CULTIVO DE *Dunaliella viridis* (VOLVOCALES: CHLOROPHYCEAE) SOB DIFERENTES REGIMES DE SALINIDADE

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RESUMO

O alimento e a qualidade da água são importantes fatores que afetam o sucesso do cultivo de larvas aquáticas. A introdução de microalgas nos tanques de cultivo pode melhorar a qualidade da água e fornecer alimento para algumas espécies de larvas cultivadas. Entretanto, várias dificuldades são encontradas na seleção de uma microalga adequada, principalmente para organismos oligohalinos. Neste estudo, a microalga *Dunaliella viridis* foi submetida a diferentes regimes de salinidade (35, 30, 25, 20, 15, 10, 5 e 0) com a finalidade de testar a viabilidade da mesma no cultivo de diferentes organismos de água doce e marinhos (oligohalinos). Anteriormente aos experimentos, as cepas algais foram cultivadas em recipientes estéreis contendo meio de cultivo Conway e água marinha com salinidade de 35, e mantidas a 28 ± 2ºC, sob iluminação contínua e uma intensidade luminosa de 1000 Lux (tubos fluorescentes). As cepas de *D. viridis* foram gradualmente aclimatadas as salinidades de 35, 30, 25, 20, 15, 10, 5 e 0. As curvas de crescimento algal foram determinadas em um período experimental de 8 dias. *D. viridis* mostrou um bom crescimento em todos os tratamentos, com densidades celulares variando entre 3,03 x 10^6 células.mL^{-1} (água doce) e 7,33 x 10^6 células.mL^{-1} (salinidade de 15). Estes resultados indicam que *D. viridis* pode ser utilizada para alimentação de diferentes tipos de organismos aquáticos cultivados sob ampla variação de salinidades.

Palavras chave: Cultivo, Microalgas, *Dunaliella viridis*, Salinidade

ABSTRACT

Culture of D*unaliella viridis* (Volvocales: Chlorophyceae) under different salinities

Food and water quality are important factors affecting the success of aquatic larval culture. The introduction of microalgae in the culture tanks may improve the water quality and also supply food for many species of cultured larvae. However, difficulties are found in the selection of an adequate microalgae, mainly for oligohaline organisms. In this study, the microalga *Dunaliella viridis* was submitted to different salinity regimes (35, 30, 25, 20, 15, 10, 5 and 0) to observe the viability of this species for the culture of different fresh water and seawater (oligohaline) organisms. Before the experiments, alga strains were cultured in sterilized flasks containing Conway medium and marine water of salinity of 35, and maintained at 28 ± 2^0 C, under continuous light exposition

and a light intensity of 1000 Lux (day-light fluorescent tubes). The strains of *D. viridis* were then gradually acclimated to the salinities of 35, 30, 25, 20, 15, 10, 5 and 0. Growth curves were obtained from an experimental period of 8 days. *D. viridis* showed rather good growth at all treatments, with cell densities ranging from 3.03 x 10^6 cell.mL^{-1} (fresh water) to 7.33 x 10^6 cell.mL^{-1} (salinity of 15). These results indicate that *D. viridis* could be used for different kind of organisms cultured at a wide range of salinities.

Key words: Culture, Microlgae, *Dunaliella viridis*, Salinity variations

INTRODUCTION

The main problems related to the utilization of microalgae in aquaculture are the scarcity of information about some of their biochemical characteristics and the nutritional requirements of the aquatic organisms (DePauw & Persoone, 1988). The introduction of some microalgae species, as live food in a larviculture tanks, without previous studies on its nutritional composition, may limit the productivity and also affect the growth and survival rates of the larvae (Oliveira *et al.*, 1998).

The culture of microalgae is a very important step integrated to aquaculture technologies for growing marine molluscs, crustaceans and fish (Fabregas *et al*., 1995) and, its production is strongly conditioned by a series of factors like chemical concentrations, irradiance, temperature, salinity and others (Guillard, 1975). The growth of larvae and adults of these organisms depends on the availability of high protein contents obtained from unicellular algae cultures.

On the other hand, microalgae also contribute to the control of the water quality by recycling inorganic nutrients, fixing carbon dioxide and supplying dissolved oxygen to the aquaculture systems by its photosynthetic activities. For example, the microalga *Nannochloropsis* sp. is usually added in the culture tanks of phyllosoma of spiny lobster species, although they are not phytoplankton feeders, to control the water quality (Kittaka, 1994).

