GROWTH AND SURVIVAL OF *Artemia franciscana* (KELLOGG) FED WITH *Chaetoceros muelleri* Lemmerman AND *Chlorella capsulata* GUILLARD.

María Concepción Lora-Vilchis [1] * and Domenico Voltolina [2]

(1) Centro de Investigaciones Biológicas del Noroeste (CIBNOR), P. O. Box 128, La Paz, BCS 23000, México.
(2) Centro de Investigaciones Biológicas del Noroeste, Laboratorio UAS-CIBNOR, Mazatlán, Sinaloa, México.
(*) Corresponding author. cony04@cibnor.mx

RESUMEN

Artemia franciscana fue alimentada por siete días con 11.7, 23.4 y 46.8 $mg \cdot l^{-1} \cdot d^{-1}$ (peso seco libre de cenizas) de *Chaetoceros muelleri* y *Chlorella capsulata*, para evaluar el valor nutritivo de estas dos microalgas. La supervivencia fue similar, cercana o mayor del 90% en todos los casos, pero hubo diferencias significativas en el crecimiento que iniciaron al tercer día. Con *C. muelleri*, el peso seco libre de cenizas final de *A. franciscana* fue siete veces mayor que con *C. capsulata* y las fases de desarrollo estuvieron entre instar 14 a 16 y 8 a 9 respectivamente. La ingestión de alimento fue similar para las dos dietas, pero las eficiencias medias de crecimiento fueron 80% con *C. muelleri* y 7% con *C. capsulata*, lo que indica una baja asimilación de *C. capsulata*.

Palabras clave: microalgas; cultivo; alimentación; *Artemia*; *Chaetoceros*; *Chlorella*..

ABSTRACT

Artemia franciscana was fed for seven days with 11.7, 23.4 and 46.8 $mg \cdot l^{-1} \cdot d^{-1}$ (ash-free dry weight) of *Chaetoceros muelleri* and *Chlorella capsulata*, to evaluate the food value of these two microalgae. Survival was similar, close to or greater than 90% in all cases, but there were significant differences in growth beginning on the third day. The final ash-free dry weight of *A. franciscana* fed with *C. muelleri* was seven times greater than with *C. capsulata* and the respective developmental stages were between instar 14 and 16 and from 8 to 9. Food ingestion was similar with the two diets, but the mean gross growth efficiencies were 80% with *C. muelleri* and 7% with *C. capsulata*, indicating a poor assimilation of *C. capsulata*.

Key words: microalgae; culture; feeding; *Artemia*; *Chaetoceros*; *Chlorella*.

There are several indirect methods for the evaluation of phytoplankton food quality for filter feeders, such as its caloric content and gross and fine chemical composition (Brown *et al*., 1997). However, at times these are misleading, because of the different nutritional requirements of each species, which in addition may vary with age and stage of development. For this reason, the food value of microalgae should be evaluated through their effect on growth and survival of the selected target organism (Yúfera and Lubián, 1990).

The brine shrimp *Artemia* is probably the most popular live diet in aquaculture. It is a continuous filter feeder which selects suspended particles only on the basis of size (Gelabert and De la Cruz, 1990), although its feeding behavior may be affected by several factors that influence its filtration, ingestion, or assimilation rates. The range of optimum particle concentrations for maximum ingestion is relatively wide, and is specific for each culture condition. In addition it varies with age, because the feeding efficiency increases with the number of functional thoracopods (Lavens and Sorgeloos, 1991).

The quality of microalgae diets for *Artemia* has been the object of several studies (Sick, 1976; Johnson, 1980; Fábregas *et al*., 1996, 1998) with different results, depending on the species of microalgae, on their culture conditions, and possibly on the species of *Artemia* used for the feeding experiments.

