GROWTH OF THE BRINE SHRIMP ARTEMIA FED ON OFFSPRING OF THE COPEPOD TISBE BIMINIENSIS VOLKMANN-ROCCO, 1973

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Abstract - Artemia nauplius is used as food for rearing shrimp and fish larvae. This study investigated the growth of Artemia fed on the copepod Tisbe biminiensis offspring (nauplii and copepodite 2), compared to those fed on the microalgae Thalassiosira fluviatilis. Artemia nauplii (45 individuals) were stocked in vessels containing 500 mL of seawater and 34 salinity. The vessels were maintained at 28°C, 13 h light/11 h dark photoperiod and provided with aeration. The culture period was 11 days. The Artemia were fed on microalgae (10,000 cells.mL⁻¹) for four days. On the 5th day two diets were supplied to the Artemia: copepod offspring (10 copepod.mL⁻¹) and microalgae (20,000 cells.mL⁻¹). No significant difference was found in mean survival between diets. The final length of the Artemia fed with copepod diet was significantly higher than Artemia fed with the microalgae diet. The final dry weight of the Artemia did not differ significantly between diets. It can be concluded that T. biminiensis offspring is an alternative food for the culture of Artemia.

Keywords: Harpacticoid copepod, culture, food, live prey

Resumo - Náuplios de artemia é um alimento usado no cultivo das larvas de camarões e peixes. Este estudo comparou o crescimento desses animais alimentados com larvas do copépodo Tisbe biminiensis, composta por náuplios e copepoditos 2, e com a alga Thalassiosira fluviatilis. Náuplios de Artemia (45 indivíduos) foram colocados em frasco contendo 500 mL de água do mar numa salinidade de 34. Os fracos foram mantidos a temperatura de 28 ºC, fotoperíodo de 13 h luz/11 h escuro e com aeração constante. O tempo de cultivo foi de 11 dias. As Artemia foram alimentadas com alga (10.000 cel.mL⁻¹) por quatro dias. No 5º dia, as Artemia foram submetidas à dieta com a prole do copépodo (10 copepodo/mL) e com alga (20.000 cel.mL⁻¹). A sobrevivência média das Artemia não foi significativamente diferente entre as dietas. O comprimento final das Artemia na dieta com as larvas do copépodo foi significativamente maior do que com alga. O peso seco final das Artemia não foi significativamente diferente entre as dietas. Conclui-se que as fases larvais do copépodo T. biminiensis é uma fonte alternativa de alimento para as Artemia.

Palavras-Chave: copépodo Harpacticoida, cultivo, alimento, presa viva
INTRODUCTION

Nauplii and adults of *Artemia* are the most used live food for larval stages of reared crustaceans and fishes. This is due to its nutritional value, size and simple technology for stocking cysts as well as the production of nauplii (Gilbert, 1996). Natural population of *Artemia* have thus far supplied the quiescent cysts for aquaculture purposes. However, over-exploitation of cyst stocks formed by the natural population of *Artemia* may result in a future problem for the supply of cysts (Evjemo & Olsen, 1999).

*Artemia* is a non-selective and filter feeder. Microalgae are the commonly used food for *Artemia*. Some substitutes for microalgae have been used as bacteria (Intriago & Jones, 1993), rice bran, corn bran and soybean pellets lactoserum (Dhont & Lavens, 1996). However, these substitutes for microalgae do not always have a consistent nutritional value (Naegel, 1999). Harpacticoid copepods are a potential food to be used in the *Artemia* cultures because they have a high content of essential fatty acids that are important for fish and crustacean growth and development (Fleeger, 2005; Lima 2011).

This study compared the growth and survival of *Artemia* fed on *Tisbe biminiensis* Volkmann-Rocco, 1973 copepod offspring to those fed only on microalgae *Thalassiosira fluviatilis*.

MATERIAL AND METHODS

MICROALGAE CULTURE

*Thalassiosira fluviatilis* diatoms were cultivated in f/2 medium (Guillard, 1975). For medium preparation, natural filtered seawater (25 and 3 µm) was used at salinity 35. Tris-HCl buffer (25% v/v and pH = 7.8) and f/2 nutrients (Table 1) were added before medium sterilization in an autoclave for 15 min at 121°C. After medium sterilization, biotin, B12 and thiamine were added just before algal inoculation. These vitamins were sterilized by filtration (0.2 µm) beforehand. The microalgae culture was incubated at 24-27°C with a 12 h light/11 dark photoperiod.

