Journal of Coastal Research	SI 39	1106 - 1109	ICS 2004 (Proceedings)	Brazil	ISSN 0749-0208

Artemia Growth Submitted to Diets of Flocculated Dissolved Organic Matter Confronted to Micro-algae Diets

S. G. Rabay and J.F. Paredes

M. Sc. Biology. Dept. Federal
University of Ceará, Fortaleza, Brazil.
sgrabay@yahoo.com.br

Ph.D. London University
CIMAR - Centro Interdisciplinar de Investigação Marinha
e Ambiental, University of Porto, Porto, Portugal
R. Macapá 170 / 102, Ondina, CEP 40.170-150 Salvador
Brasil, Phone: 5571 235 1430
jfalcaoparedes@hotmail.com

ABSTRACT

RABAY, S. G. and PAREDES, J.F., 2006. *Artemia* growth submitted to diets of flocculated dissolved organic matter confronted to micro-Algae diets. Journal of Coastal Research, SI 39 (Proceedings of the 8th International Coastal Symposium), 1106 - 1109. Itajaí, SC, Brazil, ISSN 0749-0208.

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 puper.

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 molluscs, gaining each time more prominent in the world aquaculture (SORGELOOS, 1979; SORGELOOS et al., 1980a, b). *Artemia* which in mean is 10 mm long, 3 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 KLEIN, 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 KLEIN (1997) report, in mangrove area of hipersaline lagoon on the literal North, DOM concentrations as much as 4000 mg/1. Thus, these authors suggest this kind of natural source, when submitted, to flocculation process, as an alternative kind of food, not only for *Artemia*, but also for the estuarine sedentary fauna. Indeed, the flocculation is a natural process occurring in the estuarine waters.

The purpose of this research was based in experiment described by PAREDES (1993) who maintained *Mugil* specimens in filtered sea water and fed exclusively with flocculated matter obtained with iron sulphate ($FeSO_4$) with objective to prove that the organo-metallic complexes have not deleteric effects on fishes, and might be used by them. Therefore, the objective of this experimental research was to test, if that geochemical approach would be also available for the *Artemia* growing in intensive culture regime, without any degeneration process keeping, even so, a suitable quality, as food. On the other hand, it was considered important to test the best feeding regime for *Artemia*. Thus, it performed the comparative study on induced flocculation of dissolved organic matter (DOM) as an alternative to minimized the algal food cost, concerning to three micro-algal species as feed. This comparative study was based on biochemical composition and growth rates, obtained from the *Artemia* adult biomass.

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 1cm pathway 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.

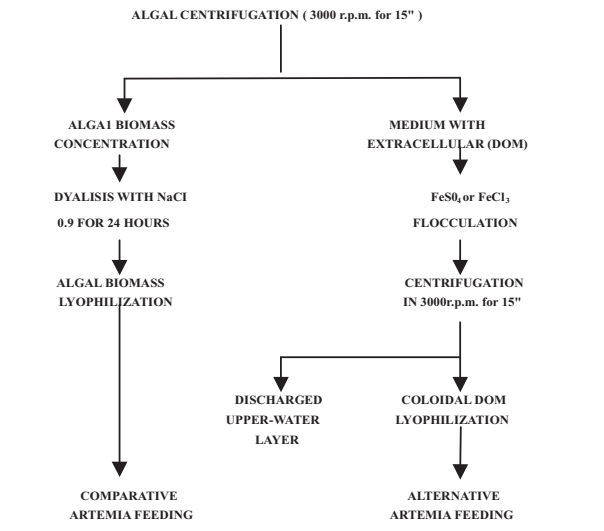

Figure 1 - Lay-out to obtain the lyophilization of algal biomass and flocculation of extracellular organic matter supplied by the *Chaetoceros gracilis* and *Dunaliella salina* cultures to promate *Artemia* intensive growth.

No damage in algal structures was verified. The algal bulk, as it shown in the Fig. 1 outline was submitted to a dialysis process against 0.9 % saline solution for 24 hours under refrigeration with solution changes each 3 hours. After dialysis the algal bulk was lyophilized, weighted and conveniently stored until to be supplied to Artemia, in the cases of exclusive algal diets.

The water medium firstly separated from the algal bulk, was then submitted to flocculation process with 15 mg/1 of $FeS0_4$, which promptly formed the reddish colloidal organic complexes from the DOM, under the actions of electrolytic agglutinating.

