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Effect of monoalgal diet on the growth, survival and egg production in *Nannocalanus minor* (Copepoda: Calanoida)

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The effect of monoalgal diet on the growth, survival, egg production and egg hatching succession in calanoid copepod *Nannocalanus minor* was studied under laboratory condition. There are seven different microalgae such as *Chlorella marina*, *Dunaliella salina, Isochrysis galbana, Nannochloropsis* sp., *Coscinodiscus centralis, Chaetoceros affinis* and *Skeletonema costatum* were tested for their efficacy on survival of *N. minor* at two different algal cell concentrations *viz.* 10,000 and 20,000 cells/ml. Among the six diets tested, *Chlorella marina* shows the extensive survival in both lowest and highest algal concentrations where the 100% survival extends for 7th and 9th days of experiment while the least survival was obtained in diatom *Skeletonema costatum.* Likewise, copepod *N. minor* grew faster at *C. marina* than other algal feed tested presently. The egg production (32±1.52 eggs/female/day) and hatching succession (93.75%) both are proportionally increased with increasing algal concentration (20,000 cells/ml) while at low algal concentration (1000 cells/ml) it was recorded as 3±1 eggs/female/day and 44.33% respectively. The study provides a realistic basis for formulating suitable algal food and algal concentration required for copepod *N. minor* to achieve utmost growth, survival and fecundity in captive condition. This information can help in developing the culture technology on copepod *Nannocalanus minor* for it use in larval fish culture.

[**Keywords:** Copepod, microalgae, *Nannocalanus minor, Chlorella marina***,** survival, egg production]

Introduction

Calanoid copepods are the most abundant and probably the most ecologically significant animals at the first consumer level of the marine food web. Calanoids plays an important role in the energy transfer between primary producers and pelagic fish populations, and it is thus a key factor influencing fish production[1-3]. Nowadays, most reared marine fish larvae are fed on rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.). *Artemia* is widely used in many countries that practice commercial aquaculture due to its easiness of use. But these live feeds failed to prove their quality nutritional profile in fish and shrimp larvae. However, copepods are excellent foods with high nutritional value for zooplanktivorous fishes and shrimps [4-5]. Furthermore, copepods provides wide size ranges (6 nauplii and 6 copepodite including adult) for fish larvae based on their mouth sizes. Moreover, the unique movement of copepods attracts the fish larvae to feed on them [6]. Copepods have a high content of both docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA), and their nutritional feasibility judged by larval growth rate, survival, pigmentation and successful metamorphosis has been well documented[7,6].

In recent decades, copepods culture paid a vast attraction due to their superior suitability in culturing fish and shrimp larvae. The problem in culturing copepods is failure in continuous mass production compared to *Artemia* and rotifer. Reproduction and growth rates in copepods have been restricted by numerous factors including temperature, salinity, food, food concentration and nutritive value of foods [8, 9]. Some foods may be readily ingested by female copepods and can promote high egg production, but low egg hatching success [10]. Decrease in food concentration resulted that decrease in the reproductive success of a copepod [11]. The identification of food which is suitable for continuous copepod production is necessary one. The present study aimed to provide an idea to optimize the influence of different microalgae diet on growth, survival, egg laying and egg hatching of the calanoid copepod *Nannocalanus minor.*

Materials and Methods

Collection, identification and culture of copepod

Copepod samples were collected from Muthupet lagoon (Lat. 10° 20’N and Long 79° 35’E) using plankton mesh (158µm) and transported to the laboratory with aeration. From the samples, *Nannocalanus minor* was identified under microscope using the key of Kasturirangan [12]. After the conformation of species, 300 numbers of individuals that includes male and female of the healthy adults of *N. minor* were isolated and stocked in an oval shaped, flat-bottomed FRP (0.54 m dia, 0.81 m length) tank containing 80 litre filtered seawater of ambient salinity (32‰) with vigorous aeration. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of: 26-30^o C; 28-32 ‰; 7.5-8.5; 5.0-7.5 ml/l respectively (during rearing period) fed with a daily ration of mixed algae *viz., Chlorella marina*, *Dunaliella salina, Isochrysis galbana, Nannochloropsis* sp., *Coscinodiscus centralis, Chaetoceros affinis* and *Skeletonema costatum* in the concentration of 20,000cells/ml. The light intensity used for copepod culture system was 500 lx.

