low food concentration showed lower metamorphosis (Figure 1). Two-way ANOVA indicated that rearing temperature, food concentration and the interaction of both, significantly influenced the metamorphosis rate (Table 1).

Instar and total naupliar duration

The larvae reared at 20°C showed longer instar duration when compared to those reared at 30°C (Figure 2A&B). At 20°C, the instar duration increased with the advancement in development. The duration of instar II at low food concentration was two days and for instar VI it was 6.6 days. At higher food concentration, the VI instar duration was short (3.4 days) (Figure 2A). From II to V

Table 1. Two-way ANOVA of the effect of temperature and food concentration on percentage metamorphosis, instar duration and total naupliar duration of larvae.

Factor	df	Percentage metamorphosis			II instar			III instar			IV instar		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Temperature	1	1918	1917	1.03×10^4 ****	4.32	4.32	191.5****	1.6	1.6	29.5****	5.14	5.14	16.9****
Food conc.	2	18 311	9155	4.9×10^4 ****	0.04	0.02	0.82 ^{n.s.}	0.006	0.003	0.05 ^{n.s.}	7.59	3.8	12.5****
Temp.*food conc.	2	675	337	1820****	0.04	0.02	0.82 ^{n.s.}	0.004	0.002	0.04 ^{n.s.}	1.07	0.53	1.7 ^{n.s.}
Within subgroup err.	12	2	0.18		0.27	0.022		0.65	0.5		3.64	0.3	
Total	17	20 906			4.67			2.26			17.4		
Factor	df	V instar			VI instar			Total naupliar duration					
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Temperature	1	8.2	8.2	28.98****	57.8	57.8	56.07****	196.5	196.5	122.3****			
Food conc.	2	6.6	3.3	11.6****	42.7	21.36	20.7****	98.4	49.2	30.6****			
Temp.*food conc.	2	1.85	0.92	3.24*	20.6	10.33	10.02***	21.8	10.89	6.8**			
Within subgroup err.	12	3.41	0.28		12.3	1.03		19.3	1.6				
Total	17	20.06			133.8			336					

****, $P \leq 0.001$; ***, $P \leq 0.005$; **, $P \leq 0.05$; *, $P \leq 0.05$; n.s., not significant.

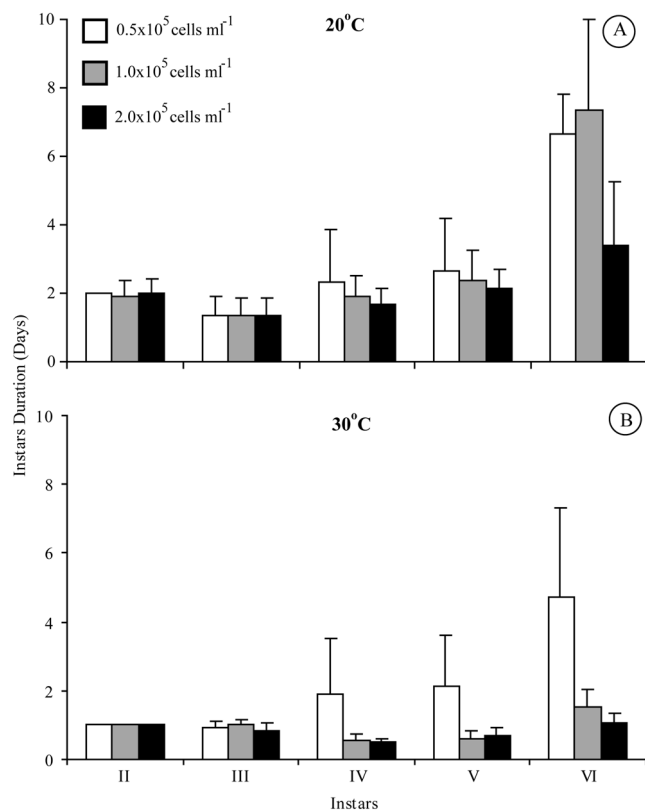


Figure 2. Variations in the naupliar instar duration of the larvae reared at different food concentrations and temperatures. (A) 20°C and (B) 30°C. Vertical lines indicate standard deviation from mean.