Diatoms are considered good sources of highly-unsaturated fatty acids, especially of 20:5ω-3. Among these, *Chaetoceros muelleri* is considered one of the best diets for brine shrimp culture (Naegel, 1999; De Micco and Hubbard, 2001). In contrast, chlorophytes are rich in C16 and C18 fatty acids (Brown *et al*., 1997; Dunstan *et al*., 1992), and in particular *Chlorella* has also a high content of carotenoids and ascorbic acid (Czygan, 1968; Merchie *et al.*, 1995), which might be of importance for growth and especially for long-term enhancement of the food quality of *Artemia*. The food value of some *Chlorella* species has been evaluated with *Artemia salina* (Sick, 1976, Yunzhen *et al.*, 1998) but not with the commercially important *A. franciscana*.

The aim of this study was to evaluate the food value of the marine chlorophyte *Chlorella capsulata* and of the diatom *Chaetoceros muelleri*, comparing the growth and development of *Artemia franciscana* during the first seven days after hatching

MATERIALS AND METHODS

The algae *Chaetoceros muelleri* (strain CHM-1) and *Chlorella capsulata* (strain CLC-1), of the collection of the Centro de Investigaciones Biológicas del Noroeste, were kept in quadruplicate, semi-continuous cultures in f/2 medium (Guillard, 1972), with daily replacements of 50 and 37% of the volume of each culture with fresh medium. The cultures were used when cell concentrations remained stable for at least five days before the feeding experiments. Culture conditions for the two algae were 36‰ salinity, 22 ± 1°C (*C. muelleri*) and 27 ± 1°C (*C. capsulata*), and pH was maintained at 8.0 ± 0.5 by continuous addition of CO_2 to the compressed air used for stirring.

The ash-free dry weight (AFDW) and gross chemical composition of both algae were determined in triplicate samples obtained on three alternate days from each culture, using the analytical methods described in Lora-Vilchis and Doktor (2001). The results were used to calculate their energy content employing the caloric equivalents of Gnaiger (1983).

The feeding experiment lasted seven days and was run in triplicate 18-l aquaria for each diet, using commercial cysts of *Artemia franciscana* (INVE Aquaculture, Belgium, Argentemia Bp 1207 N) hatched as in Van Stappen (1996). Seawater was continuously aerated and kept at 36‰ salinity, 28 °C, and in semidarkness. The initial nauplii density ranged between 1.7 to 2.0 org·ml^{-1}.

Feeding started 24 hours after hatching and algae were supplied in daily rations of 0.3, 0.6 and 1.2 x 10^6 cells·ml^{-1} of *C. muelleri* and 0.75, 1.5 and 3.0 x 10^6 cells·ml^{-1} of *C. capsulata*. In either case the AFDW of these rations was 11.7, 23.4, and 46.8 mg·l^{-1}·d^{-1}, which are equivalent to 42.5, 85 y 170% of the 27.5 mg·l^{-1}·d-1, which was found adequate for *A. franciscana* optimum growth (Evjemo and Olsen, 1999). In all cases the ration was divided in two parts, the first supplied in the morning after total water exchange and the second approximately 10 hours later.

Daily observations showed differences between diets, in length as well as in instar stage, after the second day. For this reason, samples of 20 organisms were obtained on day 3 from each culture and fixed, as described in Correa Sandoval and Bückle Ramírez (1993), to determine their mean length and developmental stage according to the instar scale of Schrehardt (1987). The total AFDW of these organisms, determined with a microbalance following Sorokin (1973), was used to obtain the average individual weight in each culture. Similar samples, taken at the end of the experiment, were used to compare the effect of the two diets and of the different rations.

The ingestion rate was calculated as in Albentosa *et al.* (1997) from the data obtained in six separate incubation chambers for each diet, with 1-2 organisms·ml^{-1} depending on the developmental stage. For each ration, two controls without organisms were used to correct the ingestion values due to possible changes in algal density. Cell concentrations were determined with a Neubauer chamber at the beginning of each experiment and after between 3 and 12 h, depending on the age and size of the organisms.

