Cultivation of the microalgae *Chaetoceros gracilis* to feed the rotifer *Brachionus plicatilis*

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Received 21-IX-2012 Corrected 29-I-2013 Accepted 20-II-2013

ABSTRACT
The microalgae *Chaetoceros gracilis* was cultivated to feed the rotifer *Brachionus plicatilis*. Microalgae and rotifers were grown separately, each for 10 days in a growing system volume of 62,5ml to 20L (1st phase). This was followed by 10 days in 250L (2nd phase), after 10 days in 1 500L (3rd phase). The growth rate exponential population at intervals of 24h for *C. gracilis* was 0,72, 0,30, and 0,28cells/ml to three phases of cultivation. Within 24h the arithmetic growth rate for the population of *B. plicatilis* was 330, 1 858 and 13 912rotifers/day in three phases of cultivation. Twenty-four hour intervals counts from the increasing number of organisms in the cultivation of microalgae, allows the prediction of a rotifer production necessary to feed a certain number organisms.

KEY WORDS
Rotifer culture, population growth, fecundity, *Chaetoceros* culture

RESUMEN
La microalga *Chaetoceros gracilis* fue cultivada para alimentar al rotífero *Brachionus plicatilis*. Las microalgas y rotíferos fueron cultivados por separado, cada uno por 10 días en un sistema de volumen creciente de 62,5 ml a 20L (1ª fase). Esto fue seguido por 10 días en 250L (2ª fase), luego de 10 días en 1 500L (3ª fase). La tasa de crecimiento exponencial de la población a intervalos de 24h para *C. gracilis* fue 0,72, 0,30 y 0,28cel/ml para las tres fases de cultivo. En 24h la tasa de crecimiento aritmética para la población de *B. plicatilis* fue de 330, 1 858 y 13 912rotíferos/día en las tres fases del cultivo. A intervalos de 24h los conteos del creciente número de organismos en el cultivo de microalgas, permite la predicción de una producción de rotíferos necesaria para alimentar un número determinado de organismos.

PALABRAS CLAVE
Cultivo de rotíferos, crecimiento de la población, la fecundidad, cultivo de *Chaetoceros*

In the aquatic natural ecosystems the continuity of the species depends on the balance established between the different levels of the food plot. Thus, the development and survival of larvae and juvenile depends on the presence of organisms that make up the phytoplankton and zooplankton, who in turn occur in the presence of adequate nutrients (Nieves et al., 1996).

Commercial mariculture of many marine and fresh water organisms has been limited by several factors including inconsistent larvae production. This is partially due to the difficulty of producing great quantities of high-quality live foods, especially microalgae and rotifers (Horstmann, 1985; Fulks & Main, 1991).

Valenzuela, Gendrop, Pérez and Wilburn (1999) mentions that the use of environment nutrients derived from fertilizers, nitrogen and phosphorous F/2 is a viable alternative to the cultivation of *C. muelleri* as food for larvae of *Litopenaeus vannamei*. The diatom, *Chaetoceros gracilis* (F. Schütt, 1895), is extensively used as a nutritive food organism in the rearing of prawn larvae (Chu, 1989). Two of the best microalgae for aquaculture feeds are Isochrysis galbana and *C. gracilis*. Helm, Bourne and Lovatelli (2004) mentioned that among some cultured phytoplankton species used in bivalve mollusk and fish hatcheries is *C. gracilis*. Their effectiveness is due in part to their small size and n-3 HUFA content (Barclay & Zeller, 1996).

Rotifers fed on microalgae such as *C. gracilis* showed better viability, larger size and low ciliate contamination (Knu, 2004).

The purpose of this study was to culture up to 1 500L of the microalgae *C. gracilis* to feed 1 500L culture of rotifers (*Brachionus plicatilis*) (Müller, 1786). For subsequent use as live food for different organisms.