instars, the duration remained similar for all the food concentrations at 20°C (Figure 2A). The VI instar showed the longest duration among all the instars, irrespective of the food concentration (Figure 2A). In the case of larvae reared at 30°C, at medium and high food concentrations, all instar durations were similar. Whereas, only at low food concentration, IV to VI instar

durations were comparatively longer (Figure 2B). The power function regression analyses showed positive slopes for II and III instar duration at 20°C, with an increase in the food concentration. For IV ($m = -0.1714$), V ($m = -0.089$) and VI ($m = -0.8464$) instars the slopes were negative, indicating a decrease in the instar duration with an increase in food concentration. At 30°C, regression analyses showed a significant decrease in the instar duration (III instar, $m = -0.1379$; IV instar, $m = -0.569$, $P \leq 0.001$; V instar, $m = -0.9179$, $P \leq 0.001$ and VI instar, $m = -0.832$, $P \leq 0.001$) with an increase in food concentration. Two-way ANOVA indicated that early instars (II and III) were significantly influenced by rearing temperature only, whereas advanced instars (IV to VI) were influenced by temperature and food concentration as well (Table 1).

Total naupliar duration decreased with an increase in the food concentration, irrespective of the rearing temperature. At 20°C, the TND was longer compared to 30°C at all three food concentrations. The TND was longest (15 days) for the larvae reared at low food concentration and at high food concentration it was 11 days at 20°C. Larvae reared at 30°C, medium and high food concentration showed almost similar TND (5 and 4.1 days respectively). At low food concentration it was 10.7 days, which was almost double than those reared at similar temperature at higher food concentration. The PFR analyses showed negative slopes indicating a significant decrease in TND with an increase in food concentration both at 20°C ($m = -0.363$, $P \leq 0.001$) and 30°C ($m = -0.5353$, $P \leq 0.001$) temperatures. Two-way ANOVA also indicated a significant influence of temperature and food concentration on the TND of the larvae (Table 1).

Larval size

The nauplii reared at 30°C were bigger in size when compared to those reared at 20°C (Figure 3). With a

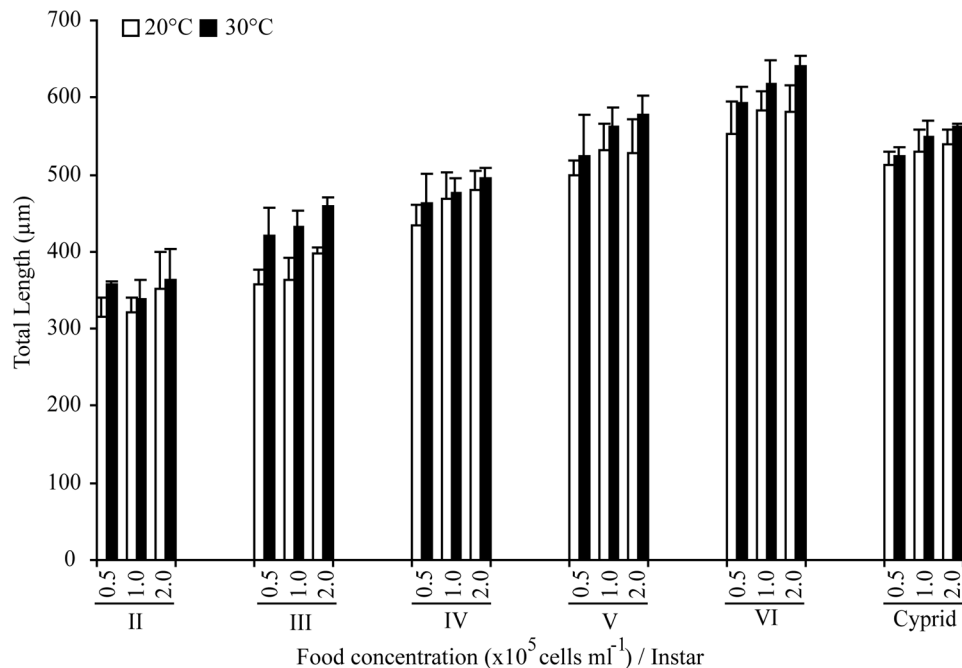


Figure 3. Variations in size (total length) of the nauplii in each instar and the cyprids reared under different temperatures and food concentrations. Vertical lines indicate standard deviation from mean.

Table 2. Two-way ANOVA of the effect of temperature and food concentration on total length of the nauplii and cyprid.

