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Cultivation of the Microalga *Thalassiosira weissflogii* to feed the Rotifer *Brachionus rotundiformis*

Abstract

The microalga *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle, 1977 was cultivated to find its adequate concentration to feed the rotifer *Brachionus rotundiformis* Tschugunoff, 1921. The microalgae and rotifers were reared separately; microalgae cultures used Guillard and Ryther F/2 medium in transparent 19 L plastic bottles, yielding 16 L of the useful crop; agitation was kept constant by bubbling compressed air filtered at 1 µm; lighting was continuous through six white daylight fluorescent lamps. The temperature ranged between 23.1° and 24.9°C, and disinfection was with commercial sodium hypochlorite at5%, which was eliminated at a rate of 0.06 g•L^{-1} sodium thiosulfate, then the F/2 nutrients were added. Rotifers in 15 L plastic containers were fed daily on *T. weissflogii* at 2.469 x 10^5cells·mL^1; Crops were harvested every third day (48 h). *T.weissflogii* cultures maintained for 48h achieved concentrations adequate to feed these rotifers.

Keywords: Cultivation; Food of rotifers; Nutrient addition; Microalgae density; *Thalassiosira weissflogii*

Short Communication

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AA Ortega-Salas[1]* and Pedro Flores Nava[2]

[1]*Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, México*

[2]*Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, México*

***Corresponding author:** AA Ortega-Salas, Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, UNAM. Calzada Joel M, México, Email: ortsal@ola.icmyl.unam.mx

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Introduction

Rotifers are a useful source of food in aquaculture [1]. Their nutritional content is enhanced if they are fed on algae, particularly in terms of vitamins and essential fattyacids. The algal genera most frequently used for this purpose are *Chaetoceros, Thalassiosira, Isochrysis, Nannochloropsis* and *Tetraselmis* [2]. The diatom *T. weissflogii* (size 6-15µm x 20 µm) is used as food for shrimp and in the production of bivalve larvae. This alga is considered by several hatcheries to be the best for growing shrimp larvae since it is a rich source of eicosapentaenoic acid (EPA) and docosahexaenoic acid DHA, essentials for growth and development of marine organisms [3-6].

Emmerson [3] reported that *Penaeus indicus* larvae at the zoea 1 stage ingested *T.weissflogii* at a rate of 2.5x10^3cells•ind^{-1}•h^{-1}.

In this work, the microalga *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle, 1977 was cultivated under conditions that sought to improve its production rate and to achieve a concentration adequate to feed the rotifer *Brachionus rotundiformis* Tschugunoff, 1921under laboratory conditions.

Materials and Methods

The diatom *T.weisflogii* (collection keyTH-W-1) was obtained from the collection of the Department of Aquaculture, Center for Scientific Research and Higher Education, Ensenada, Baja California. Culture used F/2 medium [5] with twice metasilicate. Seawater was pumped from the Bay of Mazatlán and passed through a filter system in series, with progressive particle retention of 10,5 and 1µm; an additional activated carbon filter removed organic compounds.

The temperature ranged between 23.1 and 24.9±0.52°C. The water was disinfected with1 mL•L^{-1} commercial sodium hypochloriteat 5%, at least24 h before use. Free chlorine residues were removed with 0.06g of sodium thiosulfate per liter of water, accelerating the process of neutralization by vigorous shaking aeration for 20 to25 minutes. Subsequently, the absence of free chlorine was confirmed by the traditional colorimetric technique using a commercial kit with ortho-toluidineasa indicator. Nutrient solutions were added until the F formulation was achieved.

In order to have the required daily amount of microalgal biomass, *T.weissflogii* cultures were maintained in F medium for 48h to obtain adequate concentrations of microalgae to feed rotifers. Cultures were performed in clear plastic bottles of 19L, with16 L of a useful crop; stirring was kept constant by bubbling with filtered compressed airat1 micron. This served to keep the algae in suspension, and encouraged mixing, which was intended to accelerate the exchange of gases between the culture medium and the atmosphere, removing excess photosynthetic oxygen and facilitating the dissolution of CO_2 in the medium. Lighting was continuous through six white day light fluorescent lamps.

