Artemia sp. biomass production using three different microalgae (Pinnularia sp., Porphyridium sp., and Dunaliella sp.) with yeast supply

Castro MJ, Castro MG, Flores GAF, Tinoco LPI, Salvat NKA

DOI: https://doi.org/10.22271/fish.2023.v11.i5c.2864

Abstract
Artemia plays an important role for fish and crustacean fed. The Artemia culture system was made in 160 L culture medium at 60 gL\(^{-1}\) salinity. Three experimental diets were made: a) 6 L Pinnularia sp; b) 6 L Porphyridium sp.; and c) 6 L Dunaliella sp. Growth curves were obtained from each experiment. A Life Table was made from each one to obtain the reproductive potential. The highest density value was found in the Dunaliella sp. diet with 85928 ± 233 org 160 L-1, Ro value with 42.25. The lowest density value was obtained with Porphyridium sp. diet con cero value until 39 culture days. Their Ro value was 8.62. The Tc values were similar to the Dunaliella sp. and Pinnularia sp. diet in comparison with the Porphyridium sp. diet. This experiment shows that is necessary to make mixed diets between Pinnularia and Dunaliella to obtain better results of organism densities. The Porphyridium sp. diet cannot be used as the only diet source for Artemia. We suggest that is necessary for Porphyridium sp. diet supply with brown or green microalgae from 21 culture days to complement the nutritional value of the food diet to obtain the biggest rates of survival.

Keywords: Artemia, Pinnularia, Dunaliella, Porphyridium, Bio-mass

1. Introduction
Zooplankton culture was a bottleneck for all aquaculture projects, principally for good management of crustacean larval and fry fish stages. One of these zooplankton groups was the resource Artemia sp. which is widely distribution in many hypersaline waters reservoirs or was introduced, in most cases successfully, allowing the establishment of new populations that were commercially exploited \(^1\). Actually, not only the nauplius stages were used, but also juvenile, preadult, and adult stages were used as biomass resources to feed fishes and crustaceans. This organism is important because it can supply the highest energetic value through the supplied polyunsaturated fatty acids which itself contain or can be incorporated by the bioencapsulation process during all their life cycle stages \(^2, 3, 4, 5, 6\).

In another way, Artemia can be cultured easily because of their high salinity tolerance until 300 g L\(^{-1}\). It is a wide-range oxygen-tolerant organism because it can resist concentrations below 1 mgL\(^{-1}\) of dissolved oxygen until saturation range. It is a non-selective filter organism because it can feed organic matter with proper size (< 50µm) and microalgae like Dunaliella sp. (green microalgae) which contributes with protein and pigments content, as same as Pinnularia sp. (brown microalgae) which contributes with carbohydrates and fatty acids content, and Porphyridium sp. (red microalgae), which contributes with minerals like iron. These microalgae were easy to produce in laboratory conditions, but Porphyridium sp. microalgae did not have as many research studies as Porphyridium sp. and Dunaliella sp. \(^1, 2, 7, 8\) and yeast supply which contributes with B12 vitamin.

Corresponding Author
Castro MJ
Universidad Autónoma Metropolitana
Unidad Xochimilco, Life Food and Biofloc production laboratory, Depto. El Hombre y su Ambiente, División de Ciencias Biológicas y de la Salud, Calz. del Huaso No. 1100, Col. Villa Quitud. Ciudad de México, 04960, Alcaldía Coyuacán, México

Salvat NKA
Universidad Autónoma Metropolitana
Unidad Xochimilco, Life Food and Biofloc production laboratory, Depto. El Hombre y su Ambiente, División de Ciencias Biológicas y de la Salud, Calz. del Huaso No. 1100, Col. Villa Quitud. Ciudad de México, 04960, Alcaldía Coyuacán, México

Corresponding Author
Castro MJ
Universidad Autónoma Metropolitana
Unidad Xochimilco, Life Food and Biofloc production laboratory, Depto. El Hombre y su Ambiente, División de Ciencias Biológicas y de la Salud, Calz. del Huaso No. 1100, Col. Villa Quitud. Ciudad de México, 04960, Alcaldía Coyuacán, México
2. Materials and Methods

2.1 Microalgae culture

The three microalgae used in this experiment were from the Life Food Production Laboratory at Universidad Autónoma Metropolitana-Xochimilco, México cultured in a Petri dish with bacteriological agar. They were inoculated in a 500 mL container for growth and then, inoculated in a 10 L container with fertilizer. The microalgae culture media was maintained in continuous light and aeration [9, 10, 11].

2.2 Artemia cysts

Six grams of Artemia sp. cysts were decapsulated and in three 10 L containers and hatched nauplii were fed with rice bran for three days. All meta nauplii stages were collected and introduced in a 200 L plastic container.