Factor	df	II instar			III instar			IV instar			V instar			VI instar			Cyprid		
		SS	MS	F _s	SS	MS	F _s	SS	MS	F _s	SS	MS	F _s	SS	MS	F _s	SS	MS	F _s
Temperature	1	2520	2520	2520*	18818	18818	1.8×10 ⁴ *	1300	1300	1300*	5618	5618	5618*	8844	8844	8844*	1458	1458	1458*
Food conc.	2	2412	1203	1206*	5004	2502	2502*	4503	2251	2251*	5791	2895	2895*	4827	2413	2413*	3259	1629	1629*
Temp.*food conc.	2	688	344	344*	28	14	14*	309	154	0.04 ^{n.s.}	511	255	255*	511	255	255*	129	64	65*
Within subgroup err.	12	12	1		12	1		12	1		12	1		12	1		12	1	
Total	17	5632			23862			6124			11932			14194			4858		

*, $P \leq 0.001$; n.s., not significant.

decrease in the rearing food concentration size of the nauplii decreased. Nauplii attained maximum size when reared at higher food concentration and temperature resulting in cyprids with the maximum size (Figure 3). Two-way ANOVA results indicated a significant influence of rearing temperature and food concentration on the size of all the naupliar instars and of cyprids (Table 2).

Dry weight of larvae

Changes in individual larval dry weights were observed with the rearing conditions. The newly-hatched larvae had a dry weight of $5 \pm 1.5 \mu\text{g larva}^{-1}$ and this increased with the development (Figure 4). Instar VI nauplii reared at high food concentration showed higher dry weight ($18 \pm 2 \mu\text{g larva}^{-1}$) when compared to those reared at medium food concentration ($16.8 \pm 3 \mu\text{g larva}^{-1}$) before metamorphosing to cyprid (Figure 4). The dry weight of the nauplii that had longer development duration under medium food concentration, was less than those reared

at high food concentration. Cyprids on ageing showed negligible reduction in their dry weights (Figure 4).

Organic carbon, nitrogen content and percentage metamorphosis

The rearing food concentration had a clear influence on the organic carbon and nitrogen content of the nauplii and cyprids (Figure 5). The amount of accumulated carbon increased with the instars. At higher food concentration, VI instar nauplii gained maximum carbon and nitrogen and the cyprids appeared on day four (Figure 5A&B). Nauplii having longer development duration (five days) at medium food concentration, did not gain the same amount of carbon as gained by the nauplii reared at higher food concentration. All the instars showed higher organic carbon content at higher food concentrations and this difference was more prominent in the advanced instars (IV to VI instar) (Figure 5). A decrease in the content of carbon and nitrogen was observed when the VI instar nauplii metamorphosed into the cyprid instar (Figure

