Artemia Growth Submitted to Diets of Flocculated Dissolved Organic Matter Confronted to Micro-algae Diets
S. G. Rabay and J.F. Paredes

ABSTRACT

Reports to innovation method on Artemia growing fed with colloidal particules obtained from marine dissolved organic matter by electrolytic flocculation carried out with iron sulphate and further lyophilized. This method has like reference the algal feeding of Artemia at the same environmental conditions which results are shown in the paper.

ADDITIONAL INDEX WORDS: Artemia growth, innovation system, flocculation dissolved matter.

INTRODUCTION

The micro-crustaceous Artemia sp. is considered actually the pattern food source in several larvae-culture systems not only of fishes but also of crustaceous and mollusces, gaining each time more prominent in the world aquaculture (Sorge loos, 1979; Sorge loos et al., 1980a, b). Artemia which in mean is 10 mm long, 1 mg weigh, has a high nutritive value, supplying proteins with a large spectrum of essential amino-acids, poli-unsaturated lipids, vitamins and essential mineral elements (Kurupu et al., 1995 and Kleine, 1993; 1995 a,b). Therefore more than 85 % of marine animal species consume, in some phase of their culture, newly-born nauplius from the rest cysts.

These considerations induce not only to infer an each time larger demand of this crustaceous, but also its crescent intensive production in bases either of quality, or of available cost.

Paredes and Kleine (1997) report, in mangrove area of hipersaline lagoon on the literal North, DOM concentrations as much as 4000 mg/l. Thus, these authors suggest this kind of natural organic matter by electrolytic flocculation carried out with iron sulphate and further lyophilized. This method has like reference the algal feeding of Artemia at the same environmental conditions which results are shown in the paper.

METHODS

Marine Micro-algae Culturing

The elected algal species for this experimental study were: Chaetoceros gracilis and Dunaliella salina, being the last one natural escort of Artemia in hipersaline ecosystems. The Spirulina sp. appointed by its proteic value, as available Artemia food, was also used in our experiment as referential diet. However it was not cultured, being used the commercial powder of this alga (Ciferri, 1983), obtained in chemistry shop. The mono-algal lineages as Chaetoceros gracilis and Dunaliella salina were supplied by Taiwan, Tung Kang Marine Laboratory (Yamashita and Pinto, 1948). These were maintained in Guillard and Ryther artificial standard medium, with 70 % salinity and pH 8.0-8.2. Its composition is shown in the Table 1. All aseptic conditions were always verified in cultures and in experiments management. The Figure 1 shows the culture conditions of micro-algae till to obtain the enough algal biomass to feed Artemia. The 500 ml and 20 litres flasks were permanently aerated.

The algal growths were assessed by the following methods:
— Cellular density performed in a haemacitometer (Newbauer chamber) each two days.
— Optical density at the 600 nm obtained in 1 cm path way tubes, each two days, at DR/2000 HACH spectrophotometer.

This last method was used to know when the algal growth in the 20 litres flasks reached the maximum density and when it should be interrupted to begin the cellular biomass concentration, carried out in a SORVAL RC 5B Plus centrifuge at 3000 rpm for 15 minutes.

Figure 1 - Lay-out to obtain the lyophilization of algal biomass and flocculation of extracellular organic matter supplied by the Chaetoceros gracilis and Dunaliella salina cultures to promote Artemia intensive growth.
Artemia Culture and Growth

In every experiment was used 100 specimens of Artemia grown in 1 litre of 70 % sea water medium. Each one of this triplicate experiment was submitted to five different diets as was above referred. These experiments were repeated 6 times therefore, the results totalize 30 means rates since they were performed in triplicate. All experiments were made up with 500 mg of cysts desacapsulated in sodium hypochlorite 25 % solution for 5-10 minutes of washing continuous aeration and further exhaustive washing with fresh water till the cysts suspension became hypochlorite - free. The eggs were then transferred to one litre of 35 % filtered sea water (pH 8.2), being permanently aerated until the nauplius were counted with tip free pipette and transferred to the culture containers made up from transparent plastic of commercial 2 litres Coca-Cola bottles without bottom. The unfish size of the bottles made easy the detritus accumulation and their further cleaning.

The nutrition was supplied in progressive quantities of food reported in Table 2, and based in a cellular mean weight of 0.03 µg/cel. and in ingestion speed of 1-5 cells/10³, according to Artemia growing stage and weight. Each two days, till adult stage of Artemia the following parameters, in the triplicate test, were measured: pH and temperature. During the bottom cleaning, the fecal pallets and food excess, were removed, as well as the dead specimens which were counted to estimate the feeding efficiency.