The mean gross growth efficiency (K_1) for each ration of the diets was evaluated from the data of total ingestion calculated with the results of the short-term daily evaluations of clearance rates. The mean developmental stage was determined on days 3 and 7 using the index suggested by Villegas and Kanazawa (1979) for shrimp larvae:

$$D.I. = \sum A/N$$

where A is the instar stage of each organism and N is the number of organisms in the sample.

Because the data were not normal, the gross biochemical composition of algae in percent of microalgal AFDW was compared with a Mann-Whitney test for each biochemical component. Mean survival, mean lengths, and mean D.I. and individual AFDW were compared for days 3 and 7 to determine the effectiveness of each diet and ration, using two-way ANOVA and Tukey's multiple comparison tests with α=0.05, after log transformation when the data were not normal or homoscedastic (Zar, 1999).

RESULTS

The higher individual AFDW of *C. muelleri* and the higher carbohydrate content of *C. capsulata* were the only significant ($P < 0.05$) differences between the two microalgae (Table 1). At the end of the experiment, mean *A. franciscana* survival ranged from 89 to 100% for *C. muelleri* and *C. capsulata* (Table 2), without any difference between diets and rations ($P > 0.05$). However, there were several diet and ration-related differences in growth and rate of development.

Statistical comparisons confirmed that, by the third day, there were no significant differences ($P > 0.05$) in length, development, and AFDW of *A. franciscana* fed the three rations of *C. capsulata*. In all cases, the organisms fed *C. muelleri* were significantly larger and heavier, with no effect of the ration on total length or developmental index, although those receiving the intermediate food concentration had a significantly higher organic body weight than those fed the other rations ($P < 0.05$ in all cases).

At the end of the experiment, diets and rations had significant effects. *A. franciscana* grew better with the two higher rations of *C. muelleri* without significant differences in length and AFDW between the intermediate and the highest ($P > 0.05$). In all cases, the lowest values were with the daily dose of 11.7 $mg \cdot l^{-1}$ of *C. capsulata* ($P < 0.01$).

The developmental index was higher ($P < 0.05$) and related to ration with *C. muelleri*, with which *A. franciscana* reached post-larval stages by the end of the experimental period, whereas the organisms fed *C. capsulata* only reached post-metanauplius stages, without significant differences due to the amount of food supplied (Table 2).

The mean total ingestion was similar for the same ration levels of both diets; however, the weight gain calculated from the mean final values corrected for the initial AFDW of the nauplii (2.5 $\mu g \cdot ind^{-1}$) was, in all cases, significantly higher with *C. muelleri,* with food conversion indices and mean gross growth efficiencies one order of magnitude better than those calculated for *C. capsulata* (Table 3).

DISCUSSION

The three ration levels were chosen under the assumption that eventual differences in food value of the two diets would have been more evident under different degrees of food availability, from highly or slightly limiting to higher than that required for optimum growth, with differences in mean survival and in rates of development and body growth.

Whereas the energy obtained by *A. franciscana* from the three rations of both algal diets was enough to sustain its standard metabolism, as indicated by the high and equal survival, the excess directed to somatic growth and development was different, as shown by our results which indicate deficiencies in the food value of *Chlorella*, starting on the third day.

On this date, in addition, the higher AFDW of the specimens fed the second ration level of *C. muelleri* indicates a lower assimilation efficiency with the highest ration, probably due to excessive food availability for this developmental stage. On the other hand, the final values show a significant effect of the amount of food supplied, with progressively better growth and development with the increase of the ration levels.

In view of the similar amounts of food ingested, the only possible explanations for the different effects of these diets are a lower absorption efficiency or a toxic effect of *C. capsulata*. Sotolongo (1988) found that 1.14×10^6 $cells \cdot ml^{-1}$ of *Chlorella* sp. was an adequate ration for biomass production of *Artemia*, but there are reports of low assimilation rates and low digestibility of *Chlorella* sp. and *Chlorella conductrix* by *Artemia salina* (Sick, 1976; Yunzhen *et al.*, 1998) and a toxic effect of *Chlorella* was suggested by Ryther (1954).