In the last decades much attention has been given to the genus *Dunaliella* (Volvocales, Chlorophyta) due some of the intrinsic characteristics (Spectorova *et al*., 1982; Fabregas *et al*., 1995 and references therein). The absence of cellular wall with cells enclosed within a thin elastic protoplasmic membrane improve digestibility and maximize assimilation by the animals (Oliveira *et al*., 1980; Spectorova *et al*., 1982). Besides, this microalga present a high adaptability to extreme conditions and have been also used in the production of chemicals such glycerol, β-carotene and as raw material for the vitamin industry (Spectorova *et al*., 1982; Ben-Amotz & Avron, 1973,1978,1980; Gibbs & Duffus, 1976; Ben-Amotz *et al*., 1982).

Thus, this genus include a group of unicellular species which are widely used for culture of various species of larvae due its high nutritional value and its efficient growth under a large variety of different culture medium (Payne & Rippingale, 2000; Payne *et al*., 1998; Keesing *et al*., 1999).

In the present work, we report the growth of *Dunaliella viridis* in response to a series of salinity regimes (35, 25, 20, 15, 10, 5 and 0). It is suggested that mass culture of the *D. viridis* could improve mass cultures of oligohaline organisms that grow in different salinity concentrations in which literature data are very scarce.

METHODS

The alga selected was a no axenic strain of the Chlorophyta *Dunaliella viridis* Teodoresco obtained from the LABOMAR, Ceará-Brasil (Laboratório de Ciências do Mar).

Before the experiments, all stocks were maintained in 30mL flasks containing Conway medium (Walne, 1974) with seawater at 35 salinity, and maintained in a controlled environment chamber at 28 ± 2°C, under continuous light exposition and a light intensity of 1000 Lux by day-

light fluorescent tubes. Stocks were kept in exponential growth phase and were transferred to a new culture medium every week. Nine days experiments with different salinity regimes were carried out after acclimation of the stocks to respective experimental conditions.

Growth experiments

Growth curves (during a period of 8 days) of *Dunaliella viridis* were determined after acclimation to the salinities of 35, 30, 25, 20, 15, 10, 5 and 0, respectively. Each experiment was conducted under same light intensity and temperature described before for the acclimation of the stocks.

For each treatment, three replicas of 3L were maintained in glass flasks under batch cultures and kept with no forced aeration. Initial culture volumes used to obtain the desired cell concentrations at the beginning of the experiments (2×10^5 cell.mL^{-1}) were calculated according to Barbieri & Ostrensky (2001).

Cell concentrations and growth curves were determined from subsamples collected daily from which, aliquots of 0.0018mL were counted to determine cell densities. For this, the Neubauer chamber was used, counting the number of cells with the help of the binocular optic microscope, and expressing them in average values of cell.mL^{-1}.

The contamination with protozoan organisms was avoided by the use of a Copper Sulphate solution at 0.2g.l^{-1}. After the preparation of this solution, it was added to the culture medium giving 10% of the total culture volume (Klein & Sebastien, 1998).

Statistical analysis

The obtained cell densities were $\log_{10}$ transformed and a factorial analysis was carried out to verify interactions between salinity regime and days of culture.

Experimental design was randomly outlined and variance analysis for the factorial scheme 8 x 9 (8 salinity regimes and 9 count of cells) was applied to a confidence level of 95% according to Sampaio (1998). ANOVA test was used to verify differences between means.

RESULTS AND DISCUSSION

In the present study *Dunaliella viridis* showed active growth from the first to the eighth day of experiments at 35 salinity. Maximal cell density was achieved in the last day with 5.95×10^6 cell.mL^{-1}.

Lag phase was also not observed at 30 and 25 salinities. Maximal cell densities registered for these treatments were 6.03×10^6 (eighth day) and 5.90×10^6 (sixth day), respectively (Figure 1). These results are in agreement with others authors, which reported that *D. viridis* has a wide optimal range of salinities and support extreme salinity conditions (McLachlan, 1964). Mass culture of this species is almost always conditioned to high salinity concentrations giving cell densities that oscillate from 10^6 to 10^8cell.mL^{-1} (Spectorova *et al*., 1982; Fabregas *et al*., 1995; Ginzburg, 1987).

Intermediate salinities showed maximal cell densities of 6.19×10^6 cell.mL^{-1} and 7.33×10^6cell.mL^{-1} at the seventh day for salinities of 20 and 15, respectively (Figure 2). The lower limits of growth experiments with this species are close to these salinities (Spectorova *et al*., 1982), although McLachlan (1964) refers to *D. tertiolecta* as presenting similar good growth rates at salinities raging from 1.4 to 58.

