GROWTH AND BIOMASS PRODUCTION OF Chaetoceros muelleri IN MASS OUTDOOR CULTURES: EFFECT OF THE HOUR OF THE INOCULATION, SIZE OF THE INOCULUM AND CULTURE MEDIUM.

J.A. López-Elias 1 *, F. Enríquez-Ocaña 1, M.N. Pablos-Mitre 1, N. Huerta-Aldaz 1, S. Leal 2, A. Miranda-Baeza 3, M. Nieves-Soto 4 and I. Vásquez-Salgado 1

(1) Department of Research DICTUS of the University of Sonora, Rosales y Niños Héroes s/n Col. Centro, CP 83000, Hermosillo, Sonora, México.
(2) Centro de Investigaciones Marinas, Universidad de La Habana, Calle 16 No. 114, CP 11300, Playa, Ciudad Habana, Cuba.
(3) Centro de Estudios Superiores del Estado de Sonora (CESUES), Laboratorio de Tecnologías de Cultivo de Organismos Acuáticos, Carr. a Huatabampo, Km 5, CP 85800, Navojoa, Sonora, México.
(4) Laboratorio de Ecolisiología y cultivo de organismos acuáticos, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen s/n, CP 82000, Mazatlán, Sinaloa, México.
(*) Autor corresponding: Email: jalopez@guayacan.uson.mx

ABSTRACT

Microalgae was cultivated in mass outdoor culture with an inadequate control during the inoculation of the mass outdoors tanks, and at the harvest, different concentration and cell quality are obtained. In this study we evaluate the effect of the hour of the inoculation, size of the inoculum and culture medium on the growth and biomass production. This experiment was made in the outdoor experimental units of 250 liters with constant aeration three times during spring. The treatments were: initial concentration of 0.2 and 0.4 x 10^6 cells.mL^-1, culture medium the f and f/2 (Guillard, 1975) and hour of the inoculation at 06:00 and 12:00 h. Temperature and illumination strongly fluctuated, and the pH increased with the time. At the harvest the cellular concentration varied from 1.21 to 2.83 x 10^6 cells.mL^-1, although the grown cultures at 06:00 h and inoculum of 0.4 x 10^6 cells.mL^-1 in the f medium were the highest values. The variability in the cultures at the harvest was very low, due to the standardization size of the inoculum number-cell-based. The maximum growth rate was constant (0.82 to 1.27 div.days^-1), while in the accumulated rate it was higher in the inoculated cultures with 0.2 x 10^6 cells.mL^-1 (2.59 – 3.09 divisions). The dry and organic biomass obtained was slightly higher in the cultures initiated with the higher inoculum. In general, the cultures with a higher production of cellular density and biomass, were those ones that initiated at 06:00 h with a major inoculum and in the f medium.

Key words: microalgae; culture; inoculum; culture medium, Chaetoceros muelleri.

RESUMEN

Las microalgas fueron cultivadas en cultivo al aire libre con un control inadecuado durante la inoculación. Esto hace que en la cosecha se obtengan diferentes concentraciones y calidad de las células. En este estudio fue evaluado el efecto de la hora de inoculación, tamaño del inóculo y medio de cultivo en crecimiento y producción de biomasa. Estos experimentos fueron hechos en unidades experimentales a la intemperie de 250 litros, con tres repeticiones durante la primavera. Los tratamientos fueron: concentraciones iniciales de 0.2 y 0.4 x 10^6 cel.mL^-1, medio de cultivo f y f/2 (Guillard, 1975) y horas de inoculación a las 06:00 y 12:00 h. La temperatura y la iluminación fluctuaron grandemente y el pH se incrementó en el tiempo. La concentración celular en la cosecha varió entre 1.21 y 2.83 x 10^6 cel.mL^-1 y los mejores resultados se obtuvieron cuando se inoculaba a las 06:00 h y con tamaño de inóculo de 0.4 x 10^6 cel.mL^-1 con el medio f. La velocidad de crecimiento máxima fue constante (0.82 y 1.27 div.día^-1), mientras la velocidad acumulada fue mayor en los cultivos inoculados con 0.2 x 10^6 cel.mL^-1 (2.59 – 3.09 divisiones). La biomasa seca y orgánica fue ligeramente mayor en los cultivos iniciados con el mayor tamaño de inóculo. En general, los cultivos con alta densidad celular y producción de biomasa fueron los inoculados a las 06:00 h, con el mayor tamaño de inóculo y el medio f.