Microalgal Culture

Monocultures of seven marine microalgae such as *C.marina*, *D.salina, I.galbana, Nannochloropsis* sp., *C.centralis, C.affinis* and *S.costatum* stock cultures were obtained from Central Institute of Brackishwater Aquaculture (ICAR, Govt. of India, Chennai) and maintained separately in 1 and 2 liter conical flasks containing filtered seawater at 20-23^{o}C temperature, 30‰ salinity and 7000-9000 light intensity (lux) fertilized with Conway's medium or Walne's medium[13].

Effect of algal concentration on survival of *N. minor*

A 200 individuals including male and females of the healthy adults of *N. minor* were isolated and kept overnight in 250 ml beakers containing filtered seawater (1µm) of ambient salinity (32‰) with vigorous aeration for starving prior to the experiment. For survival experiment, ten individuals of *N. minor* were maintained separately in each glass bowl containing 100 ml of sterile seawater with each of seven types of live algae in different concentrations (10,000 and 20,000 cells/ml) were used. For each concentration of algal food, a separate bowl was maintained and fed up to 10 ml of mono algae. The algal concentration was diluted using sterile seawater in different ratios and quantified under compound microscope using a Sedgewick counting chamber. The daily mortality of copepod was recorded carefully. The experimental sets were maintained at 28±1^o C till the death of all animals.

Effect of monoalgal diet on the growth of *N. minor*

The experiment on effect of different algal diet on *N. minor* growth was assessed according to Spiros and Gerard (1990). In brief, the nauplii, copepodite and adult copepods were fed with different microalgae were collected from the respective culture flasks. The total length of the different stages of copepods were measured under a microscope at a magnification of x10 using ocular and stage micrometers, from the tip of the prosome to the end of the caudal rami, excluding the caudal setae.

Effect of algal concentration on fecundity of *N. minor*

The effect of algal concentration on the egg production of copepod was determined by incubating male and female *N. minor* in Pyrex test tubes in the ratio of 1:1. The mixed microalgae containing *C.marina*, *D.salina, I.galbana, Nannochloropsis* sp., *C.centralis, C.affinis* and *S.costatum* was given at the following concentrations: 1000, 5000, 10,000 and 20,000 cells/ml. To estimate the hatching rate, laid eggs were siphoned out from the bottom of the culture flask and incubated in test tubes along with filtered seawater for 96 hours with the algal food concentration mentioned above, after that the hatched out nauplii were counted using a counting chamber under binocular microscope. Triplicates were made for each experiment. For the growth, survival and fecundity experiments, the culture system was maintained in static non-renewal condition.

Statistical analyses

The results obtained were statistically analyzed using simple correlation and analysis of variance (ANOVA) between algal concentration and copepod survival and algal concentration and fecundity.

Results and Discussion

Survival of *Nannocalanus minor* in low algal concentration

The present study inferred that the survival of *N. minor* was depending on the algal types and concentrations. In lowest algal concentration (10,000 cells/ml), 100% survival was noticed up to 2-7 days. Among the algae tested, *C.marina* shows high survival even in lowest algal concentration where 100% survival was occurred up to 7th day, 49% survival observed on 15th day and total mortality occurs on 17th day onwards. However, the *S.costatum* results the poor survival than rest of the algae tested where 100% survival observed up to 2nd day only, after that survival was declined to 48% on 10th day and total mortality was reported on 14th day onwards as shown in Fig.1.