At the end of each experiment the Artemia was retained at a 100 µm net where the specimens were counted and further weighted after a quick washing with filtered sea water followed by dehydration with 50 % alcoholic solution (wet-weighted). The Artemia biomass was again weighted, till steady dry-weight, with checkings between 12 hours. Then each biomass treated in this way was kept into desiccator in dry and cool conditions till to be analysed. The experiments were reported to five different kinds of diets:

1) Dry powder of Spirulina: its known biochemical composition, induce us to consider it as referential diet (Ciferri, 1983)
2) Chaetoceros gracilis
3) Dunaliella salina
4) DOM from Chaetoceros gracilis culture
5) DOM from Dunaliella salina culture

Analytical Methodology

Each triplicate test concerning to the referred five diets, were submitted to the following methods showed in the table:

Table 1. Showing the analytical methods to quantify the Artemia growth relatively to differents diets.

<table>
<thead>
<tr>
<th>Component</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity and ashes</td>
<td>Gravimetric weights of wet and dry biomass and this one assessed against that one oxidated at 550° C (Dubois et al., Method 1956), known as acid phenol sulphric method</td>
</tr>
<tr>
<td>Total proteins</td>
<td>Kjeldahl Method, Baethgen (1899)</td>
</tr>
<tr>
<td>Total Carbohydrates</td>
<td>Kjeldahl Method, Baethgen (1899)</td>
</tr>
</tbody>
</table>

RESULTS

The micro-algae Chaetoceros gracilis and Dunaliella salina grown in experimental tests to give nutritial support to Artemia growth reaching, in 20 litres flasks the mean values of cellular of 570 x 103 and 230 x 10³ cel/ml, respectively as it is shown in Table 2. These cellular densities, corresponded respectively to the following optical densities at 600 µm: 0.298 and 0.164 (Table 2).

Chaetoceros gracilis exhibited the best cellular density on the 10th culture day, while the Dunaliella salina, with a slowly growth rate, reached the best cellular density only on the 12th culture day.

However Dunaliella salina exhibited a lyophilized mean biomass of 2490 mg, while at the same time, Chaetoceros gracilis reached only 2170 mg, therefore with a differential value of 320 mg (Table 3).

Regarding to the extracellular dissolved organic matter (DOM), the flocculated biomass, as it is reported in the same Table 3, Chaetoceros gracilis contributed with more flocculate organic matter, reaching 1740 mg, while Dunaliella salina responded with a smaller value of 1120 mg. Therefore the differential value was, in this case, twice higher (620 mg) than the difference relatively to cellular densities. Then, we may infer that the Chaetoceros gracilis had, at the same conditions, a larger contribution in extracellular contents regarding to Dunaliella salina, what is confirmed in Table 4.

Table 2. Growth means of Chaetoceros gracilis and Dunaliella salina cultivated in laboratory.

<table>
<thead>
<tr>
<th>Micro algae species</th>
<th>Initial concentration (Cel/ml)</th>
<th>Final concentration (Cel/ml)</th>
<th>Optical density final concn. (600mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros</td>
<td>320,000</td>
<td>570,000</td>
<td>0.298</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>175,000</td>
<td>230,100</td>
<td>0.164</td>
</tr>
</tbody>
</table>

Table 3. Amount of biomass and extracellular organic matter (MOD).

<table>
<thead>
<tr>
<th>Micro algae species</th>
<th>Culture Volume (Liter)</th>
<th>Algae Biomass Lyophilized (Mg)</th>
<th>Flocculated Organic Matter Lyophilized (Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetoceros</td>
<td>08</td>
<td>2,170</td>
<td>1,740</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>08</td>
<td>2,490</td>
<td>1,120</td>
</tr>
</tbody>
</table>

Table 4. Growth, and survive rates of Artemia sp. cultivated in different diets.