Palabras clave: microalgas; cultivo; inóculos; medios de cultivo, Chaetoceros muelleri.

One of the most important aspects to consider in the shrimp post larvae productions is the nutrition. During the first larvae stages it is regularly used living food, like microalgae, and the nauplii of Artemia spp. (Merchie et al., 1997; Boeing, 2005).

The microalgae commonly used as food of penaeids larvae are of different genus, like Chaetoceros spp., Tetraselmis spp. and Dunaliella spp. (Apt and Behrens, 1999; Voltolina and López-Elias, 2002). This genus are considered as a nutritious food,
because it contains all the requirements for the peneids larvae (Rodríguez et al., 1994; D’Souza and Kelly, 2000).

In the commercial hatcheries, high variations of light and temperature are present because the microalgae is commonly cultivated in outdoor tanks; in those conditions the levels of both variables depend on the environmental conditions and can affect the microalgae growth.

In some shrimp postlarvae hatcheries, the main objective is to produce enough quantities of microalgae biomass to satisfy the necessities of the larvae cultures of shrimp. Therefore the improvements in the cultures of microalgae have been made by empiric observations and no scientific principles for bioassays are used. The production system used in commercial hatcheries is the sequential static culture (López-Elías et al., 2005a). The inconvenience of this system is that it requires a lot of infrastructures and varies the quality and quantity of the biomass produced (Coutteau and Sorgeloos, 1992; López-Elías et al., 2003).

The factors to be considered for these cultures; are the type and form of the recipient, the location of it and the production routine (Becker, 1995). Researches conducted in different commercial laboratories in Sinaloa and Sonora, states of Mexico, suggested that a real standardization in the massive production of microalgae does not exist, due primordially, to the variation in the cellular concentrations used as inoculum, which are between 0.1 and 0.6 $\times 10^6$ cells.mL$^{-1}$. Because the inoculum is based on the volume, independently of the cell density, at the end of the cultures the cell numbers are different in the outdoor tanks (López-Elías et al., 2005a).

In mass outdoor culture of microalgae in shrimp hatcheries of Mexico doesn’t measure the cell number until the end of the culture, generally at 72 hours, and all the information of the development of the cultures are unknown. For this reason, the phase and biochemical composition of the cultures are different among the culture tanks. It is important to consider that the cultures are initiated until noon (12:00 h) and not during the morning, because it’s when the solar radiation can be used in a major way for the cells.

The $f/2$ medium of Guillard is common used in commercial hatcheries, but it is important to verify if the quantity of nutrients are adequate. This medium is used until intermediate level and for mass culture generally used agriculture fertilizers.

In this work we evaluate the growth and production of dry biomass in two inoculum concentrations, initiated at 06:00 and 12:00 h, in two cultures mediums, $f$ and $f/2$ of Guillard.

**MATERIALS AND METHODS**

The selected specie was *Chaetoceros muelleri* (Lemmerman, 1898) because it is the basic food given in most of the commercial laboratories of peneids (Voltonina and López-Elías, 2002). It has a high growth rate (Trujillo-Valle and Voltolina, 1994) and it can grow in the outdoor conditions with a wide interval of temperature and illumination (Nelson et al., 1992).