2.3 Experimental design

The Artemia culture was made in 200 L plastic containers, one for each experimental treatment. The plastic containers were filled with 160 L of saline water (60 g L⁻¹), with continuous light and aeration, at 24±2°C, and a pH value between 7 to 8, for 60 days. Six liters of microalgae were added to plastic containers. The three experimental diets were: a) Pinnularia sp; b) Porphyridium sp; and c) Dunaliella sp. Also, was added to each treatment 40 mL of yeast (2 g 1000 mL⁻¹ of salty water) once a week (Fig. 1).

2.4 Density sampling

Each container was inoculated with 20 g of Artemia sp. adult biomass and every third day a sample of 1 L was obtained to take 10 subsamples to make a total density counting to obtain a mean value and extrapolate to 160 L.

2.5 Processing data

All data were introduced in an Excel 2019 database to obtain descriptive statistical processes to obtain de growth curves. A Life Table was made to obtain the reproductive potential with the following formulas.

Reproduction rate (Ro)

\[ Ro = \sum^{n}_{i=0} lx * mx \]

Where

- \( lx \) = Survival proportion of organisms produced in each sampling phase
- \( mx \) = Organisms produced per each survival female in each sampling phase

Cohort generation time (Tc)

\[ Tc = \sum x * \frac{lx * mx}{Ro} \]

Where

- \( x \) = sampling phase
- \( lx \) = Survival proportion of organisms produced in each sampling phase
- \( mx \) = Organisms produced per each survival female in each sampling phase
- \( Ro \) = Reproduction rate

Instantaneous growth rate = \( r \)

\[ r = \frac{\log_e Ro}{Tc} \]

Where

- \( Ro \) = Reproduction rate
- \( Tc \) = Cohort generation time

3. Results

Table 1 shows density organisms per each experimental treatment and Fig.2 shows growth curves with each formula. (Insert Table 1)

Table 1 shows the highest density was found in Dunaliella sp. treatment with 85928±233 org 160 L⁻¹, meanwhile, the lowest density was found in Porphyridium sp. treatment because of the organisms’ dye at 39 culture days. Between Dunaliella sp. and Pinnularia sp. treatment showed significant differences (p<0.05) in density value.

Table 2 shows the potential reproductive values obtained per each treatment. It can be observed that Dunaliella sp. treatment obtained the highest values with respect Ro with 42.25 org female⁻¹, and the lowest value was found with Porphyridium sp. treatment with only 8.62 org female⁻¹. Tc and r values with Dunaliella sp. and Pinnularia sp. treatments were close concerning Porphyridium sp. treatment. (Insert table 2)

Concerning produced biomass per each experimental treatment, Dunaliella sp. and Pinnularia sp. diets show values of 859.28 g and 796.7 g respectively, Porphyridium sp. diets only reach 72.087 g. Porphyridium sp. biomass value was obtained with 24 culture day value.

4. Discussion

At Artemia sp. culture system was used as diet live microalgae, dry microalgae, yeast, industries food sub-products like rice bran, wheat bran, and soy pellets [12,13,14,15], but live microalgae were found as better fed source because they support better survival and growth rates as was mentioned in Dhont and van Stappen [13] research, according with our results with Pinnularia sp. with a production of 2,347 org 160L⁻¹, and Dunaliella sp. diet with 2,031 org 160 L⁻¹.

According to [7] mentioned that survival rates in Artemia sp. culture with Dunaliella sp. and Chaetoceros sp. diets were above 66±1%. According to [16] mentioned that Artemia sp. cultured with Dunaliella sp. reach the adult stage early with respect Chaetoceros sp. and Spirulina sp. monoculture diets. According to [17] evaluated the culture system of Artemia sp. using different live microalgae and concluded that the use of Dunaliella bardwill obtain better results. These authors mentioned that Porphyridium cruentum microalgae can be used to feed Artemia sp. but, our results showed better results only until 24 culture days were reached with only 202 org 160L⁻¹ until they dye at 39 culture days.

Although this research wanted to observe Artemia sp. growth at different mono-cultured live microalgae like Pinnularia, Dunaliella, and Porphyridium, and supply with yeast which was rich with vitamin B12 complex, it is important to mention that [18], which work with two different fed types of mixed diets Tetraselmis suecica and Dunaliella salina diets, both with wheat bran. Better results were shown in Tetraselmis suecica and wheat bran. Also, according to [19,20] suggest the use of Pinnularia sp. mixed with Tetraselmis sp. for biomass production in the laboratory with Artemia franciscana.
According to [21] mentioned when a mix of green and brown microalgae was used it can obtain better results, because green microalgae supply proteins and pigments, and brown microalgae supply carbohydrates and fatty acids. According to [19, 20] who worked with a mixed diet of *Isochrysis galbana* and *Tetraselmis suecica*, mentioned better results when they used it at the same proportion (1:1). According to [22, 23] mentioned that wall cells of microalgae were different and the juvenile stages of *Artemia* sp., in many cases, cannot ingest rigid cell walls and the growth process can be affected not only by the microalgae size, but for his compositions and structural cell walls too. Perhaps, this condition occurred with *Porphyridium* sp. diet.