<table>
<thead>
<tr>
<th>Diets</th>
<th>pH</th>
<th>Temp. (°C)</th>
<th>Survive (%)</th>
<th>Length (Mm)</th>
<th>Artemia Final (N°)</th>
<th>Growth days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirulina</td>
<td>8</td>
<td>26</td>
<td>80</td>
<td>10</td>
<td>240</td>
<td>12</td>
</tr>
<tr>
<td>Chaetoceros</td>
<td>8</td>
<td>26</td>
<td>97</td>
<td>12</td>
<td>291</td>
<td>14</td>
</tr>
<tr>
<td>Flocculated (DOM)</td>
<td>8</td>
<td>26</td>
<td>52</td>
<td>10</td>
<td>156</td>
<td>12</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>8</td>
<td>26</td>
<td>81</td>
<td>10</td>
<td>243</td>
<td>14</td>
</tr>
<tr>
<td>Flocculated (DOM)</td>
<td>8</td>
<td>26</td>
<td>47</td>
<td>10</td>
<td>141</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 5. Basic biochemical composition of Artemia sp. cultivated in different kinds of diets.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Control group</th>
<th>From Chaetoceros</th>
<th>From Dunaliella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity (%)</td>
<td>91.21</td>
<td>81.8 ± 1.7</td>
<td>91.24</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>90.6 ± 0.5</td>
<td>19.6 ± 1.0</td>
<td>90.5 ± 1.0</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>30.6 ± 0.5</td>
<td>13.0 ± 0.4</td>
<td>32.2 ± 1.1</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>0.52</td>
<td>11.7 ± 1.1</td>
<td>28.0 ± 1.3</td>
</tr>
<tr>
<td>Ashes (%)</td>
<td>0.62</td>
<td>28.0 ± 1.3</td>
<td>32.2 ± 1.1</td>
</tr>
</tbody>
</table>

The best nutritional response as it is shown in Table 4 was given by Chaetoceros gracilis, with the best survival rate (97 %), and the biggest length (12 mm), while concerning to Dunaliella salina and Spirulina, the survival rates and the length of Artemia, when fed with these species, reached only 81 %, 80 % and 10 mm respectively (Table 4), results that seem rather conspicuous.

The 4th and 5th alternatives, which established the main objective of this research are those related with the FeSO₄, flocculation of dissolved organic matter accumulated into algal cultures. These responses with minor survival rates, reaching only 52 % and 47 %, values which are still acceptable, since no other nutrient source was supplied to Artemia (Table 4).

Regarding to nutritional efficiency the assessment as total proteins, relatively to several diets, the most remarkable result was exhibited by the alga Spirulina, with 81.8 ± 1.7. All the others concentrations remained around 50.9 % and 56.5 % (Table 5) so, much lower rates, relatively that one.

Concerning to carbohydrates the most remarkable concentration was given by the Artemia sustained with Chaetoceros gracilis reaching 30.6 ± 0.5 % when the other nutrition conditions varied in the rate band of 13.0 - 19.6 % (Table 5) being the minor values concerned to flocculated DOM nutrition. Spirulina and Chaetoceros gracilis constituted the Artemia diets, which less promoted the lipids accumulation exhibiting only rates of 0.02 % and 11.7 % respectively, while the others diets drawn up their rates to the band of 28.0 - 32.2 % (Table 5). It is, however, worth of note that the lipids rates were estimated by difference.

The humidity and the ashes contents rates were characterized by an evident uniformity, although the Artemia fed with flocculated organic matter and by Spirulina replied with higher rates of 1.2 % and 1.3 %, while the Chaetoceros gracilis and Dunaliella salina did not go over 1.1 - 1.0 % respectively (Table 5).

**DISCUSSION OF RESULTS**

Our results concerning to cellular densities of the algal cultures for nutritional support of Artemia growth were similar to those ones obtained by YAMASHITA and PINTO (1984). However YAMASHITA and MAGALHAES (1984a and 1984b) report cellular densities in the band of 300 - 400 x 10⁶ cell/ml in three experiments, while in our algal experiments that range was larger (230 - 570 x 10⁶ cell/ml).

SORGEOLOS et al., (1980b) refers a mean rate of 60 % of proteins in Artemia. However, this rate according to kind of the diets referred above, varied between 51 - 82 %.

KLEIN (1983b) grew Artemia with two kinds of NE Brazilian native plants, with which this author obtained protein rates of 49.5 % and 47.1 %, values which reach rates near those ones of the lower limits of the variation band of our experiments and even so, somewhat lower of proteins rates reached by DOM flocculation nutrition.

After CIFERRI (1995) the proteic contents exhibited by Spirulina are as much as 64 - 77 %, justifying so the high rate found in Artemia fed on this alga. Nevertheless CIFERRI (1995) advarts that Spirulina growing in open systems its protein contents may fall to the band of 60 - 61 % what should be related with others variables that may occur in open systems. This author refers to the Spirulina carbohydrates rates as much as 16.9 % of its dry weight, just the same value obtained in our experiments, and fairly near of those ones found in Artemia when was fed with DOM flocculated, which varied between 13.1 and 14.2 %.

The lipids in Artemia displayed a conspicuous variation relatively to the different kind of diets, which go from 0.02 % when fed with Spirulina until 32 % when fed with flocculated DOM from Dunaliella salina. These results must be concerned with the reserves type, since the Spirulina reserve is starch, while the others cases that exhibited higher rates (28 - 32 %) would be related with the osmotic regulation on account of 70 % of salinity used in their culture medium. Thus, the osmotic regulation is maintained by the algal glycerol production, a soluble compound in water, occurring mainly during the dialysis procedure (AVROW and AMOTS, 1990). However, after CIFERRI (1983) Spirulina grown in laboratory cultures can reach perceptual rates of lipids as much as 4 - 14 %, although when grown in open environment (out doors) that band can arise to 9 - 14 %.

