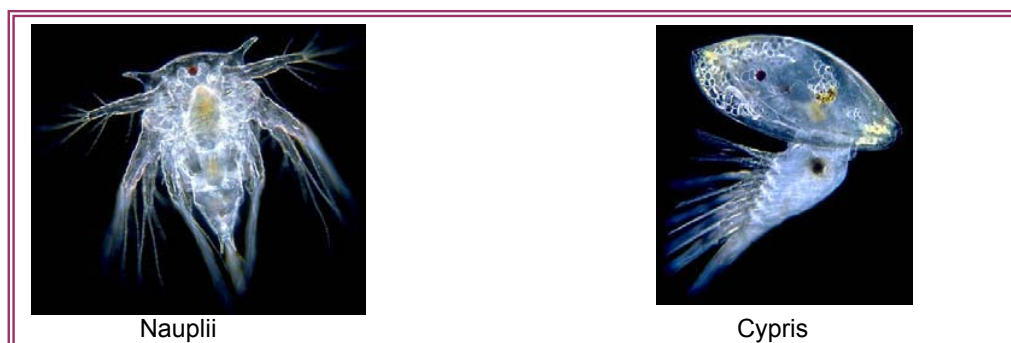


Chapter 4
Larval Ecology

4.1 Introduction

Barnacles have drawn the attention of many investigations in marine ecology owing to their easy accessibility on the rocky intertidal regions and also some species are dominant in marine fouling (Barnes 1957; Connell 1961a,1985; Strathmann et al. 1981; Sutherland 1984,1990; Crisp 1988; Holm 1990; Raimondi 1990,1991; Bertness et al. 1991; Anil et al. 1995). *B. amphitrite* (Darwin), an acorn barnacle, occurs in warm and temperate seas throughout



the world (Karande 1999). In Indian waters, breeding of this species is observed round the year. Its capability to withstand desiccation for long duration enables its distribution to the fringes of marine habitats. It can be easily maintained in the laboratory and larval phase includes six naupliar instars followed by a non-feeding pre-settling cyprid instar. Under optimal rearing conditions, naupliar development in the laboratory is completed in 5 days. This species has been widely used in studies related to larval development, metamorphosis, influence of different chemical cues and antifouling assays (Rittschof et al. 1984; Maki et al. 1988,1990; Clare et al. 1994; Anil et al. 1995; Anil and Kurian 1996; Anil and Khandeparker 1998).

The role of the non-feeding cyprid larva is to explore a suitable substratum to settle and metamorphose into juvenile, thus this larva is extensively used in

carrying out antifouling assays. The energy content of the cyprid will depend upon the naupliar processes during planktotrophic naupliar development (Pechenik 1987; Anil et al. 2001). Hence a study on the various environmental parameters and the food availability on the larval development during planktonic phase become necessary.

Key factors for larval survival include predation, starvation or limited food resources and oceanographic conditions that may transport larvae to unfavorable environments (Hirano 1962; Crisp 1976; Thorson 1950; Olson and Olson 1989; Anil et al. 1995; Anil and Dattesh 1997). It is reported that barnacles synchronize spawning with the phytoplankton blooms (Barnes 1957; Crisp 1962; Starr et al. 1990,1991). However spatial and temporal variations in the concentration of the available food particles such as phytoplanktons can lead to considerable difference in the feeding opportunities for the planktotrophic marine invertebrate larvae (Mackas et al. 1985; Pitcher et al. 1991; Franks 1992; Litaker et al. 1993). Starvation has also been considered as an important cause of reproductive loss in marine invertebrate larvae. Studies have also underlined the importance of starvation in the recruitment ecology (Anil et al. 1995). Crustacean larvae are highly sensitive to starvation (Olson and Olson 1989). Lang (1979) indicated that starvation could arrest larval development to second instar nauplii in most of the barnacles. In general, lack of suitable food in early development of planktotrophic larvae increases mortality and prolongs larval development duration (Anger and Dawirs 1981; Anger et al. 1981; Dawirs 1984). Anger and Dawirs (1981) while studying the 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 condition can recover and complete metamorphosis. Earlier studies investigating the effect of starvation underline the effects of short-term starvation on larval development (Qiu et al. 1997). The present work highlights the influence of starvation on the larval development and explores the ultimate recovery point (URP; denoting the starvation point in hours at the end of which larvae can recover and continue development) at different starving temperatures.

Effects of starvation have been reported to vary with the species, age of the larvae and on environmental variables (His and Seaman 1992; Fenaux et al. 1988; Dattesh and Anil 2000). Biordon-Metairon (1995) in her review on larval nutrition cites that temperature is a major variable that controls metabolism, thus influencing larval growth, development and survival as well as rates of reserves' utilization (Anger et al. 1981a; Olson 1985; Olson et al. 1987; Pechenik 1987; Pechenik et al. 1990; Bhaud 1988). Larval development is dependent upon the feeding efficiency.

Recruitment is considered as an important link between the adult and larval ecology. Planktotrophic larval developmental processes have a strong effect on recruitment success, which can determine the adult population and abundance. Growth and survival of marine invertebrates in relation to food and feeding situations are of great importance in larval ecology. Thus recruitment largely depends upon the abundance of larval food.

Laboratory studies on benthic or sessile marine invertebrates have shown that food availability affects the growth, development and survival (Sulkin 1975; Bigford 1978; Pechenik and Fischer 1979; Lucas 1982; Pauley et al. 1985;

Anil and Kurian 1996; Qiu et al. 1997). It has been suggested that larval experiences other than predation may have an impact on metamorphosis and recruitment. The cyprid larva is non-feeding and has to depend upon the energy reserves incorporated during the earlier planktotrophic naupliar development.

Nucleic acids play an important role in growth and development. Variation in cell number in an organism may be estimated by its DNA content, as its content per nucleus is constant in somatic cells of a given species (Leslie 1995) and each nucleus will correspond to a cell. The amount of RNA is directly proportional to the rate of protein synthesis. Several studies have shown that the ratio of RNA:DNA is a reliable and sensitive index of nutritional status (Buckley 1980; Lucas and Beninger 1985; Wright and Hetzel 1985; Jurss et al. 1986,1987; Wang and Stickle 1986; Anger and Hirche 1990; Clemmesen 1993,1996). This has been routinely used for estimating growth rates or nutritional conditions of larval fish. Only few studies have applied this technique to crustaceans and their larvae (Dagg and Littlepage 1972; Sulkin et al. 1975; Anger and Hirche 1990; Wagner et al. 1998). The influence of rearing temperature and food concentration on larval development, and ensuing changes in the content of RNA and DNA was examined.

The average food concentration in the natural environment is often considered less than what is required for maximum growth and survival (Mullin and Brooks 1976). In the laboratory, larvae are reared with a rich supply of mono-algal food and the variations in environmental parameters are also minimum, whereas the larvae in the field may have to grow against a

wide variation in the environmental parameters as well as nutritional levels. Inappropriate and insufficient food supply can cause starvation in natural populations. Experiments were carried out to evaluate the implications of starvation and comparison of the nutritional status of the laboratory and the field reared larvae. RNA:DNA ratio was used as an indicator of starvation.

4A. The role of food type, temperature and starvation on larval development.

4A 2 Materials and Methods

4A 2.1 Rearing of *B. amphitrite* nauplii

The larval phase of *B. amphitrite* consists of six naupliar instars followed by a pre-settling cyprid instar. The I instar nauplius is non-feeding and molts into II instar within a short interval. The development of II-VI instar is phytoplanktivorous. These II instar nauplii were used in carrying out different experiments. Nauplii were mass reared in 2L glass beakers (larvae ml⁻¹) using filtered seawater of 35‰ salinity, on a daily diet of *S. costatum* and *C. calcitrans* separately at a cell concentration of 2x10⁵ cells ml⁻¹. These cultures were maintained at 25° C (±1° C) at 12h light: 12h dark cycle.

4A 2.2 Chlorophyll a and Carbon content of diatoms

The chlorophyll a content of *S. costatum* and *C. calcitrans* was estimated using fluorometric technique described by Parsons et al. (1984). The carbon content of the cells was calculated using the carbon/chlorophyll ratio (Banse 1977).

4A 2.3 Filtration and grazing rates

Filtration and grazing rates were evaluated by comparing mean cell counts of the control wells and those with nauplii separately using the method described by Omori and Ikeda (1984). Five replicates of control (feed without larvae) and feed (2×10^5 cells ml^{-1}) with larvae were used for the evaluation of filtration and grazing rate. Larvae were reared at 2ml of medium larva⁻¹. The experiment was carried out till the larvae reached the cyprid instar. The growth coefficient (k) for the growth of *S. costatum* and *C. calcitrans* was calculated separately as

$$C_t = C_0 e^{kt}$$

[C_0 and C_t are cell concentrations (cells ml^{-1}) in control well after time t (6h)]

Feeding coefficient (f) was calculated using the change in cell concentrations in experimental bottles with nauplii as

$$C_{tf} = C_0 e^{(k-f)t}$$

$$F = [((\ln C_0 - \ln C_{tf})/t) + k]$$

[C_0 and C_{tf} are cell concentrations in control well after time t (6h)]

Using the value of f, the filtration rate F (volume of water filtered animal⁻¹ h⁻¹) was calculated as

$$F = Vf / N$$

[V- volume of sea water in the experimental bottle (200 ml), N – number of larvae in the bottle (N = 100)]. This was used as the larvae were reared in the multiwells at 2ml larva⁻¹.

Using the F value, the grazing rate (G, number of cells eaten animal⁻¹ h⁻¹) was calculated

$$G = (Vf/N) * [(C_{tf} - C_0) / (k-f)t]$$

4A 2.4 Starvation of *B. amphitrite* nauplii

The newly hatched nauplii (zero day old) were subjected to starvation for different durations at 5, 15 and 25° C. From each of the starving temperatures nauplii (n=24) were removed out at an interval of 12h and reared individually in the multiwells (24 well Nunclon Delta, 1 43982) at 25 (±1° C) temperature, using either *S. costatum* or *C. calcitrans* at cell concentration of 2×10^5 cells ml^{-1} , to evaluate percentage metamorphic success of nauplii to cyprids, larval instar duration and total naupliar duration (TND). Each larva was reared with 2ml of media with antibiotic dosage of 0.3ml Crystamycin l^{-1} (300mg Penicillin G and 500mg Streptomycin in 4ml distilled water) (Anil et al. 1995). The observation on the larval molts was taken everyday prior to change of food. For the starvation of other age groups (1 to 5 day, as on day 6 cyprids were appeared), nauplii from the mass rearing beakers (from both feed types) were removed out at every 24 h interval and subjected to starvation in filtered seawater at 5, 15 and 25° C. From each of the starving temperatures nauplii (n=24) were sampled out at 12h interval and reared individually in multi-wells with *S. costatum* or *C. calcitrans* at 2×10^5 cells ml^{-1} food and 25° C temperature. Rearing of these nauplii was carried out till they reached the cyprid instar.

4A 2.5 Statistical Analysis

The Power function regression (PFR) analysis were carried out to see the influence of variables such as temperature and starvation hours (V) on duration (D) as $D = bv^m$ (where b - intercept; m - slope). We only used the data of the nauplii that successfully reached the cyprid instar in the analysis. Three-way ANOVA (Sokal and Rohlf 1981) was carried out to evaluate the

combined interaction of variables such as starving temperature, starvation hours and larval age on the metamorphic success, instar duration and total naupliar duration. These analysis were carried out for 5 and 15° C and for 5, 15 and 25° C separately, as the comparison between the starvation duration could be achieved till 72h (at 15 and 25° C) whereas at all the three temperatures this could be obtained only till 24h of starvation.

4A 3 Results

4A 3.1 Percentage Metamorphosis

The metamorphic success of nauplii to cyprid instar, which were starved for different duration at different temperatures is shown (Fig. 4A.1) (*S. costatum*) and (Fig. 4A.2) (*C. calcitrans*). The non-starved larvae (Control) had a metamorphic success of about 90% for both the feed types. The larvae starved at 5° C could withstand longer starvation duration (maximum of 216 h) compared to 15 and 25° C (maximum of 108 and 60 h respectively). In case of larvae starved at 5° C and fed with *S. costatum*, the URP gradually decreased with the larval age (zero day 204 h to five day 156 h). Whereas for those starved at 15° C, URP did not vary for all the age groups except for four day old larvae (108 h) (Fig. 4A.1k). At 25° C, the URP increased with the larval age with a maximum of 60h for five day old larvae (Fig. 4A.1r). In the case of larvae fed with *C. calcitrans* the maximum URP (216 h) was observed from two day old larvae starved at 5° C with a metamorphic success of 8% (Fig. 4A.2c), which decreased gradually to a level of 144 h (29% of metamorphosis rate) for five day old larvae (Fig. 4A.2f). At 15 and 25° C starvation, a gradual increase in the URP was observed with the larval age (Fig. 4A.2).

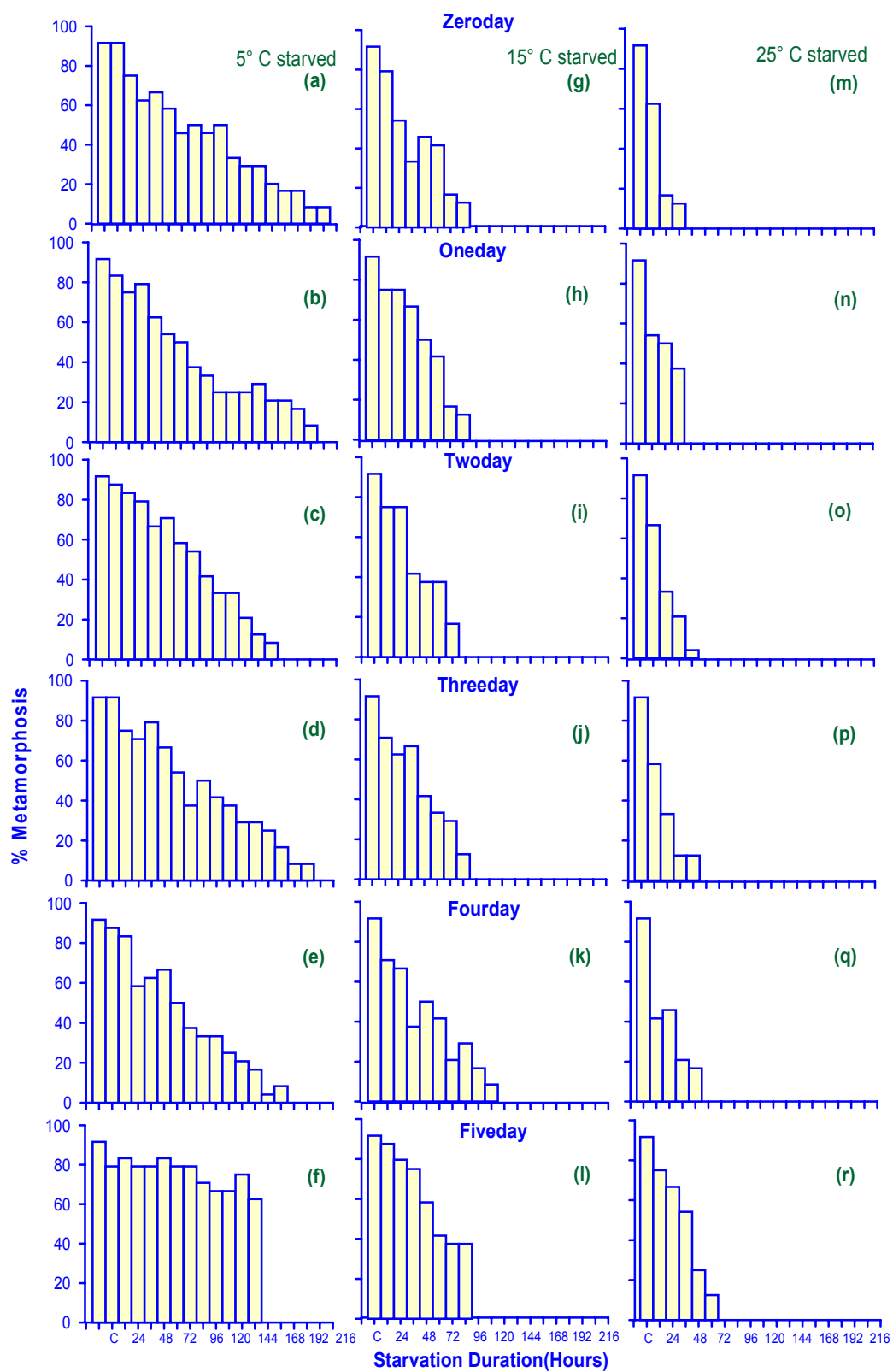


Fig. 4A.1 Percentage metamorphosis of 0-5 day old larvae starved at 5, 15 and 25°C and fed with *S. costatum*

