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Influence of temperature on the starvation threshold of nauplii of barnacle *Balanus amphitrite* (Cirripedia: Thoracica)

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An evaluation of starvation threshold of the newly hatched *Balanus amphitrite* nauplii (instar; II) has been made at three different temperatures (5, 15 and 25°C). Earlier studies on starvation in crustacean larvae demonstrated Point of No Return (PNR) as an index of starvation. PNR is the state from which no larvae exposed to stress conditions can recover and complete metamorphosis. In this study Ultimate Recovery Point (URP) has been used as a new index of starvation threshold. URP denotes the number of hours of starvation after the end of which larvae can recover and continue development. Larvae starved at 5 and 25 °C had URP of 204 h and 24 h respectively. The larvae starved at 5°C for 12 h showed reduced II instar duration (1 d) compared to control II instar duration (1.8 d). At 15 and 25°C reduction in instar duration was not evident.

Balanus amphitrite is a dominant species among barnacles which inhabits even the fringes of marine environment like supralittoral zone. The species possesses euryhaline and eurythermal survival and breeding capabilities, even at 15°C temperature[1-3]. This species breeds throughout the year in Indian waters[4]. The instar I nauplius is non-feeding and has a short instar duration of few hours. Instar II to VI are planktotrophic and their development is determined by various environmental factors such as temperature, salinity and availability of food. The three major causes amongst others of larval mortality are predation, starvation and offshore transport[5-6]. Starvation has been found to arrest larval development at stage II nauplii in most of the barnacle species[7]. Earlier studies underline importance of starvation factor in recruitment ecology[8]. Among barnacles, *Balanus amphitrite* has gained prominence in studies relevant to larval metamorphosis, influence of different chemical cues and mechanism of their perception. Selection of this species for such studies is based on the fact that it is easily maintainable in laboratory and larval development to the cypris is typically complete within five days under optimal conditions. The assays have relied on traditional rearing techniques that ensure adequate food supply to the growing larvae. It is understood that the results obtained with the reared larvae can hardly be extrapolated to real situations in the sea. Temperature is one major variable that influences metabolic pathways and its influence on larval development of *Balanus amphitrite* has been reported earlier[8]. The starvation threshold that is found to vary with temperature has been evaluated in this study at 5, 15 and 25°C temperature.

Materials and Methods

Individuals of *Balanus amphitrite* (Cirripedia: Thoracica) collected from Dona-Paula, Goa (15°, 25′ N and 73°, 45′ E) were immersed in laboratory tanks after exposure to air for some time. The larvae released by several individuals were used in the present experiments.

The newly hatched larvae were subjected to starvation for different durations at different temperatures (5, 15 and 25°C). During these experiments the test larvae were kept in the incubators set at 5, 15 and 25°C temperature in filtered seawater without any supply of food. From these 24 nauplii were picked up at an interval of 12 h and reared individually in multiwells (24 well, Nunclon Delta, 1 43982) at 25°C temperature using *Chaetoceros calcitrans* an unicellular diatom at a concentration of 2×105 cells/ml. Each larva was reared in 2 ml of seawater containing food and antibiotic dose of 0.4 ml/l of Crystamycin[9,10] and the observations were recorded only if the larva achieved the cyprid stage. Three observations namely instar duration, total naupliar duration and metamorphic success leading to cyprid stage were recorded.

The influence of starvation duration (hours) on the larval development period (D) at different temperatures was evaluated using power function regression, $D = bv^m$, (where b; is the intercept , m; is the slope

obtained by regression of the ln-transformed data). Two-way ANOVA has been applied to evaluate the combined interaction of the variables such as starvation duration and starving temperature on the instar duration as well as total naupliar duration[11].

Results

The data on metamorphic success of nauplii to cyprids starved for different duration at 5, 15 and 25°C are presented in (Fig. 1). It is observed that 29% of the larvae starved for 204 h at 5°C metamorphosed to cyprid stage. Whereas 8% success was achieved when they were starved for 60 h at 15°C. In the case of larvae starved for 24h at 25°C, 25% metamorphic success was achieved. The lower temperatures were thus found to have increased the starvation threshold limit of the larvae.