The study was conducted during the spring (April), in the Experimental Kino Unit of the University of Sonora laboratory. The outdoor cultures were developed in experimental tanks of 250 liters of capacity per triplicate. The treatments were: initial concentration of $0.2 \times 10^6$ cells.mL$^{-1}$ in the $f/2$ medium, inoculated at 06:00 (a) and 12:00 h (b); initial concentration of $0.2 \times 10^6$ cells.mL$^{-1}$ in the medium, inoculated at 06:00 (c) and 12:00 h (d); initial concentration of $0.4 \times 10^6$ cells.mL$^{-1}$ in the $f/2$ medium at 06:00 (e) and 12:00 h (f) and initial concentration de $0.4 \times 10^6$ cells.mL$^{-1}$ in the $f$ medium at 06:00 (g) and 12:00 h (h).

The culture medium was the Guillard $f$ (1975), broadly used for the microalgae cultivation.

The temperature was measured with a conventional mercury thermometer, the illumination was registered in luxes with a portable photometer of Fisher brand, the luxes were converted to $\mu$mol.m$^{-2}$.s$^{-1}$, where $1 \, \mu$mol.m$^{-2}$.s$^{-1} = 78.3$ lux (Lüning, 1981) and the pH was measured with a portable potentiometer that was previously calibrated with buffer solution of pH of 7 and 10, in all cases, the variables were measured every six hours.

The samples of the cell count were taken each 12 hours, since the inoculum until the harvest of the culture. The samples were fixed with lugol solution and were counted immediately after the sampling in a microscope composed by a hemocytometer of 0.1 mm of depth (Andersen, 2005).

To determinate the dry biomass, the samples (100 to 200 mL) were taken at the end of the cultures, and filtered in previously calibrated Whatman GFC fiberglass filters of 47 mm of diameter. The samples were burned in a conventional oven at 75°C for 8 hours and then were ashed in a muffle furnace to 480°C per 12 hours and weighed until
constant weight in an analytical balance to obtain
the inorganic weight. The organic matter was
calculated by difference between these values
(López-Elías et al., 1995).

With the information of the cultures, we estimated,
of the 48 and 72 hours the cell concentration, the
maximum \((µ_{max} = (\log_2 B_2 - \log_2 B_1)/t_2-t_1)\) and
accumulated growth rate \((µ=µ_1+µ_2+µ_3)\) and
biomass; all cultures inoculated at 06:00 h (a, c, e
and g) were compared with the ones initiated at
12:00 h (b, d, f and h) with t Student test, when
the premise of normality and homoscedasticity of
variances were fulfilled and with the Mann Winney
test, when the data did not fulfill the premises.

Later, an ANOVA of two ways was made to
compare the parameters obtained of the hour of
the inoculum and the culture medium (Zar, 1984).

RESULTS AND DISCUSSION

The temperature fluctuated from 20 to 33ºC, the
minimum values were registered at 06:00 h and
the highest at 16:00 h, with a fast increment from
06:00 to 14:00 h (Fig. 1). In previous studies
developed in the Northwest of Mexico, these
temperatures have been recorded in the spring.

The illumination varied form 347 to 1584 \(µ\text{mol.m}^{-2}.s^{-1}\), with values above the 1500 \(µ\text{mol.m}^{-2}.s^{-1}\) from
10:00 to 16:00 h (Fig. 2). When contrasting with
other investigations carried out in the same region,
it was found that the values are very similar
(López-Elías et al. 2005 a, b).

It was observed that pH tends to increase
depending on the age of the culture, additional
that with fluctuations are presented during the

cycle of day and night. The higher values were
registered at the beginning of the day and the
lower at the end (Fig. 3). In general, the bicarbonate and CO₂ extinguish when the culture
grows, so the carbonates are left with an alkaline
pH (Richmond, 2004); mean while the fluctuations
between days and nights are due to the photosynthesis and respiration processes (Riley
and Chester, 1971).