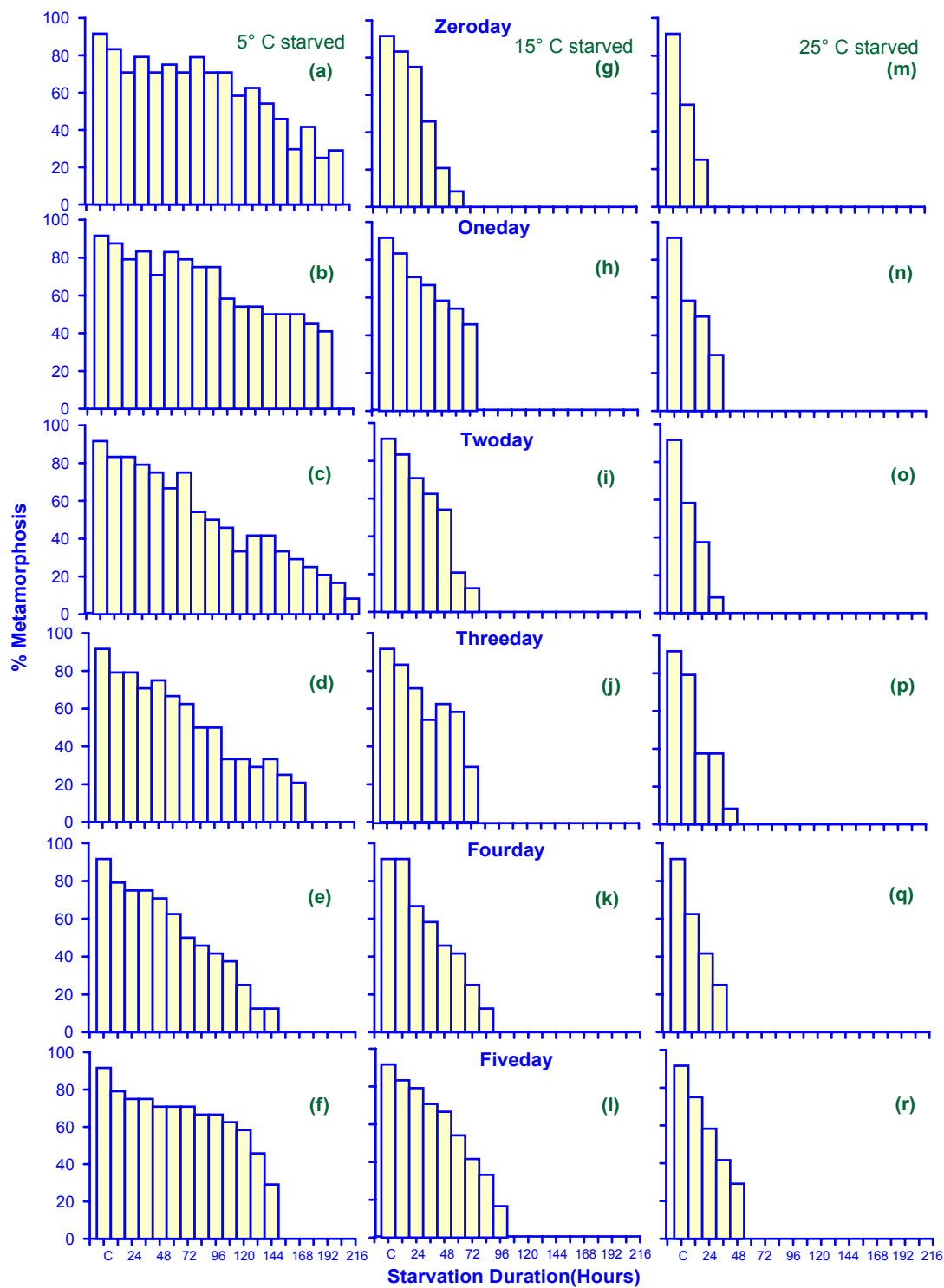


Fig. 4A.2 Percentage metamorphosis of 0-5 day old larvae starved at 5, 15 and 25°C and fed with *C. calcitrans*

The zero to four day old larvae starved at 5 and 15° C and fed with *S. costatum* showed almost similar metamorphic success (8%) from their respective URP. In case of five day old larvae, metamorphic success from the URP was higher when starved at 5 and 15° C (62.5 and 37.5% respectively) (Fig. 4A.1f,l). Zero day old larvae starved at lower temperature (5° C) when fed with *C. calcitrans* showed higher metamorphic success (29%) from URP (Fig. 4A.2a). Similar is the case with one day old larvae, which had a metamorphic success of 41% (Fig. 4A.2b). The URP and metamorphic success of zero to two day old larvae starved at lower temperature was higher for *C. calcitrans* when compared to *S. costatum*. However in the aged larvae (three to five day) the URP and metamorphic success was higher in the larvae fed with *S. costatum*. Three-way ANOVA results indicated that when all the three temperatures are considered, the interaction of starvation hours * starvation temperature and starvation hours * larval age jointly influenced the metamorphic success irrespective of food types (Table 4A.1 and 4A.2). A comparison of 5 and 15° C temperatures indicated that the interaction of starvation temperature and starvation hours influence metamorphic success for both the food types whereas significant influence of starvation temperature larval age is seen only for larvae fed with *C. calcitrans*.

Table 4A.1 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on percentage metamorphosis of larvae reared with *S. costatum*.

Factor	5 & 15° C starved				5, 15 & 25° C starved			
	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	1	6319	6319		2	9396	4698	
B (St. hour)	6	22798	3799		3	16552	5517	
C (Larval Age)	5	1576	315		5	1987	397	
A*B	6	2319	386	9.2*****	6	3685	614	14*****
A*C	5	284	56	1.3ns	10	881	88	2*
B*C	30	2034	67	1.6ns	15	2289	152	3.5****
A*B*C	30	1257	41		30	1314	43	

(*****p≤0.001, ****p≤0.005, *** p≤0.01, **p≤0.025, *p≤0.05, ns- not significant)

Table 4A.2 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on percentage metamorphosis of larvae reared with *C. calcitrans*

Factor	5 & 15° C starved			5, 15 & 25° C starved			Fs	
	df	SS	MS	df	SS	MS		
A (St. temp.)	1	6919	6919	2	7101	3550		
B (St. hour)	6	18064	3010	3	20263	6754		
C (Larval Age)	5	2320	464	5	3206	641		
A*B	6	5123	853	15****	6	3299	549	8.3****
A*C	5	1433	286	5.1****	10	913	91	1.3ns
B*C	30	2065	68	1.2ns	15	5436	362	5.5****
A*B*C	30	1666	55		30	1973	65	

(****p≤0.001, ****p≤0.005, *** p≤0.01, **p≤0.025, *p≤0.05, ns- not significant)

4A 3.2 Naupliar Instar Duration

The non-starved larvae had instar duration of 1day (III to VI instar) irrespective of the food type. The II instar duration is 1.8d in case of *S. costatum* and 1d in the case of *C. calcitrans* fed larvae.

The zero day old larvae starved at different temperatures for different duration resulted in increased II and III instar duration for both the food types (Fig. 4A.3a,b and f,g). The effect of starvation when they were zero day old persisted in IV to VI instar in the case of *S. costatum* (Fig. 4A.3c,d and e). However such was not the case with the larvae fed with *C. calcitrans* (Fig. 4A.3; Table 4A.3a).

Similar results were evident in case of one day old larvae where reflection of starvation of III and IV instars (Fig. 4A.4b,c) lead to an increased duration of the V and VI instars only in the case of larvae fed with *S. costatum* (Fig. 4A.4d,e; Table 4A.3b).

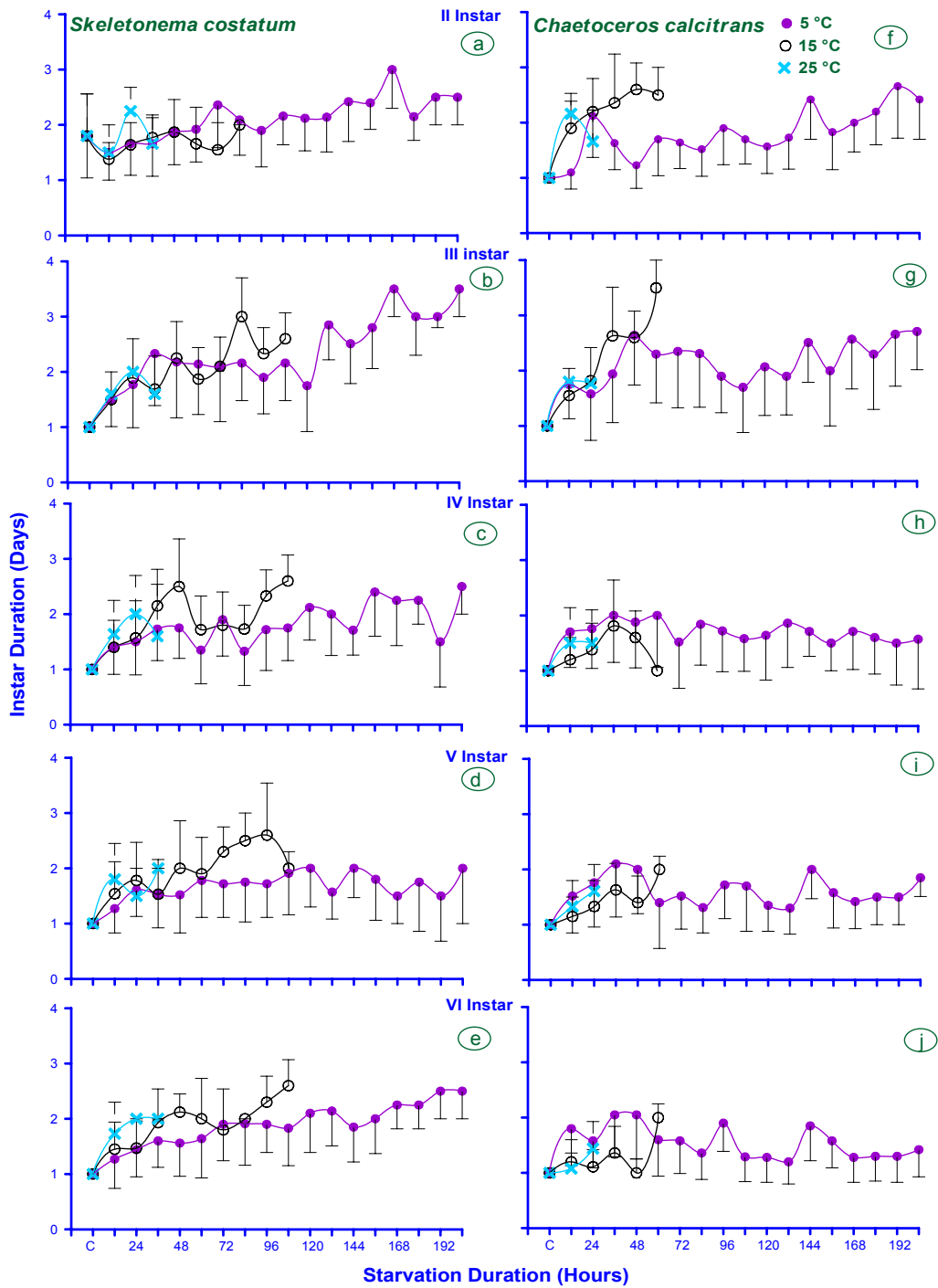


Fig. 4A.3 Instar duration of the zero day old larvae starved at 5, 15 & 25° C and reared with *S. costatum* and *C. calcitrans*

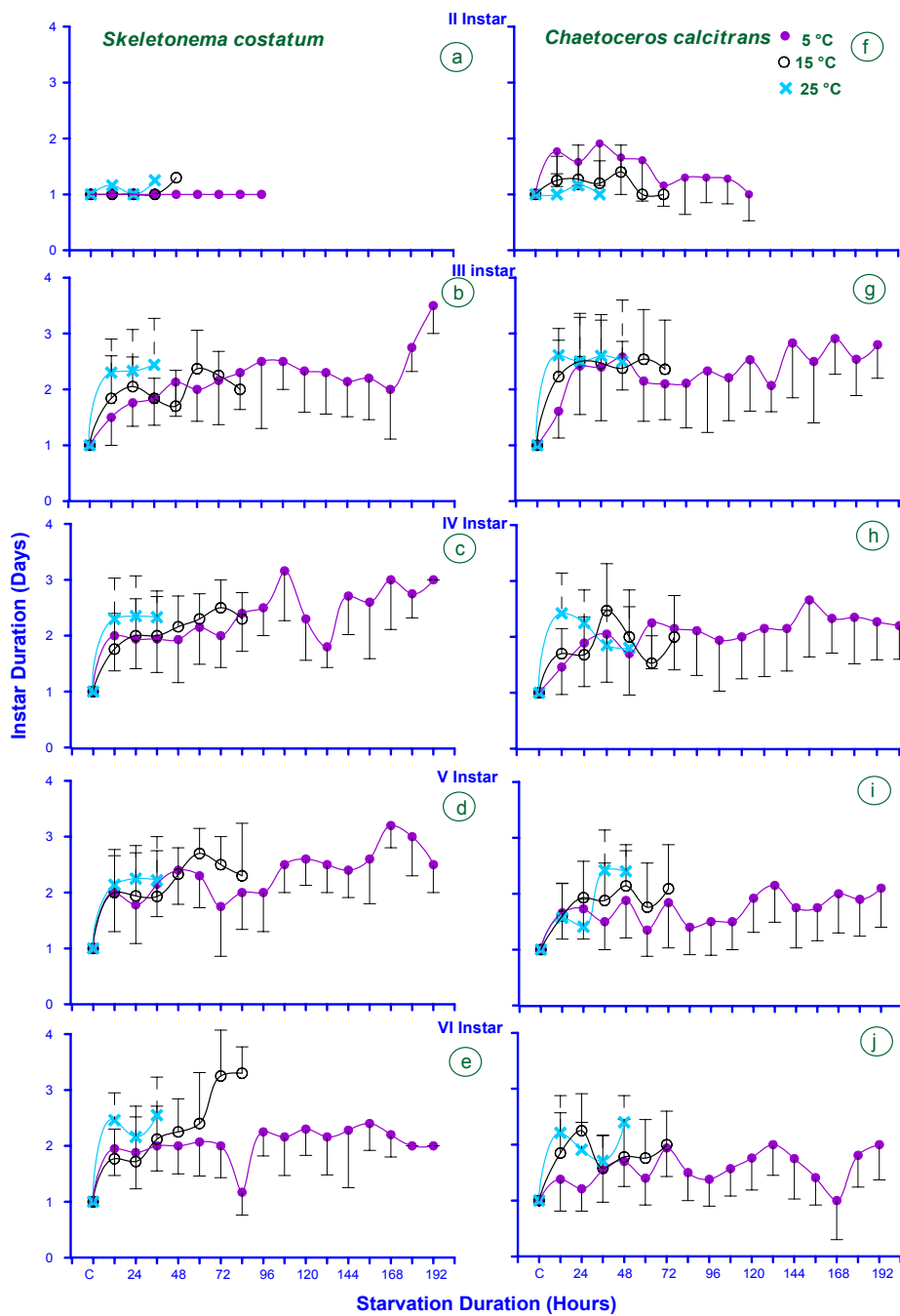


Fig. 4A.4 Instar duration of the one day old larvae starved at 5, 15 & 25° C and reared with *S. costatum* and *C. calcitrans*

Table 4A.3a Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for zero day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Chaetoceros calcitrans</i>			
	b	m	r	p	b	m	r	p
II instar								
5° C	1.07	0.14	0.51	≤0.001	1.04	0.10	0.37	≤0.001
15° C	1.14	0.09	0.39	≤0.001	1.12	0.19	0.68	≤0.001
25° C	1.16	0.11	0.47	≤0.001	1.14	0.23	0.81	≤0.001
III instar								
5° C	1.04	0.16	0.50	≤0.001	1.19	0.11	0.32	≤0.001
15° C	1.05	0.15	0.55	≤0.001	1.10	0.17	0.58	≤0.001
25° C	1.10	0.14	0.56	≤0.001	1.17	0.14	0.46	≤0.001
IV instar								
5° C	1.03	0.11	0.40	≤0.001	1.3	0.04	0.13	Ns
15° C	1.02	0.14	0.50	≤0.001	1.1	0.06	0.24	≤0.05
25° C	1.08	0.11	0.47	≤0.001	1.13	0.07	0.29	Ns
V instar								
5° C	1.01	0.10	0.40	≤0.001	1.13	0.06	0.19	Ns
15° C	1.0	0.15	0.55	≤0.001	0.97	0.09	0.45	≤0.001
25° C	1.04	0.16	0.64	≤0.001	1.0	0.09	0.52	≤0.001
VI instar								
5° C	1.04	0.11	0.41	≤0.001	1.29	0.03	0.1	Ns
15° C	1.10	0.12	0.46	≤0.001	1.04	0.03	0.16	Ns
25° C	1.16	0.14	0.57	≤0.001	1.01	0.08	0.45	≤0.01

(Ns- Not significant)

Table 4A.3b Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for one day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Chaetoceros calcitrans</i>			
	b	m	r	p	b	m	r	p
II instar								
5° C	1.34	-0.7	0.61	≤0.001	1.54	-0.04	0.12	Ns
15° C	1.34	-0.1	0.47	≤0.01	1.07	0.02	0.08	Ns
25° C	1.39	-0.1	0.38	Ns	0.99	0.01	0.14	Ns
III instar								
5° C	1.1	0.14	0.52	≤0.001	1.19	0.14	0.52	≤0.001
15° C	1.22	0.12	0.49	≤0.001	1.25	0.17	0.58	≤0.001
25° C	1.23	0.19	0.67	≤0.001	1.21	0.21	0.7	≤0.001
IV instar								
5° C	1.09	0.16	0.57	≤0.001	1.07	0.13	0.4	≤0.001
15° C	1.06	0.17	0.63	≤0.001	1.17	0.11	0.29	≤0.001
25° C	1.09	0.22	0.77	≤0.001	1.37	0.11	0.42	≤0.001
V instar								
5° C	1.02	0.17	0.61	≤0.001	1.15	0.08	0.27	≤0.001
15° C	1.01	0.19	0.64	≤0.001	1.1	0.13	0.45	≤0.001
25° C	1.02	0.23	0.79	≤0.001	1.24	0.15	0.56	≤0.001
VI instar								
5° C	1.19	0.12	0.50	≤0.001	1.06	0.08	0.3	≤0.001
15° C	1.09	0.17	0.57	≤0.001	1.19	0.1	0.36	≤0.001
25° C	1.16	0.22	0.78	≤0.001	1.16	0.16	0.61	≤0.001

(Ns- Not significant)

In two day old larvae the III instar was seen only up to 72 h when larvae were fed with *C. calcitrans* (Fig. 4A.5e) and 144 h when fed with *S. costatum* (Fig. 4A.5a) suggesting larvae had longer III instar when fed with *S. costatum*. The URP was 156h at 5° C for *S. costatum* and 216 h for *C. calcitrans* (Fig. 4A.5b and 4A.5f) for IV instar. At similar starvation duration (156h) the instar duration was longer in case of larvae fed with *S. costatum* (IV, V and VI instar duration was 4, 6 and 4 days respectively) (Fig. 4A.5b, c, d).

