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 28ºC, 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 5 th dia, as *Artemia* foram submetidas à dieta com a prole do copépodo (10 copépodo/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 Harpaticoida, cultivo, alimento, presa viva

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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 121ºC. 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 24-27ºC 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 ºC, 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*.

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Table 1. Composition of the f/2 medium (Guillard, 1975) for one litre seawater.

Nutrients	Quantity
$NaNO_3$	75 mg (883 µM)
$NaH_2PO_4.H_2O$	5 mg (36.3 µM)
$NaSiO_3.9H_2O$	30 mg (107 µM)
Traces metals	
$Na_2.EDTA$+	4.36 mg (11.7 µM)
$FeCl_3.6H_2O$+	3.15 mg (11.7 µM)
$CuSO_4.5H_2O$	0.01 mg (0.04 µM)
$ZnSO_4.7H_2O$	0.022 mg (0.08 µM)
$CoCl_2.6H_2O$	0.01 mg (0.05 µM)
$MnCl_2.4H_2O$	0.18 mg (0.9 µM)
$NaMoO_4.2H_2O$	0.006 mg (0.03 µM)
Vitamins	Quantity
Tiamina	0.01 µg
Biotina	0.5 µg
B12	0.5 µg

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 ± 1ºC, 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 ± 1ºC, 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 60ºC for 24 h and weighed again. The *Artemia* weight was caulculated usind the equation: weight of *Artemia* on the 4 th day of culture – weight of *Artemia* on the 11 th 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

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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-Smironov 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 11th 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).

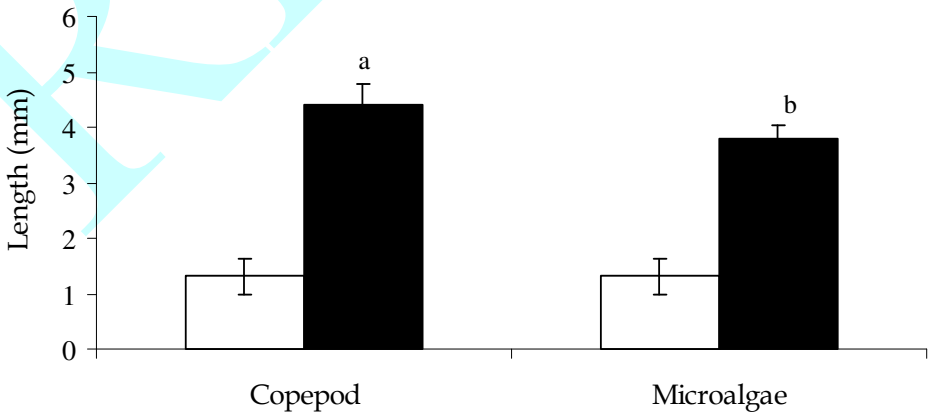

Figure 1. Length (mm) of *Artemia* (□, n= 52) fed microalgae *Thalassiosira fluviatilis* during 1rd to the 5th day of culture. Length of *Artemia* fed microalgae *T. fluviatilis* (■, n=85) or *Tisbe biminiensis* copepod offspring (■, n=83) during 6 th to the 11th day of culture. Different letters indicate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

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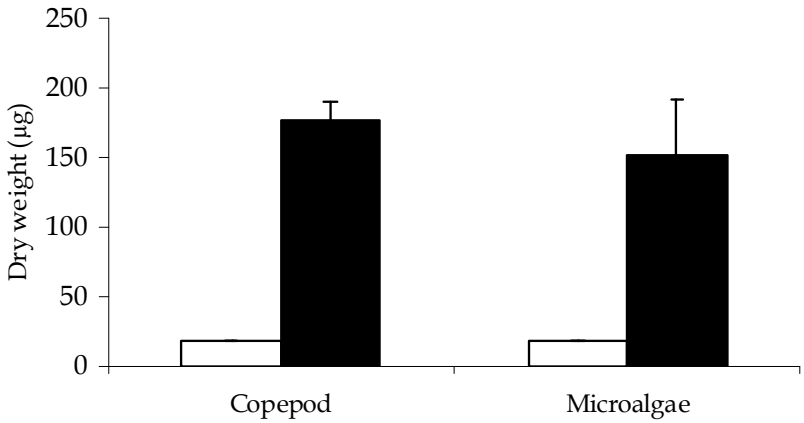

Figure 2. Dry weight (µg) of *Artemia* fed microalgae *Thalassiosira fluviatilis* (□, n= 52) during 1rd to the 5th day of culture. Dry weight of *Artemia* fed microalgae *T. fluviatilis* (■, n=85) or the *Tisbe biminiensis* copepod offspring (■, n=83) during 6 th to the 11th day of culture (ANOVA, P>0.05).

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 10^3 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.

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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 10^4 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 microalga 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 mocroalgae *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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