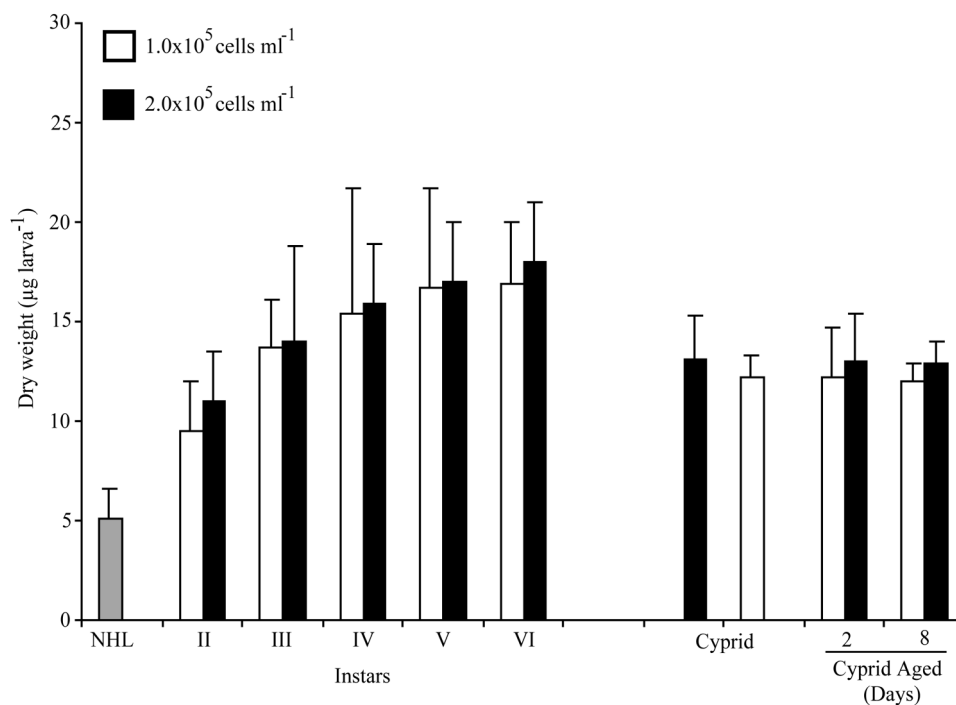


Figure 4. Variations in the dry weight of the nauplii and cyprids reared at different food concentrations and aged cyprids. Vertical lines indicate standard deviation from mean. NHL, newly hatched larvae.

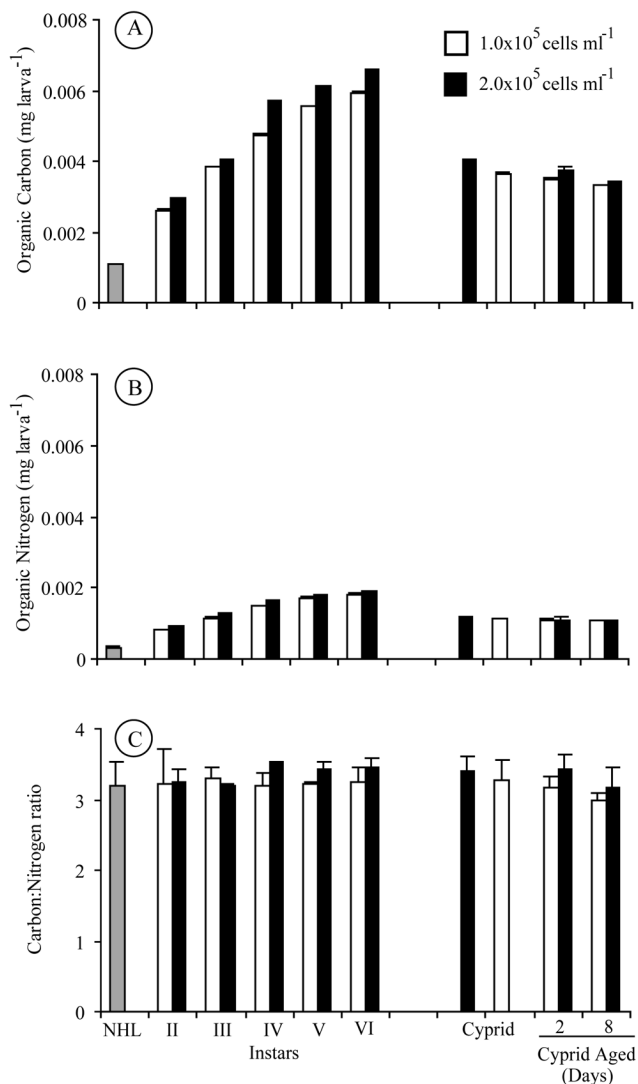


Figure 5. Variations in the (A) organic carbon; (B) organic nitrogen; (C) C:N ratio for the nauplii, cyprids and aged cyprids reared at different food concentrations. Vertical lines indicate standard deviation from mean. NHL, newly hatched larvae.

5A&B). The cyprids on ageing at 5°C showed a progressive decrease in their carbon and nitrogen content. The C:N ratio, which is considered as an indicator of the lipid:protein ratio, was higher for the larvae raised at high food concentration (Figure 5C). The cyprid metamorphosis assay results evaluating the influence of ageing showed higher metamorphosis for the cyprids raised at higher food concentration (Figure 6A&B). It was positively influenced by natural biofilms. An increase in the metamorphosis rates was observed with the age of the cyprids as well as the days of observation (i.e. from day 1 to day 4). The 8-d aged cyprids showed highest metamorphosis (Figure 6B).