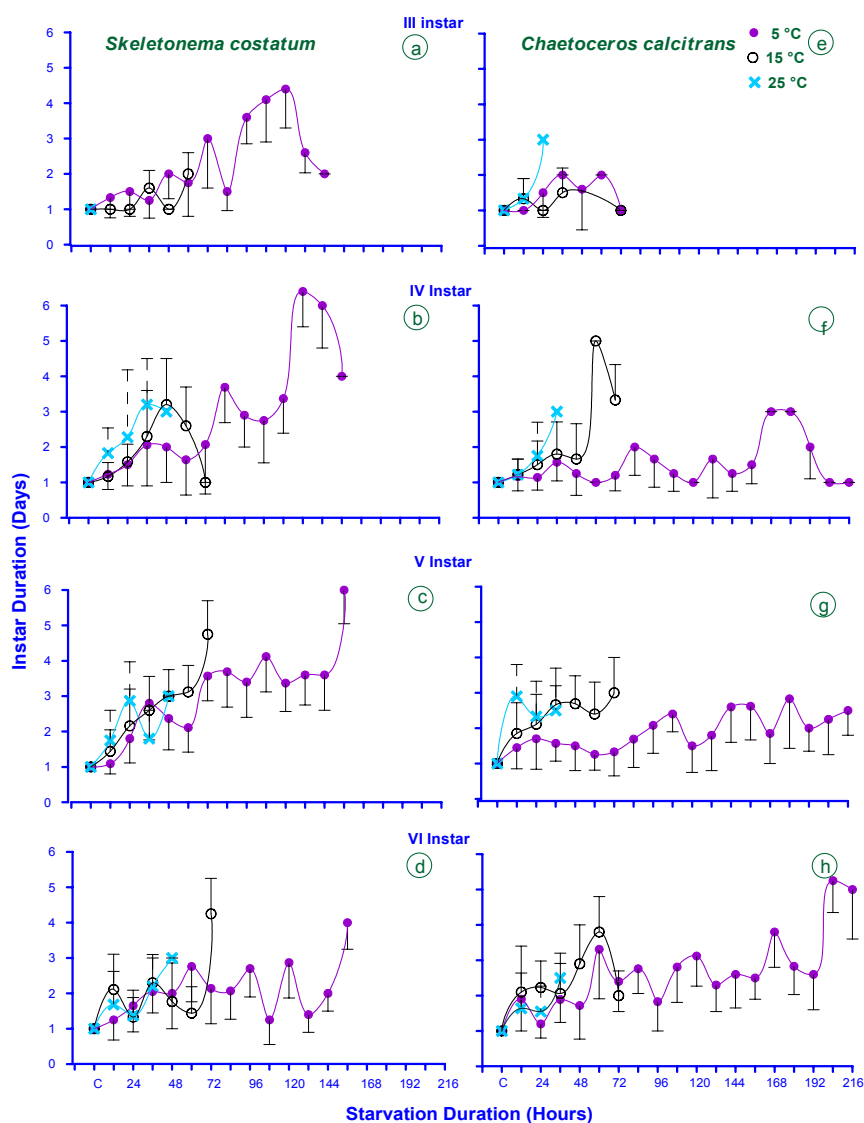


Fig. 4A.5 Instar duration of the two day old larvae starved at 5, 15 & 25° C and reared with *S. costatum* and *C. calcitrans*

Table 4A.3c Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for two day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Chaetoceros calcitrans</i>			
	b	m	r	p	b	m	r	p
III instar								
5° C	0.37	0.42	0.57	≤0.001	0.96	0.1	0.45	Ns
15° C	0.49	0.29	0.81	≤0.001	1.01	0.04	0.28	Ns
25° C	0.81	0.28	0.65	≤0.05	0.97	0.18	0.66	≤0.05
IV instar								
5° C	0.58	0.31	0.58	≤0.001	1.01	0.05	0.25	≤0.01
15° C	0.71	0.26	0.58	≤0.001	0.98	0.13	0.44	≤0.001
25° C	0.79	0.30	0.67	≤0.001	1.01	0.11	0.47	≤0.01
V instar								
5° C	0.98	0.22	0.50	≤0.001	1.29	0.05	0.14	Ns
15° C	1.18	0.17	0.45	≤0.001	1.4	0.12	0.36	≤0.001
25° C	1.31	0.12	0.38	≤0.01	1.5	0.15	0.39	≤0.01
VI instar								
5° C	1.74	-0.01	0.002	≤0.001	1.08	0.14	0.39	≤0.001
15° C	1.99	-0.06	0.18	Ns	1.17	0.17	0.43	≤0.001
25° C	1.99	-0.07	0.24	Ns	1.16	0.07	0.24	Ns

(Ns- Not significant)

In case of three day old larvae the recovery from starvation at all the temperatures when fed with *S. costatum* or *C. calcitrans* was almost identical in IV instar (Fig. 4A.6a,d). This uniformity was not seen in V instar where the duration was longer in *S. costatum* (at 5, 15 and 25° C starvation the instar duration was 4, 3.6 and 3.6 days respectively) (Fig. 4A.6b). Interestingly this did not remain similar in VI instar and the instar duration increased in the case of larvae fed with *C. calcitrans* (Fig. 4A.6f). The PFR results indicate that while the regression slopes of IV instar are almost identical, the regression slopes of V instar is greater in *S. costatum* and for VI instar in case of larvae fed with *C. calcitrans* (Table 4A.3d).

Table 4A.3d Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for three day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Chaetoceros calcitrans</i>			
	b	m	r	p	b	m	r	p
IV instar								
5° C	0.93	0.09	0.27	≤0.05	1.27	0.03	0.11	Ns
15° C	1.02	0.07	0.28	Ns	1.17	0.06	0.19	Ns
25° C	0.98	0.04	0.31	Ns	1.19	0.04	0.19	Ns
V instar								
5° C	1.04	0.15	0.40	≤0.001	1.54	0.01	0.03	Ns
15° C	1.07	0.14	0.46	≤0.001	1.39	0.06	0.16	Ns
25° C	1.06	0.11	0.41	≤0.01	1.48	0.07	0.24	≤0.1
VI instar								
5° C	1.39	0.09	0.06	≤0.001	1.48	0.19	0.46	≤0.001
15° C	1.5	0.06	0.19	Ns	1.48	0.21	0.37	≤0.001
25° C	1.6	0.04	0.12	Ns	1.17	0.24	0.57	≤0.001

(Ns- Not significant)

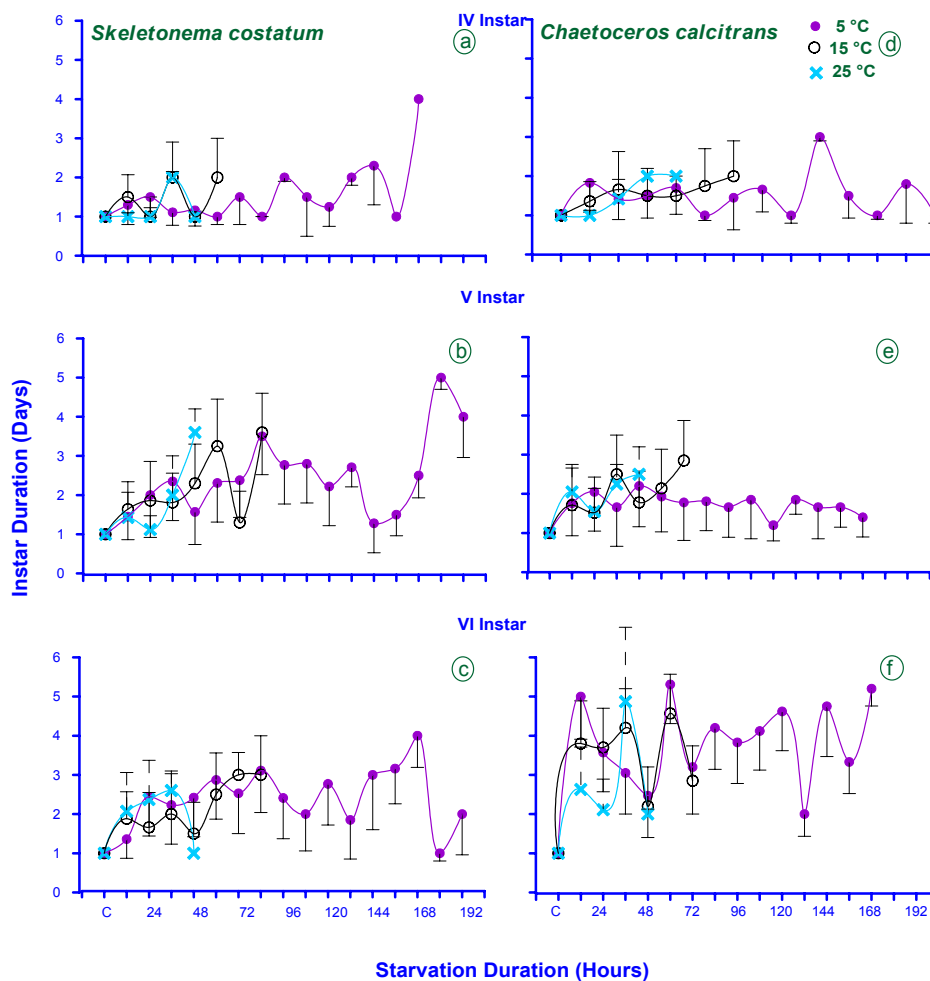


Fig. 4A.6 Instar duration of the three day old larvae starved at 5, 15 & 25° C and reared with *S.costatum* and *C. calcitrans*

Four day old larvae when subjected to starvation had an instar index of 4.7 (the average instar number of 10 different larval instars observed everyday before subjecting to starvation). Hence the influence on IV instar was evident only till 72 h beyond which they molted into V instar (Fig. 4A.7a,d). On V instar larvae the effect of starvation was more when they were fed with *C. calcitrans*, which had, lower URP (144h at 5° C) and longer instar duration (7±3d) (Fig. 4A.7e). This effect was prominent at 15 and 25° C (Fig. 4A.7e; Table 4A.3e). In the case of VI instar duration the effect was similar for both the food types but at 25° C the instar duration was longer (6±1 d) in case of *C. calcitrans* fed larvae (Fig. 4A.7f; Table 4A.3e).

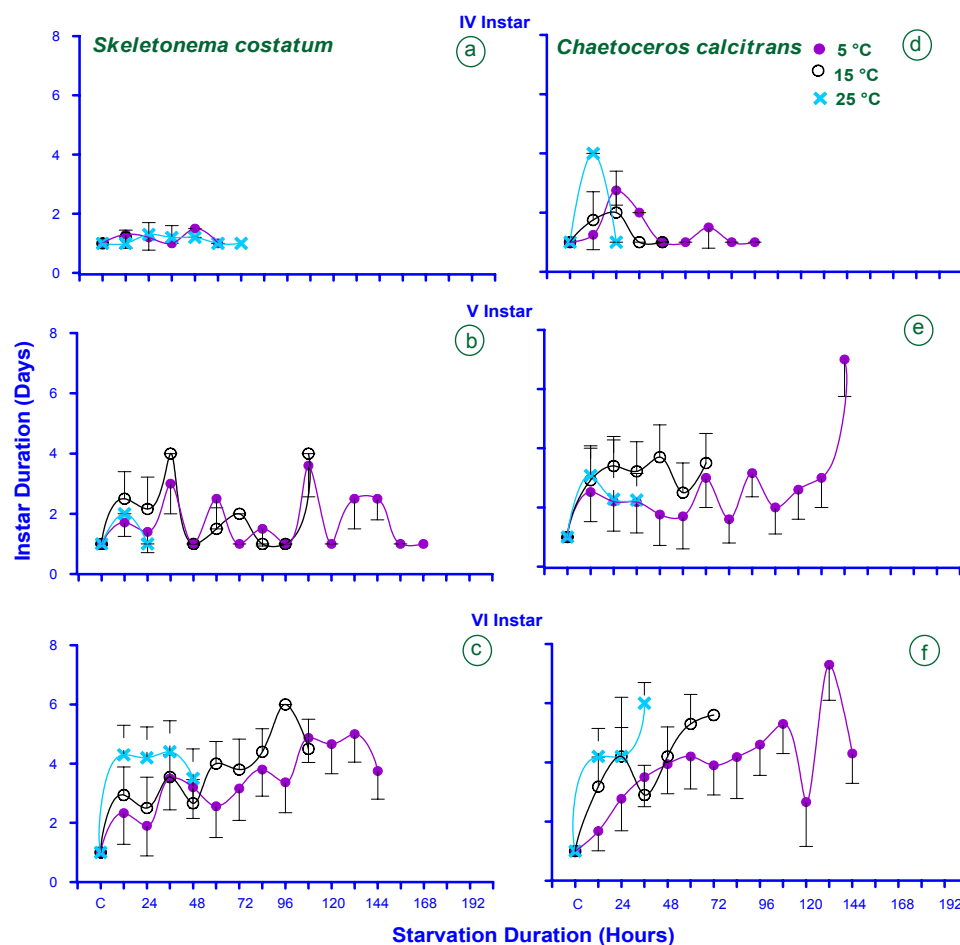


Fig. 4A.7 Instar duration of the four day old larvae starved at 5, 15 & 25° C and reared with *S. costatum* and *C. calcitrans*

Table 4A.3e Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for four day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Cheatoceeros calcitrans</i>			
	b	m	r	p	b	m	r	p
IV instar								
5° C	1.26	-0.03	0.09	Ns	1.15	0.06	0.21	Ns
15° C	1.49	-0.09	0.37	Ns	1.09	0.05	0.19	Ns
25° C	1.64	-0.11	0.21	Ns	1.04	0.19	0.52	Ns
V instar								
5° C	1.07	0.1	0.3	≤0.01	1.14	0.13	0.30	≤0.001
15° C	1.05	0.19	0.60	≤0.001	1.04	0.28	0.59	≤0.001
25° C	1.01	0.10	0.57	≤0.01	1.06	0.23	0.55	≤0.001
VI instar								
5° C	0.97	0.27	0.60	≤0.001	0.98	0.27	0.49	≤0.001
15° C	1.05	0.28	0.68	≤0.001	1.08	0.28	0.51	≤0.001
25° C	1.09	0.37	0.79	≤0.001	1.04	0.42	0.72	≤0.001

(Ns- Not significant)

In five day old larvae the effect of starvation was more on the larvae fed with *C. calcitrans* compared to *S. costatum* (Fig. 4A.8). Both V and VI instar indicated comparatively greater slopes for the larvae fed with *C. calcitrans* (Table 4A.3f).

Table 4A.3f Power function regression values for the instar duration ($D=bv^m$), for the influence of temperature and the starvation hours (V) on the duration (D) for five day old larvae reared with *S. costatum* and *C. calcitrans*

	<i>Skeletonema costatum</i>				<i>Chaetoceros calcitrans</i>			
	b	m	r	p	b	m	r	p
V instar								
5° C	2.05	-0.01	0.02	Ns	1.55	0.11	0.30	≤0.001
15° C	1.45	0.08	0.20	≤0.02	1.28	0.17	0.50	≤0.001
25° C	1.27	0.12	0.29	≤0.02	1.19	0.19	0.52	≤0.001
VI instar								
5° C	1.37	0.07	0.25	≤0.01	1.30	0.11	0.39	≤0.001
15° C	1.22	0.14	0.54	≤0.001	1.11	0.14	0.52	≤0.001
25° C	1.15	0.15	0.55	≤0.001	1.07	0.22	0.84	≤0.001

(Ns – Not significant)

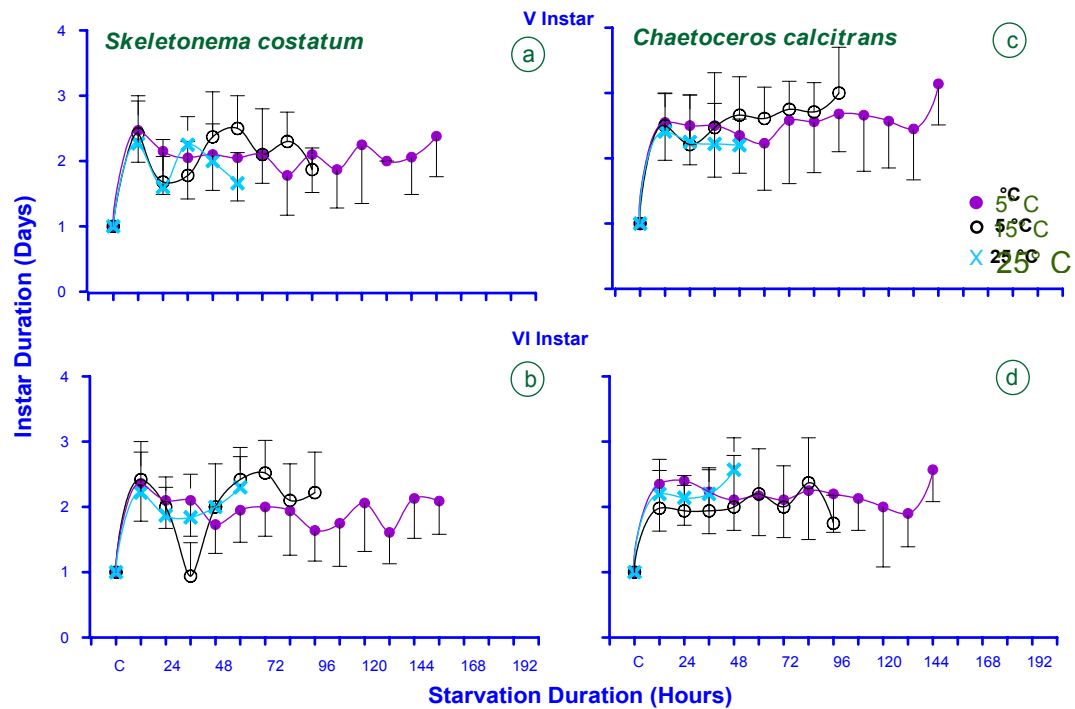


Fig. 4A.8 Instar duration of the five day old larvae starved at 5, 15 & 25° C and reared with *S. costatum* and *C. calcitrans*

Three-way ANOVA carried out by considering 5 and 15° C, indicated that the interaction of starvation temperature * larval age significantly influenced the IV instar duration in case of larvae fed with *S. costatum*. Whereas when fed with *C. calcitrans* all the instar durations (II to VI) were significantly influenced. The interaction of starvation hours with larval age significantly influenced the II, IV and V instar of *S. costatum* fed and II, V and VI instar of *C. calcitrans* fed larvae (Table 4A.3 and 4A.5). When all the three temperatures are considered the interaction of starvation temperature * larval age influenced the III, IV and VI instar of *S. costatum* fed larvae and V and VI instar of *C. calcitrans* fed larvae (Table 4A.4 and 4A.6).