The total naupliar duration for different starvation hours and temperatures are given in (Fig. 2). In the case of larvae starved at 5°C total naupliar duration ranged from 8 to 10 days (204 h; Ultimate Recovery Point, which denotes the number of hours of starvation after the end of which larvae can recover and continue development). Larvae starved for 60 h (URP) at 15°C indicated a mean duration of 10 days. The mean total naupliar duration in the control ones as well as after 24 h starvation at 25°C was 8 days.

The instar durations of the larvae subjected to starvation at different temperatures are presented in (Fig. 3). The mean second instar duration for the larvae starved ranged from 1.7 to 2.5 days. The mean third instar duration ranged from 1.5 to 3.5 days. The fourth instar duration ranged from 1 to 1.6 days. The fifth instar duration ranged from 1.6 to 2 days and sixth instar duration ranged from 1.4 to 2 days. The slopes of the power function regression which reflected the influence of temperature and starvation

Table 1 — Power function regression (D=bvm) values for the influence of starvation hours (v) on instar and total naupliar duration (D) , (where b; is the intercept and m; is the slope)

		b	m	r	p
II instar	5°C	0.792	0.164	0.327	<0.001
	15°C	0.912	0.258	0.370	<0.01
	25°C	1.874	0.516	0.092	Ns
III instar	5°C	1.262	0.096	0.158	<0.10
	15°C	0.421	0.469	0.553	<0.001
	25°C	1.053	0.176	0.112	Ns
IV instar	5°C	1.946	–0.05	0.086	Ns
	15°C	0.713	0.192	0.257	<0.05
	25°C	1.256	0.036	0.026	Ns
V instar	5°C	1.226	0.096	0.158	<0.10
	15°C	0.563	0.260	0.389	<0.01
	25°C	0.800	0.176	0.149	Ns
VI instar	5°C	2.443	–0.116	0.225	<0.02
	15°C	1.206	–0.018	0.035	Ns
	25°C	0.087	0.971	0.856	<0.001
Total Duration	5°C	8.308	0.009	0.036	Ns
	15°C	3.580	0.255	0.729	<0.001
	25°C	4.785	0.203	0.366	<0.10

ns: not significant

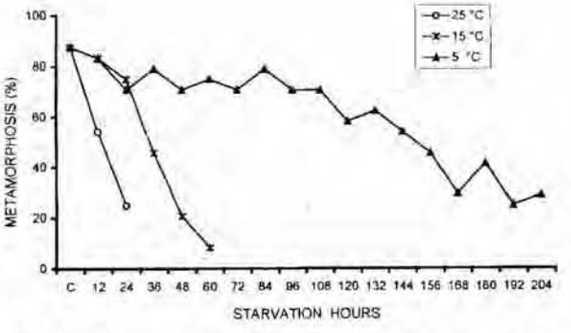

Fig. 1 — Percentage naupliar metamorphosis to cyprid instar starved for different duration at 5, 15 and 25°C temperature