DISCUSSION

Recruitment of larvae is considered to be an important link between adult and larval ecology. Growth and survival of marine invertebrate larvae in relation to food and feeding situations and environmental parameters are of great importance in larval ecology. In the present investigation it was observed that food concentration and

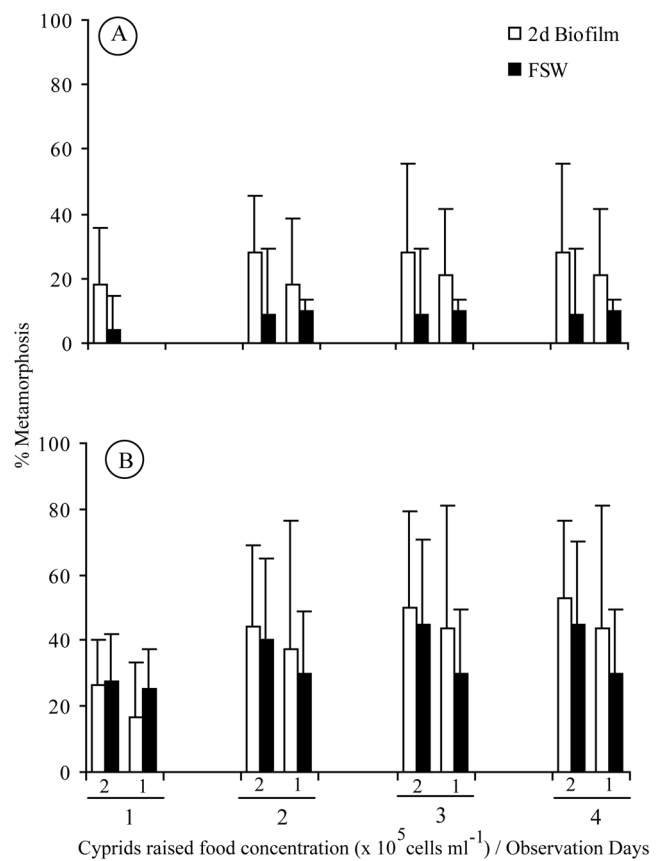


Figure 6. Influence of ageing of cyprids at 5°C on the percentage metamorphosis for (A) 2-d aged cyprids; and (B) 8-d aged cyprids. Vertical lines indicate standard deviation from mean.

temperature played an important role in the larval development and on the energy buildup through feeding. Results indicated a reduced metamorphosis rate of nauplii to cyprids at 20°C when compared to 30°C, indicating that higher temperature favoured the development of this species.

In the study area, larvae of *Balanus albicostatus* occur in the plankton during the summer months (June to October), when average seawater temperature is 25 to 28°C. Whereas, in the winter (cooler) months the temperature falls below 17°C and can reach as low as 12°C. Studies on the larvae of *Balanus amphitrite* from a tropical environment have also reported higher metamorphosis success of nauplius to cyprid at higher temperatures (Anil et al., 2001).

At 20°C, metamorphosis of nauplii to cyprids increased with an increase in food concentration, whereas at 30°C, it was low at lower food concentration. In general, the percentage metamorphosis was more at 30°C compared to 20°C at any given food concentration. Also, at 20°C, the total naupliar duration was longer compared to 30°C at all food concentrations. These results indicate that at lower temperature, even the maximum food concentration could not compensate for faster development and higher survival rate. Scheltema & Williams (1982) stated that for the larvae of *Balanus eburneus*, the survival rate and time to reach the cyprid stage are similar at middle and high food concentrations at 30°C. Anil & Kurian (1996) also reported similar results with the larvae of *B. amphitrite*. However, there was a difference in the dry weight and

C:N ratio of the larvae reared at different food concentrations at 30°C, indicating that although successful development can be achieved, the quality (growth and the energy status) of the larvae and the resultant cyprids might vary with the rearing conditions. The size of the nauplii and cyprids also varied with the food concentration. It was reported in the case of *B. eburneus* that the rearing conditions influenced the larval size (West & Costlow, 1987). Studies carried out with the larvae of other balanid species also indicated that food availability and temperature jointly determine larval development and energy allocation (Scheltema & Williams, 1982; Anil & Kurian, 1996). Olson & Olson (1989) reported that recruitment is largely dependent upon the abundance of food. Holm (1990) indicated that temporal variation in cyprid behaviour may be a result of changes in larval culture conditions and/or maternal effects. Later, Holm et al. (2000) tested for genetic, maternal and ontogenetic effects on permanent attachment of cyprids of the barnacle *Balanus amphitrite* (Darwin) and indicated that the same genetic or maternal factors may affect attachment in each treatment. Mullin & Brooks (1976) stated that the average food concentration is often less in the natural environment than is required for maximum growth and survival of larvae. The larvae in nature are nutritionally compromised or experience a nutritional stress when compared to those reared in the laboratory (Dattesh & Anil, 2002). The chlorophyll-*a* data from this study locality revealed high concentration during June to October and then declined during November to May (Kawamura, 2003), which indicates lower phytoplankton abundance during the cooler months. Thus, extrapolating the results of the present study to natural conditions, the absence of larvae during the low temperature period can be attributed to the combined effect of temperature and low food concentration.