COPEPOD CULTURE

The copepod *T. biminiensis* has been reared in laboratory in 500 mL or 5L vessels with seawater at a salinity 31-33, filtered through 25 µm and 3 µm. Culture was maintained at 29-31 °C, a 13h light/11h dark photoperiod and with aeration (Souza-Santos et al., 2006). Copepods were fed on the diatom *T. fluviatilis* and commercial fish food (Alcon Basic®). Every other day, the seawater was completely changed. The collection of the offspring was carried out with a couple of sieves with 63 µm and 250 µm meshes. The 250 µm sieve retained adult copepods and egg-bearing females, while the 63 µm sieve retained nauplii and copepodites, that were used as food for *Artemia*.
Table 1. Composition of the f/2 medium (Guillard, 1975) for one litre seawater.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>75 mg (883 µM)</td>
</tr>
<tr>
<td>NaH₂PO₄·H₂O</td>
<td>5 mg (36.3 µM)</td>
</tr>
<tr>
<td>NaSiO₃·9H₂O</td>
<td>30 mg (107 µM)</td>
</tr>
<tr>
<td>Traces metals</td>
<td></td>
</tr>
<tr>
<td>Na₂·EDTA+</td>
<td>4.36 mg (11.7 µM)</td>
</tr>
<tr>
<td>FeCl₃·6H₂O+</td>
<td>3.15 mg (11.7 µM)</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.01 mg (0.04 µM)</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.022 mg (0.08 µM)</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.01 mg (0.05 µM)</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.18 mg (0.9 µM)</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>0.006 mg (0.03 µM)</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Tiamina</td>
<td>0.01 µg</td>
</tr>
<tr>
<td>Biotina</td>
<td>0.5 µg</td>
</tr>
<tr>
<td>B12</td>
<td>0.5 µg</td>
</tr>
</tbody>
</table>

Artemia Culture

Cysts of *Artemia* were hydrated for 1h in freshwater with aeration. The cysts were then transferred to a beaker with seawater at a salinity 37, at 28 ± 1°C, and with aeration for 24h. *Artemia* nauplii (60 individuals) that hatched were stocked in conic bottom plastic flasks containing 500 mL of seawater at 34 ± 1 salinity. Ten flasks were used and they were maintained at 28 ± 1°C, 13 h light/11 h dark and provided with aeration.

During the first four days of culture, *Artemia* nauplii were fed on the diatom *T. fluviatilis* at a concentration of 10,000 cells.mL⁻¹. On the 5th day of culture, 70% of the seawater was renewed and 6 *Artemia* nauplii were collected in each flask in order to measure initial length and weight. Length was measured under ocular micrometer stereomicroscope. In order to obtain the dry weight, two or three groups of 23 to 28 *Artemia* were put in aluminium envelopes, which were previously weighed on an analytical balance (0.0001 g). These envelopes with *Artemia* were then dried at 60°C for 24 h and weighed again. The *Artemia* weight was calculated using the equation: weight of *Artemia* on the 4th day of culture – weight of *Artemia* on the 11th day of culture.

On the 5th day of culture, *Artemia* was submitted to two different diets each with 5 replicates, and containing 45 *Artemia: T. biminiensis* copepod offspring, composed of nauplii and copepodite in the proportion of 23.7% and 76.3%, respectively, and other diet diatom *T. fluviatilis*. Every other
day, the copepod offspring group was offered randomly at a density of 10 copepod mL\(^{-1}\), while the diatom group was offered a daily density of 20,000 cells mL\(^{-1}\).

Every other day, about 70\% of the seawater in the experimental flasks was renewed using a 125 µm mesh. At the end of the culture of 11 days, Artemia survival was observed. The animals were then preserved in formalin 4\% v/v for further measurement of their length and weight.

**STATISTICAL ANALYSIS**

To compare the mean final survival, length and dry weight of *Artemia* the ANOVA was used, after testing normality of data (Kolmogorov-Smirnov Test) and variance homogeneity (Cochran’s C Test). The non-parametric test of Kruskal-Wallis was used when the data was not normal or variance was not homogenous. The significance level was 0.05 for all tests (Satatgraphics; Zar, 1999).

**RESULTS**

On the 11\(^{th}\) day, all the *Artemia* had metamorphosed into juveniles. The mean survival was 51\% ± 23.84 for those on the microalgal diet and 47\% ± 17.55 for the copepod offspring diet; there was no significant difference between both diets (ANOVA, P>0.05).

At the end of the experiment, the length of *Artemia* was significantly higher with copepod offspring diet (4.40 ± 0.39 mm) in comparison with microalgal diet (3.80 ± 0.38 mm) (Kruskal-Wallis, P<0.05) (Figura 1).

The final dry weight had no significant difference between diets (ANOVA, P>0.05). The mean final dry weight of the *Artemia* was 177 ± 13.7 µg for the copepod offspring diet and 151.2 ± 40.4 µg for the microalgal diet (Figure 2).