Table 4A.3 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on instar duration and total naupliar duration of the larvae reared with *S. costatum* (Larvae starved at 5 and 15° C temperature)

	II Instar				III Instar				IV Instar			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	1	0.3	0.3		1	0.4	0.4		1	0.008	0.008	
B (St. hour)	6	0.64	0.1		6	5.9	0.9		6	3.4	0.57	
C (Larval Age)	1	3.97	3.9		2	2.3	1.1		4	13.4	3.3	
A*B	6	0.96	0.1	5.9**	6	1.3	0.2	0.8ns	6	0.3	0.05	0.3ns
A*C	1	0.01	0.01	0.5ns	2	0.9	0.4	1.7ns	4	2.04	0.51	3.4**
B*C	6	1.46	0.2	8.9***	12	0.3	0.03	0.1ns	24	7.8	0.33	2.2**
A*B*C	6	0.16	0.02		12	3.1	0.26		24	3.5	0.15	

	V Instar				VI Instar				Total Duration			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	1	1.1	1.1		1	2.9	2.9		1	0.5	0.5	
B (St. hour)	6	14.3	2.3		6	38.5	6.4		6	108.6	18.1	
C (Larval Age)	4	3.7	0.9		5	13.4	2.6		5	51.9	10.3	
A*B	6	0.6	0.1	0.5ns	6	13.4	2.2	2.1*	6	6.1	1.0	2.4*
A*C	4	0.5	0.1	0.8ns	5	5.3	1.0	1.02ns	5	4.3	0.8	2.05*
B*C	24	13.9	0.5	3.3****	30	29.4	0.9	0.9ns	30	48.8	1.6	3.8****
A*B*C	24	4.1	0.1		30	31.1	1.0		30	12.6	0.4	

Table 4A.4 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on instar duration and total naupliar duration of the larvae reared with *S. costatum* (Larvae starved at 5, 15 and 25° C temperature)

	II Instar				III Instar				IV Instar			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	2	0.1	0.05		2	0.3	0.1		2	0.5	0.25	
B (St. hour)	3	0.66	0.2		3	2.2	0.7		3	5.5	1.8	
C (Larval Age)	1	1.31	1.31		2	4.8	2.4		4	3.9	0.9	
A*B	6	0.06	0.01	0.4ns	6	0.3	0.05	0.5ns	6	0.5	0.1	1.7ns
A*C	2	0.003	0.001	0.1ns	4	2.7	0.7	6.9****	8	0.8	0.1	2.1*
B*C	3	0.6	0.19	7.4**	6	1.7	0.29	2.9*	12	2.8	0.2	4.8****
A*B*C	6	0.15	0.02		12	1.1	0.09		24	1.1	0.05	

	V Instar				VI Instar				Total Duration			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	2	0.5	0.23		2	1.7	0.8		2	4.8	2.4	
B (St. hour)	3	12	4.1		3	19.5	6.5		3	98	32	
C (Larval Age)	5	1.2	0.24		5	11.5	2.3		5	54	10	
A*B	6	1.2	0.2	0.9ns	6	0.9	0.2	2.4*	6	3.8	0.6	2.04ns
A*C	10	4.1	0.4	1.8ns	10	2.9	0.3	4.3****	10	4.7	0.5	1.5ns
B*C	15	3.2	0.2	0.96ns	15	5.6	0.4	5.5****	15	31	2.1	6.8****
A*B*C	30	6.7	0.2		30	2.0	0.1		30	9.3	0.3	

(****p<0.001, ****p<0.005, ***p<0.01, **p<0.025, *p<0.05, ns- not significant)

Table 4A.5 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on instar duration and total naupliar duration of the larvae reared with *C. calcitrans* (Larvae starved at 5 and 15° C temperature)

	II Instar				III Instar				IV Instar			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	1	0.05	0.05		1	0.03	0.03		1	0.04	0.04	
B (St. hour)	6	5.6	0.92		6	10	1.6		6	4.1	0.7	
C (Larval Age)	1	0.23	0.23		2	6.9	3.4		4	3.2	0.8	
A*B	6	0.3	0.05	0.4ns	6	0.3	0.05	0.2ns	6	0.6	0.1	0.2ns
A*C	1	1.4	1.4	12***	2	1.6	0.8	4.1*	4	5.1	1.3	3.3**
B*C	6	2.4	0.41	3.4ns	12	7.1	0.6	3.1**	24	14	0.6	1.5ns
A*B*C	6	0.72	0.12		12	2.3	0.2		24	9.1	0.4	

	V Instar				VI Instar				Total Duration			
	df	SS	MS	Fs	df	SS	MS	Fs	SS	MS	Fs	
A (St. temp.)	1	2.2	2.2		1	0.3	0.3		1	5.2	5.2	
B (St. hour)	6	12	1.9		6	28	4.7		6	107	17	
C (Larval Age)	5	12.4	2.5		5	53	10		5	93	18	
A*B	6	1.3	0.2	2.3*	6	0.6	0.1	0.5ns	6	2.3	0.4	1.04ns
A*C	5	3.1	0.6	6.7*****	5	3.2	0.6	3.4***	5	15	3.1	8.1*****
B*C	30	8.8	0.3	3.1*****	30	29	0.9	5*****	30	160	5.4	14*****
A*B*C	30	2.8	0.1		30	5.6	0.2		30	11	0.4	

(*****p≤0.001, ****p≤0.005, *** p≤0.01, **p≤0.025, *p≤0.05, ns- not significant)

Table 4A.6 Three-way ANOVA of the effect of starvation temperature, starvation hours and larval age on instar duration and total naupliar duration of the larvae reared with *C. calcitrans* (Larvae starved at 5, 15 and 25° C temperature)

	II Instar				III Instar				IV Instar			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	2	0.1	0.07		2	0.1	0.04		2	0.4	0.2	
B (St. hour)	3	3.7	1.2		3	5.6	1.8		3	5.1	1.7	
C (Larval Age)	1	0.04	0.04		2	5.6	2.8		4	3.3	0.8	
A*B	6	0.3	0.05	0.5ns	6	1.6	0.3	1.01ns	6	1.1	0.2	0.6ns
A*C	2	0.6	0.3	2.6ns	4	0.2	0.1	0.1ns	8	0.6	0.1	0.2ns
B*C	3	3.6	1.2	11*****	6	5.9	0.9	3.7**	12	14	1.2	4.2*****
A*B*C	6	0.6	0.1		12	3.1	0.3		24	6.7	0.3	

	V Instar				VI Instar				Total Duration			
	df	SS	MS	Fs	df	SS	MS	Fs	df	SS	MS	Fs
A (St. temp.)	2	0.4	0.2		2	0.9	0.4		2	1.8	0.9	
B (St. hour)	3	12.8	4.2		3	24	8.2		3	104	34	
C (Larval Age)	5	12	2.4		5	42	8.6		5	136	27	
A*B	6	1.0	0.1	2.4*	6	3.7	0.6	1.7ns	6	2.7	0.5	1.05ns
A*C	10	1.8	0.2	2.8***	10	8.3	0.8	2.3**	10	12	1.2	2.8***
B*C	15	8.7	0.6	9*****	15	23	1.5	4.4*****	15	162	11	25*****
A*B*C	30	1.9	0.06		30	10	0.3		30	12	0.4	

(*****p≤0.001, ****p≤0.005, *** p≤0.01, **p≤0.025, *p≤0.05, ns- not significant)

4A 3.3 Total Naupliar Duration (TND)

The total naupliar duration of the larvae at different starvation temperatures and larval age fed with *S. costatum* and *C. calcitrans* is shown in Fig. 4A.9. The non-starved larvae had total naupliar duration of 6d (Fig. 4A.9a; C-Control). The TND of zero to three day old larvae starved at 5° C was more for the larvae fed with *S. costatum* compared to *C. calcitrans* (Fig. 4A.9a,b,c). The PFR analysis also indicated greater slopes for *S. costatum* fed larvae. Whereas results were vice-versa for four and five day old larvae. The PFR slopes were also high for *C. calcitrans* fed larvae. In case of zero and one day old larvae starved at 15 and 25° C, TND was similar for both the food types (Fig. 4A.9g,h and m,n). The TND of two day larvae fed with *S. costatum* was slightly longer. In case of three and four day old larvae, TND was longer for the larvae fed with *C. calcitrans* at 15 and 25° C (Fig. 4A.9j,k and p,q). Five day old larvae starved at 25° C indicated almost similar TND (Fig. 4A.9r), whereas larvae starved at 5 and 15° C the TND of *C. calcitrans* fed larvae was slightly higher (Fig. 4A.9f,i). Three-way ANOVA carried out with 5 and 15° C indicated that interaction of starvation temperature * starvation duration influenced the TND of the larvae fed with *S. costatum*. Whereas the interaction of starvation temperature * larval age and starvation duration * larval age influenced the TND for both the food types. When all the three temperatures are considered the interaction of starvation duration * larval age influenced the TND of *S. costatum* fed larvae and the interaction of starvation temperature * larval age and starvation duration * larval age influenced the TND when fed with *C. calcitrans* (Tables 4A.3 to 4A.6, Total duration).

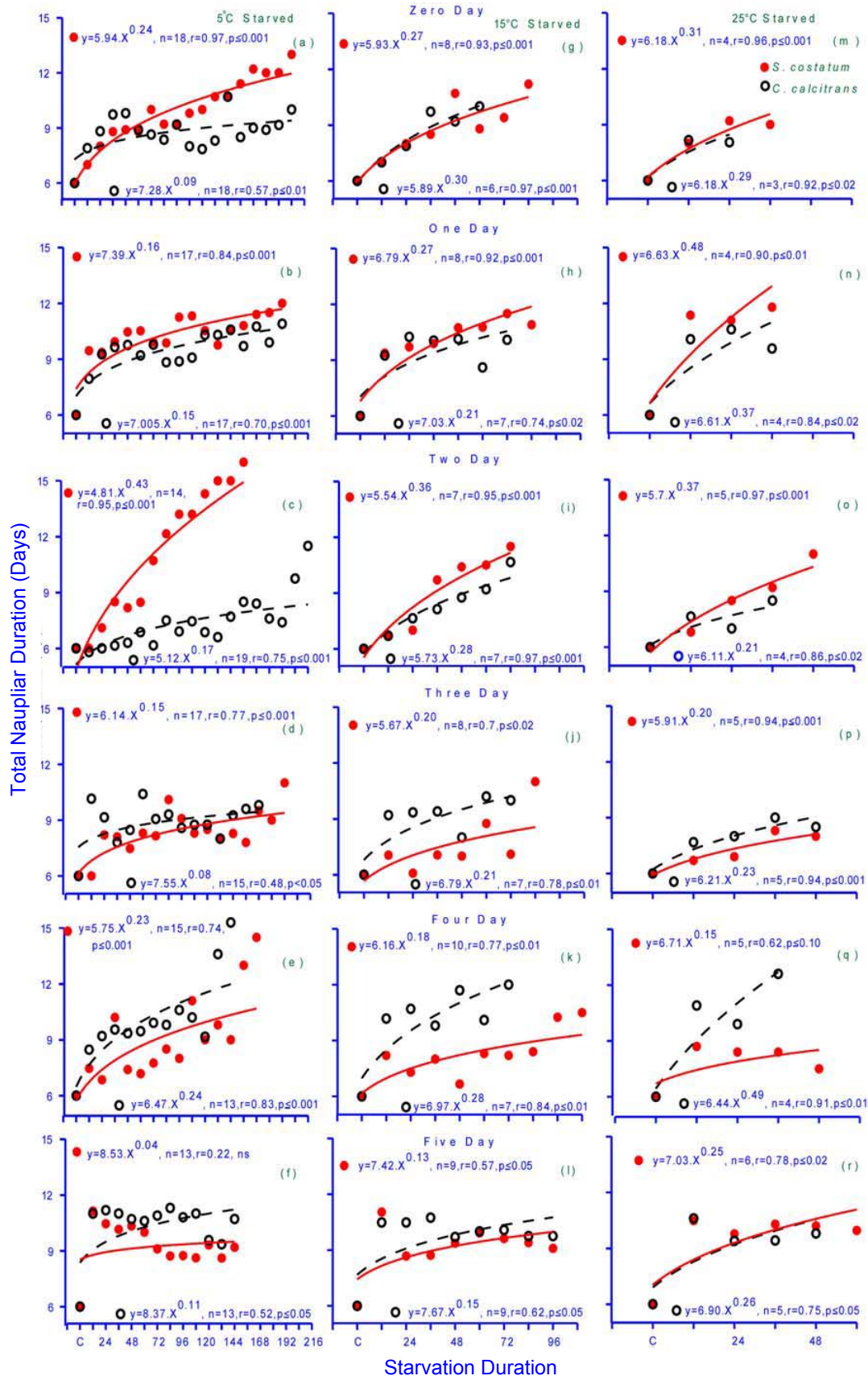


Fig. 4A.9 Total naupliar duration of the larvae starved at 5, 15 and 25° C and reared with *S. costatum* and *C. calcitrans*

4A 3.4 Filtration rate and grazing rate

Regression analysis between the filtration rate and larval age indicated insignificant results. However slopes obtained were positive for both *S. costatum* and *C. calcitrans* (Fig. 4A.10a). The grazing rates indicated that with *C. calcitrans* the slopes obtained were insignificant whereas in the case of *S. costatum* fed larvae the slopes were positive and significant (Fig. 4A.10b). The filtration rate ranged from 0.05 ± 0.03 (Zero day) to 0.09 ± 0.02 (five day) ml animal⁻¹ h⁻¹ when fed with *S. costatum* whereas with *C. calcitrans*, filtration rate ranged from 0.02 ± 0.01 (zero day) to a maximum of 0.07 ± 0.04 ml animal⁻¹ h⁻¹ (two day), further a decrease in the filtration rate was observed from three to five day larvae (Fig. 4A.10a). The grazing rate for the larvae fed with *S. costatum* ranged from 400 cells animal⁻¹ h⁻¹ (zero day) to 3500 cells animal⁻¹ h⁻¹ for four day old larvae. The advanced instars showed higher grazing rates compared to early instars. In the case of larvae reared with *C. calcitrans* the grazing rate was found maximum for two day old (4000 cells animal⁻¹ h⁻¹) larvae which decreased with larval age. The five day old showed a grazing rate of 2000 cells animal⁻¹ h⁻¹ (Fig. 4A. 10b).

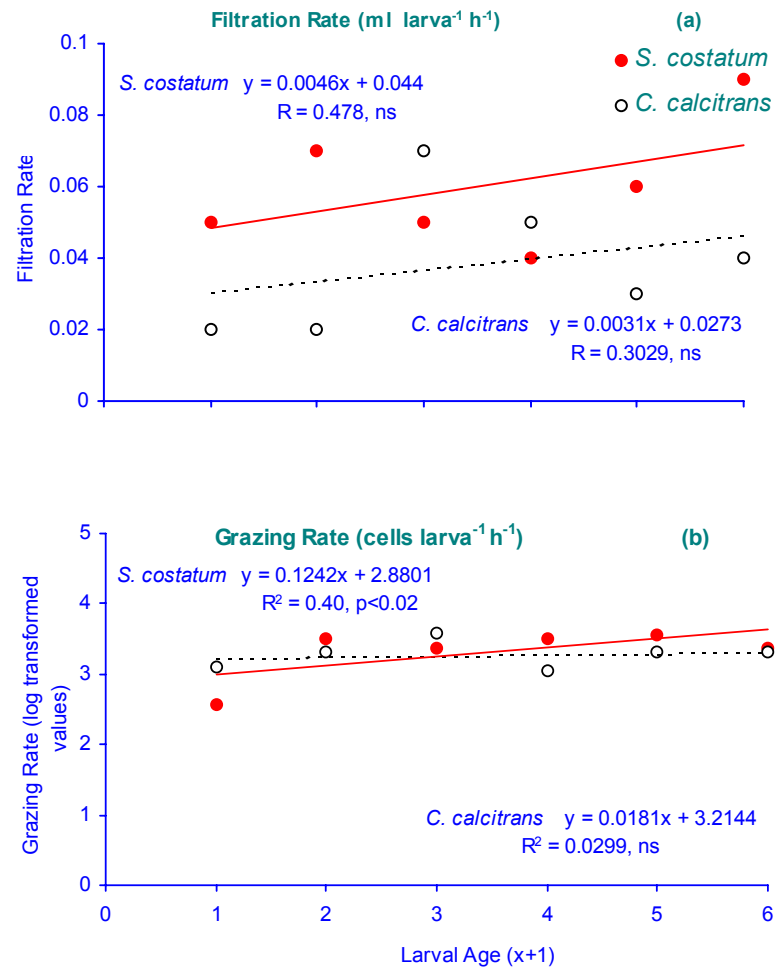


Fig. 4A.10 Filtration and grazing rate of the larvae reared with *S. costatum* and *C. calcitrans*

4B Significance of food concentration, temperature and nucleic acids on larval development

4B.2 Materials and Methods

4B 2.1 Rearing of *B. amphitrite* larvae

Larval development consists of six naupliar instars and a non-feeding cypris instar. The first nauplius stage is non-feeding and is of short duration. Nauplii obtained from *B. amphitrite* were mass reared in 2 l. glass beakers (1 larva ml⁻¹) on a daily diet of *C. calcitrans*, a unicellular diatom, at a concentration of 1 x10⁵ and 2 x10⁵ cells ml⁻¹. The cultures were maintained at 20° C & 30° C (±1°C) in an incubator (12h light:12h dark cycle). The larval density and food concentrations were maintained by monitoring mortality of the larvae every day. The rearing experiments for each of the conditions were carried out three times.

From each rearing experiment larvae (~10) were sub-sampled every day for making observations on the different larval instars. The average instar number of this observation is presented as the instar index, and utilized in determining progression in larval development at a particular rearing condition. Simultaneously measurement of size in terms of total length and total breadth of these larvae was carried out using a microscope equipped with an ocular micrometer.