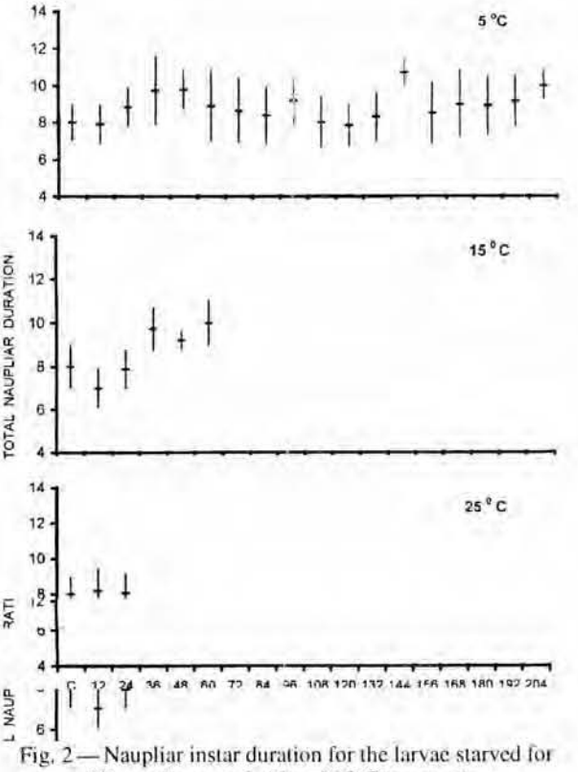

Fig. 2 — Naupliar instar duration for the larvae starved for different hours at 5, 15 and 25°C temperature

hours on instar and total naupliar duration (Table 1) revealed positive slopes in most of the cases at 5 and 15°C, indicating an increase in the duration with starvation. However the same was not evident at 25°C starvation temperature wherein URP was only 24 h. The only positive influence of starvation at 25°C was observed in the sixth instar duration, wherein 5 and 15°C batches showed negative slopes. Analysis of the result of larval instar duration and total naupliar duration for the larvae starved at 5 and 15°C by applying 2-way ANOVA for starvation duration of 12, 24, 36, 48 and 60 h was done. The results of this analysis indicated that starvation temperature significantly influenced II and IV instar duration (Table 2).

Discussion

The most critical factor that determines the larval growth and recruitment success is mortality. Major

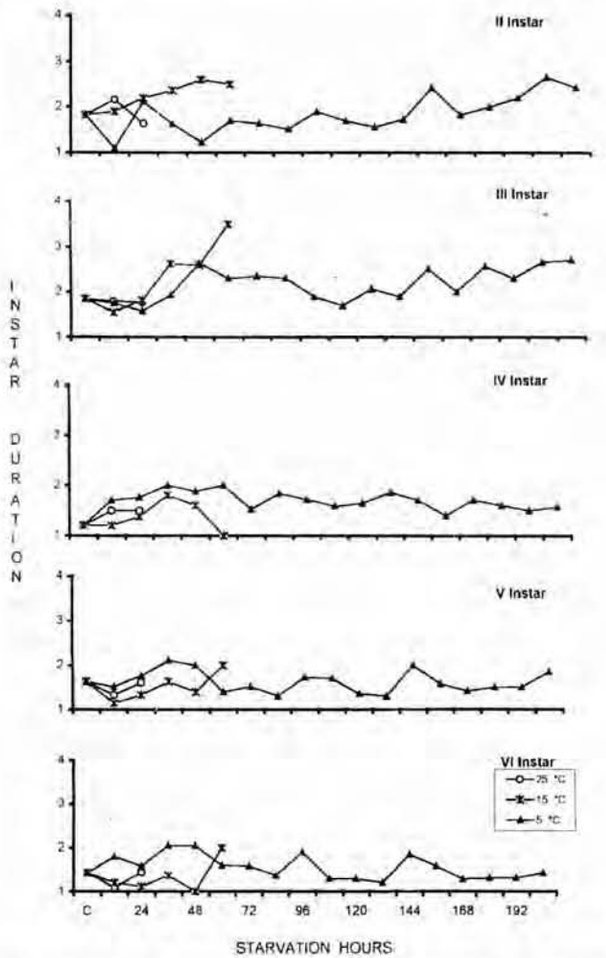

Fig. 3 — Total naupliar duration of the larvae starved at 5, 15 and 25°C temperature. Vertical lines indicate standard deviation from the mean

Table 2 — Two-way ANOVA for the evaluation of interaction of starvation hours and temperature on instar duration and total naupliar duration (df, degree of freedom; SS, sum of the squares; MS, mean of the squares; Fs, Fischer constant