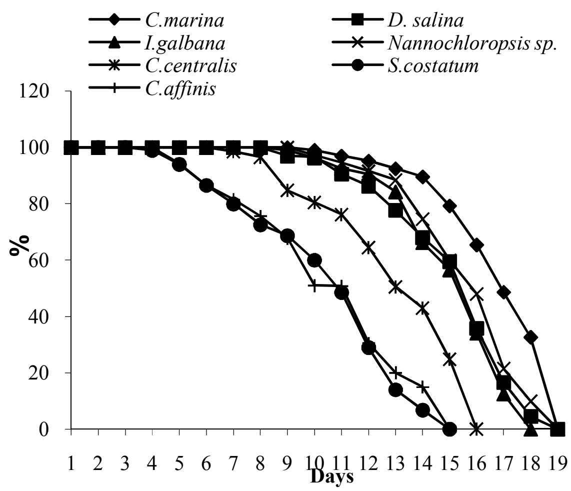

Fig. 1 Effect of low algal food concentration on survival of *Nannocalanus minor*

Survival of *Nannocalanus minor* in high algal concentration

In high algal concentration, the 100% survival was renowned up to 3-9 days. The maximum copepod survival was noticed in *C.marina* which shows100% survival up to 9th day, 48.6% on 17th day and total mortality on 19th day onwards while the *S.costatum* exhibits the poor survival than rest of the algal diets studied. Here, 100% survival was observed up to 3rd day only, after that survival decline to 48.5% on 11th day and complete mortality was procured on 15th day onwards (Fig. 2).

Growth of *Nannocalanus minor*

Copepod *N. minor* shows least growth at *S.costatum* where the Nauplii I (NI) grew to 0.094mm and Nauplii VI (NVI) reached 0.188mm. The length of Copepodite I (CI) and adult female (AF) and adult male (AM) were 0.39mm, 1.906mm and 1.743mm respectively. The growth of *N. minor* was comparatively higher in *C.marina.* Here the Nauplii (NI) was hatched after 24hrs with the length of 0.107mm while the body length of NVI was 0.275mm. The length of CI and CV were 0.427mm, 1.563mm (Female) and 1.266mm (male). Adult female and male copepod length was 1.963 and 1.756mm. The daily average growth of *N. minor* fed with different algal diets was given in Table 1 & 2.

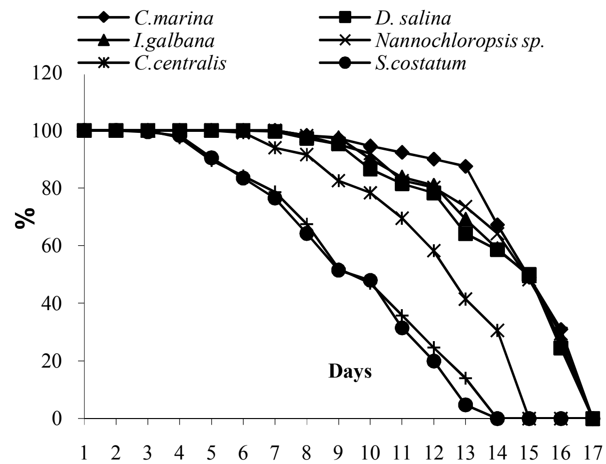

Fig. 2 Effect of high algal food concentration on survival of *Nannocalanus minor*

Effect of algal concentration on egg production of *N. Minor*

The egg production rate increased with increasing food concentration (Table 3). The utmost mean egg production (32±1.52 eggs/female/day) was achieved with the maximum algal cell concentration (20,000 cells/ml) while the lowest (3±1 eggs/female/day) egg production was noticed in lowest algal concentration of 1000cells/ml. Algal food concentration was positively correlated with egg production of copepod (r value = 0.97217). One way ANOVA between algal concentration and egg production was found highly significant with F Value 0.056318 (Table 4).

Effect of algal concentration on egg hatching of *N. minor*

The lowest algal cell concentration (1000cells/ml) results the lowest hatching (44.33%) whereas at high cell concentration (20,000cells/ml.) it was found as 93.75% (Table 3) as evidenced by correlation matrix where the correlation between hatching rate and algal food concentration found to be highly significant (P) with 'r' value of 0.98776.