4B 2.2 Estimation of nucleic acids in *B. amphitrite* larvae

The technique described by Clemmesen (1988,1993), which is based on fluorimetric determination of RNA and DNA content of an individual fish larva, was adopted for estimation of nucleic acids in the larvae of *B. amphitrite*. All

the chemicals used were analytical grade. Ethidium bromide was obtained from BDH laboratories and Ribonuclease A was obtained from Merck. Yeast ribonucleic acid (RNA) and Calf thymus deoxyribonucleic acid (DNA) were procured from Hi media. Sodium dodecyl sulphate was procured from Sigma chemicals (USA). Standard curves of DNA and RNA were prepared. Larval homogenate (10 in no) was prepared and then spiked with known amounts of standard DNA and RNA to evaluate the precision of the method which was found to be $\pm 5\%$.

4B 2.3 Statistical analysis

Influence of rearing temperature and food concentration on the size (total length and total breadth) of nauplii and cyprids, DNA and RNA content and RNA:DNA ratio were evaluated by linear regression analysis and Two-way ANOVA (Sokal and Rohlf 1981). For the analysis of naupliar instars vs. DNA and RNA content an average of the replicates ($n=5$) from each of the 3 sets was used.

4B 3 Results

4B 3.1 Larval mortality and development

Larval mortality was minimal at 30° C using both food concentration (5% and 10% at 2 and 1×10^5 cells ml^{-1} respectively). Whereas at 20° C, mortality differed with the food concentration (Fig. 4B.1). At 2×10^5 cells ml^{-1} the mortality was 21% and at 1×10^5 cells ml^{-1} it was 46%.

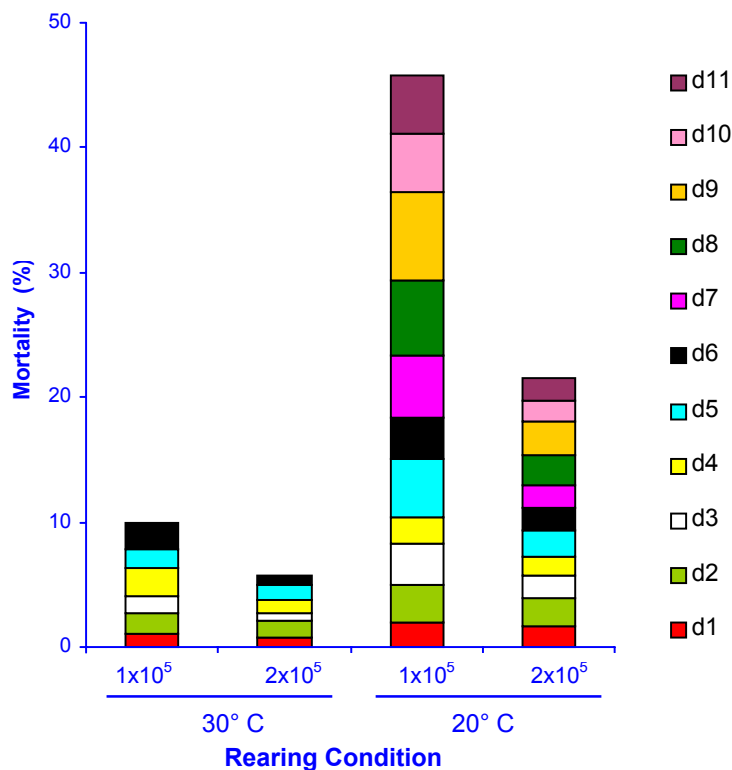


Fig. 4B.1 Percentage mortality of nauplii under different rearing conditions

Naupliar development was completed in 6 (2×10^5 cells ml^{-1}) or 7 days (1×10^5 cells ml^{-1}) and cyprids were obtained as early as 4 days at 30° C rearing temperature, whereas at 20° C it was up to 12 days. The first cyprid at 20° C appeared on day 9. Average instar index on different rearing days is shown in Fig. 4B.2a,b.

Temperature influenced the larval length of all the instars significantly, whereas the food concentration influenced the larval length of instars V and VI (Table 4B.1a to d). Variation in the breadth of the larvae was not significantly influenced by food concentration and temperature. Exceptions were the

observed influence of food concentration on instar III and food concentration and temperature on the IV instar.

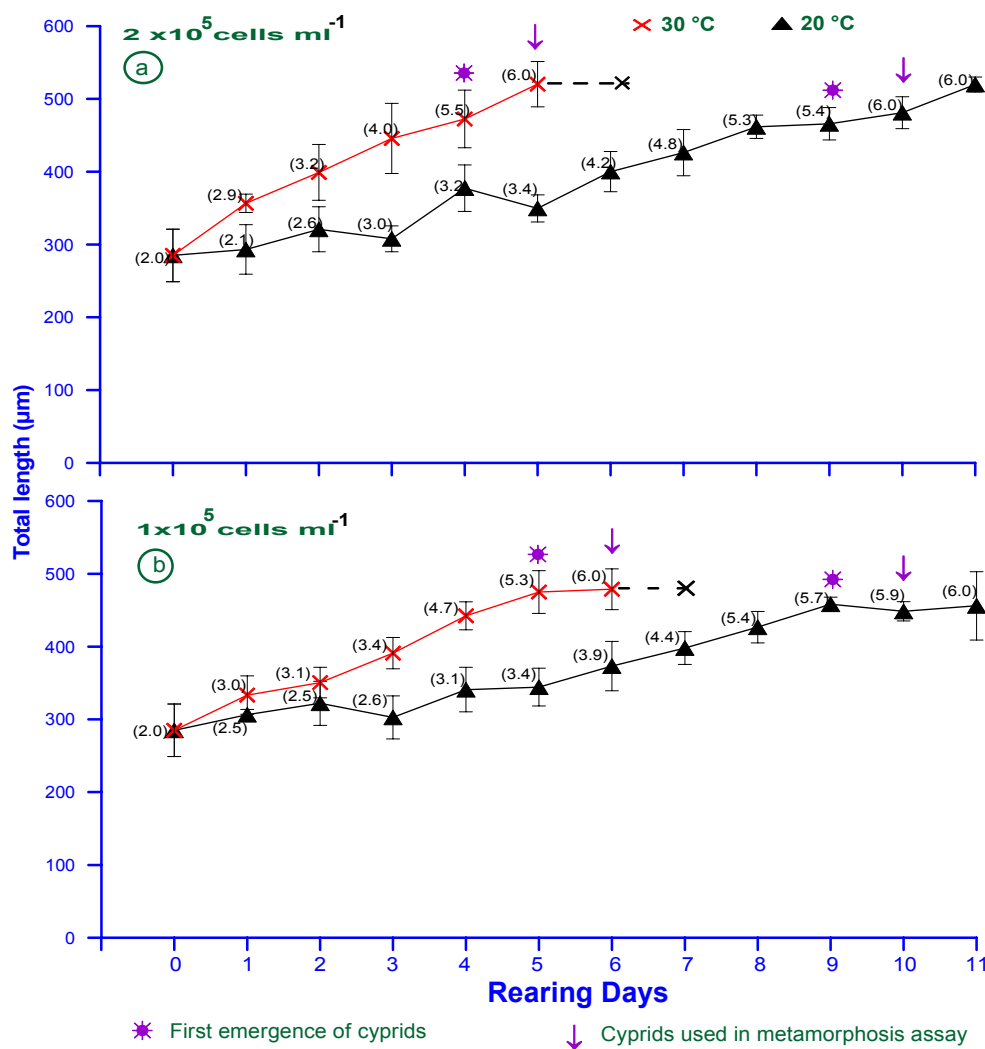


Fig. 4B.2 Variations in the length of the nauplii reared at different temperatures (30 and 20° C) and food concentrations. (a) 2×10^5 cells ml^{-1} (b) 1×10^5 cells ml^{-1} . Numbers in the parentheses indicate the naupliar instar index and the bars represent the standard deviation

Table 4B.1a Two-way ANOVA of the influence of food concentration and temperature on total length and total breadth of the III instar nauplii reared under different conditions

Factor	df	Total Length			Total breadth		
		SS	MS	F _s	SS	MS	F _s
Food Conc.	1	1178	1178	1.35ns	7801	7801	6.1**
Temperature	1	7163	7163	8.24***	1173	1173	0.9 ns
Food conc.x Temp	1	14603	14603	16.8*****	81	81	0.06 ns
Within sub. Gr.err.	36	31276	868		46019	1278	
Total	39	54220			55074		

(***** $p \leq 0.001$, *** $p \leq 0.01$, ** $p \leq 0.025$, ns- not significant)

Table 4B.1b Two-way ANOVA of the influence of food concentration and temperature on total length and total breadth of the IV instar nauplii reared under different conditions

Factor	Total Length				Total breadth			
	df	SS	MS	Fs	SS	MS	Fs	
Food Conc.	1	2924	2924	2.39ns	7188	7188	4.9*	
Temperature	1	5731	5731	4.7*	7188	7188	4.9*	
Food conc.x Temp	1	3756	3755	3.0ns	28	28	0.01 ns	
Within sub. Gr.err.	36	43874	1218		51796	1438		
Total	39	56285			66200			

Table 4B.1c Two-way ANOVA of the influence of food concentration and temperature on total length and total breadth of the V instar nauplii reared under different conditions

Factor	Total Length				Total breadth			
	df	SS	MS	Fs	SS	MS	Fs	
Food Conc.	1	6875	6875	8.75***	1433	1432	1.64 ns	
Temperature	1	15024	15024	19.14*****	549	549	0.63 ns	
Food conc.x Temp	1	-9.31E-10	-9.31E-10	-1.19E-12	393	393	0.45 ns	
Within sub. Gr.err.	36	28253			31281	869		
Total	39	50251			33656			

Table 4B.1d Two-way ANOVA of the influence of food concentration and temperature on total length and total breadth of the VI instar nauplii reared under different conditions

Factor	Total Length				Total breadth			
	df	SS	MS	Fs	SS	MS	Fs	
Food Conc.	1	10712	10712	12.06****	637	637	0.65 ns	
Temperature	1	27825	27825	31.33*****	208	208	0.21 ns	
Food conc.x Temp	1	140	140	.158 ns	117	117	0.12 ns	
Within sub. Gr.err.	36	31963	888		34855	968		
Total	39	70640			35817			

(***** $p \leq 0.001$, **** $p \leq 0.005$, *** $p \leq 0.01$, * $p \leq 0.05$, ns- not Significant)

Variations in total length and breadth of the nauplii raised under different rearing conditions are shown in Fig. 4B.3a,b. Larger larvae developed at 30° C, 2×10^5 cells ml⁻¹ (larval length; $Y = 43.7x + 253.9\mu\text{m}$, $r = 0.84$, $p \leq 0.001$; larval breadth; $Y = 36.07x + 179.3\mu\text{m}$, $r = 0.82$, $p \leq 0.001$) and smallest ones at 20° C, 1×10^5 cells ml⁻¹ (larval length; $Y = 39.8x + 222.5\mu\text{m}$, $r = 0.86$, $p \leq 0.001$; larval breadth; $Y = 40.2x + 133.3\mu\text{m}$, $r = 0.8$, $p \leq 0.001$). Larvae raised at 30° C, 1×10^5 cells ml⁻¹ (larval length; $Y = 50.5x + 191.6\mu\text{m}$, $r = 0.84$, $p \leq 0.001$, larval breadth;

$Y=36.5x+154.6\mu\text{m}$, $r=0.81$, $p\leq 0.001$) and 20°C , $2 \times 10^5 \text{ cells.ml}^{-1}$ were more similar in size (larval length; $Y=41.8x+226.2\mu\text{m}$, $r=0.82$, $p\leq 0.001$, larval breadth; $Y=30.8x+183.5\mu\text{m}$, $r=0.66$, $p\leq 0.001$).

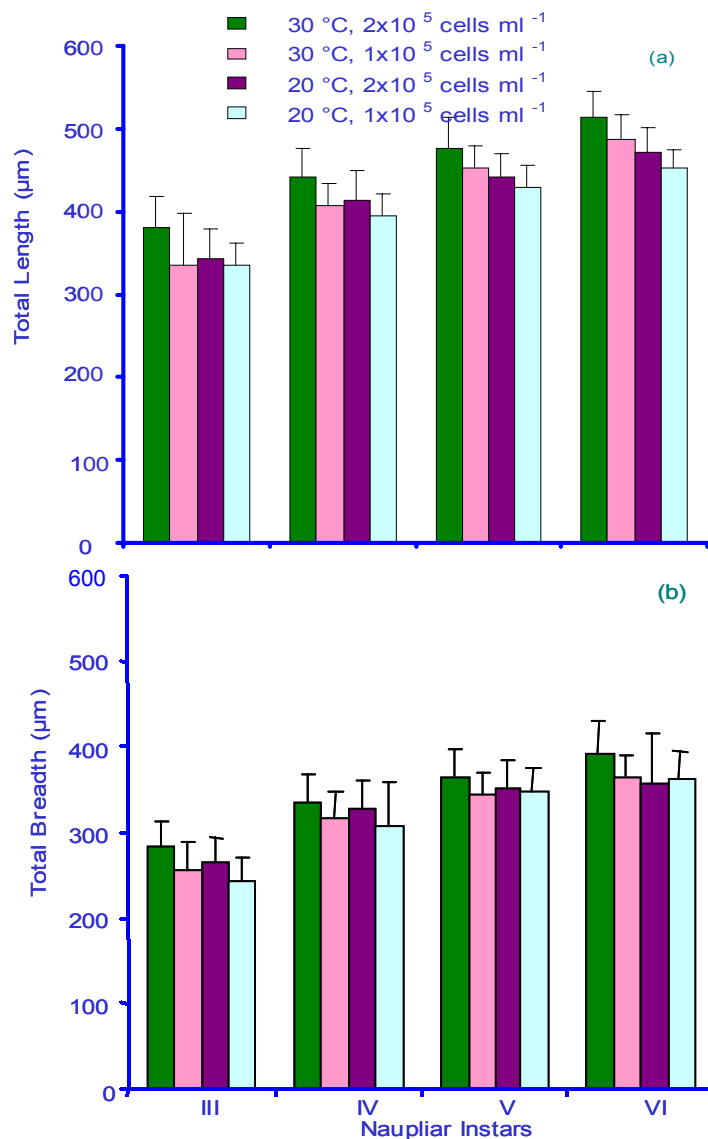


Fig. 4B.3 Variations in the size of the naupliar instars reared under different temperature and food concentrations (a) Total length (b) total breadth

4B 3.2 Nucleic acid content

The newly hatched nauplii were either as the I or II instars. First instar duration is short and lasts for ~ 2 hours. Estimation of nucleic acid was

initially carried out with II instar larvae. The DNA and RNA content in newly hatched larvae (II instar) averaged to $1.3 \pm 0.3 \mu\text{g larva}^{-1}$ and $1.9 \pm 0.4 \mu\text{g larva}^{-1}$ respectively and the mean RNA:DNA ratio (II instar) was found to be 1.5 ± 0.6

(Fig. 4B.4c, f).

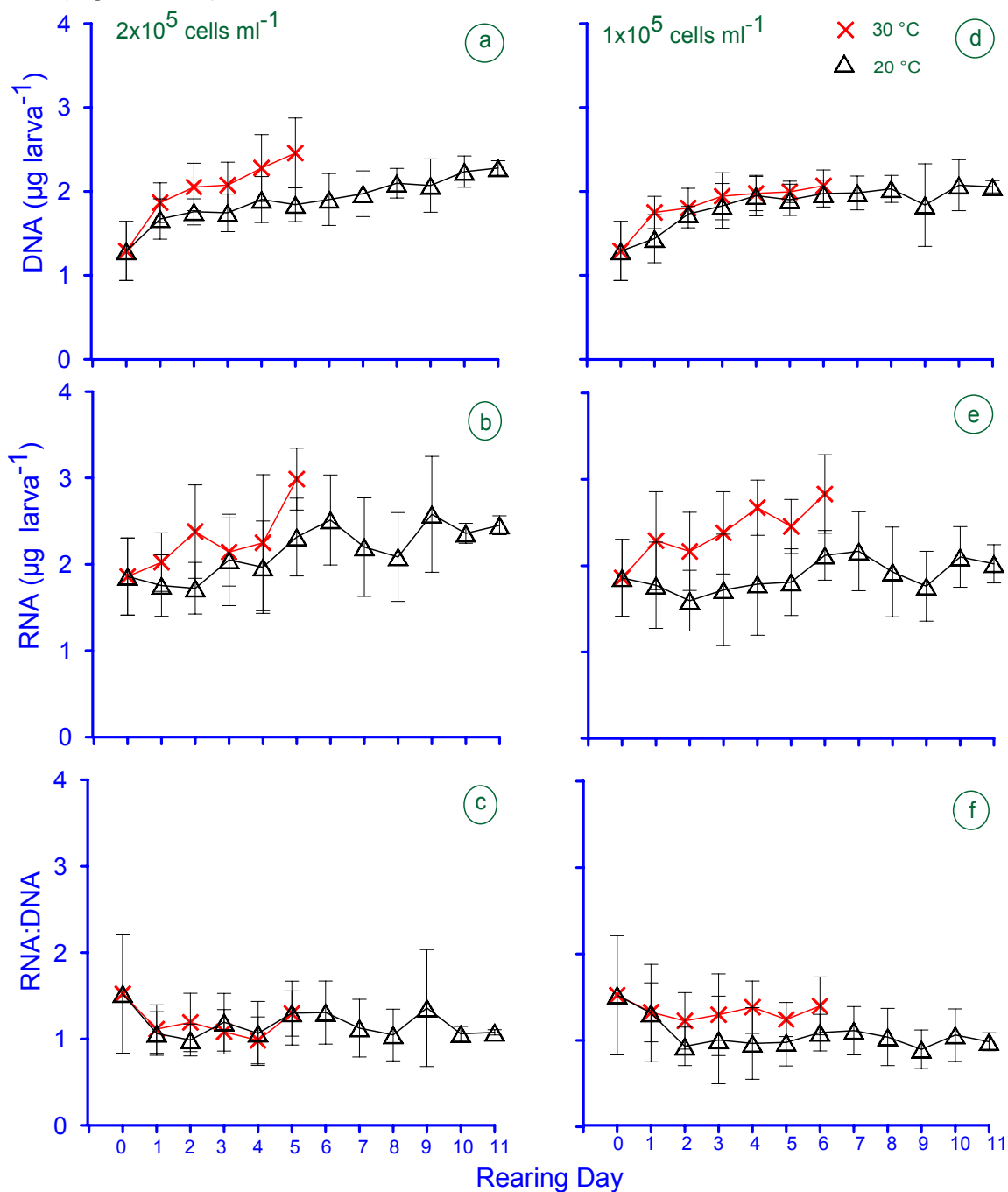


Fig 4B.4 Variations in the DNA, RNA content and the RNA:DNA ratio with reference to rearing days at 30° C and 20° C. (a), (b) & (c) refer to $2 \times 10^5 \text{ cells ml}^{-1}$ and (d), (e) and (f) refer to $1 \times 10^5 \text{ cells ml}^{-1}$ food concentration. Vertical bars represent the standard deviation

The DNA and RNA content of the five day old larvae at 30° C (2×10^5 cells ml⁻¹) with an instar index VI averaged 2.5 ± 0.4 & 2.9 ± 0.3 $\mu\text{g larva}^{-1}$ respectively (Fig. 4B.4a,b). The larvae reared at 30° C (1×10^5 cells ml⁻¹) reached instar index VI on day 6 and had an average DNA content of 2.1 ± 0.2 $\mu\text{g larva}^{-1}$ and RNA content of 2.8 ± 0.5 $\mu\text{g larva}^{-1}$ (Fig. 4B.4d,e).

