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, *C. 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 its 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 play an important role in the energy transfer between primary producers and pelagic fish populations, and it is thus a key factor influencing fish production. 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. Furthermore, copepods provide 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. 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.

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. Decrease in food concentration resulted in decrease in the reproductive success of a copepod. 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 confirmation 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°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 20,000 cells/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°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 at 20-23°C temperature, 30‰ salinity and 7000-9000 light intensity (lux) fertilized with Conway’s medium or Walne’s medium 13.

The daily mortality of copepod was recorded carefully. The experimental sets were maintained at 28±1°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.

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

<table>
<thead>
<tr>
<th>Rearing period in hours</th>
<th>Stages</th>
<th>C.marina</th>
<th>D.salina</th>
<th>Igalbana</th>
<th>Nannochloropsis sp.</th>
<th>C.centralis.</th>
<th>S.costatum.</th>
<th>Caffinis</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>NI</td>
<td>0.107±0.002</td>
<td>0.096±0.005</td>
<td>0.104±0.004</td>
<td>0.107±0.001</td>
<td>0.094±0.003</td>
<td>0.094±0.002</td>
<td>0.096±0.002</td>
</tr>
<tr>
<td>0.4</td>
<td>NIH</td>
<td>0.127±0.001</td>
<td>0.107±0.001</td>
<td>0.113±0.003</td>
<td>0.118±0.001</td>
<td>0.106±0.002</td>
<td>0.108±0.013</td>
<td>0.106±0.002</td>
</tr>
<tr>
<td>0.42</td>
<td>NII</td>
<td>0.136±0.002</td>
<td>0.118±0.003</td>
<td>0.125±0.004</td>
<td>0.127±0.003</td>
<td>0.115±0.004</td>
<td>0.115±0.003</td>
<td>0.116±0.003</td>
</tr>
<tr>
<td>0.34</td>
<td>NIV</td>
<td>0.155±0.004</td>
<td>0.139±0.001</td>
<td>0.145±0.003</td>
<td>0.155±0.004</td>
<td>0.129±0.004</td>
<td>0.124±0.004</td>
<td>0.126±0.003</td>
</tr>
<tr>
<td>0.39</td>
<td>NV</td>
<td>0.179±0.005</td>
<td>0.167±0.003</td>
<td>0.168±0.001</td>
<td>0.181±0.007</td>
<td>0.149±0.001</td>
<td>0.148±0.003</td>
<td>0.155±0.005</td>
</tr>
<tr>
<td>25</td>
<td>NVI</td>
<td>0.275±0.021</td>
<td>0.208±0.012</td>
<td>0.201±0.013</td>
<td>0.24±0.036</td>
<td>0.188±0.001</td>
<td>0.186±0.005</td>
<td>0.191±0.004</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Rearing period in hours</th>
<th>Stages</th>
<th>C.marina</th>
<th>D.salina</th>
<th>Igalbana</th>
<th>Nannochloropsis sp.</th>
<th>C.centralis.</th>
<th>S.costatum.</th>
<th>Caffinis</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.67</td>
<td>CI</td>
<td>0.427±0.009</td>
<td>0.394±0.003</td>
<td>0.414±0.005</td>
<td>0.456±0.041</td>
<td>0.384±0.013</td>
<td>0.39±0.003</td>
<td>0.383±0.012</td>
</tr>
<tr>
<td>30.19</td>
<td>CII</td>
<td>0.55±0.026</td>
<td>0.445±0.006</td>
<td>0.481±0.025</td>
<td>0.527±0.010</td>
<td>0.449±0.013</td>
<td>0.440±0.015</td>
<td>0.472±0.032</td>
</tr>
<tr>
<td>36.34</td>
<td>CIII</td>
<td>0.789±0.005</td>
<td>0.652±0.016</td>
<td>0.743±0.044</td>
<td>0.768±0.018</td>
<td>0.671±0.016</td>
<td>0.661±0.015</td>
<td>0.668±0.017</td>
</tr>
<tr>
<td>38.46</td>
<td>CIV(♂)</td>
<td>0.944±0.004</td>
<td>0.894±0.001</td>
<td>0.94±0.03</td>
<td>0.946±0.020</td>
<td>0.916±0.025</td>
<td>0.888±0.002</td>
<td>0.906±0.012</td>
</tr>
<tr>
<td>60</td>
<td>CV(♀)</td>
<td>1.563±0.020</td>
<td>1.42±0.045</td>
<td>1.493±0.085</td>
<td>1.526±0.035</td>
<td>1.366±0.050</td>
<td>1.28±0.01</td>
<td>1.4±0.07</td>
</tr>
<tr>
<td>60</td>
<td>CV(♂)</td>
<td>1.266±0.075</td>
<td>1.046±0.041</td>
<td>1.15±0.026</td>
<td>1.176±0.025</td>
<td>0.988±0.002</td>
<td>0.983±0.003</td>
<td>0.987±0.003</td>
</tr>
<tr>
<td>60</td>
<td>Adult(♀)</td>
<td>1.96±0.037</td>
<td>1.936±0.015</td>
<td>1.933±0.005</td>
<td>1.946±0.055</td>
<td>1.936±0.035</td>
<td>1.906±0.015</td>
<td>1.953±0.020</td>
</tr>
<tr>
<td>60</td>
<td>Adult(♂)</td>
<td>1.75±0.030</td>
<td>1.74±0.02</td>
<td>1.75±0.01</td>
<td>1.873±0.100</td>
<td>1.743±0.040</td>
<td>1.743±0.04</td>
<td>1.74±0.036</td>
</tr>
</tbody>
</table>

Note: ♀- Female; ♂- Male

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Egg production</th>
<th>Hatching rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algal concentration</td>
<td>0.97217*</td>
<td>0.98776*</td>
</tr>
</tbody>
</table>

Above 5000 cells/ml. algal concentration was enough for metabolic and egg production in Centropages sp. In our study, egg laying 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 Sinocalamus 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 Klepel 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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