Discussion

In our experiment, the survival of copepod *N. minor* was found to increase with increased algal

Table: 1 Influence of algal diets on growth (mm) of *Nannocalanus minor* Nauplii Stages

Rearing period in hours	Stages	*C.marina*	*D.salina*	*I.galbana*	*Nannochloropsis* sp.	*C.centralis.*	*S.costatum.*	*C.affinis*
24	NI	0.107±0.002	0.096±0.005	0.104±0.004	0.107±0.001	0.094±0.003	0.094±0.002	0.096±0.002
0.4	NII	0.127±0.001	0.107±0.001	0.113±0.003	0.118±0.001	0.106±0.002	0.108±0.013	0.106±0.002
0.42	NIII	0.136±0.002	0.118±0.003	0.125±0.004	0.127±0.003	0.115±0.004	0.115±0.003	0.116±0.003
0.34	NIV	0.155±0.004	0.139±0.001	0.145±0.003	0.155±0.004	0.129±0.004	0.124±0.004	0.126±0.003
0.39	NV	0.179±0.005	0.167±0.003	0.168±0.001	0.181±0.007	0.149±0.001	0.148±0.003	0.155±0.005
25	NVI	0.275±0.021	0.208±0.012	0.201±0.013	0.24±0.036	0.188±0.001	0.188±0.005	0.191±0.004

Table: 2 Influence of algal diets on growth (mm) of *Nannocalanus minor* Copepodite stages

Rearing period in hours	Stages	*C.marina*	*D.salina*	*I.galbana*	*Nannochloropsis* sp.	*C.centralis.*	*S.costatum.*	*C.affinis*
24.67	CI	0.427±0.009	0.394±0.003	0.414±0.005	0.456±0.041	0.384±0.013	0.39±0.003	0.383±0.012
30.19	CII	0.55±0.026	0.445±0.006	0.481±0.025	0.527±0.010	0.449±0.013	0.440±0.015	0.472±0.032
36.34	CIII	0.789±0.005	0.652±0.016	0.743±0.044	0.768±0.018	0.671±0.016	0.66±0.015	0.668±0.017
38.46	CIV(♀)	0.944±0.004	0.894±0.001	0.94±0.03	0.946±0.020	0.916±0.025	0.888±0.002	0.906±0.012
	CIV(♂)	0.931±0.006	0.820±0.005	0.906±0.005	0.921±0.006	0.813±0.005	0.833±0.020	0.847±0.034
60	CV(♀)	1.563±0.020	1.42±0.045	1.493±0.085	1.526±0.035	1.366±0.050	1.28±0.01	1.4±0.07
	CV(♂)	1.266±0.075	1.046±0.041	1.15±0.026	1.176±0.025	0.988±0.002	0.983±0.003	0.987±0.003
60	Adult (♀)	1.963±0.037	1.936±0.015	1.933±0.005	1.946±0.055	1.936±0.035	1.906±0.015	1.953±0.020
	Adult (♂)	1.756±0.030	1.74±0.02	1.75±0.01	1.873±0.100	1.743±0.040	1.743±0.04	1.74±0.036

Note: ♀- Female; ♂- Male

concentration and decrease with decreased algal diets concentration. In low algal concentration, the copepod survival was found to be low might be due to the lack of food. As it could be easily understood, because of the insufficient food supply the copepod cannot showing further metabolism and survival, so that the species become to sudden mortality. However the high survival was observed in high algal concentration could be attributed to the availability of required amount of food as agreed by Luis [14]. The obtained realistic variations in the survival of *N. minor* with different algal feed could be related to the morphology of microalgae used [15]. Presently, high survival was noticed in *C.marina,* which might be due to the favorable size and nutritional status of the prey [16, 17, 14]. Assimilation efficiency of *N. minor* was also comparatively higher in high algal diet concentration besides *C.marina* algal type because of its efficiency capacity [18]. The lowest survival was noticed in *S.costatum* could be attributed to the less consuming capability of copepod on chain forming diatoms and also the mouth parts of copepod are not facilitating the capture of larger food organisms and therefore presently least survival was observed in *S.costatum* as agreed earlier by Perumal *et al.*[19]; Castro and Santos [11]; Santhanam and Perumal [15] and Santhanam *et al.*[20].