Factor	Second instar				Third instar				Fourth instar				Fifth instar				Sixth instar				Total duration			
	df	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Starved Hours	4	0.5	0.1	1.3ns	2.4	0.6	3.8ns	0.3	0.1	1.4	0.3	0.1	0.7ns	0.3	0.1	0.4ns	7.5	1.9	5.2ns
Temperature	1	1.4	1.4	13.6**	0.4	0.4	2.2ns	0.6	0.6	10.9**	0.3	0.2	1.4ns	0.6	0.6	4.0ns	0.2	0.2	0.5ns
Starved hr* Temp	4	0.4	0.4		0.7	0.2		0.2	1.0		0.5	0.1		0.6	0.1		1.4		

**p < 0.025; ns, not significant
(0 Day larvae starved at and 15°C for 12, 24, 36, 48 and 60 h)

factors influencing larval mortality are their predation, starvation and offshore transport7. Susceptibility to starvation depends upon the species, on the stage of growth as well as on the environmental variables[3,9, 10]. Larvae starved at 5°C showed a better recovery rate of 29% with an URP of 204h (Fig.1). The increase in the starving temperature to 15 and 25°C resulted in decrease of URP to 60 and 24 h. The anterior midgut cells of cirripede nauplii contain lipid droplets and glycoprotein globules (remnants of yolk) in instars I and II, which are readily used up during the initial development process[12]. For successful metamorphosis from instar II to VI there has to be a gradual build up of lipids that are derived from the injested food. It has been observed that if early stage larvae are starved beyond their Point of No Return, renewed feeding is ineffective because of the irreversible damage caused to both mitochondria and hepatopancreas systems[13]. At 5°C larvae starved for 12 h showed reduced II instar duration (1.1 d) in comparison to control II instar (1.8 d). However the same was not evident at 15 and 25°C starvation. After prolonged starvation at 5°C moulting did not occur quickly. This indicates that initial temperature shock (5°C) reduces the instar duration. However beyond 24h of starvation at 5 and 15°C the duration of II and III instar is considerably increased. The larvae starved at 15 and 25°C that had longer II-IV instar duration compared to the control ones. However in such cases the duration of advanced instars (V and VI) was reduced. It has been reported that in advanced instars there is an exceptional rise in metabolic rate due to extensive cyprid morphogenesis[14]. Starvation of newly hatched larvae probably initiates the energy storing requirement to the early instars (stage II and IV) from the advanced instars (stage V and VI) to accomplish cyprid stage. Further experiments evaluating the starvation threshold limits of advanced instars are need to be carried out to understand this aspect in larval metamorphosis and growth.

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References

1 Crisp D J & Costlow J D, Oikos, 14(1963) 22.
2 Iwaki T, Marine Fouling, Tokyo, 3 (1981) 61.
3 Anil A C, Chiba K, Okamoto K , Biofouling, 2 (1990) 137.
4 Anil A C, Studies on marine biofouling in the Zuari estuary (Goa) west coast of India. Ph.D. thesis, Karnatak University, India, 1986.
5 Thorson G, Biol. Rev, 25 (1950) 1.
6 Olson R R & Olson M H, Ann Rev Ecol Syst, 20 (1989) 255
7 Lang W H , NOAA Tech. Report NMFS Circ. 421 (1979) 421.
8 Anil A C, Chiba K, Okamoto K & Kurokura K, Mar. Ecol. Prog. Ser, 118(1995)159.
9 Harmes J, Helgolander Meeresunters, 40(1986) 355.
10 Anil A C & Kurian J, Mar. Biol, 127(1996) 115.
11 Sokal R R & Rohlf F J , Biometry: The principles and practice of statistics in biological research , (W H Freeman & Comp, San Francisco), 1981.
12 Walley L J, Phil. Trans. R. Soc. (B).256 (1969) 237.
13 Storch V & Anger K, Helgolander Meeresunters, 36 (1983) 67.
14 West T L & Costlow J D, Mar Biol, 96 (1987) 47.