In general, lack of suitable food in early development of planktotrophic larvae increases mortality and prolongs the duration of larval development (Anger & Dawirs, 1981; Anger et al., 1981; Dawirs, 1984). Prolongation of development duration may increase the chance of larval dispersal from the settlement habitat, and might also increase the threat of larval predation. Predation, dispersal, availability of appropriate food, physical environmental factors are considered as the important factors for larval mortality in marine invertebrates (Thorson, 1950; Crisp, 1976; Olson & Olson, 1989; Anil et al., 1995; Dattesh & Anil, 2002). A significant decrease in the instar duration that was observed with an increase in the food concentration indicated faster development at higher food concentration irrespective of temperature. The results of ANOVA indicated significant influence of temperature on early instar duration (II and III), whereas both temperature and food concentration affected significantly the duration of the advanced instars (IV–VI), indicating food may not be a critical factor during the early stages of planktotrophic development in this species. In the case of *Balanus balanoides*, considerable energy reserves remain when the larva enters II instar and the absence of food may not be fatal (Achituv et al., 1980). Similar results were observed with the larvae of *B. amphitrite* indicating that starvation did not hinder the development of the II instar (Dattesh & Anil, 2002).

In an earlier study it was reported that larvae reared after starvation showed prolonged instar duration and the lengthening of these periods was viewed as an additional period necessary to accumulate enough energy to moult successfully to the next instar and on to the cyprid (Dattesh & Anil, 2004). Studies carried out on brachyuran larvae under laboratory conditions also indicated similar results (Anger & Dawirs, 1981; Anger et al., 1981). Food energy is allotted first to survival, then to moulting and development (Knowlton, 1974). In the present study, the amount of carbon gained increased with the instars. At higher food concentration, advanced instars (IV to VI) accumulated a higher amount of organic carbon when compared to those reared at medium food concentration. Whereas early instars showed almost similar organic content at both the food concentrations. This indicates that the advanced instars are more influenced by the rearing food concentration when compared to the early instars, as they need to accumulate more energy before metamorphosing into a cyprid. The observation on the larval mortality showed maximum mortality when the larvae were in VI instar just before metamorphosing into the cyprid. Thus the larvae that showed prolonged VI instar duration and died, probably might not have accumulated sufficient energy required for successful metamorphosis into a cyprid. A considerable decrease in the content of carbon and nitrogen was observed when the VI instar nauplii metamorphosed into the cyprid instar, indicating loss of energy during the process of metamorphosis.

Rearing food concentration influenced the dry weight of the nauplii and the resultant cyprids, which also affected the amount of carbon gained by them. Longer development duration at lower food concentration did not gain the same amount of carbon as those larvae reared at higher food concentration, indicating its effect on larval energetics. Lucas et al. (1979) indicated that the extent of energy reserves in cyprids depends upon the conditions under which the nauplii grow. Higher metamorphosis at higher food concentration also indicates that food concentration during planktotrophic development plays an important role in larval development and also in settlement and recruitment of cyprids. Cyprids showed a decrease in the carbon content with ageing indicating loss of energy. Results of the metamorphosis assay showed higher metamorphosis by 8-d aged cyprids when compared to 2-d aged cyprids and it was higher for the cyprids resulting from higher food concentrations. Maki et al. (1988) showed from their experimental results on inhibition of attachment of *B. amphitrite* to bacterial films, 4-d-old cyprids show increased cyprid attachment when compared to 2-d-old cyprids. Earlier experiments, which evaluated the effect of ageing on metamorphosis of *B. amphitrite* cyprids also showed that the metamorphosis rate peaked when the cyprids were aged for more days (12–16 days) (Anil et al., 2001). The results of the present investigation show variations in the naupliar development with the rearing conditions and subsequently on cyprid energy content and metamorphosis rate, indicating that the naupliar rearing conditions have probably the greatest impact upon the energy allocation, settlement and recruitment.

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