In the case of larvae reared at 20° C, instar index VI was observed on day 10 (2×10^5 cells ml⁻¹) and on day 11 at 1×10^5 cells ml⁻¹ food concentration. The corresponding DNA and RNA contents varied from 2.2 ± 0.2 , 2.4 ± 0.1 at 2×10^5 cells ml⁻¹ (Fig. 4B.4a,b) and 2.1 ± 0.3 , and 2.1 ± 0.3 $\mu\text{g larva}^{-1}$ at 1×10^5 cells ml⁻¹ respectively (Fig. 4B.4d,e).

The regression of DNA and RNA content with reference to larval instars at different rearing conditions revealed that the slope is greater at 30° C, 2×10^5 cells ml⁻¹ (DNA; $Y=0.15x+1.46\mu\text{g}$, $r=0.54$, $p \leq 0.02$ and RNA; $Y=0.27x+1.11\mu\text{g}$, $r=0.81$, $p \leq 0.001$) when compared to those larvae reared at 30° C, 1×10^5 cells.ml⁻¹ (DNA; $Y=0.08x+1.58\mu\text{g}$, $r=0.69$, $p \leq 0.001$ and RNA; $Y=0.15x+1.84\mu\text{g}$, $r=0.51$, $p \leq 0.02$). Similar is the case when a comparison is made between the two food concentrations at 20° C (at 2×10^5 cells ml⁻¹ DNA; $Y=0.13x+1.34\mu\text{g}$, $r=0.79$, $p \leq 0.001$ and RNA; $Y=0.18x+1.44\mu\text{g}$, $r=0.59$, $p \leq 0.001$ and at 1×10^5 cells ml⁻¹ DNA; $Y=0.05x+1.76\mu\text{g}$, $r=0.57$, $p \leq 0.01$ and RNA; $Y=0.02x+1.85\mu\text{g}$, $r=0.07$, not significant).

Two-way ANOVA indicated food concentration to influence DNA content of III and VI instar significantly and the variable temperature to influence IV and V instar. The RNA content of IV to VI instars was significantly influenced by

variable temperature, whereas food concentration influence was not significant for any of the instars (Table 4B.2a,b,c,d).

Table 4B.2a Two-way ANOVA of the influence of food concentration and temperature on DNA, RNA content and RNA:DNA ratio of the III instar nauplii reared under different conditions

Factor	df	DNA			RNA			RNA:DNA		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Food Conc.	1	0.36	0.36	4.7*	0.02	0.02	0.055 ns	0.17	0.17	1.5 ns
Temperature	1	0.05	0.05	0.7 ns	0.423	0.42	1.15ns	0.15	0.15	1.3 ns
Food conc.x Temp	1	0.006	.006	0.08 ns	0.003	0.003	0.008 ns	0.0005	0.0005	0.004 ns
Within sub. Gr.err.	36	2.77	0.77		13.23	0.367		4.27	0.11	
Total	39	3.207			13.68			4.60		

Table 4B.2b Two-way ANOVA of the influence of food concentration and temperature on DNA, RNA content and RNA:DNA ratio of the IV instar nauplii reared under different conditions

Factor	df	DNA			RNA			RNA:DNA		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Food Conc.	1	0.04	0.04	0.64 ns	0.39	0.39	2.59 ns	0.04	0.04	0.33 ns
Temperature	1	0.51	0.51	7.07***	0.56	0.56	3.72*	0.25	0.25	1.97 ns
Food conc.x Temp	1	0.08	0.08	1.14 ns	0.07	0.07	0.48 ns	0.47	0.47	3.73*
Within sub. Gr.err.	36	2.61	0.07		5.44	0.15		4.62	0.12	
Total	39	3.26			6.47			5.39		

Table 4B.2c Two-way ANOVA of the influence of food concentration and temperature on DNA, RNA content and RNA:DNA ratio of the V instar nauplii reared under different conditions

Factor	df	DNA			RNA			RNA: DNA		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Food Conc.	1	0.11	0.11	1.60 ns	0.04	0.04	0.08 ns	0.02	0.02	0.38 ns
Temperature	1	0.94	0.94	12.8*****	5.73	5.73	11*****	1.24	1.24	17.4*****
Food conc.x Temp	1	0.50	0.50	6.90**	1.12	1.12	2.19 ns	0.003	0.003	0.05 ns
Within sub. Gr.err.	36	2.65	0.07		18.53	0.51		2.57	0.07	
Total	39	4.22			25.44			3.85		

Table 4B.2d Two-way ANOVA of the influence of food concentration and temperature on DNA, RNA content and RNA:DNA ratio of the VI instar nauplii reared under different conditions

Factor	df	DNA			RNA			RNA: DNA		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
Food Conc.	1	0.63	0.63	10.73****	0.25	0.25	1.72 ns	0.005	0.005	0.10 ns
Temperature	1	0.08	0.08	1.53 ns	2.46	2.46	16.41*****	1.04	1.04	18.87*****
Food conc.x Temp	1	0.03	0.03	0.58 ns	0.19	0.19	1.31 ns	0.12	0.12	2.29 ns
Within sub. Gr.err.	36	2.11	0.05		5.39	0.14		1.99	0.05	
Total	39	2.86			8.31			3.17		

(*****p<0.001, ****p<0.005, ***p<0.01, *p<0.05, ns- not Significant)

4C Comparison of nutritional status of laboratory and field reared larvae

4C 2 Materials and Methods

4C 2.1 Rearing of *B amphitrite* larvae

Larval development in barnacle consists of six naupliar instars and a presettling cyprid instar. The I instar nauplius is non-feeding and molts into II instar within a short interval. II to VI instars are phytoplanktivorous. Nauplii obtained from *B. amphitrite* were mass reared in 2 L glass beakers (larva ml⁻¹) on a daily diet of *C. calcitrans*, a unicellular diatom at two different cell concentrations of 1x10⁵ cells ml⁻¹ and 2x10⁵ cells ml⁻¹. The cultures were maintained at 20 and 30° C (±1° C) temperature in the incubators at 12h light:12h dark photoperiod. Larval density and food concentration were maintained by monitoring mortality of the larvae every day, prior to changing of food. Depending upon the number of larvae the food concentration and the volume (larva ml⁻¹) of the medium was maintained. The rearing experiments for each of the conditions were carried out three times.

From each of the rearing experiment larvae were sub-sampled every 24 h interval (n=10) for making observations on the different larval instars. The average instar number of this observation is presented as the instar index, and utilized in determining progression in larval development at a particular rearing condition. Simultaneously measurement of size in terms of total length and total breadth of these larvae were carried out using a microscope equipped with an ocular micrometer.

4C 2.2 Rearing of *B. amphitrite* larvae in the field

The field rearing was carried out in the month of May '2000 at Dona-Paula Bay, West Coast of India (15° 27'N, 73° 48'E). The walls as well as the bottom of 1 L plastic sampling bottles were cut and then sealed with 100 μ mesh. Before suspending the bottle it was assured that the larvae could not escape from the bottle. The newly hatched larvae (larva ml⁻¹) were introduced into the bottle by opening the lid and suspending it in the field at a depth of 1m below the lowest low tide mark. At every 24h interval, nauplii (~n=25) were removed from the bottle and used for the estimation of nucleic acids and for measurement of stage and size.

4C 2.3 Starvation of nauplii

The newly hatched nauplii were subjected to starvation for different duration at 30 and 20° C. From each of the starvation temperatures, nauplii were removed out every 24 h interval for the estimation of nucleic acid content. For the starvation of other age groups (1-6 days at 30° C and 1-9days at 20° C rearing) nauplii from the mass rearing beakers (30 and 20° C, 1x10⁵ cells ml⁻¹ and 2x10⁵ cells ml⁻¹) were removed out at every 24 h interval and subjected to starvation in filtered seawater at 20 and 30° C temperature. From these starvation batches nauplii were sampled out at 24 h interval till the larvae recover from starvation for the estimation of nucleic acids. Starvation of all the age groups of larvae was carried out till the nauplii from the mass rearing beakers reached the cyprid instar in each of the rearing conditions.

4C 2.4 Estimation of nucleic acids in *B. amphitrite* nauplii

The method described above as mentioned in section 4B 2.2, was utilized to evaluate the nucleic acid content of the laboratory and field reared larvae.

4C 3 Results

4C 3.1 Nucleic acid content of fed nauplii

The DNA and RNA content of the fed larvae increased with age. Newly hatched larvae had average DNA and RNA content of 1.3 ± 0.3 and 1.9 ± 0.4 μg larva⁻¹, respectively (Fig. 4C.1a,b). The increase in the RNA content per larva varied with the rearing condition. The RNA:DNA ratio did not show much variation. Generally the RNA:DNA ratio of the newly hatched nauplii were highest for all the instars (Fig. 4C.1c).

4C 3.2 Nucleic acid content of starved nauplii

A comparison of DNA, RNA content and RNA:DNA ratio of fed larvae with food deprived larvae is presented in Figures 4C.1,2,3 and 4. The II instar larvae when subjected to starvation showed that DNA content increased from 1.3 ± 0.4 μg larva⁻¹ to 1.7 ± 0.4 μg larva⁻¹ at 30° C and 1.6 ± 0.1 μg larva⁻¹ at 20° C starvation respectively (Fig. 4C.1a,d). The instar index increased from 2.0 to 2.6 at 30° C and 2.4 at 20° C starvation indicating a progression in development. However RNA content and RNA: DNA ratio decreased after starvation at both the temperatures (Fig. 4C.1b,c,e,f). The increase in the DNA content of II instar nauplii was also reflected by increase in their size from 273 μm to 308 μm (total length) and 189 μm to 231 μm (total width), and

yielded a positive slope in the regression analysis (Table 4C.1). However the slopes for RNA content and RNA:DNA ratios were negative (Table 4C.1). The results of two-way ANOVA indicated that starvation resulted in decrease in the RNA content and RNA:DNA ratio. The size of the larvae (II instar) was also influenced by the duration of starvation ($p \leq 0.001$) and starving temperature ($p \leq 0.001$) and larval breadth was influenced by starvation duration ($p \leq 0.001$) (Table 4C.2).

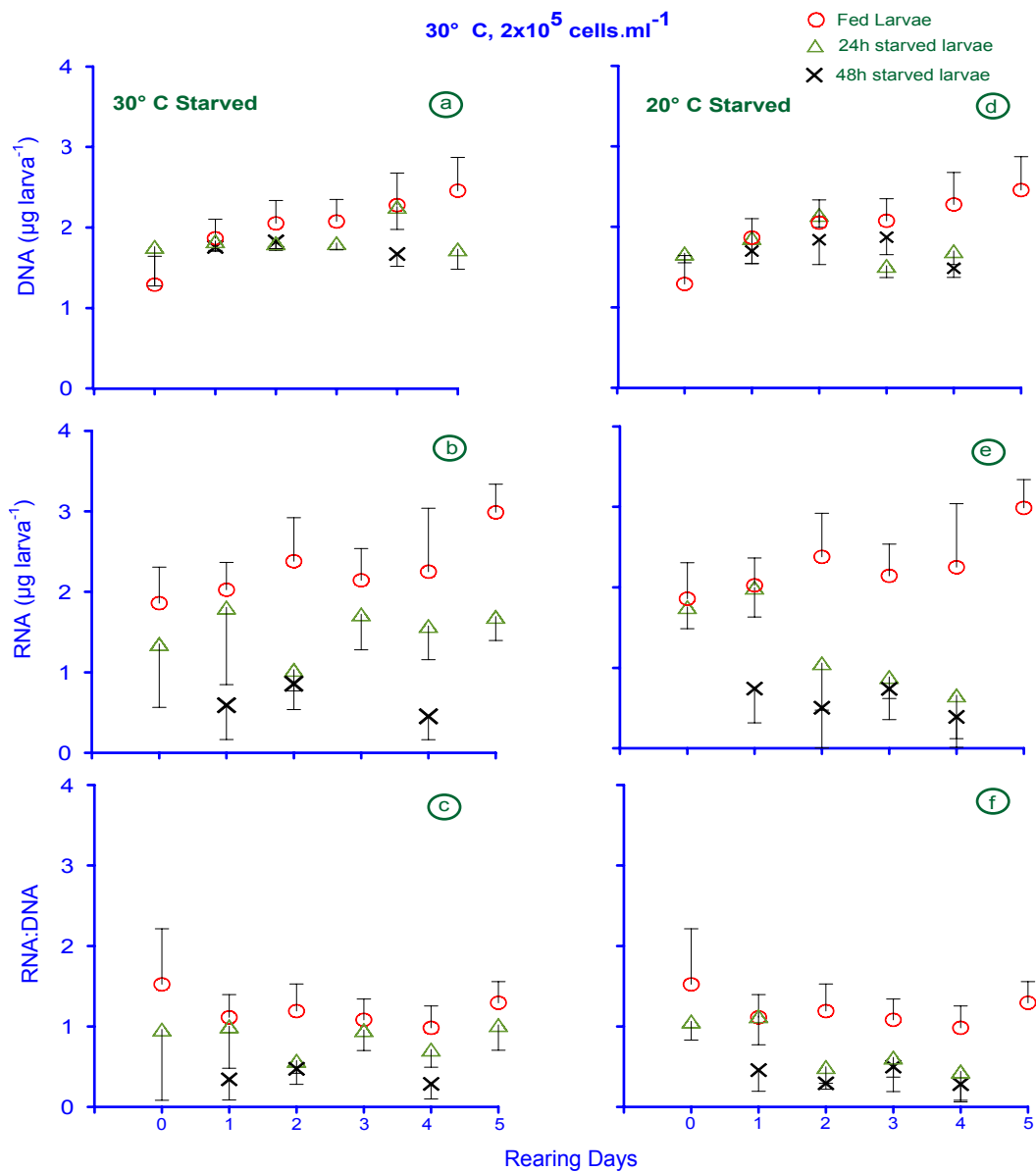


Fig. 4C.1 Influence of rearing condition (30°C , 2×10^5 cells ml⁻¹) and starvation (a, b and c starved at 30°C and d, e and f at 20°C) on the DNA, RNA and RNA:DNA ratio of the larvae

The influence of starvation on the other larval age groups (1-5 day old in case of 30 °C reared and 1-9 day old in case of 20° C reared) varied with rearing and starving temperature. The nauplii reared at 30° C tolerated a maximum of 48h of starvation at 20 and 30° C temperature (Figs. 4C.1 and 4C.2).

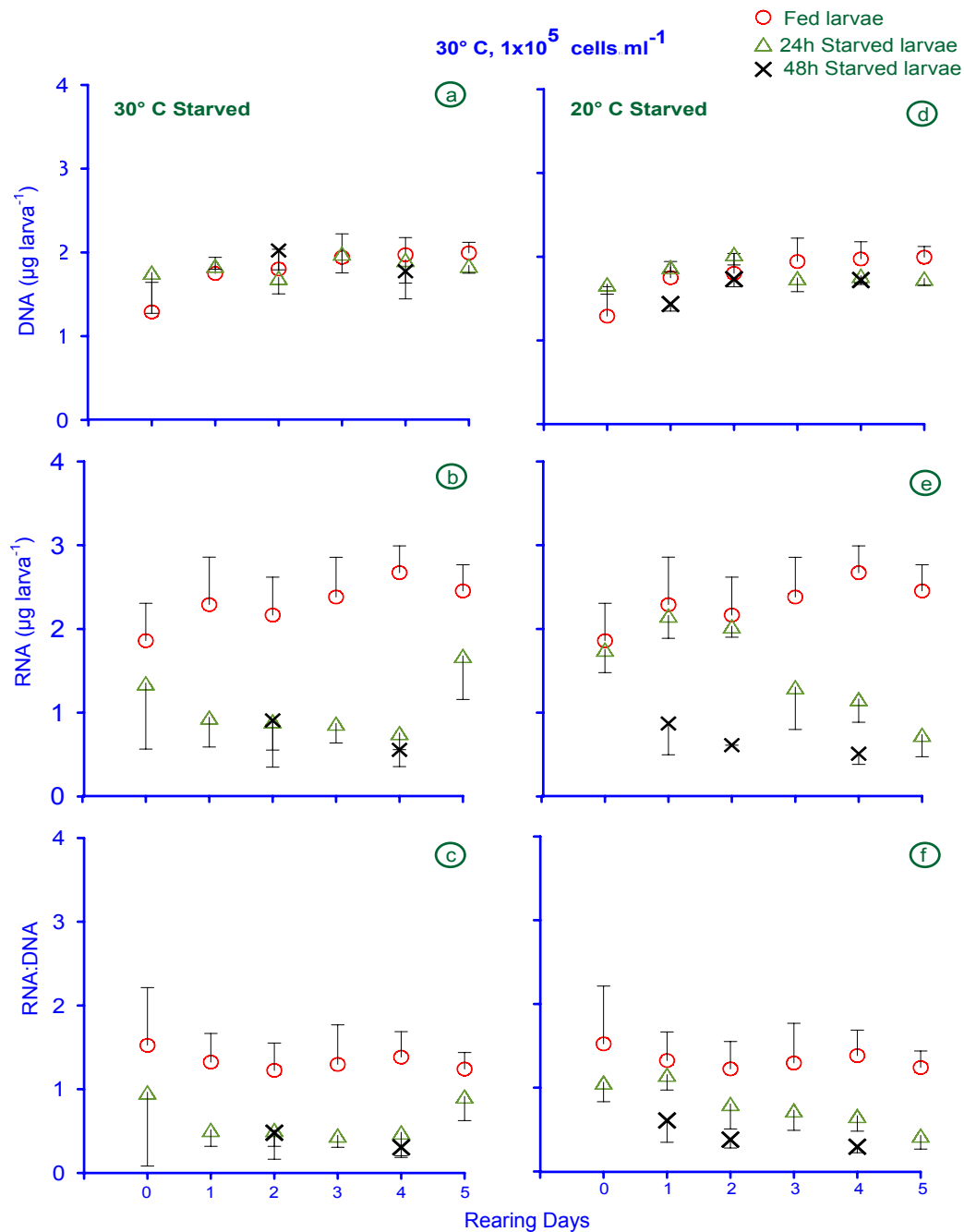


Fig. 4C.2 Influence of rearing condition (30°C , 1×10^5 cells ml^{-1}) and starvation (a, b and c starved at 30°C and d, e and f at 20°C) on the DNA, RNA and RNA:DNA ratio of the larvae

However for the nauplii reared at 20° C temperature, rearing food concentration influenced starvation tolerance. It was 72 h in the case of 2×10^5 cells ml^{-1} and 48 h in the case of 1×10^5 cells ml^{-1} when starved at 20° C. The larvae raised at 20° C temperature when starved at 30° C the differentiation in the starvation limits with reference to rearing food concentration was not evident (Figs. 4C.3 and 4C.4).