Another variable that many authors according were about *Artemia* sp. survival and culture density growth was the supply of microalgae culture growth phase as food for this crustacean. This important variable is according to [24] work, which use seven different marine microalgae species at three different growth phase stage, and the study according to [9] which evaluate the growth of different larval stages of *Artemia franciscana* fed with two different proportion of microalgae. They concluded that using the microalgae stage in their exponential phase influences *Artemia* sp. growth and survival because, in different stages of the culture microalgae phase, they produced different concentrations of proteins, fatty acids, carbohydrates, and lipids. This condition was maintained in this experiment. The microalgae culture was harvested every third day to maintain the microalgae cultures in this exponential phase.

![Fig 1: Experimental design of *Artemia* sp. culture at three experimental treatments.](image1)

![Fig 2: Growth density curves of cultured *Artemia* sp. experimental treatment.](image2)
Table 1: Organisms density of *Artemia* sp. produced per each experimental treatment.

<table>
<thead>
<tr>
<th>Culture days</th>
<th><em>Dunaliella</em></th>
<th><em>Pinnularia</em></th>
<th><em>Porphyridium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2000±31</td>
<td>2000±83</td>
<td>2000±77</td>
</tr>
<tr>
<td>3</td>
<td>1412±68</td>
<td>2940±31</td>
<td>13039±60</td>
</tr>
<tr>
<td>6</td>
<td>2315±79</td>
<td>4877±80</td>
<td>4537±89</td>
</tr>
<tr>
<td>9</td>
<td>2843±40</td>
<td>5998±75</td>
<td>1137±100</td>
</tr>
<tr>
<td>12</td>
<td>3134±74</td>
<td>6486±42</td>
<td>994±95</td>
</tr>
<tr>
<td>15</td>
<td>3322±19</td>
<td>6527±25</td>
<td>2579±29</td>
</tr>
<tr>
<td>18</td>
<td>3542±49</td>
<td>6308±51</td>
<td>4684±56</td>
</tr>
<tr>
<td>21</td>
<td>3930±20</td>
<td>6013±22</td>
<td>6418±40</td>
</tr>
<tr>
<td>24</td>
<td>4622±91</td>
<td>5828±57</td>
<td>7209±126</td>
</tr>
<tr>
<td>27</td>
<td>5753±119</td>
<td>5938±107</td>
<td>6803±80</td>
</tr>
<tr>
<td>30</td>
<td>7459±227</td>
<td>6529±132</td>
<td>5265±92</td>
</tr>
<tr>
<td>33</td>
<td>9875±145</td>
<td>7786±102</td>
<td>2979±150</td>
</tr>
<tr>
<td>36</td>
<td>13136±166</td>
<td>9894±112</td>
<td>646±158</td>
</tr>
<tr>
<td>39</td>
<td>17379±166</td>
<td>13040±112</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>22738±168</td>
<td>17408±113</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>29349±173</td>
<td>23184±169</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>37348±174</td>
<td>30553±188</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>46869±181</td>
<td>3970±201</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>58050±205</td>
<td>50812±203</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>71024±207</td>
<td>64074±209</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>85928±233</td>
<td>79670±211</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Organisms production values of *Artemia* sp. per each experimental treatment.

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Reproduction rate (Ro)</th>
<th>Cohort generational time (Te)</th>
<th>Instantaneous growth rate (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dunaliella</em> sp.</td>
<td>42.25</td>
<td>15.24</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Pinnularia</em> sp.</td>
<td>39.18</td>
<td>15.36</td>
<td>0.23</td>
</tr>
<tr>
<td><em>Porphyridium</em> sp.</td>
<td>8.62</td>
<td>21.90</td>
<td>0.13</td>
</tr>
</tbody>
</table>

5. Conclusions

Although *Artemia* sp. culture density growth was obtained with microalgae *Pinnularia* and *Dunaliella*, it is necessary to make mixed diets between them to obtain better results at final density and biomass. *Porphyridium* sp. microalgae cannot be used as a mono-culture food source in the culture system after 21 culture days. It is necessary to apply other microalgae like green or brown. To supply nutritional value.

6. References