MATERIALS AND METHODS

Culture of *C. gracilis* and *B. plicatilis* (Tables 1 and 2) was initially indoors in sea water at 24°C (±1) and aerated by an electric pump. Volume was increased over the course of 10 days at 24h intervals increase, from 60,5ml to 20L under continuous artificial light from twelve 30 w fluorescent tubes. Two subsequent stages, held at 250L and 1 500L respectively, were outdoors, with 13h natural light and 11h darkness, which consisted of EDTA-Fe together with a metal solution Co, Zn, Cu, Mo (Nutrafin Plant Gro).

For *C. gracilis*, these subsequent stages used medium F as described by Guillard (1973), and followed the batch culture method (Coutteau, 1996). Natural sea water was filtered through 5µm then 1µm; then 0,25ml of commercial chlorine for 24h was used, and 0,25ml of sodium thiosulfate at 10% normal solution was applied to neutralize the commercial chlorine.

A number of the culture volume of microalgae harvested from 20L was used to seed the 250L volume, after 10 days a number of the microalgae harvested were used to seed the 1 500L volumes, and after 10 days these microalgae harvested were used to feed the rotifers (Table 1). At 24h intervals, a microalgae sample (1-10ml) was preserved with lugol and counted under a light microscope with a Neubauer hematocimeter to calculate the concentration (Odum, 1972). Population growth rate was calculated by the least-square method using the following formula: Number of cells = $a\ e^{b\ (d)}$, where a is a constant, b is the slope, and d is duration of culture (days).

Culture of *B. plicatilis* was similar to that of the microalgae (Table 2). Rotifers were cultured in sterile sea water and fed at 24h intervals with at 1 x 106cells/ml microalgae. The total number of rotifers (nr) was calculated by adding the total number of rotifers to the total number of eggs/ml (nr = nr + ne) (Ramírez-Sevilla, 1991). Population growth (ml) was determined at 24h interval counts of 10-20 groups of rotifers. Daily population growth rate was calculated by the least-squared method using the following formula: Number of rotifers = $a + b^{(d)}$ where (d) is duration of culture (days), a is constant, and b is slope.

RESULTS

Table 1 shows that in 62,5ml the total average cells/ml initiated with 1 800 000 and finalized in 20L with 3 283 300cells/ml. In 250L initiated with 250 000 and it finalized with 3 866 700cells/ml. In 1 500L initiated with 200 000 and finalized with 2 850 000cells/ml. Table 2 shows that in 62,5ml the total average rotifers initiated with 35,7 and finalized in 20L with 151rotifers/ml. In 250L initiated with 42,7 and it finalise with 120rotifers/ml. In 1 500L initiated with 34,7 and finalized with 119rotifers/ml.

In general, population growth rate was exponential for microalgae and arithmetic for rotifers (Table 3). Also at growing volume culture of 62,5ml to 20L at 24h intervals exponential population growth rate could be expressed as 0,84 since the determination coefficient was 0,9.

DISCUSSION

When Vega-Pérez (1991), Sapién and Leal (1992) used Guillard's F medium, in a continuous culture of *Tetraselmis suesica* and *Chaetoceros* sp., they found that the production of microalgae was proportionally quantitative to the quantity of supplied nutrients. In present paper the microalgae growth rate results were similar. Also Reyes-Bustamante (1999), found that F medium obtained the highest yields of the microalgae *Kirchneriella obesa, Scenedesmus quadricauda*, and *Chlorococcum infusorium*, although its cost was a limiting factor. The rate of microalgae population growth, in media based on commercial agricultural fertilizers is comparable to the results with the F medium, but at lower cost. Use of F medium for microalgae culture enhances exponential without any toxic effects, since the constituent metals are necessary for synthesis of enzymatic growth cofactors used in oxy-reduction cycles (Round, 1973; Fogg, 1975).

As a food source, *B. plicatilis* favors *C. gracilis*, possibly because of the relatively small individual size of the cells (4 x 4µm) compared with *Dunaliella* sp. (8 x 14µm) or *Tetraselmis* sp. (7 x 12µm) (Ortega, 1984; Trujillo & Voltolina, 1994). The density of rotifers in commercial cultures is generally between 100 and 200/ml (Hirata, 1980). Helm, Orhun, Bourne and Lovatelli (1991), reported that in culture volumes of 8 500L, 540 *B. plicatilis*/ml were maintained for periods of 8 to 60 days using bread yeast supplemented with microalgae.