Before rotifers were fed, the adequate cell concentration of the *T. weissflogii* was verified by direct counts in a compound microscope using a hemocytometer 0.1 mm deep, equipped with a Neubauer rule. Every 24 h, samples of the culture were taken to check their condition and their stability by optical density reading sina Hach DR 5000spectrophotometerata wavelength of 550 µm. The viability of the culture was monitored by pH readings of each culture vessel with a portable potentiometer rCHEK-MITE Corning model pH-10, pre-calibrated with buffers of 7.0 and10.0units; this was done in order to prevent high pH values due to a rapid use of bicarbonates, leading to a limitation of substrates for photosynthesis and hence to maturation of the crop before harvest time, or a switch to the stationary phase or death. Cells were counted at 24 and 48 h and were harvested at 48 h when the cultures were in the exponential growth phase.

Results

An initial concentration of 0.101x 10^5cells·mL^{-1} increased to 0.723x10^5cells·mL^{-1} at 24h and to 2.469x10^5cells·mL^{-1} at 48h, the end of each set of cultures (Table 1). Crops were harvested every third day. These cell concentrations indicated that 2.854 *T.weissflogii* cell divisions had occurred in the first 24h, and a further 1.772 at 48h, a total of 4.626 at the time of harvesting.

Table 1: Culture of *T.weissflogii*: cell concentration (CC, 10^5cells·mL^{-1}), pH and optical density (OD).Values: average ± standard deviation.

Hours	CC	pH	OD
0	0.101 ± 0.008	8.094 ± 0.253	0.044 ± 0.003
24	0.723 ± 0.164	8.422 ± 0.171	0.062 ± 0.004
48	2.469 ± 0.522	9.084 ± 0.183	0.110 ± 0.006

The cultures from which the samples were obtained to estimate the weight of the microalgae had a cell concentration of1.903x10^5cells·mL^{-1}; these crops produced at the time of harvest at 48 ha dry biomass of 114.131mg·mL^{-1}, and the organic content was 66.681mg·mL^{-1}. With the above values, the unit dry biomass is estimated at 603.363±52.896pg·cells^{-1}, and unit organic content of352.801±29.697pg· cells^{-1}, and 58 511% of the dry weight was organic matter (Table 2).

B.rotundiformis cultures were maintained at a temperature of 26.6±0.771 and 27.8±0.830°C. The pH values in all recipients ranged from 7.85 to 8.19, with a mean value of 8.03. Average daily rotifer density obtained was 131.5±14.153rot·mL^{-1}; this amount was enough to feed three stages of mysislarvae of *Litopenaeus vannamei* (Boone, 1931). pH values increased from 8.094 at 0 h to 9.084 at 48 h. The optical density increased from 0.044 at 0 h to 0.110 at 48 h.

Table 2: *Thalassiosira weissflogii* sampled after 48 h culture for weight determinations: cell concentration(CC, 10^5cells·mL^{-1}), dry weight (DW) and organic weight (OW) inmg·mL^{-1}, dry weight per unit (DWU) and organic weight unit(OWU) pg·cells^{-1}, percentage of organic matter in the dry weight(OW/DW).Values: Average ± standard deviation.

CC	DW	OW	DWU	OWU	OW/DW
10^5cells·mL^{-1}	µg·mL^{-1}	µg·mL^{-1}	pg·cells^{-1}	pg·cells^{-1}	%
1.903 ± 0.171	114.131 ± 6.645	66.681 ± 2.200	603.363 ± 52.896	352.801 ± 29.692	58.511 ± 1.687

Discussion

Bermúdez-Lizárraga [1] also used *T. weissflogii* to feed rotifers, but the present study has confirmed that after culture for 48 h under the conditions described here, the crop is of sufficient concentration to feed rotifers and larvae of shrimp.

Flores-Nava [4] found that the density of *T.weissflogii* supplied to zoe a larvae of *L.vannamei* did not influence the growth, survival, and development of the larvae.

The diatom *T.pseudonana* (Cleve, 1873) is the only diet that has been found to produce rotifers with the required complement of n^3 polyunsaturated fatty acids suitable for larval fish rearing [6], but here we have used *T. weissflogii* to feed rotifers with good results.

Conclusion

T.weissflogii cultures maintained for 48h achieved adequate concentrations to feed the rotifer *B. rotundiformis* which later can be used to feed shrimp larvae.

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Conflict of Interest

None.

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