In the present experiment, the growth of copepod *N. minor* was affected by different algal foods. The maximum growth in copepod was achieved in *C.marina* while the least growth was obtained at *S.costatum*. It is clear that the food limitation may effectively act as a filter for small copepods [21]. The size and structure of algae might be a reason for slow growth noticed at *S.costatum*. The chain forming nature and larger size of *S.costatum* might not suitable for *N. minor*. Therefore slow growth was procured in copepod as reported earlier by some workers [22-24].

Table: 3 Effect of mixed microalgal concentration on egg production and hatching of *Nannocalanus minor*

Algal concentration (Mixed algae) (cells/ml)	**Fecundity rate Eggs/female/ day**	**Hatching rate Nauplii/female/ day**	**Hatching (%)**
1000	3±1.0	1.33±0.57	44.33
5000	14±1.41	8±1.41	57.14
10000	22±1.41	19±0	86.36
20000	32±1.52	30±0.577	93.75

The egg production and hatching succession in *N. minor* was found to be highly significant with algal concentration with the ‘r’ values of 0.97217 and 0.98776 respectively. Different concentration of algal food results the unusual egg production in copepods [23]**.** The high algal concentration results the more egg production (32±1.52) while at the low algal cell concentration (1000 cells/ml) copepod produces the least eggs (3±1 eggs/female/day). Our result is similar to the findings of Nival *et al*. [25] who stated that the calanoid copepod, *Centropages typicus* did not lay eggs at 500 cells/ml of algal concentration its due to all of the available energy being used for survival.

Table: 4 Correlation coefficient (r) values between algal concentration and fecundity of *Nannocalanus minor*

Parameter	Egg production	Hatching rate
Algal concentration	0.97217*	0.98776*

Above 5000 cells/ml. algal concentration was enough for metabolic and egg production in *Centropages* sp. In our study, egg lying was started at 1000 cells/ml but in the case of *Centropages* sp. the egg laying was started at 5000 cells/ml. From these results, it could be inferred that *N. minor* is most suitable to culture and other physiological monitoring. Williams and Jones [26] described that the offspring production declined when the algal concentration is lower than the optimal level. The feeding history had a strong influence on egg production rate, which was much higher in females of *Calanus finmarchicus* exposed to different feeding conditions [27]. Results observed by Runge [28] and Kimoto *et al.* [29] indicate larger clutches was gained at high food concentrations in *C.finmarchicus* and *Sinocalanus tenellus* respectively. Apart from food concentration, the quality is also an important for reproductive success of copepods [30]. The factors such as particle size and species composition of food also influenced the egg production [31-32]. Arnaud *et al.* [33] suggested that the nutritive value of food also controlled the clutch and egg production of copepod. The reproductive success of copepods not only dependent on egg production rate, but also on egg hatching rate, which may not be affected by food quality in the similar mode as egg production [34]. The insufficient food supply might be probably the reason for low hatching rate obtained presently in *N. minor* as agreed by Burkhart and Kleppel [35]; Koski *et al.*[36] and Genuario and Anna [37].

Conclusion

The study provides a realistic basis for formulating ecological principles that govern food chains in the coastal and marine systems. The experiment also indicates that the levels of food concentrations and type of suitable food required for copepod *N. minor* at different trophic levels. From the study, it could be understood that the egg production increased with increasing food concentration. This experiment suggests that the food selectivity and feeding regimes may vary from the individual's grazing in relation to the availability of food and food size. Only few algal cultures serves as the best nutritional supplement to the copepods and their size, morphology plays a role in feeding strategies. Hence, to make aware of the feeding selectivity of the calanoid copepod *N. minor*, detailed experiments on all these aspects are highly important.

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