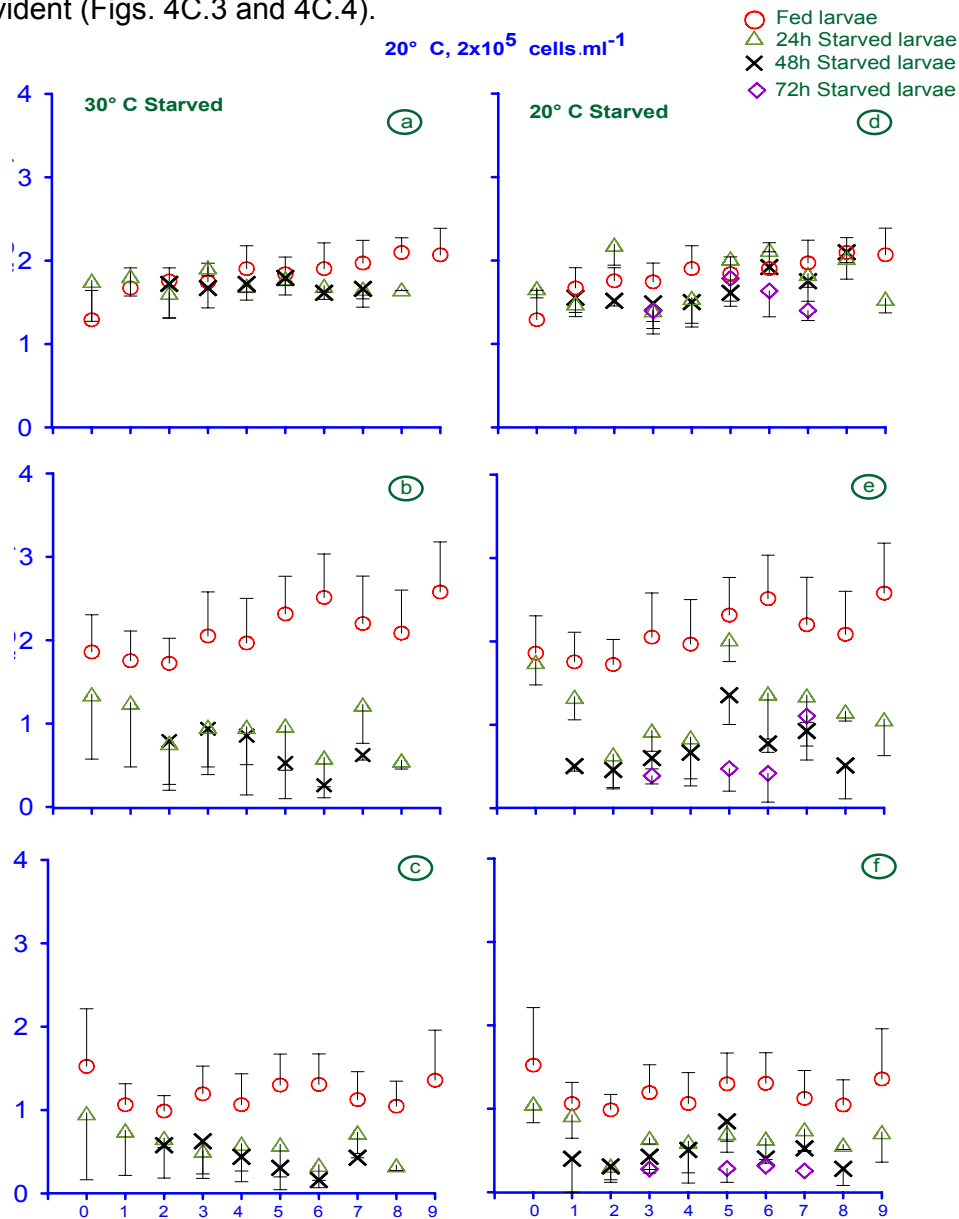


Fig. 4C.3 Influence of rearing condition (20° C, 2×10^5 cells ml^{-1}) and starvation (a, b and c starved at 30° C and d, e and f at 20° C) on the DNA, RNA and RNA:DNA ratio of the larvae

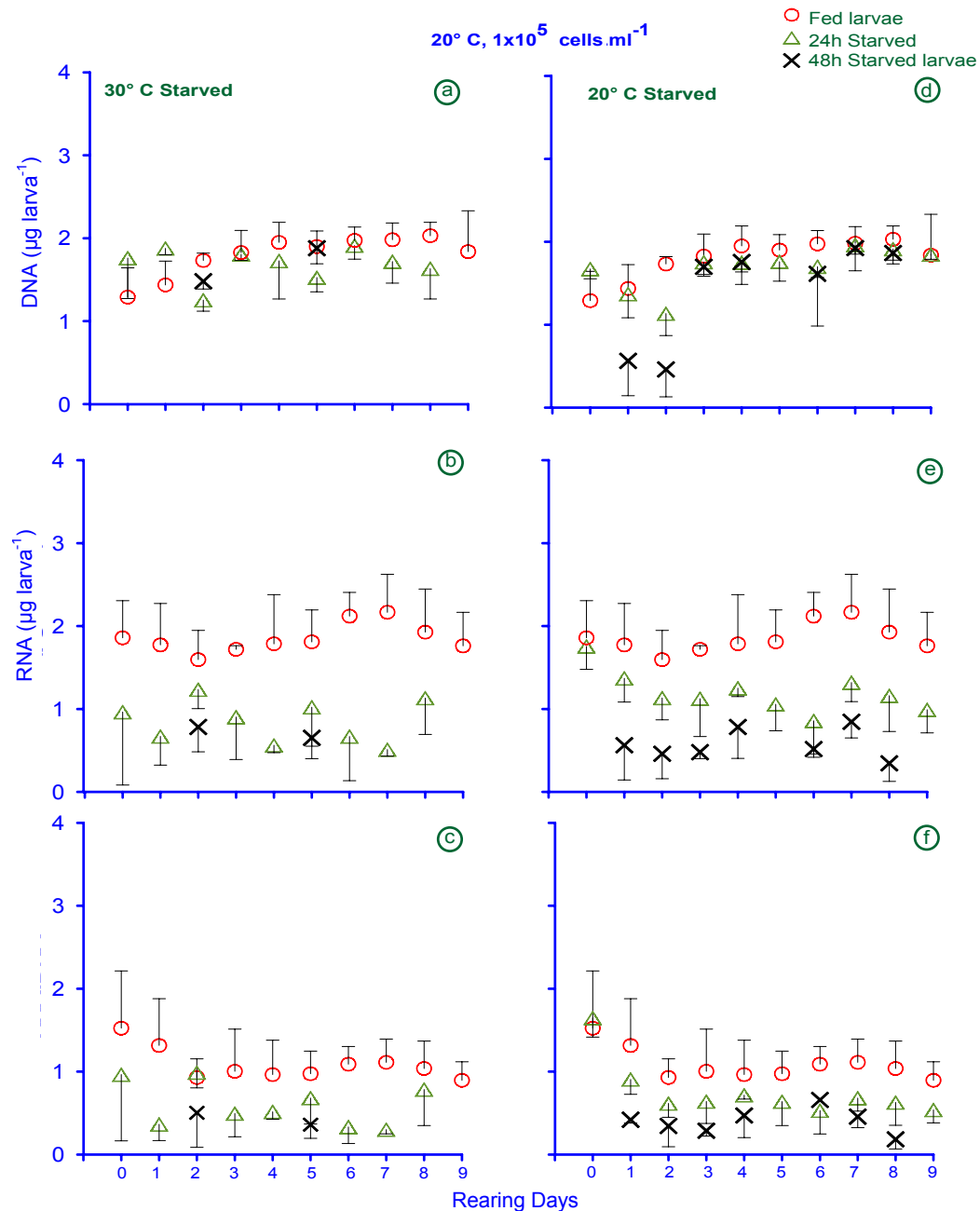


Fig. 4C.4 Influence of rearing condition ($20^{\circ}\text{C}, 2 \times 10^5 \text{ cells ml}^{-1}$) and starvation (a, b and c starved at 30°C and d, e and f at 20°C) on the DNA, RNA and RNA:DNA ratio of the larvae

Upon starvation, a decline in the RNA content and RNA:DNA ratio from the initial was observed (Figs. 4C.1,2,3 and 4). This decrease was observed with 24 h of starvation. There was a further decline in RNA content with an increase in the starvation duration (48 and 72 h). The DNA content was not significantly influenced by starvation. However catabolism of RNA during

starvation resulted in a decrease in the RNA:DNA ratio, and could be clearly distinguished from the fed larvae.

When the sixth instar nauplii reared at 30° C starved at 20° C the change in temperature triggered the metamorphosis of larvae to cyprid. Similar behavior was observed when 20° C reared larvae starved at 30° C temperature. However the results were not same when they were starved at the same temperature as that of rearing, and larvae could tolerate only 24 h starvation with no metamorphosis.

The regression analysis of the effect of starvation on the DNA, RNA and RNA:DNA ratio indicated negative slopes for RNA content and RNA:DNA ratio for all the age groups of larvae indicating starvation resulted in a decrease in RNA content and RNA:DNA ratio (Appendix 1). Two-way ANOVA was used to see the influence of starvation duration and starvation temperature on the DNA, RNA content and RNA:DNA ratio of different age groups of the larva. The results indicated DNA was not affected significantly by starvation, however RNA content and RNA:DNA ratio were significantly influenced by starvation in all the age groups of larvae (Appendix 2).

Table 4C.1 Polynomial regression results of the influence of starvation (at 20 and 30° C for 24h) duration on the DNA, RNA and RNA:DNA ratio of zero day old (instar II) larvae

Zero day larvae	Constant	R	P _≤	N
20° C st. 24 h.				
DNA	1.3475, 0.0267	0.7265	0.01	14
RNA	1.85, -0.0047	0.6312	0.01	14
RNA/DNA	1.7328, -0.0507	0.6432	0.01	14
30° C st. 24 h.				
DNA	1.3618, 0.0124	0.4986	0.05	15
RNA	1.8243, -0.0054	0.1640	Ns	15
RNA/DNA	1.7109, -0.0266	0.4701	0.1	15

(Ns; Not significant)

Table 4C.2 Two-way ANOVA of the influence of starvation temperature and starvation hours on the DNA, RNA and RNA:DNA ratio of the zero day old (II instar) larvae reared at 20 and 30° C

	df	DNA			RNA			RNA:DNA		
		SS	MS	Fs	SS	MS	Fs	SS	MS	Fs
St. temp.	1	0.06	0.06	1.5 ns	0.3	0.3	1.4 ns	0.35	0.353	7.93**
St. duration	1	1.81	1.81	47.7*****	2.3	2.3	10.1***	10.1	10.105	226.9*****
Interaction	1	0.06	0.06	1.5	0.3	0.3	1.4 ns	0.35	0.353	7.93 **
Error	8	0.30	0.04		1.84	0.2		0.35	0.044	
Total	11	2.23			4.85			11.15		

(***** $p \leq 0.001$, *** $p \leq 0.01$, ** $p \leq 0.025$, ns- Not significant)

4C 3.3 Comparison of field and laboratory reared larva

The surface temperature and salinity of the environment in which the field rearing was carried out ranged between 31 to 33° C and 33 to 35 ‰ respectively. Chlorophyll *a* values ranged from 1.9 to 4.0 $\mu\text{g L}^{-1}$. Comparison of the field rearing is made with the larvae reared at 30° C, which were given a food supply of 1×10^5 cells ml^{-1} . Even at this food concentration the chlorophyll *a* content would amount to $60 \mu\text{g L}^{-1}$.

4C 3.4 Larval development

The naupliar development was completed in 7 days in the field, as compared to 5 days in the lab (30° C, 1×10^5 cells ml^{-1}). The total length of the laboratory reared larvae was larger than the field reared larvae (Fig. 4C.5a).

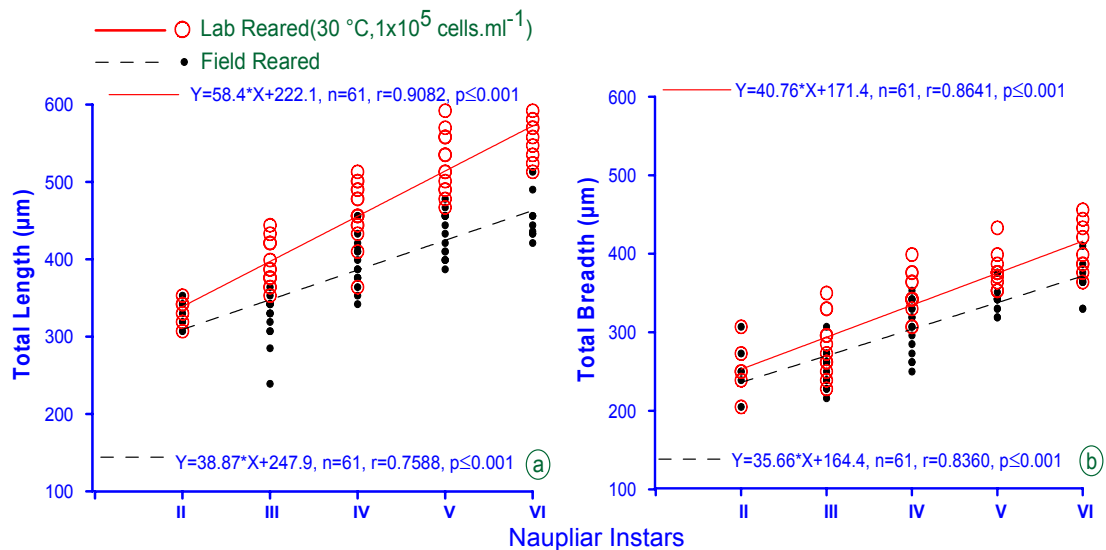
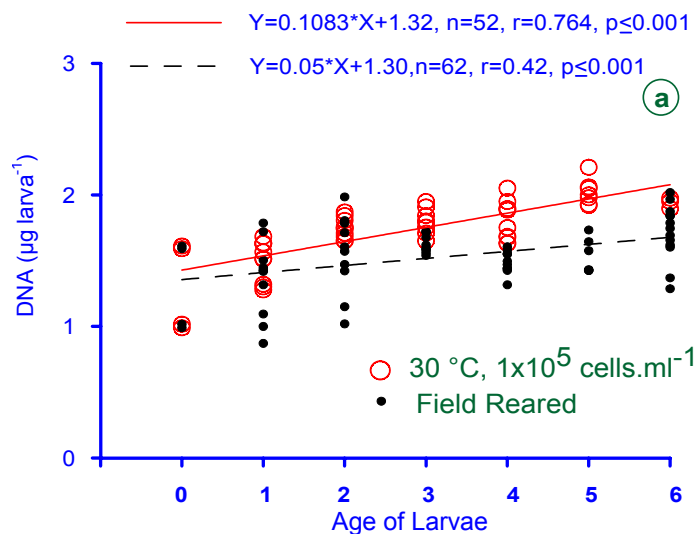


Fig. 4C.5 Relationship between the naupliar instars and the size (µm) of the laboratory and the field reared larvae. (a) Total length (b) Total breadth

4C 3.5 Nucleic acid content

The DNA, RNA content and RNA:DNA ratio of field-reared nauplii compared with laboratory reared (30° C temperature, at 1×10^5 cells ml⁻¹) is presented in Figure 4C.6a,b,c. The DNA content of the field-reared larvae was lower than laboratory reared larvae. The RNA content of the laboratory reared larvae increased with the age. However negative slopes were obtained for field-reared larvae



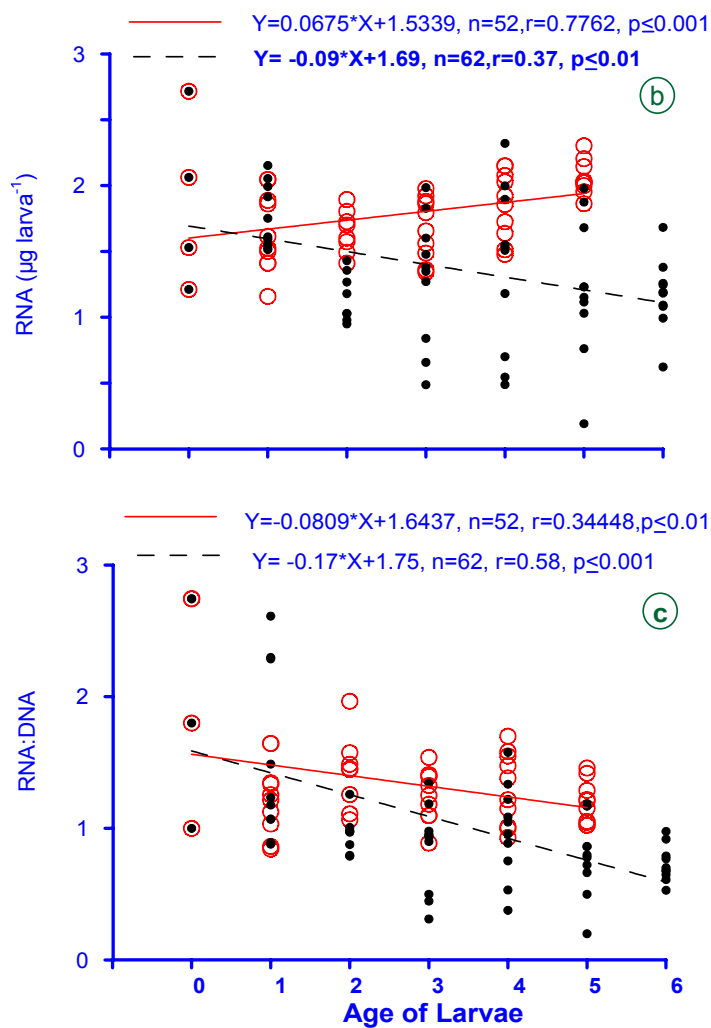


Fig. 4C.6 Relationship between the age of the larvae and the DNA, RNA and the RNA:DNA ratio of the laboratory reared (30°C , 1×10^5 cells ml^{-1}) and the field reared larvae (a) DNA (b) RNA (c) RNA:DNA ratio

The RNA:DNA ratio of larvae reared in the laboratory at 30°C and 20°C (1×10^5 cells ml^{-1}) compared with the 24 h starved larvae at respective temperature and the RNA:DNA ratio of the field reared larvae is presented in Figure 4C.7. Results indicate that RNA:DNA ratio of the field-reared larvae

was similar to that of fed larvae during the first 3 days and thereafter it decreased and in a range similar as that of 24h starved larvae (Fig. 4C.7).