Density of rotifers obtained in the present study was lower than those observed by other researchers (Hirata, 1980; Schluter & Groeneweg, 1981; Meralgemene, 1985; Hirayama, 1987). Maeda and Hino (1991) also mentioned

TABLE 1
Growth of the population *C. gracilis* cultivated for three consecutive periods of 10 days

Day	V (ml)	A/ml	SD
1	62,5	1 800 000	0
2	125	2 300 000	336,60
3	250	2 700 000	216,02
4	500	2 966 700	205,48
5	1000	4 200 000	294,39
6	2000	2 800 000	294,39
7	5000	2 800 000	81,65
8	10000	2 400 000	216,02
9	20000	2 900 000	81,65
10	20000	3 283300	209,50
1	250000	250 000	0
2	250000	416 670	23,57
3	250000	750 000	40,82
4	250000	1 116 700	62,36
5	250000	1 516 700	124,72
6	250000	1 916 700	102,74
7	250000	2 416 700	84,98
8	250000	2 733 300	563,22
9	250000	3 650 000	40,82
10	250000	3 866 700	478,42
1	1 500 000	200 000	0
2	1 500 000	333 330	32,57
3	1 500 000	423 330	20,548
4	1 500 000	606 670	49,216
5	1 500 000	673 330	55,578
6	1 500 000	933 330	224,85
7	1 500 000	1 266 700	379,33
8	1 500 000	1 600 000	294,39
9	1 500 000	2 316 700	385,86
10	1 500 000	2 850 000	533,85

The increase in the average number of cells (A/mL) volume (V), and standard deviation (SD).

TABLE 2
Population growth of *B. plicatilis* cultivated during three consecutive periods of 10 days

Day	V (ml)	A/ml	SD
1	62,5	35,7	2,49
2	125	48,3	6,24
3	250	51,7	12,5
4	500	64,7	6,60
5	1 000	85,3	8,99
6	2 000	105,0	4,11
7	5 000	111,0	5,35
8	10 000	127,0	11,3
9	20 000	138,0	12,6
10	20 000	151,0	14,7
1	250 000	42,7	6,18
2	250 000	56,3	2,49
3	250 000	60,7	6,13
4	250 000	72,0	9,09
5	250 000	83,7	6,55
6	250 000	84,0	6,98
7	250 000	86,3	4,99
8	250 000	92,3	3,68
9	250 000	104,0	3,30
10	250 000	120,0	4,99
1	1 500 000	34,7	5,44
2	1 500 000	42,7	5,79
3	1 500 000	54,7	3,30
4	1 500 000	56,7	10,50
5	1 500 000	72,0	6,48
6	1 500 000	78,3	9,18
7	1 500 000	86,3	8,96
8	1 500 000	99,0	10,00
9	1 500 000	108,0	7,12
10	1 500 000	119,0	12,20

Increase in the average numbers of eggs (ne) more rotifers (nr) (A/mL) times volume (V), and standard deviation (SD).

TABLE 3
Regression parameters of population growth of *C. gracilis* and *B. plicatilis*, at three culture volumes

Volume	Constant	Slope	r^2
MICROALGAE			
62,5 mL-20 L	0,0743	exp 0,7232	0,98
250 L	67,3	exp 0,2961	0,94
1 500 L	260,8	exp 0,2828	0,99
ROTIFERS			
62,5 mL-20L	-1019,5	330,06	0,72
250 L	9 816,6	1 857,6	0,95
1 500 L	36 133	13 912,1	0,99

that bacteria and protozoa are important biotic factors that affect the rate of growth of the rotifer population.

Once the production of microalgae is continuous, production of rotifers is continuous, and it is possible to feed larvae, reduce their natural mortality, and predict the number of larvae that will grow.

ACKNOWLEDGEMENTS

We appreciate the technical support of A. Nuñez P. and S. Rendón R.

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