Salinities below to 15 (i.e. salinities from 10 to 0) showed higher maximal cell concentrations at the seventh day for 10 (6.83×10^6 cell.mL^{-1}), 5 (7.32×10^6 cell.mL^{-1}) and 0 (3.03×10^6cell.mL^{-1}), respectively (Figure 3). Although data on the growth rates of *Dunaliella* sp.

under these salinity regimes are very scarce, our results confirm those obtained by McLachlan (1964).

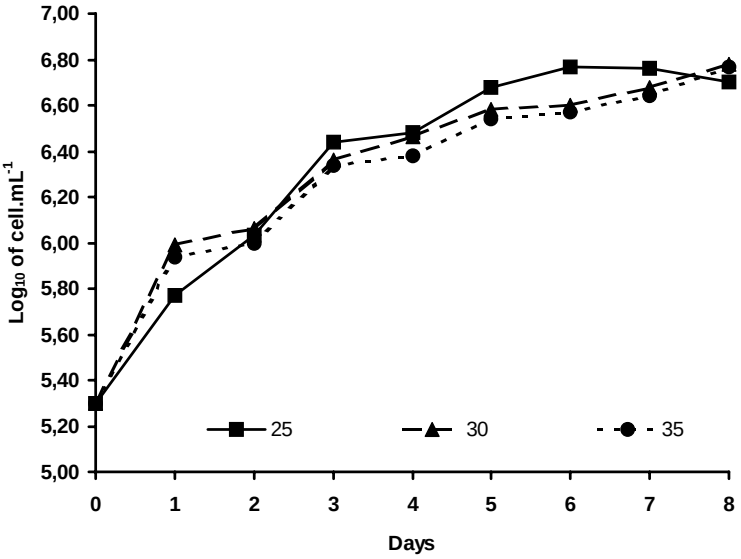

Figure 1. Growth curves of *Dunaliella viridis* under the highest salinity regimes used during the experiments.

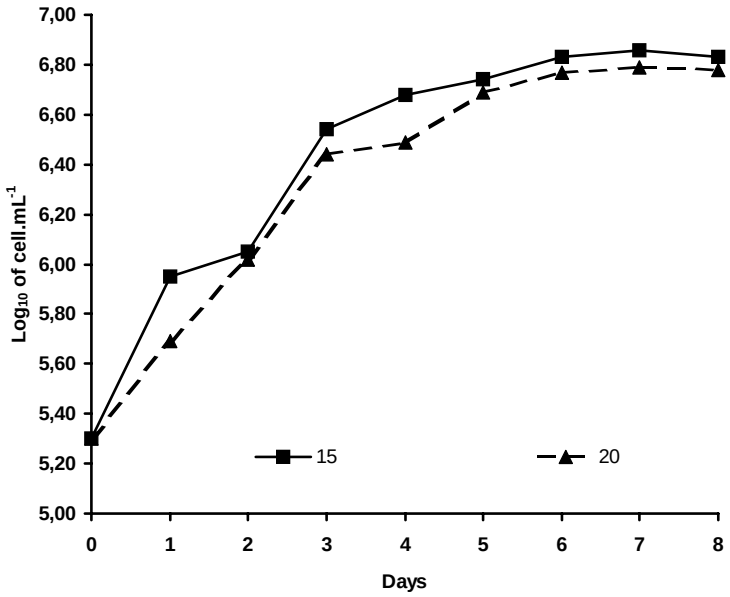

Figure 2. Growth curves of *Dunaliella viridis* under intermediated salinities used during the experiments.

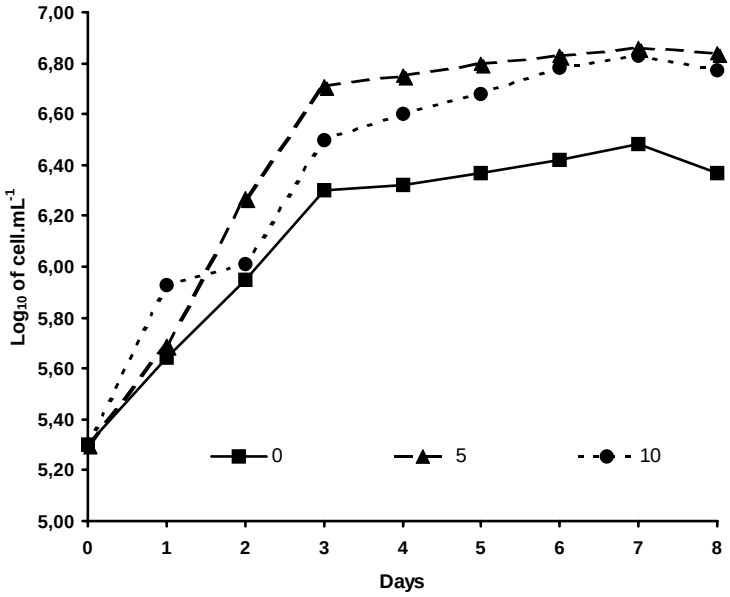

Figure 3. Growth curves of *Dunaliella viridis* under the lowest salinities used during the experiments.