The decantation process was left, under refrigeration for 24 hours. The colloidal bulk decantated was then centrifuged for 15 minutes under speed of 5000 rpm, and ressuspended at a 0.9 % NaCl solution, during 24 hours, under refrigeration with solution changes each 3 hours. This particulated bulk with DOM, was also straightway lyophilized, weighted and stored until to be supplied to *Artemia* in experiments that had this daily kind of food as sole diet. As we have grown two mono-algal cultures, we should emphasize that we obtained, by this approach, two kinds of flocculated DOM: one from the *Chaetoceros gracilis* and another one from *Dunaliella salina*.

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 differents 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 descapsulated in sodium hypochlorite 25 % solution for 5-10 minutes of washing continuous aeration and further exhaustive washing with fresh water till the cysts suspensation 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 funil shape 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/10", 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 considerer 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 different diets.*

Humidity and ashes assessement analysis	Gravimetric weights of wet and dry biomass and this one against that one oxidated at 550º C
Total proteins Analysis	Kjedahl Method, Baethgen and Alley (1989)
Total Carbohydratantes Analysis	Dubois et al., Method (1956), known as acid phenol sulphric method

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 10^3 cel/ml, respectively as it is shown at 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.*

Micro algae species	Initial concentration (Cel/ml)	Final concentrarion (Cel/ml)	***Optical density final concen. (600mµ)***
Chaetoceros	320,000	570,000	0.298
Dunaliella	175,000	230,100	0.164

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

Micro algae species	Culture Volume (Liter)	Algae Biomass Lyophilized (Mg)	Flocculated Organic Matter Lyophilized (Mg)
Chaetoceros	08	2,170	1,740
Dunaliella	08	2,490	1,120

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

Diets	pH	Temp. (°C)	Survive (%)	Length (Mm)	Artemia Final (N°)	Growth days
*Spirulina**	8	26	80	10	240	12
Chaetoceros	8	26	97	12	291	14
Flocculated (DOM)**	8	26	52	10	156	12
Dunaliella	8	26	81	10	243	14
Flocculated (DOM)***	8	26	47	10	141	12

Table 5. *Basic biochemical composition of Artemia sp. cultivated in different kinds of diets.*

Different diets	Constituents				
	Humidity (%)	Protein (N x 6,25) (%)	Carbohydrate (%)	Total Lipidis (%)	Ashes (%)
*Spirulina**	91.21	81.8 ± 1.7	16.9 ± 0.6	0.02	1.2
Chaetoceros	91.58	56.5 ± 5.0	30.6 ± 0.5	11.7	1.1
Flocculated (DOM)**	93.82	56.4 ± 4.3	14.2 ± 0.3	28.0	1.3
Dunaliella	91.34	50.9 ± 1.7	19.6 ± 1.3	28.5	1.0
Flocculated (DOM)***	91.62	53.5 ± 3.6	13.0 ± 0.4	32.2	1.2

* Control group; ** From *Chaetoceros;* *** From *Dunaliella*

The best nutritial 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 $FeS0_4$ flocculation of dissolved organic matter accumulated into algal cultures. These responses with minor survival rates, reaching only 52 % and 47 %, values, which still are acceptable, since no other nutritial source was supplied to *Artemia* (Table 4).

Regarding to nutricial 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 among 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 nutritial support of *Artemia* growth were similar to those ones obtained by YAMASHITA and PINTO (1984). However YAMASHITA and MAGALHÃES (1984a and 1984b) report cellular densities in the band of 300 - 400 x 10^3 cel/ml in three experiments, while in our algal experiments that range was larger (230 - 570 x 10^3 cel/ml).

SORGELOOS *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) adverts that the *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, witch 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 %.

SORGELOOS (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-alga whose reserve is lipids (JOLY, 1963).

SORGELOOS (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 parthenogenetic 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.

SORGELOOS (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.

VANHAECKE and SORGELOOS (1986) submitted two *Artemia* breeds from Macau-Brazil, to the following nutritial 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 nutritial 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 nutritial perspectives than that on obtained from the uni-algal 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 $FeS0_4$. 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-metalic 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 phecal 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

BAETHGEN, W. E.; ALLEY, M. 1989. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Communication in Soil Science. V. 2, p. 961-969.

BASIL, J. A . and NAIN, A. J. 1995. Laboratory studies on the culture of the shrimp *Artemia* using organic wastes. Bio Resource Technology, 51: 265-267.

CIFERRI, O. 1983. Spirulina, the Edible Microorganism. Microbiological Reviews, p. 551-578, Dec.

DUBOIS, M.; GILLES, A.; HAMILTON, J. K.; REBERS, P. A. and SMITH, F. 1996. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chem. V. 28, n. 28, p. 350-356.

FABREGAS, J.; OTERO, A.; MORALES, E.; CORDERO, B. and PATINO, M. 1996. *Tetraselmis suecica* cultured in different nutrient concentrations varies in nutritional values to *Artemia*. Aquaculture, 143(2): 197-204.