In this case, the toxic effect is improbable, because the different ration levels had similar effects on survival. In view of this and of the similar gross composition and energy content of both algae, the poor growth of *A. franciscana* fed *C. capsulata* may be explained only by the low absorption of this diet, probably because of the thick cell wall of *Chlorella*. As a consequence of its poor absorption, this microalga cannot be considered as a suitable diet for *A. franciscana*, because the amount of energy obtained is not sufficient to sustain normal growth. For the same reason, it is highly unlikely that its use would enhance the food quality of brine shrimps.

Table 1. Mean values ± standard error of size, AFDW and proximate composition (in % of AFDW) of *Chaetoceros muelleri* and *Chlorella capsulata.* The differences (Mann-Whitney test) are marked by different letters (n = 12 in all cases).

MICROALGAE	SIZE μM	AFDW PG·CELL^{-1}	PROTEIN %AFDW	CARBOHYDRATE %AFDW	LIPIDS %AFDW	ENERGETIC EQUIVALENT J·MG^{-1}
C. muelleri	6.6 x 5.2	40.0 ± 2.57b	39.7 ± 2.1 a	13.5 ± 1.2a	46.8 ± 5.3a	30.4
C. capsulata	4.4 x 3.2	15.2 ± 1.18a	31.8 ± 3.0a	27.4 ± 3.0b	41.3 ± 3.9a	28.8

Table 2. Mean values and standard error of survival, length, developmental index (DI),and AFDW, of *Artemia franciscana* after 3 and 7 days of culture with three rations of *C. capsulata* and *C. muelleri.* Equal or common letters indicate lack of significant differences (two-way ANOVA and Tukey multiple comparison test. α = 0.05).

DIET	AGE (DAYS)	RATION (MG·L^{-1}·D^{-1})	MEAN SURVIVAL %	MEAN LENGTH (MM)	D I	AFDW (μG·IND^{-1})
C. capsulata	3	11.7	100 (2)a	0.91 (0.01)a	4.2 (0.3)a	3.18 (0.04)a
		23.4	98 (1)a	0.97 (0.02)a	5.0 (0.7)ab	3.24 (0.22)a
		46.8	99 (3)a	0.99 (0.02)a	5.0 (0.0)ab	3.79 (0.21)a
C. muelleri	3	11.7	99 (2)a	1.67 (0.03)b	7.0 (0.7)b	12.85 (0.03)b
		23.4	98 (4)a	1.66 (0.04)b	7.0 (1.4)b	17.12 (0.07)d
		46.8	99 (2)a	1.75 (0.03)b	7.0 (0.7)b	15.45 (0.14)c
C. capsulata	7	11.7	93 (2)a	0.99 (0.02)a	7.4 (0.7)a	4.90 (0.71)a
		23.4	89 (3)a	1.18 (0.02)b	7.8 (0.7)a	10.01 (0.61)b
		46.8	98 (3)a	1.37 (0.03)c	8.4 (0.3)a	14.71 (0.73)b
C. muelleri	7	11.7	99 (2)a	3.44 (0.07)d	14.3 (0.3)b	35.98 (4.18)c
		23.4	94 (1)a	3.56 0.11)de	14.9 (0.1)c	73.01 (10.01)d
		46.8	98 (3)a	3.78 (0.09)e	15.5 (0.2)d	104.93 (5.08)e

Mean values of three replicates for each ration. n=3 for weights and 60 for length and DI.

Table 3. Total food supplied, mean values and standard error of total ingestion and of in increase in AFDW, food conversion index and gross growth efficiency (K_1) of *A. franciscana* after seven days of culture with the three rations of *C. capsulata* and *C. muelleri.* Equal or common letters indicate lack of significant differences (two-way ANOVA and Tukey multiple comparison test. α=0.05).