In all treatments, the lag phase was undetectable
and the log phase was registered between 24 and
48 hours, after that, the growth decreased slightly,
but all cultures were harvested at 72 hours (Fig.
4), because the majority of the commercial shrimp
hatcheries harvest the microalgae cultures to feed
shrimp larvae’s at this time (López-Elías et al.,
2005a).
The growth of the cultures initiated at 06:00 and 12:00 h had an ascending tendency in the different treatments of inoculum and the culture medium. In general, the cultures, reached by the end of the culture (72 h) an average cellular concentration between 1.21 and 2.83 x 10^6 cells.mL^{-1} (Table 1). The variation coefficient was very low in all treatments (< 10%) in virtue that the inoculum was established by a number-cell-based, not based on a pre established volume, which is routine in commercial laboratories of shrimp larvae. In these laboratories the variations get between a 9 and 72% with an average of 28% (López-Elías et al. 2005a).

No significant differences were found in the cellular concentration (at 48 hours) between the cultures inoculated at 06:00 and 12:00 h (z=0.46, p >0.67), although with an extra day (72 hours) of growth it was found that the cellular concentration of the cultures initiated at 06:00 h was partially higher that the cultures initiated at 12:00 h (z =2.37, p<0.05).

The density of inoculum was determinant to establish significant differences between the cellular densities reached at 48 hours between the treatments. The cellular density was higher in the cultures inoculated with 0.4 x 10^6 cells.mL^{-1} in the f medium, followed by the cultures initiated with the same inoculum, but with the f/2 medium (Table 1). The cellular density reached in this time is similar to the commercial culture laboratories of the Northwest of Mexico (López-Elías et al., 2005a, b).

In the last two days of the culture (48 and 72 h), it was found that the higher cellular density corresponded to the treatments with higher inoculum and with the f medium, although in these same conditions, they were also recorded lower accumulated growth rate, due to the "auto-shading" of the same microorganisms. In comparison with some cultures of Sinaloa and Sonora laboratories that harvest at the 72 hours (López-Elías et al., 2005a), the density was higher in all the treatments of this research, because the inoculum was number-cells-based and a possibly effect of the kind of recipients, in this case were tanks of 250 liters whereas in the commercial laboratories the recipients are tanks of 2 500 and 4 000 liters.
Table 1. Average cellular concentration and standard deviations at 48 y 72 hours, maximum growth rate ($\mu$) and accumulated and standard deviation in the cultures growth at 06:00 h and 12:00 h to inoculums de 0.2 y 0.4 x 10^6 cells.mL^-1 in the f and f/2 medium. Different letters indicate significant differences (two way analysis of variance, $\alpha = 0.05$) a < b< c.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (x 10^6)</th>
<th>Rate maximum</th>
<th>Rate accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 h</td>
<td>72 h</td>
<td></td>
</tr>
<tr>
<td>Inoculum: 06:00 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 x 10^6 f/2</td>
<td>0.75 ± 0.01^a</td>
<td>1.52 ± 0.11^a</td>
<td>1.24 ± 0.17^b</td>
</tr>
<tr>
<td>0.2 x 10^6 f</td>
<td>0.82 ± 0.04^a</td>
<td>1.71 ± 0.17^a</td>
<td>0.90 ± 0.07^a</td>
</tr>
<tr>
<td>0.4 x 10^6 f/2</td>
<td>1.31 ± 0.13^b</td>
<td>1.75 ± 0.02a</td>
<td>1.15 ± 0.16^ab</td>
</tr>
<tr>
<td>0.4 x 10^6 f</td>
<td>1.55 ± 0.04^c</td>
<td>2.83 ± 0.07^b</td>
<td>0.96 ± 0.05^ab</td>
</tr>
<tr>
<td>Inoculum: 12:00 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 x 10^6 f/2</td>
<td>0.73 ± 0.18^a</td>
<td>1.21 ± 0.11^a</td>
<td>0.82 ± 0.36^a</td>
</tr>
<tr>
<td>0.2 x 10^6 f</td>
<td>0.92 ± 0.05^ab</td>
<td>1.41 ± 0.17^b</td>
<td>1.10 ± 0.12^a</td>
</tr>
<tr>
<td>0.4 x 10^6 f/2</td>
<td>1.13 ± 0.12^bc</td>
<td>1.39 ± 0.02^ab</td>
<td>1.21 ± 0.21^b</td>
</tr>
<tr>
<td>0.4 x 10^6 f</td>
<td>1.28 ± 0.03^c</td>
<td>2.78 ± 0.07^c</td>
<td>1.27 ± 0.07^a</td>
</tr>
</tbody>
</table>

In all the treatments, the maximum division rate fluctuated around one division per day (Table 1), this rate was in the interval reported for other massive exterior cultures (López-Elías et al., 2005b).