**Figure 1.** Length (mm) of *Artemia* (■, n=52) fed microalgae *Thalassiosira fluviatilis* during 1\(^{st}\) to the 5\(^{th}\) day of culture. Length of *Artemia* fed microalgae *T. fluviatilis* (■, n=85) or *Tisbe biminiensis* copepod offspring (■, n=83) during 6\(^{th}\) to the 11\(^{th}\) day of culture. Different letters indicate significant difference (Kruskal-Wallis, P<0.05).
Figure 2. Dry weight (µg) of Artemia fed microalgae Thalassiosira fluviatilis (□, n=52) during 1st to the 5th day of culture. Dry weight of Artemia fed microalgae T. fluviatilis (■, n=85) or the Tisbe biminiensis copepod offspring (■, n=83) during 6th to the 11th day of culture (ANOVA, P>0.05).

Discussion

The survival obtained in this study after 11 days of culture was lower than the survival obtained for the A. franciscana which was 75-93% (Lora-Vilchis et al., 2004), for the A. saline which was 84-68% (Sick, 1976) and for the Artemia which was 80% (Naegel, 1999). In all these studies, Artemia were submitted to diets of microalgae and the total water exchanges were daily. This low survival rate can be explained by the low rate of seawater renewal in culture (70% every other day), which probably promoted the decrease of seawater quality (Hoff & Snell, 2004).

The final length reached by Artemia on the copepod diet was greater than with the microalgal diet. This result indicated the T. biminiensis copepod offspring can sustain Artemia growth. This fact may be explained by the high nutritional value of T. biminiensis copepod, and it could be related to the known capacity of some species of Tisbe to synthesize highly unsaturated fatty acids (Nanton & Catell, 1998). The Artemia lack certain essential polyunsaturated fatty acids (Léger et al., 1987). To supply this deficiency, they were often enriched with polyunsaturated fatty acids (Barreto & Cavalcanti, 1997; Hoff & Snell, 2004).

The final length of Artemia were similar to those reported to A. saline fed on Chlamydomonas sphagnicola, Nitzschia closterium, Platymonas elliptica and Dunaliella viridis after 12 days of culture (Sick, 1976), Artemia fed on Chaetoceros after 11 days of culture (Naegel, 1999) and A. franciscana fed on Isochrysis on the 6th day. However, A. franciscana fed on Chaetoceros muelleri (1,100 x 10³ cells.mL⁻¹) reached a larger length of 6 mm (Lora-Vilchis et al., 2004) than in this study, which can be explained by different culture regimes and supplied microalgae species.
The dry weight of Artemia in this study was not different to that reported by Evjemo & Olsen (1999) as 195 µg for A. franciscana reared for 11 days using Isochrysis galbana as food.

Growth and development of Artemia are dependent on type, quality and quantity of food ingestion (Sick, 1976). The density of the microalgae T. fluviatilis used in this study was lower when compared with previous studies. Sick (1976) used C. sphagnicola (17 to 21 x 10^4 cells.mL^-1), N. closterium (62 to 74 x 10^6 cells.mL^-1), P. elliptica (11 to 16 x10^4 cells.mL^-1) and D. viridis (22 to 30 x 10^4 cells.mL^-1) to feed Artemia nauplii. Lora-Vilchis et al. (2004) used Isochrysis at 1,300 x10^3 cells.mL^-1 and C. muelleri at 1,100 x 10^3 cells.mL^-1. However, it can be noted that if the microalgae has a larger volume it can be used in lower concentration than small ones, and that is the case of T. fluviatilis. The size of T. fluviatilis is about 11 to 17 µm (Barbieri-Jr & Ostrensky-Neto, 2001) while Isochrysis and C. muelleri range from 3 to 5 µm (D’Souza & Loneragen, 1999).

According to Dhont & Lavens (1996) Artemia preferentially take up and digest alimentary particles with the maximum size of 50 µm. However, in this study the results suggested that Artemia ingested the T. biminiensis copepod offspring composed of nauplii (about 50-150 µm) and copepodite 2 (about 200-450 µm). Lima & Souza-Santos (2007) observed the ingestion of T. biminiensis copepod offspring by the larvae of Litopenaues vanamei during the stages mysis and postlarva. The offspring is also accompanied by fecal pellets, pieces of cast exoskeletons and eggs, that can also be used as food and promote bacteria growth. Bacteria can contribute for their nutritional value.

Artemia nauplii grew and developed when fed on T. biminiensis copepod offspring or with the microalgae T. fluviatilis. Thus, the copepod offspring is an alternative food for the culture of Artemia. Additionally, the copepod culture can be less costly than microalgae culture once that copepod grew fed only in ration. However, more research is needed to evaluate the nutritional value of the copepod offspring as food for Artemia.

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