KLEIN, V. L. M. 1995a. Identificação de diferentes linhagens de *Artemia* sp. do Nordeste do Brasil, através de eletroforese. In: VI Congresso Latino-americano de Ciências del Mar-1995, Mar del Plata, Argentina. Livro de Resumos, 23 a 27 October.

KLEIN, V. L. M. 1995b. Aspectos da reprodução de *Artemia* sp. de salinas do nordeste do Brasil. In: VIII Semana Nacional de Oceanografia - 1995, Rio Grande RS. Livro de Resumos. 15 a 20 October.

KLEIN, V. L. M. 1993. Contribuição ao Estudo de Diferentes Linhagens de *Artemia* sp. no Nordeste do Brasil. Universidade de São Paulo, 1-137 pp. (Tese de Doutorado).

KURUPU, M. M. and EKARATNE, S. U. K. 1995a. Characterization of brine shrimp *Artemia* from Sri Lanka. Journal of the National Science Council of Sri Lanka, 23(4): 153-160.

KURUPU, M. M. and EKARATNE, S. U. K. 1995b. Effect of temperature and salinity on survival, growth and fecundity of brine shrimp *Artemia parthenogenetica* from Sri Lanka. Journal of the National Science Council of Sri Lanka, 23(4): 161-169.

PAREDES, J. F. 1968. Some methods used to obtain bacteria-free cultures of marine phytoplankton. Mems. Inst. Invest. Cient. Mocamb., 9(A). From Ph. D. Thesis, Westfield College, University of London.

PAREDES, J. F. 1993. Evaluation of the environmental effects of the industrial effluents from Tibrás - Titânio do Brasil in the area under influence of its underwater outfalls. Revista de Geologia da UFC, 6: 105-125.

PAREDES. J. F. and KLEIN, V. L. M. 1997. Geochemical study on the lagoon (river) Camurupim (Piauí). Congresso de Geoquímica. Universidade Federal da Bahia Salvador. p. 1-6.

SORGELOOS, P. 1997. The brine shrimp, *Artemia salina*: A bottleneck in mariculture? In: FAO - Technical Conference on Aquaculture. Pillay, T. V. R., Wm. A. Dill (Eds.). Fishing News Books, Ltd., Farnham (England), 653 p.

SORGELOOS, P.; PERSOONE, G.; ROELS, O. and JASPERS, E. 1980a. New aspects of the use of inert diets for high density culturing of brine shrimp. In: G. Persoone, P. Sorgeloos, O. Roels e E. Jaspers (Eds.). The Brine Shrimp *Artemia*. V. 3. Ecology, Culturing. Use in Aquaculture. Universal Press, Wetteren, Belgium. 456 p.

SORGELOOS, P.; SORGELOOS, G.; ROELS, O. and JASPERS, E. 1980. The Use of the brine shrimp in aquaculture. In: G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds.). The Brine Shrimp Artemia. V. 3. Ecology, Culturing. Use in Aquaculture. Universal Press, Westten, Belgium. 456 p.

SORGELOOS, P.; SORGELOOS, G.; ROELS, O. and JASPERS, E. 1980c. International study of *Artemia* IV. The biometrics of *Artemia* strains from different geographical origin. In: G. Persoone, P. Sorgeloos, O. Roels, e E. Jaspers (Eds.). The Brine Shrimp *Artemia*. V. 3. Ecology, Culturing. Use in Aquaculture. Universal Press, Westtern, Belgium.

SORGELOOS, P. 1974. The influence of algal food preparation on its nutritional efficiency for *Artemia salina* L. larvae. Thalassia Yugoslavia. 10(1/2): 313-320.

VANHAECKE, P. and SORGELOOS, P. 1986. International study on *Artemia*. Growth and survival *Artemia* larvae of different geographical origin in standard culture test. Mar. Ecol. Prog. Ser., 3(4): 303-307. Apud SORGELOOS.

YAMASHITA, C. and MAGALHÃES, P. M. S. 1984a. Meios de cultura para a alga *Chaetoceros gracilis*. EMPARN Boletim de Pesquisa, 4: 1-18.

YAMASHITA, C. and MAGALHÃES, P. M. S. 1984b. Métodos simples para o cultivo da alga *Tetraselmis chuii*. EMPARN Boletim de Pesquisa, 8: 1-21.

YAMASHITA, C. and PINTO, M. F. C. M. 1984c. Uso de diferentes espécies de algas na alimentação de larvas de camarão *Penaeus brasiliensis* no estagio de zoéa. EMPARN Boletim de Pesquisa, 9: 1-18.