Contamination with protozoan organisms is very common in mass culture of microalga. Our results showed that at low salinity levels (0) contamination with these organisms was more frequent. Additions of copper sulphate to the mass cultures of *D. viridis* controlled and eliminated protozoan contaminants, although the control of contamination of lag phase cultures was more difficult.

Statistical analysis showed that cultures might be harvested at the seventh day for salinity regimes of 20, 15, 10, 5 and 0. Cultures growing at salinities of 35 and 30, and 25 must be harvested at the eighth and sixth days, respectively. Variance analysis and interactions between salinity regimes and days of culture are given in tables 1 and 2, respectively.

Table 1. Variance analysis (ANOVA) of *Dunaliella viridis* growth at different salinity regimes.
• C.V =(√0.0165/1363.44) = (0.128/6.312) = 2.03%

Source of variation	FD	SS	MS	$F_{calculated}$	F-value
Total	215	53.303			
Treatment	71	50.922	0.717	43.376	1.388
Error	144	2.381	0.0165		

Extensive works have been reported about success of larval culture of marine species in which, the species of *Dunaliella* were used in the tanks of seawater. In some of these works, using the calanoid copepod *Gladioferens imparipe*, the microalga *Isochrysis* was the most efficacious diet, followed by *Chaetoceros* and then *Dunaliella* (Payne & Rippingale, 2000). Some marine copepods were also reared and fed on *D. tertiolecta* as source of hightly unsaturated fatty acid (Payne *et al*.,

1998). Culture of *D. tertiolecta* also improved the culture of marine larvae of the starfish *Acanthaster planci* (Keesing *et al*., 1999). However, little works has related about the utilization of the *D. viridis* for marine larvae.

Table 2. Interactions between salinity regimes and days of culture during the experiments.

Sal	Log_{10} of cell.mL^{-1} Cell Counts								
	0	1	2	3	4	5	6	7	8
35	5.30^{a1}	5.94^{b1}	6.00^{b2}	6.34^{c2}	6.38^{c2}	6.54^{c2}	6.57^{d2}	6.64^{d2}	6.77^{d1}
30	5.30^{a1}	5.99^{b1}	6.06^{b2}	6.36^{c2}	6.46^{c2}	6.58^{d2}	6.60^{d2}	6.68^{d1}	6.78^{d1}
25	5.30^{a1}	5.77^{b2}	6.03^{c2}	6.44^{d2}	6.48^{d2}	6.68^{e1}	6.77^{e1}	6.76^{e1}	6.70^{e1}
20	5.30^{a1}	5.69^{b2}	6.02^{c2}	6.44^{d2}	6.49^{d2}	6.69^{e1}	6.77^{e1}	6.79^{e1}	6.78^{e1}
15	5.30^{a1}	5.95^{b1}	6.05^{b2}	6.54^{c1}	6.68^{c1}	6.74^{c1}	6.83^{d1}	6.86^{d1}	6.83^{d1}
10	5.30^{a1}	5.93^{b1}	6.01^{b1}	6.50^{c2}	6.60^{c1}	6.68^{c1}	6.78^{d1}	6.83^{d1}	6.77^{d1}
5	5.30^{a1}	5.69^{b2}	6.27^{c1}	6.71^{d1}	6.75^{d1}	6.80^{d1}	6.83^{d1}	6.86^{d1}	6.84^{d1}
0	5.30^{a1}	5.64^{b2}	5.95^{c3}	6.30^{d2}	6.32^{d2}	6.37^{d3}	6.42^{d3}	6.48^{d2}	6.37^{d2}

*The same letters within lines and numbers within columns means no statistical differences.

Our work pointed out the potential of this *Dunaliella viridis* (chlorophyceae) as live food for species cultured under low levels of salinity at aquaculture plants. However, we think that biochemical studies are also important to determine the carbohydrate and protein contents of *D. viridis* at these low salinity regimes to prove its viability as nutritive food source under these conditions.

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