SORGEOLOS (1980b) imputed to adult Artemia an average of 10 % in the lipids contents, what is concerned with our experiment when Artemia was fed with Chaetoceros gracilis (11.7), micro-algo whose reserve is lipids (JOLY, 1963).

SORGEOLOS (1974) points survival rates of 87 - 98 % in Artemia when fed with Dunaliella salina. In this condition the nauplius exhibits, lengths among 2.15-2.57 mm.

On the other hand KURUPU and EKARA.TNE (1995b) culturing Artemia parthenogenetic fed with Dunaliella salina in different conditions of temperature and salinity, reached survival rates of 72 - 94 % in temperature of 25°C and in 100 - 120 % of salinities. Nevertheless, the temperature increasing till 29 - 35 °C, push down the survival rates till 78 - 82 %. In the temperature of 21°C and in 65 % salinity the survival rates fallen drastically within to 10 - 48 %. These authors demonstrated there to be a narrow relationship between temperature and salinity at least with parthenogenic species, to which suggest the bands of 25 - 30°C and 100 - 120 % as the best conditions for the success of Artemia culture.

The studies carried out by FABREGAS (1996) where this author fed Artemia with *Tetraselmis suecica* obtaining a survival rate of 85 % and the length of 8.3 mm after 19 growth days. That result fall near the survival rate on our algal feeding experiment, but regarding to its length, staid somewhat lower, which varied in the band of 10 - 12 mm after 12-14 growth days.

SORGEOLOS (1979) grew Artemia fed with rice, soy-bean and wheat, obtaining during the larval stages, the following results, as survival rates and larva sizes: with rice (10 % and 2.08 mm); with soy-bean (80 % and 4.26 mm) and with wheat (80 % and 2.08 mm). The survival rates in our experiments, concerning to inert food were, as we may see, surpassed by the test with soy-bean and wheat diets, however our results are regarding to adult stages while sorgeloos results report to the larval stages.

VANHAECCE and SORGEOLOS (1986) submitted two Artemia breeds from Macau-Brazil, to the following nutrital conditions: Dunaliella salina and rice bran. In the first case Artemia displayed a survival rate of 84 - 96 %, figures compatibles with our algal test. In the second case Artemia exhibited the remarkable survival rates of 90 - 100 %, proving how the rice bran (not the rice) can be important to Artemia culture.

Others interesting experiments performed by BASIL AND NAIN (1995) who grew Artemia with food of cabbage rubbish at 45, 60, 70 and 100% of salinities, found to be the 75% salinity that asserted the best survival rate (97 %).

In front of the results reached in this experimental research and here discussed, seems evident the availability of the nutrital alternative here proposed to use the dissolved organic matter circulating in coastal ecosystem where we may come across with high concentrations as it is reported by PAREDES and KLEIN (1997). Natural waters, should offer to Artemia better nutrital perspectives than that on obtained from the unialgal cultures. In bonus of this considerations, can be pointed
out the experience reported by PAREDES (1993), who kept a shoal of Mugil sp. during a week, fed exclusively on filtered sea water which was further flocculated with FeSO₄. This experience exhibited 100% of survival. Therefore, under this point of view, both of these considerations reinforce the merit of this research here attempted.

According to this research it is confirmed that Artemia is an excellent source of proteins, and carbohydrates when sustained by the suggested diets, even with the flocculated ones. In this latter condition was observed a minor speed in the toracopoda Artemia beating, what was inferred to be related with the irregular morphology of the colloidal organo-metallic particles.

Another reason could be a wise to save energy due to be catching sluggish inert particles, which containing iron, would be accountable for the somewhat darker colour of the phaecal pellets.

**CONCLUSIONS**

1. The experimental method to feed Artemia with flocculated DOM, proved to be an innovation available, concerning to protein rates.

2. Relatively to carbohydrates, the flocculated DOM feeding, Artemia exhibited minor rates than thoses ones fed with algal, mainly with Chaetoceros, with one was the most conspicuous.

3. Concerning to lipids the differences among the rates were not significants, except that one displayed by Artemia fed with Chaetoceros e rates of the others diets reached 27.5%.

4. We stand out that there was not difference between the diets tested relatively to Artemia length.

5. Nevertheless the survival rates somewhat minor than those ones fed with algae which ones had 14 days of growth, while the former ones had growth only for 12 days.

**LITERATURE CITED**