DIET	RATION (MG·L^{-1})	TOTAL FOOD SUPPLIED (μG·IND^{-1})	TOTAL INGESTION (μG·IND^{-1})	INCREASE IN AFDW (μG·IND^{-1})	FOOD CONVERSION INDEX	K1 (%)
	11.7	45.0	43.9 (5.2)a	2.4	18.7	12.1
C. capsulata	23.4	89.9	87.9 (13.8)b	7.5	12.0	8.5
	46.8	179.9	165.3 (24.3)c	12.2	14.7	6.8
C. muelleri	11.7	45.0	39.3 (2.4)a	33.5	1.4	85.3
	23.4	89.9	83.7 (19.7)b	70.5	1.3	84.2
	46.8	179.9	142.2 (14.3)bc	102.4	1.8	72.0

Mean values of six daily replicates for total ingestion and of three for AFDW increase.

ACKNOWLEDGEMENTS

Work in partial fulfillment towards the Ph. D. degree of the first author. Research supported by CIBNOR projects PAC38 and PAC2. Dr. E. Glazier edited the English manuscript.

REFERENCES

Albentosa, M., A. Pérez-Camacho, U. Labarta and M.J. Fernández-Reiriz (1997): Evaluation of freeze dried microalgal diets for the seed culture *of Ruditapes decussatus* using physiological and biochemical parameters. *Aquaculture* 154:315-321.

Brown, M.R., S.W. Jeffrey, J.K. Volkman and C.A. Dunstan (1997): Nutritional properties of microalgae for mariculture. *Aquaculture* 151:315-331.

Correa Sandoval, F. y L.F. Bückle Ramírez (1993): Morfología y biometría de cinco poblaciones de *Artemia franciscana* (Anostraca: Artemiidae). *Rev. Biol. trop.* 41:103-111.

Czygan, F. (1968): Sekundar-Carotinoide in Grünalgen. I. Chemie, Vorkommen und Faktoren welche die Bildung dieser Polyene beinflussen. *Arch. Mikrobiol.* 61:81-102.

De Micco, E. and R. Hubbard (2001): Plankton alternatives to *Artemia* for growth of marine shrimp *Litopenaeus vannamei* larvae: 180. *In*: *Aquaculture 2001*. World Aquaculture Society. Baton Rouge, LA.

Dunstan, G.A., J.K. Volkman, S.W. Jeffrey and S.M. Barrett (1992): Biochemical composition of microalgae from the classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. *J. exp. mar. Biol. Ecol.* 161:115-134.

Evjemo, J.O. and Y. Olsen (1999): Effect of food concentration on the growth and production rate of *Artemia franciscana* feeding on algae (T. Iso). *J. exp. mar. Biol. Ecol.* 242: 273-296.

Fábregas, J., A. Otero, E. Morales, B. Cordero and M. Patiño (1996): *Tetraselmis suecica* cultured in different nutrient concentrations varies in nutritional value to *Artemia*. *Aquaculture* 143:197-204.

Fábregas, J., A. Otero, E. Morales, B.O. Arredondo-Vega and M. Patiño (1998): Modification of the nutritive value of *Phaeodactylum tricornutum* for *Artemia* sp. in semi-continuous cultures. *Aquaculture* 169:167-176.

Gelabert, R. y A. de la Cruz, A. (1990): La selección del tamaño de partículas por *Artemia* (Branchiopoda). *Rev. Invest. Mar.* 9(1):63-69.

Gnaiger, E. (1983): Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. *In*: *Polarographic oxygen sensors*. (E. Gnaiger and H. Frostner, *eds*.) Springer Verlag, Berlin,pp: 337-345.

Guillard, R.R.L. (1972): Culture of the phytoplankton for feeding marine invertebrate larvae. *In*: *Culture of marine invertebrate animals* (W.L. Smith. and M.H. Chanley, *eds*.), Plenum Publishing Co., New York pp: 29-60

James, C.M. and T.S. Abu-Rezeq (1988): Effect of different cell densities of *Chlorella capsulata* and a marine *Chlorella* sp. for feeding the rotifer *Brachionus plicatilis*. *Aquaculture* 69:43-56.