No significant differences were found between the maximum growth rate in the cultures initiated at 06:00 h compared with the ones at 12:00 h ($t=0.4$, $p>0.69$); however the comparison of the cultures initiated at 06:00 show a significant difference between the treatment with inoculums of 0.2 x 10^6 cells.mL^-1 in the f/2 medium in comparison with the f medium. Nevertheless, when the maximum growth rates between the cultures initiated at 12:00 h were compared, no significant differences were found (Table 1).

The accumulated growth rate was different between the cultures initiated at 06:00 and the ones of 12:00 h, with the higher value average in the first ones ($z=2.08$, $p>0.04$). The lowers values corresponded to the treatments inoculated with 0.4 x 10^6 cells.mL^-1 in the f/2 medium.

The average values found varied from 36.3 to 99.1 g.m^-3, while in other commercial laboratory research in Sonora state, average values were registered between 29.0 y 69.7 g.m^-3 (López-Elías et al., 2005a).

Considering all the treatments (including the cultures initiated at 06:00 h and the ones of 12:00 h) dry biomass was higher in the treatment with the inoculum of 0.4 x 10^6 cells.mL^-1 with the f medium, followed of the inoculum of 0.2 x 10^6 cells.mL^-1 with the same f medium.

The organic content was slightly superior in the treatments with f/2 medium, specially in the cultures inoculated at 06:00 h. When the cultures are initiated during the sunrise the quantity of daily incident light increases, this improves the growth and production of dry biomass in this microalgae specie. Even more if the cultures are initiated with the higher inoculum in an f medium, although the accumulated growth rate is lower.
Table 2. Dry and organic cellular biomass average and standard deviation to the moment of harvest in the cultures growth at 06:00 h and 12:00 h to inoculums of 0.2 y 0.4 x 10^6 cells.mL⁻¹ in the f and f/2 mediums. Different letters indicate significant differences (two way analysis of variance, α = 0.05) a < b<c.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight (g·m⁻³)</th>
<th>Organic matter (g·m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inoculum: 06:00 h</td>
<td></td>
</tr>
<tr>
<td>0.2 x 10^6 f/2</td>
<td>0.087 ± 0.025 a</td>
<td>0.055 ± 0.003 a</td>
</tr>
<tr>
<td>0.2 x 10^6 f</td>
<td>0.131 ± 0.017 b</td>
<td>0.060 ± 0.029 a</td>
</tr>
<tr>
<td>0.4 x 10^6 f/2</td>
<td>0.085 ± 0.006 a</td>
<td>0.064 ± 0.004 a</td>
</tr>
<tr>
<td>0.4 x 10^6 f</td>
<td>0.186 ± 0.010 c</td>
<td>0.099 ± 0.007 b</td>
</tr>
<tr>
<td></td>
<td>Inoculum: 12:00 h</td>
<td></td>
</tr>
<tr>
<td>0.2 x 10^6 f/2</td>
<td>0.121 ± 0.012 ab</td>
<td>0.069 ± 0.016 a</td>
</tr>
<tr>
<td>0.2 x 10^6 f</td>
<td>0.155 ± 0.033 bc</td>
<td>0.065 ± 0.011 a</td>
</tr>
<tr>
<td>0.4 x 10^6 f/2</td>
<td>0.110 ± 0.003 a</td>
<td>0.066 ± 0.002 a</td>
</tr>
<tr>
<td>0.4 x 10^6 f</td>
<td>0.173 ± 0.023 c</td>
<td>0.099 ± 0.015 b</td>
</tr>
</tbody>
</table>

REFERENCES


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