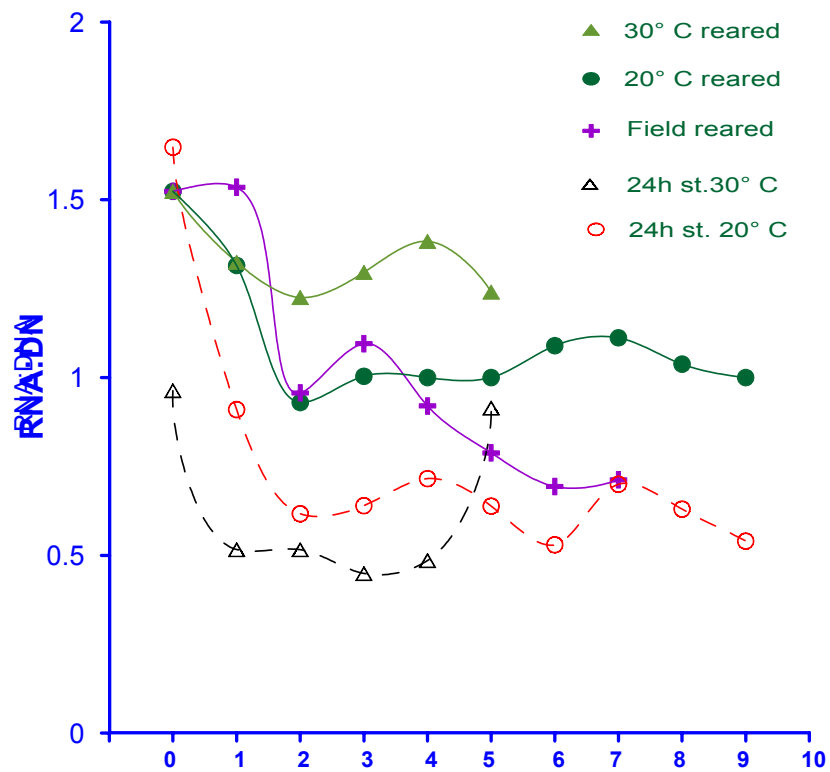


Fig. 4C.7 Variation in the RNA:DNA ratio with age of the larvae reared in laboratory (20 and 30° C, 1×10^5 cells ml^{-1}), field reared larvae and of the 24h starved larvae reared at 20 and 30° C (1×10^5 cells ml^{-1})

4.4 Discussion

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.

B. amphitrite, is a euryhaline cirripede species and is able to breed at temperatures as low as 12- 15° C (Crisp and Costlow 1963; Iwaki 1981; Anil 1991). The naupliar development of this species is planktotrophic. Under optimal rearing conditions the naupliar development is achieved in 4-6 days. However environmental 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 the 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). In the present investigation the larvae starved at 15 and 25° C had relatively lower URP than those starved at 5° C (Fig.4A.1). Higher temperature leads to faster degradation of energy reserves and the metabolic demands of the larvae are greater (Hodgson and Bourne 1988). Temperature, through its effect on physiological processes, also influences motion. Because water viscosity is physically coupled to temperature, changes in temperature can influence the activity of microscopic organisms through both physiological and physical means (Podolsky and Emlet 1993). It was observed in the present experiment 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. Yule (1984) reported that swimming rate of

the nauplii of *Balanus amphitrite* increased significantly between 20 and 30° C. He hypothesized that, if the larvae swim faster because of an increase in temperature, a greater amount of their available energy may go into swimming and the ability of the larvae to replace that energy becomes a limiting factor for continued development. Hence an increase in the temperature can be detrimental to the larvae experiencing starvation even for a short duration.

The effect of temperature on starvation varied significantly with the age of the larvae. When starved at 5° C, a decrease in the URP was observed with the larval age. The decrease in the URP at lower temperature can be attributed to a sudden change in temperature from 25 ° C, at which they were being reared. However such a decrease in the URP was not evident in case of larvae starved at 15 and 25 ° C.

The newly hatched larvae (zero day) tolerated maximum starvation at 5° C when compared to other age groups. The anterior midgut cells of cirripede nauplii contain lipid droplets and glycoprotein globules in instar I and II, which is quickly utilized during the initial development process (Walley 1969). It has also been observed with the larvae of *Balanus balanoides* that fare amount of energy reserves are left behind when larvae enter the II instar and absence of food may not be fatal to this instar (Achituv et al. 1980).

The URP and metamorphic success in case of early instar (zero to two day) larvae was higher when fed with *C. calcitrans*, whereas in case of advanced instar (three to five day) larvae the same was true when fed with *S. costatum*, irrespective of temperature at which they were starved. The starved early instar larvae showed lower TND 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 lower TND was observed when fed with *S. costatum*.

Thus 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 was also reported that *C. calcitrans* has similar food value as that of *S. costatum* (Kado 1991). Thus 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.

For many feeding larvae, food clearance rate is proportional to the length of the ciliated band (Strathmann 1971; Strathmann et al. 1972), which commonly increase with increase in body length of the larvae (Strathmann and Bonar 1976; McEdward 1984). The intersetular distance of cirripede larvae is likely to increase with successive molts (Stone 1986,1988). It is possible that this increase in mesh size can influence the size of the feed particles that the larvae can capture (Stone 1988). The grazing rate indicated that (Fig 4A.10b) early instar larvae fed better on *C. calcitrans* whereas advanced instar larvae fed better on *S. costatum*. 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. *C. calcitrans* is an unicellular diatom having a cell size of 4 μ m (diameter) whereas *S. costatum* is 7x16 μ m and is chain forming. The stage II nauplii of this species have most (~ 45%) of the setules spaced 3-5 μ apart on the antennal endopodite and exopodite. Some (~ 16%) of the setules spaced 7-9 μ m apart. Whereas in the VI instar nauplii few (~ 15%) setules are spaced 3-5 μ and most (~ 42%) of them are 9-14 μ apart. *C. caclitrans*, which is smaller in size, would be efficiently fed by the early instar larvae, compared to the advanced instars. It was reported earlier that

though there is a fringe of closely spaced hairs along the pre-axial edge of the exopodite in the stage VI nauplius, such fringes are unimportant in the feeding of cirripede nauplii (Stone 1986).

The difference in the filtration and grazing rates of *S. costatum* and *C. calcitrans* for early and advanced instar larvae necessitates the understanding of the nutritional value of both the diatoms. This was achieved by estimating the carbon and chlorophyll a content of both the diatoms. The carbon content was 20 and 23 pg C cell⁻¹ for *S. costatum* and *C. calcitrans* and the chlorophyll a content was 99 and 115 µg l⁻¹. Since the grazing rates are different the energy gained by different age group of larvae will be different. It was observed that the carbon content gained through feeding is minimum in zero day old larvae when fed with *S. costatum* and it gradually increased with the larval age (Fig. 4.4.1). However when fed with *C. calcitrans* maximum carbon content was observed in two day old larvae and it decreased with larval age (Figure 4.4.1). This substantiates that with development the utility of the feed change.

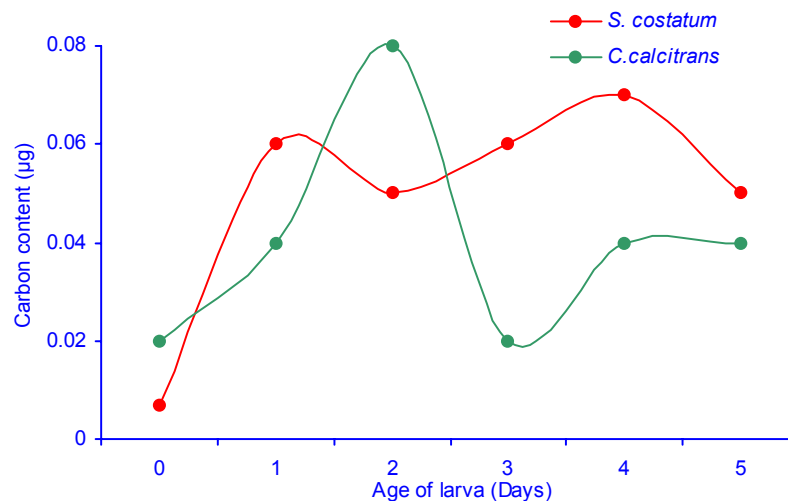


Fig. 4.4.1 Carbon content of the cells grazed by the larvae fed with *S. costatum* and *C. calcitrans*

In addition to the differences in the nutritional value of the diatoms there were differences in sinking rate that can contribute to the differences in grazing rate. The sinking rate is largely a function of cell size and chain length (Smayda and Boleyn 1965,1966a,b; Smayda and Bienfang 1983). In the present experiment the larval culture medium was not aerated and larvae had to feed before the diatoms sink to the bottom. The diatom *C. calcitrans* being unicellular and *S. costatum* being chain forming had different sinking rates. The *S. costatum* showed higher sinking rate (1.5 m d^{-1}) compared to *C. calcitrans* (0.03 m d^{-1}). Owing to slower sinking rate, *C. calcitrans* were readily available to the larvae when compared to *S. costatum*. However the results indicated that only early instar nauplii fed better on *C. calcitrans*. This can be explained on the larval photo taxis behavior. The early instar larvae of barnacles show a prominent photopositive behavior than the advanced instars (Lang et al. 1979; Barnes and Klepal 1972; Crisp and Ritz 1973). Hence the time spent by the early instar larvae at the surface would be more than the advanced instar due to which they had enough time to feed on *C. calcitrans* compared to *S. costatum*. Whereas vice-versa was observed in advanced instars.

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. These interpretations are also supported by the studies carried out on brachyuran larvae under starvation conditions (Anger and Dawirs 1981; Anger et al. 1981). Food energy allotted first to survival, than 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 starvation induced molting differed with the type of feed. This kind of molting was evidenced with *S. costatum* in case of one, three and four day

old larvae (Figs. 4A.4a, 4A.6a, 4A.7a) and in case of two day old larvae when fed with *C. calcitrans* (Fig. 4A.5e). It is evident that the carbon content of one, three and four day old larvae fed with *S. costatum* is greater than those fed with *C. calcitrans* (Fig. 4.4.1). However, in case of two day old larvae greater carbon assimilation is observed with *C. calcitrans* (Fig. 4.4.1). Thus the difference in the stress induced molting can be attributed to changes in the food quality.

Spawning activity in some marine invertebrates coincides with phytoplankton blooms (Barnes and Barnes, 1958; Starr et al. 1990). This coupling is explained as one of the survival measures offered to the planktotrophic offspring. Barnes and Barnes (1958) while examining the recruitment of *Balanus* (= *Semibalanus*) *balanoides* over a period of ten years in the Firth of Clyde, attributed recruitment failure to abnormal increase in larval predation, unusual physical parameters, presence of deleterious chemicals as well as defective sampling. Olson and Olson (1989) however felt that recruitment largely depended on the abundance of larval food. 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). In an earlier effort to evaluate the effect of food concentration, temperature and salinity on the larval development of *B. amphitrite*, it was observed that food availability and temperature jointly influence the energy allocation (Anil and Kurian 1996). The results from this investigation also reflected these observations. Naupliar development duration was prolonged at 20° C (9-11 days) when compared to that at 30° C (5-6 days). West and Costlow (1987) observed that effect of food concentration on naupliar size was slight, whereas the size of the cyprid varied directly with food concentration. In this investigation it was observed that temperature influenced the larval length of all the

instars whereas food concentration influenced only the more advanced instars (V and VI).

The larvae raised at 30° C, 1×10^5 cells ml⁻¹ and 20° C, 2×10^5 cells ml⁻¹ in the present experiments were similar in size and nucleic acid content. Using the regression constants from the equations it is evident that VI instar larvae raised at 30° C, 1×10^5 cells ml⁻¹ have an RNA content of 2.7 µg.larva⁻¹, whereas those raised at 20° C, 2×10^5 cells.ml⁻¹ have an RNA content of 2.5 µg. larva⁻¹.

Jian-Wen and Pei-Yaun (1997) found that the larvae of *B. amphitrite* from a single brood responded in the same way as those from multiple broods. They also observed that food concentration had a clear impact on larval survival and development time and growth whether they were obtained from single or multiple broods. The larvae used in the present experiments were collected from multiple broods. The DNA and RNA content in the newly hatched nauplii showed considerable variation (Fig. 4B.4a; 0 day). However, with development the variations in RNA:DNA ratio narrowed down in each of the rearing conditions (Fig. 4B.4). 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. In an earlier investigation it was documented that food availability and temperature jointly determine the energy allocation for metamorphic progress (Anil and Kurian 1996). 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 (Anil et al. 2001). Scheltema and Williams (1982) observed with *Balanus eburneus* that the concentration of food appears irrelevant and stated that the larval survival rate is similar

at 1 and 2×10^5 cells.ml⁻¹ at 30° C. However at 20° C, they found that increase in food concentration not only increased survival but also decreased the time necessary for larvae to develop into the cyprid instar. Apparently at lower temperature increasing food concentration could compensate for decreased feeding efficiency and resulted in enhanced survival and shorter development period. The sizes of the larvae were positively influenced by the increasing temperature and food concentration (Anil et al. 2001).

Barnacle release I or II instar larvae into plankton. The duration of the nauplii I to II intermolt is short and completed within 1-2 h. Changes that occur between the release of free swimming nauplius I and the nauplius II indicate the net utilization by the non-feeding nauplii I instar. It has been observed with *Balanus balanoides*, that a considerable amount of energy reserves is left behind when the larvae enter the II instar and absence of food may not be fatal (Achituv et al. 1980). It was evident from this experiment that the size, DNA content and instar index increased in starved II instar larvae substantiating that the starvation did not hinder the development of II instar.

Starvation may affect the larval development either directly leading to death or by lengthening the developmental period. Thus it increases the chance of mortality by predation or dispersal of larvae to unfavorable conditions. It was also observed that advanced instar nauplii given a temperature shock (i.e 20 to 30° C and 30 to 20° C) for subjecting to starvation triggered the metamorphosis of larvae to cyprid and can be attributed to thermal shock. In one of our earlier experiments it was observed that subjecting II instar larvae to starvation resulted in a longer II–IV instar duration, whereas the V and VI instar duration was reduced (Dattesh and Anil 2000).

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.

Many studies have suggested the use of RNA:DNA ratio as a means of differentiating between fed and starved larva and reduced RNA:DNA ratio have been observed in starved groups (Bulow 1970; Wright and Martin 1985; Clemmesen 1987; Richard et al. 1991). It was observed that starvation was followed by a decrease in the RNA content and RNA:DNA ratio. The starvation tolerance limits varied with age of the larvae and also on the condition under which the nauplii were reared. The DNA content of the starved nauplii was found almost similar to that of fed ones. The amount of DNA, the carrier of genetic information, is independent of nutritional condition and only a measure of cell number (Regnault and Luquet 1974; Dortch et al. 1983). Whereas the amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring and is directly affected by nutritional condition. In a well-fed larva the amount of RNA and the ribosomal activity is always high. Starvation will bring down this activity. Prolonged starvation will lead to degradation of ribosomes and loss of RNA.

The RNA content and the RNA:DNA ratio of the starved larvae decreased considerably (Figs. 4C.1, 4C.2, 4C.3 and 4C.4). The minimum RNA content of the larvae, which

could survive the starvation stress, was 0.4 to 0.5 $\mu\text{g larva}^{-1}$ and the RNA:DNA ratio was $0.3 \pm 0.2 \mu\text{g larva}^{-1}$.

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 $\mu\text{g L}^{-1}$. 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 (Figs. 4C.5 and 4C.6).

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. In an earlier effort (Anil et al. 2001) it was observed that cyprid raised at 30° C, 2×10^5 cells ml^{-1} and 1×10^5 cells ml^{-1} were viable till 16 and 8 d of aging at 5° C, whereas the cyprids raised at 20° C could successfully metamorphose only till 4 and 2 d (at 2×10^5 and 1×10^5 cells ml^{-1} respectively).

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.