Johnson, D.A. (1980): Evaluation of various diets for optimal growth and survival of selected life stages of *Artemia*:. *In*: *The brine shrimp Artemia* (G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers, *eds*.), Universa Press, Wetteren, Belgium, pp: 185-191.

Lavens, P. and P. Sorgeloos (1991): Production of *Artemia* in culture tanks. *In: Artemia Biology* (R.A. Browne, P. Sorgeloos and C.N.A. Trotman, *eds*.), CRC Press, Boca Raton, FL, Ann Arbor Boston, pp: 317-351.

Lora-Vilchis, M.C. and N. Doktor (2001): Evaluation of seven algal diets for spat of the Pacific Scallop *Argopecten ventricosus*. *J. World Aquac. Soc.* 32:228-235.

Merchie, G., P. Lavens, P. Dhert, M. Dehasque, H. Neils, D.A. Leenheer and P. Sorgeloos (1995): Variation of ascorbic acid content in different live food organisms. *Aquaculture* 134(3-4):325-337.

Naegel, L.C.A. (1999): Controlled production of *Artemia* biomass using an inert commercial diet, compared with the microalgae *Chaetoceros*. *Aquacult. Eng.* 21(1):49-59.

Ryther, J.H. (1954): Inhibitory effects of phytoplankton upon the feeding of *Daphnia magna* with reference to growth, reproduction and survival. *Ecology* 35:522-33.

Schrehardt, A. (1987): A scanning electron-microscope study of the post-embryonic development of *Artemia*:. *In*: *Artemia research and its applications Vol. 1. Morphology, genetics, strain characterization, toxicology* (P. Sorgeloos, D.A. Bengston, W. Decleir and E. Jaspers, *eds*.), Universa Press, Wetteren, Belgium, pp: 5-32.

Sick, L.V. (1976): Nutritional effect of five species of marine algae on the growth, development and survival of the brine shrimp *Artemia salina*. *Mar. Biol.* 35:69-78.

Sorokin, C. (1973): Dry weight, packed cell volume and optical density. *In: Handbook of phycological methods. Culture methods and growth measurement.* (J. Stein, *ed.*) Cambridge University Press, Cambridge and New York, pp: 321-343.

Sotolongo, M.E. (1988): The evaluation of various diets for optimal growth and survival of different life stages of *Artemia.. In: General Aquaculture Course*. Kanagawa International Fisheries Centre. JICA. Kanagawa, Japan, pp: 73-78.

Van Stappen, G. (1996): *Artemia*: use of cysts. *In: Manual on the production and use of live food for aquaculture* (P. Lavens and P. Sorgeloos, *eds*.), Food and Agriculture Organization of the United Nations. Fisheries Technical Paper, pp:107-123

Villegas, C.T. and A. Kanazawa (1979): Relationship between diet composition and growth of zoeal and mysis stages of *Penaeus japonicus* (Bate). *Fish. Res. J. of the Philippines* 4(2):32-40.

Yúfera, M. and L.M. Lubián (1990): Effects of microalgal diet on growth and development of invertebrates in marine aquaculture. *In: Introduction to applied phycology* (I. Akatsuka, *ed*.), SPB Academic Publishing bv The Hague, pp: 209-227.

Yunzhen, F., L. Hefang and L. Fayi (1998): Studies on polyunsaturated fatty acids of marine microalgae, III. Transfer of polyunsaturated fatty acids from microalgae to *Artemia salina. Stud. Mar. Sin. Haiyang Kexue Jikan* 40:161-166.

Zar, J.H, (1999): *Biostatistical Analysis*. Prentice-Hall, Inc., Engelwood Cliffs, N. J. USA, 663 pp.

Aceptado: 13 de octubre del 2003